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

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EXECUTIVE SUMMARY

From the mid-1980s to the present, the Department of Energy (DOE) has developed, tested, and deployed diverse bioremediation strategies for chlorinated volatile organic compounds (cVOCs). A systematic review of these projects after decades of activity provides an opportunity to identify crosscutting themes and lessons learned. The knowledge provided by a DOE bioremediation retrospective represents a resource to support current and future bioremediation operations, and future decisions related to cVOC bioremediation. This systematic review examined the design, objectives, performance and outcomes for remediation projects at DOE sites including Savannah River, Hanford, Idaho, Mound and Pinellas. The results were used to identify emergent themes to provide actionable insights. The bioremediation retrospective technical team first developed standardized criteria to support the systematic review. Then, the evaluation was performed using a sequential process that was informed by local technical experts who identified and provided the structured information that served as the basis for the evaluation. The participation of these experts was invaluable to the effort.

Importantly, DOE cVOC bioremediation efforts were implemented based on the foundational knowledge developed by U.S. Department of Defense (DoD) strategic and applied environmental technology development and certification programs, as well as technical, policy and regulatory guidance from the U.S. Environmental Protection Agency (EPA), Interstate Technology and Regulatory Council (ITRC), U.S. Geological Survey (USGS), industry, and universities. To maximize the value of the DOE cVOC bioremediation retrospective, the systematic review strategy focused on identifying important DOE-specific experiences, trends and lessons learned that would extend the knowledge available from these other key entities.

Overarching themes that were identified during the DOE cVOC bioremediation retrospective include:

1) The most successful DOE cVOC bioremediation efforts focused on transitioning the site from active treatment to passive attenuation-based remedies using Enhanced Attenuation (EA). DOE provided technical support in developing and implementing ITRC Technical and Regulatory Guidance for EA protocols in collaboration with state and federal regulators, other federal agencies, industry, and universities. A key product of that collaboration was a technical and regulatory guidance document from the Interstate Technology and Regulatory Council (ITRC). EA provides a roadmap and bridge to move from active to passive remediation. The sites that were designed and deployed using the ITRC guidance documents have performed well.
2) Designs that created structured geochemical zones to combine anaerobic and aerobic bioremediation processes provided significant benefits at several DOE case study sites – such designs leverage the complementary strengths of anaerobic biological processes to rapidly degrade parent cVOCs and aerobic biological processes to degrade daughter cVOCs, minimizing the buildup of toxic daughter products.
3) Detailed monitoring, including multiple lines of evidence, has proven effective in implementing and documenting cVOC bioremediation projects. Key performance metrics, use of DNA and other molecular biological tools (MBTs), have been particularly useful at some case study sites – demonstrating the robustness of the subsurface microbial community and their response and adaption to bioremediation amendments and bioremediation progress. Notably, the MBTs suggest that bioaugmentation (additional of supplemental and/or specialized bacteria) may not be needed at most sites. Collecting baseline and periodic data on the microbial ecology is recommended for current and future bioremediation projects.
4) Underperformance of bioremediation projects generally resulted from either poor access and delivery of amendments or from unfavorable biogeochemical conditions. Poor access and delivery were associated with physical limitations such as low permeability or well spacing that was too far apart. Innovative access techniques matched to site conditions, such as use of horizontal wells, proved beneficial at some sites. An example of unfavorable biogeochemical

conditions was use of air sparging to stimulate aerobic biodegradation in a bulk anaerobic setting – in this example, the air travelled upward through preferential pathways and did not adequately aerate the target aquifer zone resulting in limited degradation and plume spreading.
5) The most successful projects considered the balance of the direct beneficial bioremediation impacts (e.g., degradation of contaminant cVOCs) versus potential adverse collateral impacts such as generating a large area of unusable / unpalatable groundwater.
6) Over time there is a trend toward sustainability in performance, consistent with EA and structured geochemical zone strategies and designs. A notable example of this is a shift toward longer lived electron donors such as vegetable oils versus the lactate and similar reagent compounds used in early bioremediation projects.
7) Successful bioremediations were performed under both CERCLA and RCRA – most were part of a combined remedy that included other actions such as pump and treat and proposed/future monitored natural attenuation (MNA). In aerobic oligotrophic aquifers (groundwater systems with low biomass), MNA rates for the most common cVOCs are relatively slow with half lives in the range of 50 to 100 years – additional studies may be needed to confirm and refine attenuation rates.
8) Several of the overarching themes identified in the cVOC bioremediation retrospective, particularly those related to EA, structured geochemical zones, and performance metrics based on multiple lines of evidence are somewhat universal/portable/durable and would apply to bioremediation and other in situ remediation strategies at sites contaminated with various organic contaminants, inorganic contaminants, radionuclides, or comingled contaminants.

More specific technical findings from the retrospective are provided in the lessons learned section of the report and are briefly summarized below:

For Anaerobic Bioremediation

- Design Basis and Site Conditions
  - Most anaerobic bioremediation pilot and full-scale projects were designed as active bioremediations.
  - There are emerging opportunities in DOE to transition some of the full-scale anaerobic remediation projects to EA – particularly those that have used long lived electron donors. This transition can be performed by restructuring the metrics/monitoring and working with regulators and stakeholders to formally revise the governing record(s) of decision.
  - Anaerobic remediation projects have proven to be robust and relatively effective under a wide range of baseline hydrogeologic and biogeochemical conditions.
  - When deployed in an aerobic system, anaerobic bioremediation results in a shift in redox status and an associated degradation of overall groundwater quality that limits beneficial use.
- Biostimulation and Bioaugmentation
  - All tested and deployed electron donors have been shown to be effective in supporting bioremediation.
  - Over time the preferred electron donors have shifted from pure or blended reagents such as lactate, alcohols, benzoate and similar chemicals to lower cost materials such as industrial byproducts (e.g., whey) and vegetable oils. Vegetable oil amendments provide a longer performance period. In some cases, a single injection has supported effective remediation performance for ten or more years, based on cVOC concentration trends and characterization of the structure and function of the subsurface microbial community.

- Two case studies effectively deployed neat (pure) vegetable oil in the lower portion of the vadose zone to form a shield at the water table interface. This supplemental electron donor deployment strategy was developed by DOE and represents a cost-effective beneficial action.
- The evidence from the systematic review regarding the need for bioaugmentation to support anaerobic designs is equivocal. Bioaugmentation should be considered only if there is site specific data or evidence that the microbial ecology at the site is deficient in a key capability or if testing indicates that the site will experience a significant lag in the initiation of bioremediation under site-specific field conditions.

<u>For Aerobic Bioremediation</u>

- Design Basis and Site Conditions
  - DOE aerobic bioremediation pilot and full-scale projects were designed as active bioremediations.
  - DOE aerobic bioremediation projects exhibited highly variable levels of success. Compared to anaerobic bioremediation, aerobic remediation for cVOCs was found to be less robust and resilient and successful projects were limited to a narrower range of environmental conditions. For aerobic bioremediation of cVOC, cometabolism is a primary mechanism for moderately chlorinated molecules like TCE and chloroform. Less chlorinated cVOCs such as DCE and VC are subject to more efficient and more rapid direct aerobic metabolism.
  - Most of the DOE aerobic remediation projects were performed using biosparging.
  - Due to the variable performance of aerobic cVOC bioremediation, recent projects that incorporated aerobic processes into the bioremediation design were performed as combined anaerobic and aerobic structured geochemical zones.

- Biostimulation and Bioaugmentation
  - Air was used as the electron acceptor in all the documented aerobic cVOC bioremediation projects.
  - Methane was used as the cometabolite for all the DOE aerobic cVOC bioremediation projects.
  - Gas phase nutrients (ammonia and triethylphosphte) were used for the SRS aerobic cVOC bioremediation project. The phosphorus addition process was patented by DOE and licensed to industry for commercial use in bioventing, biosparging and bioremediation.

<u>For Combined Anaerobic-Aerobic Bioremediation – Structured Geochemical Zones</u>

- Design Basis and Site Conditions
  - DOE exemplars of combined anaerobic-aerobic bioremediation strategies were based on the EA design paradigm as specified in the ITRC technical and regulatory guidance.
  - Monitoring the numbers, structure and function of the subsurface microbial community has been integral to the success of the combined anaerobic-aerobic remediation projects.
  - The use of structured geochemical zones provided optimal conditions for rapid degradation of parent cVOCs in the anaerobic areas and less chlorinated daughter cVOCs in the adjacent aerobic areas.
  - Structured geochemical zones minimize the accumulation of DCE and VC and minimized the volume of the aquifer that was impacted by collateral water quality impacts, reduced costs and remediation time, and sites where this strategy was implemented can be immediately and efficiently transitioned to a passive attenuation upon completion.
- Biostimulation and Bioaugmentation
  - Electron donors with higher longevity such as emulsified and neat vegetable oils were used for creating the structured geochemical zones.

- No added electron acceptors were used between the anaerobic treatment zones.

The portfolio of cVOC bioremediation projects and project experience in DOE has yielded significant success. Two sites have formally transitioned to a passive EA remedy from pump and treat – discontinuing expensive active treatment and now moving rapidly toward closure with no further action. All wells and all constituents at both EA sites are near or below MCLs. Some of the full-scale anaerobic bioremediations are also approaching ROD reviews where they may be able to formally transition to a passive EA remedy. Within these projects DOE has developed, tested, and deployed several innovative technologies, some of which are patented and licensed. Finally, DOE led the collaboration to develop technical guidance for implementing EA for cVOCs. This 30+ year period of DOE cVOC bioremediation experience as summarized in this systematic review can serve to support DOE managers/decision makers as well as contractors in their project management, project design and field operations responsibilities for current and future cVOC bioremediation.

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AED	alternate electron donor
ACL	alternate concentration limit
ARARS	applicable or relevant and appropriate requirements
ARD	anaerobic reductive dechlorination
Bgs	below ground surface
CAB	community advisory board
CAGW	C-Area ground water
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
COC	contaminant of concern
CSM	conceptual cite model
CT	carbon tetrachloride
cVOC	chlorinated volatile organic carbon
DCE	Dichloroethane
DNAPL	dense non-aqueous phase liquid
DO	dissolved oxygen
DOE	Department of Energy
DoD	Department of Defense
DHM	*Dehalococcoides mccartyi*
DWPF	Defense Waste Processing Facility
EA	enhanced attenuation
EACO	Enhanced Attenuation Chlorinated Organics
EM	Environmental Management
EMCBC	Environmental Management Consolidate Business Center
EPA	Environment Protection Agency
ESD	Explanation of Significant Differences
EVO	emulsified vegetable oil
EZVI	emulsified zero-valent iron
Gw	Groundwater
HWMF	Hazardous Waste Management Facility
IC	institutional controls
ICP	Idaho Cleanup Project
INL	Idaho National Laboratory
ISB	In situ bioremediation
ITRC	Interstate Technology and Regulatory Council

KDEP	Kentucky Department of Environmental Protection
LM	Legacy Management
LLAZ	Lost Lake Aquifer Zone
LOE	line of evidence
MBTs	molecular biological Tools
MCLs	maximum contaminant levels
MLE	multiple lines of evidence
MNA	monitored natural attenuation
NPTF	New Pump and Treat Facility
NO_3	Nitrate
O_2	Oxygen
ORC	oxygen release compound
ORP	oxygen reducing potential
OU	Operable Unit
P&T	pump and treat
PCE	Perchloroethylene
PNNL	Pacific Northwest National Laboratory
POC	point of compliance
POE	point of entry
PRB	permeable reactive barrier
qPCR	quantitative polymerase chain reaction
RAO	Remedial Action Objective
RCRA	Resource Conservation and Recovery Act
RGA	regional gravel aquifer
ROD	Record of Decision
SCDES	South Carolina Department of Environmental Services
SCDHEC	South Carolina Department of Health and Environmental Control
SLF	sanitary landfill
SRPA	Snake River Plain Aquifer
SRS	Savannah River Site
SVE	soil vapor extraction
SWMU	solid waste management unit
TAN	Test Area North
TCE	Trichloroethylene
TD	Technology Development
TOC	total organic carbon

TNX	Technical Nuclear Explosives
TSF	Technical Support Facility
USGS	United States Geological Survey
VC	vinyl chloride
VFA	volatile fatty acids
ZVI	zero valent iron

1.0 Problem Statement

Department of Energy (DOE) Office of Environmental Management (EM) has responsibilities to manage and remediate complex groundwater plumes across the nation with contaminants that include a broad range of chemical and radiological contaminants. Notably, chlorinated volatile organic compounds (cVOCs) are a principal contaminant in many of the groundwater plumes. A variety of in situ bioremediation technologies have been tested and deployed to address DOE cVOC plumes since the mid-1908s. Bioremediation involves the use of naturally occurring microbiota/bacteria or introduction of specific bacteria species to biodegrade/transform contaminants into less toxic or more stable substance. This retrospective evaluation will focus exclusively on the application and performance of bioremediation for cVOCs and critical aspects of project development: design principles; electron donors and nutrients; bioaugmentation; subsurface access and deployment; and monitoring performance and metrics. The final report will provide an overview of cVOC bioremediation across DOE EM and DOE Legacy Management (LM) sites based on case studies from EM/LM sites. The specific case studies will be consolidated to inform a more comprehensive picture of the complexities involved in successful bioremediation.

2.0 Objectives

DOE EM, in collaboration with their field sites, have pursued numerous bioremediation projects throughout the last three to four decades of environmental cleanup. Success of these projects has varied widely, depending on the contaminant, hydrogeologic conditions, available technologies, and state of the science. EM's Office of Subsurface Closure aims to draw on the wealth of bioremediation experience across the EM complex to conduct a historical retrospective that can be used to inform future bioremediation efforts. This review of lessons learned dovetails with current DOE bioeconomy initiatives.

3.0 Introduction

Chlorinated solvents were used extensively as degreasers beginning in the 1940s and 1950s to support the U.S. Cold War mission. The accepted disposal path for these organic compounds often included deposition on porous soils or into unlined basins. As a result, unintended releases were common during this time. These factors lead to widespread organic contamination, both in soil and groundwater, across DOE sites, as well as Department of Defense (DoD) military and industrial locations.

In aerobic groundwater (typically dissolved oxygen content > 1 mg/L), chlorinated solvent plumes attenuate (reduce in concentration) slowly and the plumes can expand over large areas with large portions of the plumes having relatively low contaminant concentrations ("large and dilute plumes"). This can lead to high costs and difficulties in effectively treating the plume because of the high volumes of contaminated water and large areal footprint. Further, source solvent can migrate as a separate phase and secondary source zones (areas where residual solvent accumulates and dissolves slowly) are common even after the primary source mass is remediated. Bioremediation strategies can be applied in situ or ex situ and under aerobic (in the presence of oxygen) or anaerobic (very little or no oxygen present) conditions. Often the bioremediation of cVOCs relies on addition of an electron donor and the resulting anaerobic processes (e.g., reductive dechlorination) due to higher degradation rates of the typical solvents (e.g., perchloroethylene (PCE) and trichloroethylene (TCE)) – however this strategy can lead to the buildup of hazardous daughter products such as vinyl chloride (VC) in some situations. Complete anaerobic dechlorination of TCE and PCE results in the generation of ethylene, a non-toxic compound, in place of VC which is a known carcinogen and more toxic than TCE or PCE. A combined anaerobic/aerobic strategy (structured geochemical zones) has been used to better control and balance the rate of parent and daughter destruction rates. For the large and dilute portions of plumes in aerobic settings, enhancing aerobic co-metabolism is a primary method that has been considered for bioremediation.

Several treatment technologies are effective for chlorinated solvent remediation, but site conditions play an important role in the efficacy of each approach and must be carefully considered during the selection and implementation processes. Bioremediation approaches are often favored because they utilize natural processes to degrade chlorinated hydrocarbons and are typically considered more of a passive approach to cleanup. These approaches make use of microorganisms capable of contaminant breakdown in the subsurface through use or transformation of the contaminants. This saves time and costs over more active methods, minimizes maintenance activities, as well as limits the environmental impact of cleanup operations.

4.0 Technical Approach

4.1 Overview

This DOE cVOC bioremediation retrospective is structured as a formal systematic review. A systematic review examines the performance and outcomes of multiple separate projects/sites and then consolidates the information to provide actionable insights. A systematic analysis organizes the disparate information using various prespecified criteria and then evaluates the structured information to identify relationships, trends, and lessons learned. Several crosscutting categories and/or criteria provide a framework for interpreting the historical experiences with cVOC bioremediation across the DOE complex. The structure for the DOE cVOC bioremediation retrospective is summarized in Figure 4-1. The overarching evaluation categories include a) the remediation design strategy for the bioremediation, and b) the target/predominant biogeochemical (redox) conditions. As shown in Figure 4-1, the three bins for design strategies are active bioremediation, enhanced attenuation, and monitored natural attenuation. The three bins for redox conditions are anaerobic, aerobic and combined anaerobic-aerobic (structured geochemical zones). Note that these overarching categories can occur in any combination. For example, an active bioremediation project can be performed using either anaerobic or aerobic conditions. In addition to the overarching evaluation categories, the systematic review also considers site specific modifying factors and deployment conditions (e.g., lithology and hydrogeology) and key details of the deployment (e.g., cVOC concentrations, amendments used, subsurface access methods, size/scale, performance metrics and monitoring strategy, as well as regulatory objectives and assessment(s) of success). Some of these modifying factors change depending on the overarching bins. For example, an anaerobic bioremediation would typically include and electron donor (amendment while an aerobic bioremediation might use an electron acceptor amendment). The various design strategies, redox conditions, modifying factors and deployment details are further defined and described in more detail below. These definitions and descriptions were used to organize and interpret the information collected from the cVOC projects performed at various DOE sites across the nation.

Following development of the systematic review structure, the retrospective evaluation was performed using a sequential process. The first step was to collect the information needed to support the review. For each DOE site where a significant cVOC bioremediation project had been performed the team identified and contacted local technical expert(s)/representative(s) and provided a template for them to use to prepare for a short (e.g., 1 hour) interview (Appendix A). The template and interview were organized to rapidly and efficiently elicit the information needed to support the systematic review. The interviews were extensively documented (Appendix B-Appendix G). The information was then summarized in table form to facilitate the cross-site comparisons and development of lessons learned. The resulting information on factors, performance, and trends will assist in future EM decision making and aid DOE sites who are considering bioremediation to optimize efficiency and performance as they develop their strategies and designs.

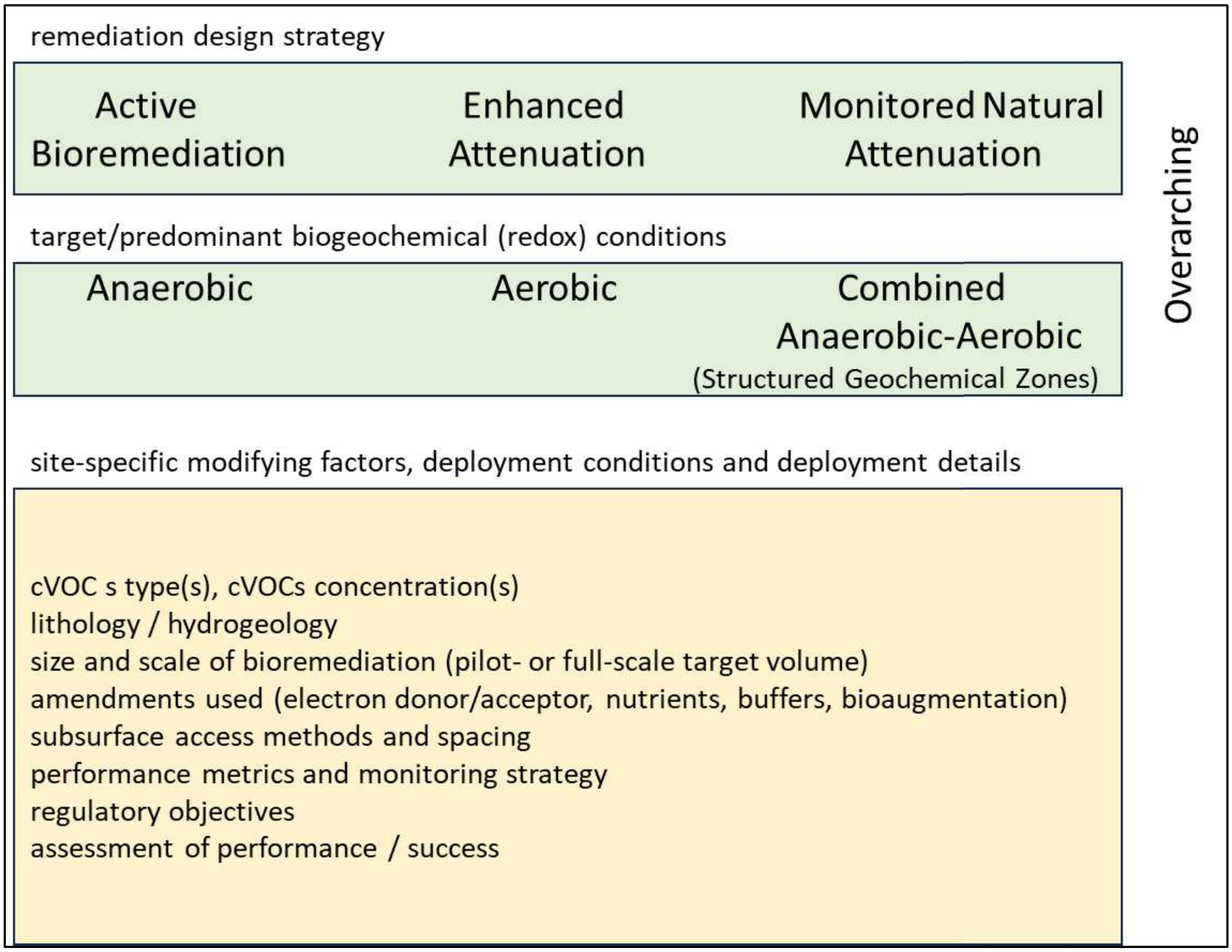

Figure 4-1. Simplified Structure of Categories used for DOE Systematic Review of cVOC Bioremediation.

4.2 Remediation Design Strategy – Design Basis

In Situ Bioremediation (ISB) is a soil and groundwater remediation approach that makes use of subsurface microbial processes to beneficially mineralize or transform contaminants, The ultimate goal for any bioremediation is reduction or elimination of contaminants and contaminant-related toxicity and risk. For purposes of the systematic review, the historical cVOC bioremediation activities in DOE have been divided into the following three bins – active bioremediation, enhanced attenuation, and monitored natural attenuation. These bioremediation bins represent a continuum that transitions from more active toward passive strategies, respectively. When deciding between active bioremediation and enhanced attenuation (which is also an active engineered process), the determining factors are the expected longevity of effectiveness, how the system will be monitored and managed, and how the bioremediation will support the regulatory process and cleanup objectives. The following sections provide a synopsis and brief description of each of the Remediation Design Strategy categories. In this and the other background sections, the descriptions rely heavily on definitions – these definitions are highlighted in boxes in the respective sections. For each project/site and for each category, the definitions were used as the basis for assigning a bin to support the systematic review.

4.2.1 Active Bioremediation

Active Bioremediation for cVOCs – An engineered treatment that uses biostimulation or/and bioaugmentation to support microbial processes to destroy cVOCs, reduce risk and accelerate progress toward remedial goals.

In situ active bioremediation for cVOCs relies on the addition of key amendments (biostimulation) or/and microorganisms (bioaugmentation) to accelerate and support bioremediation objectives. The key design basis differentiator is the expected longevity of the impacts in the subsurface and the expectation that additional amendments may be needed. The design does not focus on sustainably transforming the site but rather views the subsurface as a biogeochemical reactor that is actively managed using a traditional chemical engineering paradigm. In this paradigm, the ongoing monitoring is focused on when and how much additional amendments are needed. Active bioremediation can utilize a wide range of amendments, including those with shorter effective lifespans in the subsurface following deployment -- any amendment can be deployed as part of an active bioremediation. A typical timeframe for active bioremediation is 1 to 10 years.

4.2.2 Enhanced Attenuation

Enhanced Attenuation – 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 the remedial objectives are met in a reasonable timeframe.

Note that DOE led in developing the principles of enhanced attenuation (EA) and developed the technical basis for this design strategy – in collaboration with scientists from multiple Federal Agencies, various university, industry and with an interagency and State/Federal regulators. DOE supported the Interstate Technology and Regulatory Council (ITRC) as they developed clear and comprehensive technical guidance through the “Enhanced Attenuation Chlorinated Organics (EACO)” team (Figure 4-2). Because of DOE’s leadership role in developing enhanced attenuation for cVOCs, more detail and information is provided for this design strategy compared to active bioremediation and monitored natural attenuation. The added detail in this description is intended to serve as a resource to DOE EM managers to aid in communicating to Congress, the Executive Branch and to the public information about DOE’s contributions to the science, application and success of enhanced attenuation for cVOCs.

Selection of enhanced attenuation is based on a mass flux analysis to best assess how to alter the relationship between cVOC source strength and the attenuation capacity within the plume. EA relies on sustainable enhancements that are designed to be an effective bridge between active treatment and a monitored natural attenuation (MNA) remedy (Figure 4-2). EA uses active-engineered bioremediation actions to augment naturally occurring attenuation in the subsurface. The design basis for EA applications is different from conventional active bioremediation because EA is built on the principles of sustainability and mass balance – beneficially altering the relationship between mass loading from the source area(s) and the rate of mass attenuation (attenuation capacity) in the plume. Figure 4-3depicts the underlying mass balance conceptual model for cVOC plumes – in this paradigm, the cVOC attenuation processes (primarily biological degradation) are the engineering controls that are modified to meet a design goal of reducing flux and concentration and the associated risks as the contaminants flow through the subsurface. The mass balance relationship defines the emergent plume behavior (i.e., expanding, stable or shrinking).

Providing sustainable conditions for a stable or shrinking plume based on a mass balance evaluation is the fundamental design basis and outcome of a successful EA. Sustainability is defined as the ability of a groundwater system to maintain the attenuation mechanisms that destroy or immobilize contaminants for a sufficient time and rate to meet remedial objectives until the source mass flux is depleted. When properly designed, EA shifts the site conditions to meet the requirements of MNA. Sustainability is affected by the rate at which the contaminants are transferred from the source area and whether the protective processes are robust and renewed due to the long-term resilience of the microbial community. The core requirement of sustainability, i.e., shifting the site in a technically defensible, enduring and sustainable manner, typically requires use of longer lasting amendments and more focus on the structure and function of the subsurface microbial ecosystem. A typical performance timeframe for EA is 5 to 30 years.

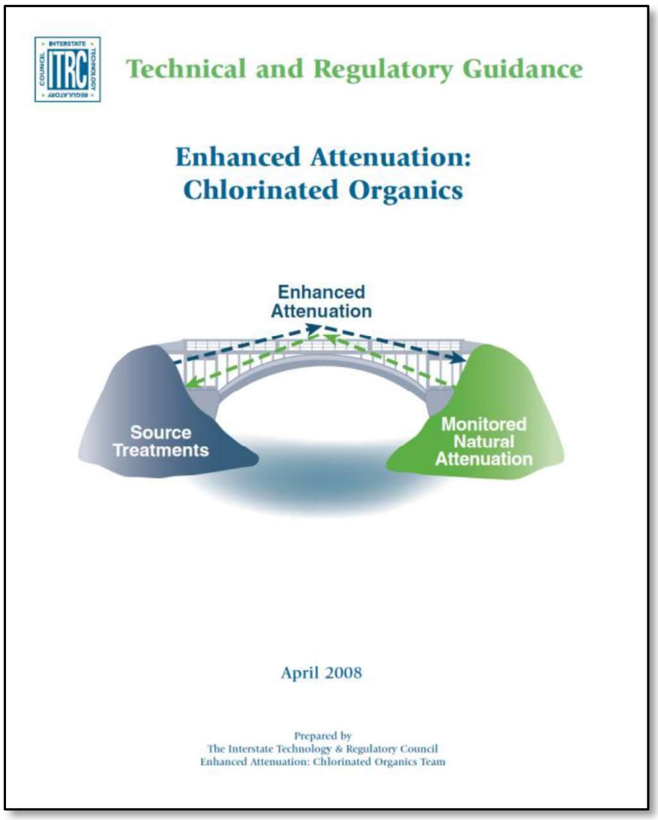

Figure 4-2. ITRC Technical Guidance on Enhanced Attenuation for Chlorinated Organics -- Enhanced attenuation provides a “bridge” between active treatment and MNA.

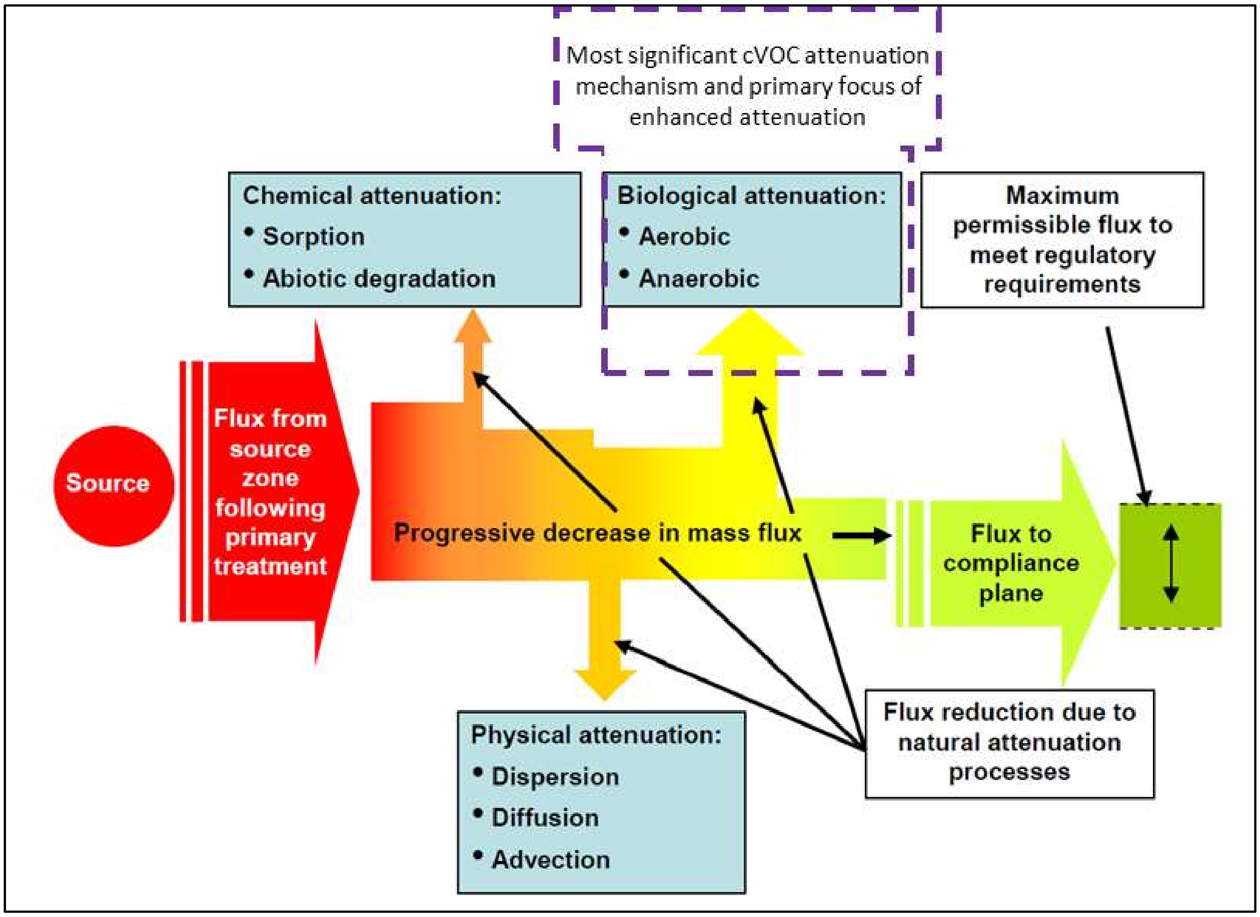

Figure 4-3. Natural Attenuation Mass Balance Paradigm (ITRC, 2008)**.**

To facilitate implementation of EA for cVOCs, the ITRC developed technical and regulatory guidance that is built around an implementation flowchart. The flowchart provides an important roadmap for decision making (Figure 4-4 and Figure 4-5). This flowchart was based on existing regulatory guidance documents and protocols. The initial efforts at a contaminated site (blue boxes I and II in Figure 4-4 and Figure 4-5) represent the initial discovery, characterization, source treatment, and active remediation. These activities result in a range of characterization data as well as decision-making information related to risk, technology performance, treatment time, and treatment cost (green circles). These criteria, in turn, are inputs to a series of questions related to the viability of MNA (yellow diamonds). This portion of the process encourages implementation of MNA according to the existing regulatory protocols with added emphasis on mass balance-based assessment of plume stability and with documentation of treatment sustainability. This ITRC specified sustainability requirement, represents an additional level of documentation and rigor compared to prior MNA protocols.

As a site is approaching MNA but does not meet the requirements of MNA, (i.e., it does not pass the yellow diamond gantlet of requirements), the decision flowchart provides an additional potential option of EA (orange assessment-implementation process). The EA path provides specific requirements to be considered in evaluating the mass balance to optimize long-term plume stability/reduction (shrinking) and in selecting and designing an EA treatment. In this case, the scientist/engineer determines whether there is a sustainable action that will modify the risk, plume stability, or remediation time frame and allow for implementation of that action. The types of enhancement evaluated, and the objectives of the enhancements are developed based on the specific issues identified in the MNA questions. For example, if the remediation time frame is determined to be too long, then enhancements that increase degradation rates will be identified; if conditions are not sustainable then enhancements to further sustain the attenuation process will be identified and evaluated. If enhancements are not viable, then traditional treatment continues. If enhancement is viable and has the potential to be more effective than the current treatment, then it is implemented and monitored to document that the desired change was achieved so that the site can transition to MNA or to identify that the desired change was not achieved such that further enhanced treatment is required. The ITRC (2008) technical guidance document provides detailed descriptions and documentation for each step in the flowchart process.

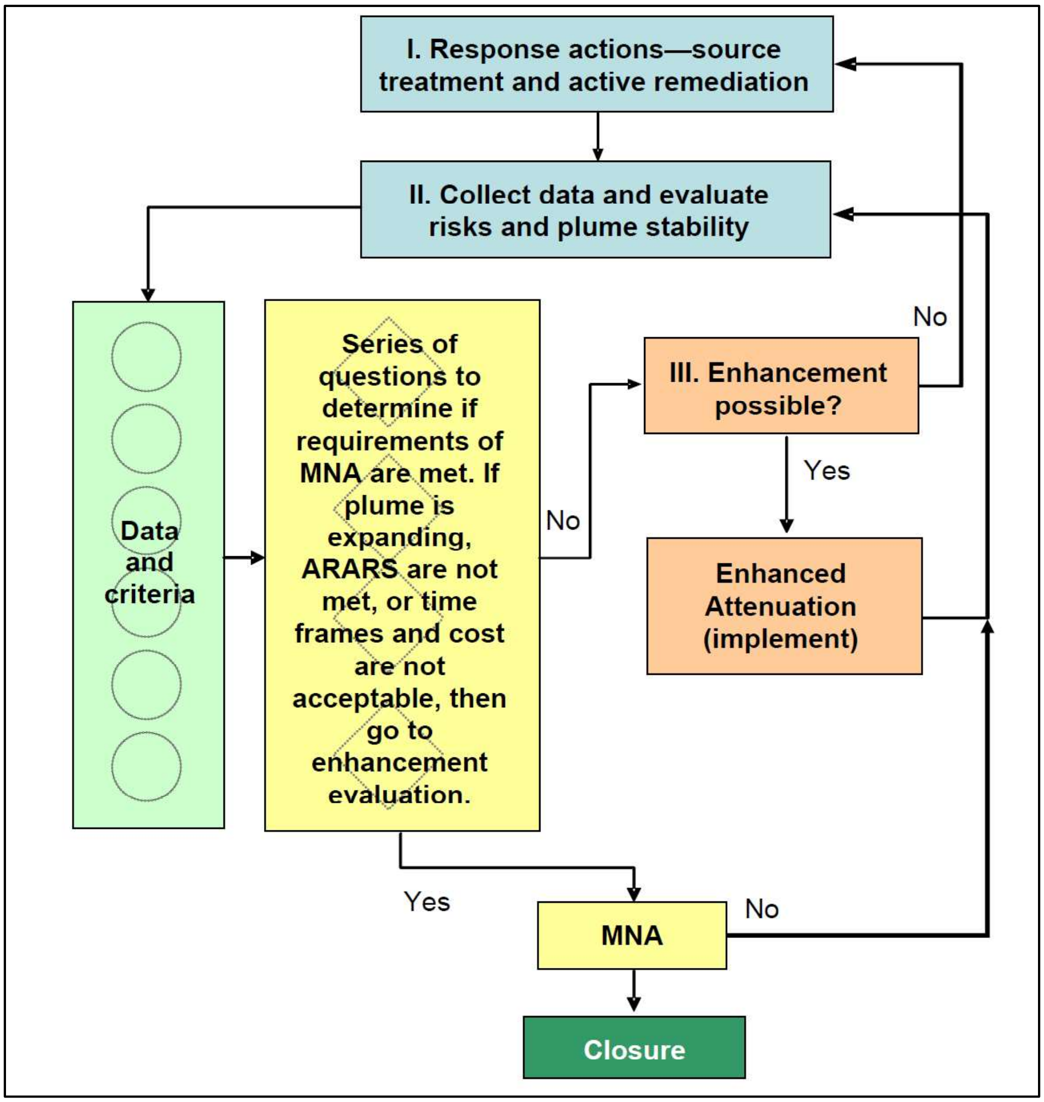

Figure 4-4. General Structure of ITRC MNA/EA Decision Flowchart (ITRC, 2008)**.**

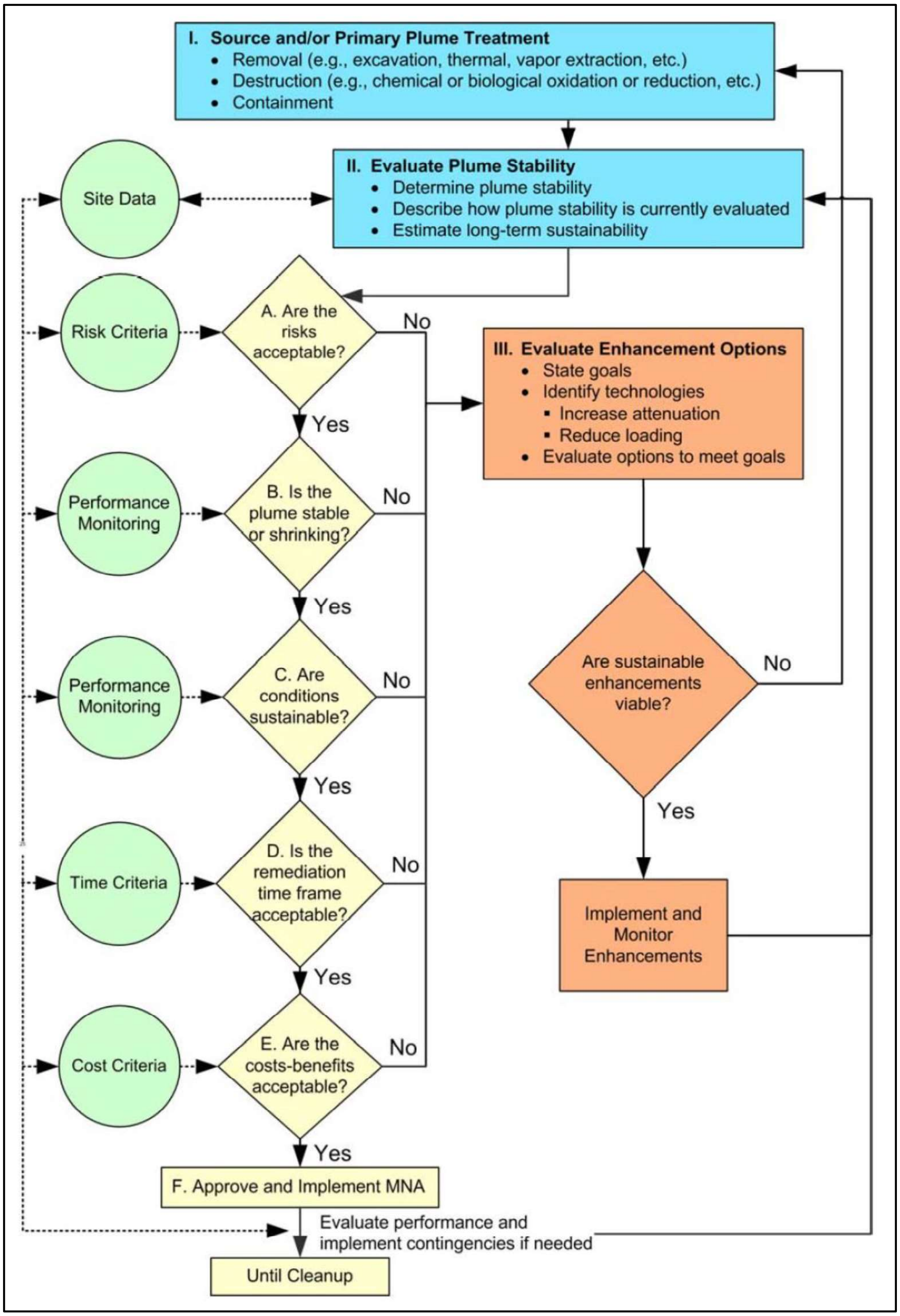

Figure 4-5. Expanded ITRC MNA/EA Decision Flowchart (ITRC, 2008)**.**

4.2.3 Monitored Natural Attenuation

MNA is an important environmental management strategy that recognizes the effects of natural mechanisms in the subsurface which stabilize or shrink a contaminant plume. During the past 30 years, MNA for chlorinated organics has advanced rapidly, supported by improved scientific information and clear policy developments. EPA formally recognized the use of natural attenuation for chlorinated solvents and the use of the term "MNA" with issuance of two documents, a protocol (EPA, 1998) and a directive (EPA, 1999). These encouraged the use of MNA, in combination with other actions, to achieve remediation goals. According to EPA (1999), the processes that contribute to MNA include "a variety of physical, chemical, or biological processes that under favorable conditions, act without human intervention to reduce mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater." EPA guidance for MNA typically relies on multiple lines of evidence (MLE). In summary, the requirements for environmental strategies that rely on natural attenuation include the following:

- documenting that the plume poses minimal risk
- documenting that the plume is stable or shrinking
- monitoring to ensure environmental protection
- triggers to implement contingency plans as needed

MNA is a remedial strategy that specifies no human intervention. It has been described as "watchful waiting"; however, MNA remedies are intended to move toward remediation goals that minimize risks at an acceptable rate. There are a variety of issues and challenges to broader implementation of MNA of cVOCs, including:

- limited understanding of site-specific natural attenuation processes
- limited characterization, including site-specific geochemical conditions
- unreasonably long remediation time frames
- insufficient natural attenuation rates relative to the mass loading entering the plume from the source area(s)
- inability to collect information due to ongoing operations of an active remedy such as pump-and-treat (note that this challenge is a primary basis for the development of EA as a technically defensible bridge between active and passive remedies).

A typical timeframe for MNA is 10 to 50+ years. In cases where natural attenuation mechanisms are not sufficient to achieve remediation goals— because of risk/exposure to receptors, plume growth, or long time-frames — additional actions are required. Targeted approaches are necessary to overcome the conditions(s) that cause MNA alone to be inadequate for site remediation. Such actions can include active treatments or EA.

4.3 Target-Predominant Biogeochemical (Redox) Condition

To support specific bioremediation objectives, the system needs to meet target redox conditions and associated biogeochemistry (e.g., pH, macronutrients and micronutrients). In particular, the redox state serves as a primary master variable that controls the types and rates for microbial degradation of cVOCs. The range of relative redox potentials necessary for reduction of chlorinated compounds to occur varies depending on the type of contaminant. Further, the presence of comingled contaminants can play a role in determinations related to stimulating or inhibiting the targeted contaminant breakdown. The presence and concentration of competing electron acceptors and donors may also impact performance. Other baseline conditions like pH and temperature can also impact microbial activity and community growth. The three overarching redox categories are anaerobic, aerobic, and combined anaerobic-aerobic (structured geochemical zones). The first two redox categories are straightforward and can be described using traditional definitions.

4.3.1 Anaerobic

Conditions for growth or metabolism whereby microorganisms use an element other than oxygen as an electron acceptor. These alternate electron acceptors include nitrate, sulfate, iron, carbon dioxide, and (importantly) other organic compounds (including cVOC contaminants). Anaerobic processes have been a primary focus of cVOC attenuation research since the early 1990s because the rates for anaerobic degradation processes for typical industrial cVOCs (e.g., PCE, TCE, CT and chloroform) are typically faster that the degradation rates for aerobic processes (e.g., nominal half-life of <1 to 10 years versus decades). However, the cVOC degradation rates typically decrease for daughter compounds that contain fewer chlorine atoms. To encourage anaerobic conditions and enhance degradation rates, the redox condition at a site can be manipulated by addition of an electron donor amendment. The primary microbial-enzymatic pathway for anaerobic degradation is reductive dichlorination by which chlorine atoms are sequentially removed from the parent compound and replaced with hydrogen. This can be summarized for chloroethenes as follows:

PCE → TCE → DCE → VC → methane and inorganic compounds

Risks for both the parent and daughter cVOCs are important considerations for anaerobic sites and the toxicity and potential buildup of daughter products such as vinyl chloride (VC) are factored into the design process and performance metrics.

4.3.2 Aerobic

Conditions for growth or metabolism in which the organism is sufficiently supplied with molecular oxygen. Aerobic respiration, the process whereby microorganisms use oxygen as an electron acceptor to generate energy. Note that the degradation rates for typical industrial cVOCs (e.g., PCE, TCE, CT and chloroform) is typically slow and fully ("per-") chlorinated cVOCs are typically assumed to have very low or negligible degradation rates for aerobic conditions. Importantly, there are two major microbial and enzyme pathways for aerobic degradation of cVOCs: cometabolism and direct metabolism. Cometabolism relies on the poor specificity of some of oxidative enzymes that are targeted to different substrates (like methane or toluene) to fortuitously degrade cVOC contaminants. Cometabolism does not typically result in direct benefits for the organism (i.e., does not supply energy for growth and reproduction). Direct metabolism relies on enzymes that are targeted to a specific cVOC and which typically result in providing energy for growth and reproduction. An advantage of aerobic process is that the reaction pathways to mineralization (forming nontoxic inorganic byproducts) is more direct compared to anaerobic pathways with less buildup of daughter products.

4.3.3 Combined Anaerobic-Aerobic (Structured Geochemical Zones)

Similar to EA, DOE led the development of systems that rely on a combined anaerobic-aerobic structured geochemical zone approach. Additional background is provided below as a resource for DOE management. Structured geochemical zones conditions rely on groundwater flow through a succession of anaerobic and aerobic conditions. The anaerobic zones stimulate relatively rapid degradation of the original cVOC compounds while the downgradient/surrounding aerobic areas encourage relatively rapid degradation of daughter products as well as enhanced cometabolism stimulated by the release of methane and other reduced hydrocarbons from the anaerobic zones (Figure 4-6 and Figure 4-7).

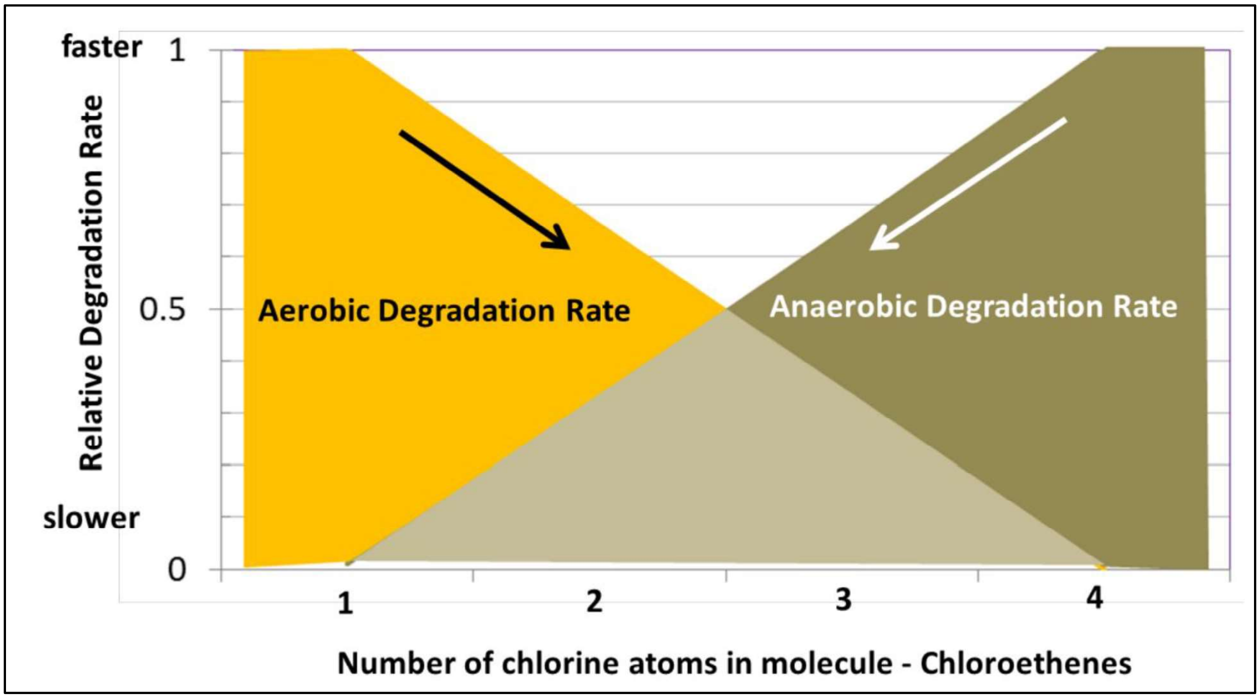

Figure 4-6. Simplified Depiction of the Relative Rates of Chloroethene Degradation under Anaerobic and Aerobic Conditions (DOE, 2020).

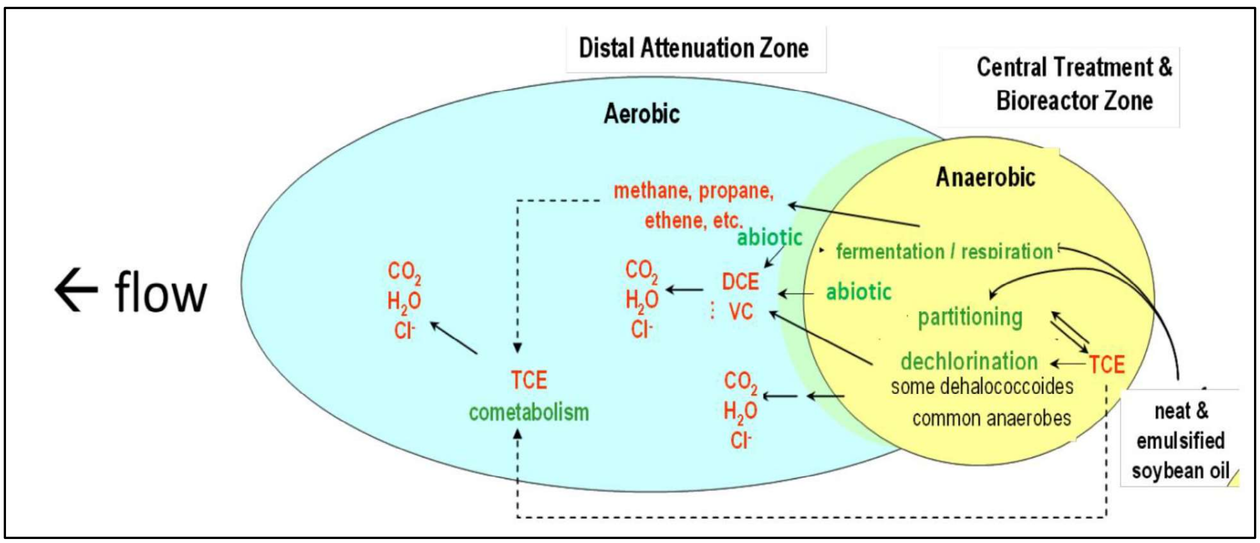

Figure 4-7. Example Depiction of the Relationship between Anaerobic and Aerobic Treatment Areas – A structured geochemical zone design would include additional coupled areas along the plume flow path (DOE, 2020).

4.4 Site-Specific Modifying Factors, Deployment Conditions and Deployment Details

A thorough and accurate site characterization informs an effective remediation strategy and design of a bioremediation approach relies on knowledge of the site and contaminant plume. Characterization is guided by the conceptual site model (CSM), which informs the remedy design, implementation, and operation. For example, physical factors like site hydrology, groundwater flow paths, and lithology impact substrate selection and delivery method. For enhanced in situ reductive dechlorination of cVOCs specifically, subsurface conditions must be conducive to introduction and distribution of an electron donor so that a reactive zone can be created. The following categories of sites-specific factors were elicited during the site interviews to support the systematic review process.

4.4.1 cVOCs Type(s)

List of the site-specific predominant cVOCs that need to be addressed to mitigate risks and meet remedial objectives.

4.4.2 cVOCs Concentration(s)

The starting contaminant concentration will also inform monitoring activities and future assessments of the bioremediation once implemented. This technology has been shown effective for groundwater plumes with concentrations from 0.01 to 100 mg/L (Parsons, 2004). Sites with residual or sorbed dense non-aqueous phase (DNAPL) at concentrations > 100 mg/L can also potentially be treated with bioremediation approaches, but areas with significant DNAPL source zones may not be able to be remediated in a realistic timeframe (Parsons, 2004). This factor was set relative to solubility of the particular cVOC to account for the potential presence of residual (undissolved) cVOC dense nonaqueous phase solvents (DNAPLs). All bioremediation strategies are significantly adversely impacted by the presence of DNAPL. The case studies will be used to help assess relative success of various bioremediation strategies used at varying concentration levels. For purpose of the structured review the following definitions and log-based bins were developed:

- Likely DNAPL >10% solubility up to recovered solvent
- Very High >1% solubility up to <10% solubility (there is potential for near-field DNAPL in this bin)
- High >0.3% solubility up to <1% solubility
- Medium >0.03% solubility up to <0.3% solubility
- Low <0.03% solubility

For some of the common DOE cVOCs these bins (bounds organized from high to low) are as follows:

- TCE (solubility ≅1000000 µg/L → 100000, 10000, 3000, 300)
- PCE (solubility ≅200000 µg/L → 20000, 2000, 600, 60)
- CT (solubility ≅800000 µg/L → 80000, 8000, 2400, 240)
- Chloroform (solubility ≅8000000 µg/L → 800000, 80000, 24000, 2400)

4.4.3 Lithology/Hydrogeology

Typical descriptors were used – for example clayey, silty, sandy, layered, homogeneous, anisotropic and similar for sedimentary or granular media, and the nature of fractures in solid/rock/limestone media. Baseline geochemical conditions also inform whether a bioremediation approach is a viable remediation option for a site. For example, natural oxidizing/reducing conditions and/or aerobic/anaerobic conditions will influence which type of approach(es) may be effective for a system.

4.4.4 Size and Scale of Bioremediation (pilot- or full-scale target volume)

Based on interview and project objectives.

The extent of the plume and how it was used for the bioremediation strategy design and implementation process. The scale is used in determining the needed radius of influence of treatment, as well as the required microbial ecology.

4.4.5 Amendments Used

This is a significant factor that influences the performance of the bioremediation. Amendments will be assessed in several subcategories: redox modifier (electron donor/acceptor), bioaugmentation, and nutrients/buffers.

The two major types of redox modifiers are electron donors (to enhance anaerobic conditions and processes) and electron acceptors (to enhance aerobic conditions and process). Redox modifiers are notable because they can be selected based on desired effect and longevity. Shorter longevity is normally associated with a more intensive bioremediation enhancement but with the potential need for more frequent reapplication. More intensive amendments are sometimes classified as "hot" while very long-lived amendments are classified as "cool". A summary of available electron donors and electron acceptors that are used for bioremediation is provided below.

Most electron donors are applied as liquids, but gases and solids are also available. Available electron donors are summarized in Figure 4-8. These are arranged on a continuum that moves (left to right) from hot to cool and the approximate amendment longevity is annotated on the bottom of the figure. On the left are hydrogen, sugars and other easily oxidizable materials/hydrocarbons. The center of the chart includes more complex hydrocarbons (including commercial byproducts, glycerin and vegetable oils). The right portion of the diagram includes natural organic matter such as mulch, humic/fulvic acids and peat. Inorganic electron acceptors such as zero valent iron (various particle sizes) have also been used for help support bioremediation projects. Early studies often used relatively expensive reagent-based amendments such as lactate, however the industry as moved to lower cost materials such as molasses and vegetable oil – often containing key nutrients and buffers.

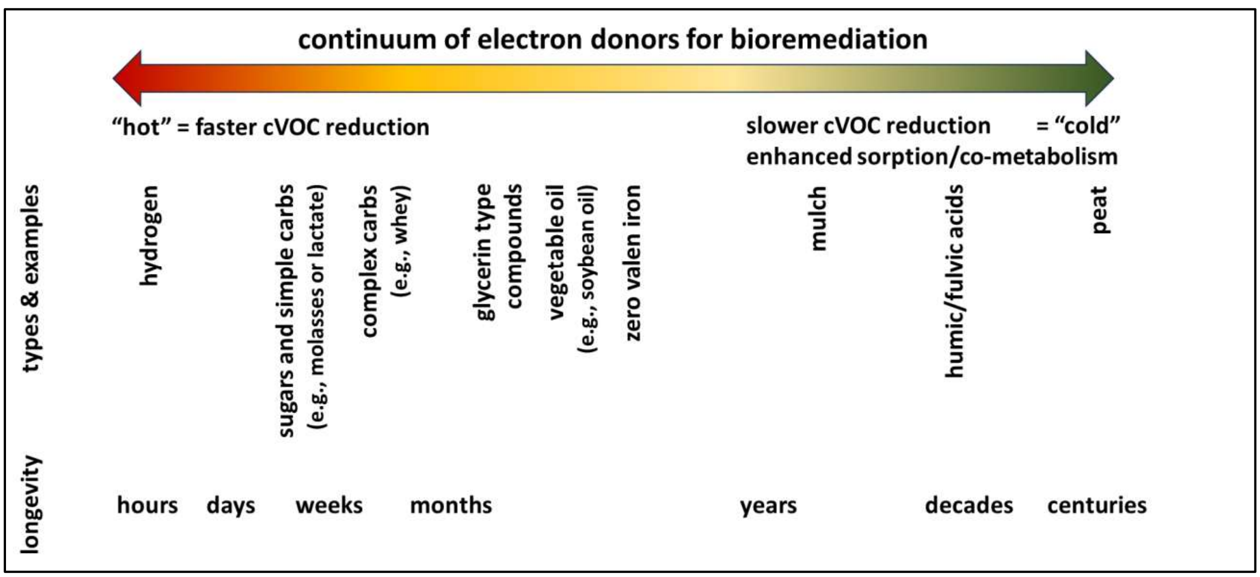

Figure 4-8. Summary of Electron Donors used for Anaerobic Bioremediation.

The scope of materials currently in use for *electron acceptors* to support bioremediation is more limited. A key challenge for electron acceptors is that the delivery of electron acceptor is constrained by the low solubility of oxygen in water. Many of the available electron acceptors are gases. The most common electron acceptor to support bioremediation is air (i.e., biosparging). Compared to air, "hotter" gas phase electron acceptors include oxygen and ozone (ozone is typically not used because it is so reactive that it can adversely impact the microbial community). Water in equilibrium with air typically contains oxygen at approximately 10 mg/L and water in equilibrium with oxygen gas contains about 40 mg/L. In both cases, the ability of water to deliver oxygen throughout subsurface systems that have high biomass (high oxygen demand) is limited and such scenarios are poorly matched to aerobic bioremediation. Solid electron acceptor amendments (such as calcium or magnesium peroxide (i.e., similar to oxygen bleach)) deployed in wells/boreholes or fractures are also in use. Like oxygen, these solids generate dissolved oxygen concentrations in the vicinity of the deployment location in the range of 40 mg/L. Liquid electron acceptor amendment such as nitrate have seen limited use.

Bioaugmentation is an important design decision that may impact performance. Bioaugmentation typically adds one or more key microorganisms that has documented ability to degrade the target cVOCs. The objective of bioaugmentation is to assure that the microbial community will have the ability to perform the bioremediation and to minimize the potential for a lag time in developing the desired biodegradation reactions. Most of the bioaugmentation has been performed for anaerobic biodegradation using carefully selected cultures of *Dehalococcoides*. Notably, a number of other organisms can contribute to biodegradation and characterization/monitoring techniques to assess the naturally occurring microbial community and ecology are increasingly being used – sometimes eliminating the need for bioaugmentation. Table 4-1 provides a synopsis of organisms and genes that commonly contribute to cVOC bioremediation.

Table 4-1. Synopsis of Organisms/Genes that Contribute to Bioremediation (DOE, 2020)**.**

	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 chlorocoercia (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)	Tolune Dioxygenase (TOD)	Phenol Hydroxylase (PHE)	Tricholorobenzene Dioxygenase (TCBO)	Toluene Monooxygenase 2 (RDEG)	Toluene Monooxygenase (RMO)	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

Notes:

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 chorinated ethenes, ethanes, methanes, phenols, benzenes and propanes.

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

3 Dehalogenimonas (DHG) is confirmed to reductively degrade chorinated 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 chorinated ethenes, ethanes, phenols, and propanes.

5 Dehalobium chlorocoercia (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.

Amendments can also include nutrients or buffers. These impacts are considered based on the interview.

4.4.6 Subsurface Access Methods and Spacings

Various types of access strategies (vertical or horizontal wells, drilling, direct push, fracturing, trenches, or other methods) and geometries have been used for bioremediation projects. The distance between injection or application point/lines has been a significant factor in performance.

4.4.7 Performance Metrics and Monitoring Strategies

Effective monitoring of remedial performance is a crucial element in the design and implementation of bioremediation. This assessment will focus on the selected metrics – for example only tracking concentration versus multiple lines of evidence. If multiple lines of evidence are used the interview/evaluation will assess the alignment of the metrics with EPA or ITRC guidance such as the lines of evidence used for MNA. The review will also assess potential adverse collateral impacts (plume spread, accumulation of toxic daughter products, release of volatile contaminants, etc.).

4.4.8 Regulatory Objectives

Regulatory objectives were assessed based on the interview. The nature of the regulatory interactions and project objective impacts how the project fits into overall remedial strategy decision-making. For example, if a pilot study, what information was collected to help determine if full scale deployment is warranted? If formally adopted as a remediation, does the bioremediation project lead to final (or interim) remedial objectives?

4.4.9 Assessment of Performance/Success

Assessment of performance was based on the site interview and project objectives. Did bioremediation lead to success in achieving remedial objectives? Was it used at other sites? Were there lessons learned? A cost/benefit assessment, if available, was used as a further metric for success.

When using a more passive approach to remediation, particularly for complex sites, multiple lines of evidence (MLE) are often used to assess viability and performance. Lines of evidence in an MLE approach typically incorporate both short- and long-term monitoring. For remediation of chlorinated solvents, example lines of evidence may include cVOC concentration trends, biogeochemical conditions that support a known attenuation mechanism, and confirmation of the targeted bio-attenuation mechanism through molecular and biological tools. These lines will individually support or not support components of effective remediation. If the multiple lines agree, this supports overall success of the treatment approach and demonstrates the efficacy.

Regulatory and stakeholder groups often impose additional metrics of success. Stakeholders can include the public, investors, regulators, technology providers, etc. and these metrics can stem from regulatory approval processes (RCRA, CERCLA, EPA, etc.) or from stakeholder criteria. For groundwater specifically, metrics are typically based on maximum contaminant levels (MCLs) or similar criteria.

4.5 Other Terms and Definitions

Table 4-2. Key Terms and Definitions.

Approach	Application location	Targeted treatment zone	Description
Bioventing	In situ	Source treatment	Drawing (or forcing) air through the vadose zone air to deliver oxygen (O_2) to stimulate biodegradation in contaminated unsaturated soils (primarily used for petroleum – not chlorinated – hydrocarbons)
Biosparging	In situ	Groundwater	Injection of air below the water table to promote aerobic biodegradation/volatilization
Biostimulation	In situ	Groundwater	Bioremediation strategy that used redox modifier(s) such as electron donor or electron acceptor, along with nutrients, buffers and other conditioning reagents to accelerate biodegradation
Bioaugmentation	In Situ	Groundwater	Bioremediation strategy that adds microbial culture to assure that the subsurface microbial community has the capabilities needed to support the target bioremediation. Design objective is to minimize lag time in early stages

			of bioremediation and to maximize rates and performance
Phytoremediation	Near Surface	Groundwater Treatment	Relies on plant communities to enhance the degradation and or uptake of cVOC contaminants. Most of the effort in DOE has focused on the enriched microbial communities that are present in the root zone of plants (the "rhizosphere") and tracked degradation occurring as cVOC plumes approaches surface water receptors such as streams, rivers or lakes. No DOE full scale or permitted phytoremediation case studies were identified.
Permeable Reactive Barriers (PRBs)	In Situ	Groundwater Treatment	Bioremediation PRBs have seen successfully implementation in DoD and for industrial sites. The PRBs typically organic substrates such as mulch or blends of amendments as opposed to abiotic PRBs that do not rely on microbial processes and use granular zero valent iron and similar amendments. PRBs are intended to provide cVOC destruction and serve as a barrier limiting the plume extent. No DOE full scale or permitted cVOC bioremediation PRB case studies were identified.
Land treatment	Ex situ	Source treatment	Contaminated soil, sediment, or sludge excavated, applied to lined beds, and periodically turned over/tilled to aerate- can also add amendments to contaminated media in beds
Composting	Ex situ	Source treatment	Contaminated soil excavated then mixed with bulking agents and organic amendments
Biopiles	Ex situ	Source treatment	Excavated soils mixed with soil amendments and placed in aboveground enclosures; aerated static pile to form compost that is aerated with blowers or vacuum pumps
Slurry-phase treatment	Ex situ	Source treatment	Aqueous slurry created by combining soil, sediment, or sludge with water and additives; slurry mixed to keep solids suspended and microorganisms in contact with contaminants (usually in series of tanks)

5.0 Site Data

DOE sites with known, historical use of bioremediation for cVOC remediation were contacted, specifically, SRS, Hanford, Idaho, and DOE LM (Mound and Pinellas) were asked to provide further information about the use, effectiveness, and associated challenges/lessons learned from these activities. A combination of reference review and discussion with site contacts allowed for the development of a comprehensive overview for each site. These sites are broadly representative of the cVOC bioremediation implemented across the DOE complex and the knowledge gained from this activity provides a thorough look at the application of this bioremediation within the DOE complex. Condensed summaries for each site are provided below, while a detailed case study for each site is presented in Appendix B-Appendix G.

Table 5-1 summarizes key study information.

1. **SRS Sanitary Landfill**

   The Sanitary Landfill (SLF) began receiving waste in 1974 and the main area of the landfill was filled by 1987. Since then, portions of the landfill that received the RCRA waste were approved for a geosynthetic closure cap initiated in 1996 under a RCRA Post Closure Part B Permit. Initial characterization activities showed vinyl chloride migrating towards the southern end of the landfill. A biosparging remedy was initiated in 1999 as an in-situ bioremediation corrective action where TCE and VC were the targets of the remediation. Corrective action operations were stopped in 2005 based on the regulatory agreement (RCRA permit). Groundwater monitoring by SRS is ongoing (quarterly) to ensure that no further remediation of the groundwater is needed. The RCRA permit was renewed in 2024 after the previous permit expired, but no changes to monitoring frequency were made. The original chemistry of the subsurface was primarily aerobic and pH was typical for a landfill. More anaerobic activity was seen as VC was generated. After biosparging, lower concentrations of cVOCs were consistently observed, and ACLs were reached at the point of compliance (POC) wells and MCLs were achieved at point of entry (POE) wells. Constituents remained below MCLs after biosparging was completed.

2. **SRS C-Area Operable Unit**

   Groundwater in the C-Area Groundwater Operable Unit is contaminated with tritium and VOCs, primarily TCE, due to releases associated with reactor operations. A non-time critical (NTC) removal action was to be conducted to ensure human health and environmental protection per the EPA and SCDES. This removal action included the injection of an emulsified oil base, a buffer, and vitamin C to remove oxygen from the water, with vitamin B injected to promote microbial activity. Two rows of injections and a one-time deep push injection were performed with application of heat near the source. This remediation is ongoing and expected to last 3-5 years. TCE started breaking down after 3 years. Concentrations in three surface water stations have dropped below 5 ug/L and MNA will be the preferred remedy going forward if water concentrations for TCE and tritium drop/remain below the MCLs. No additional injections are planned, but alternative approaches are being considered for future implementation. It was recommended that in the future, oil be injected prior to a second injection of buffer and nutrients to support microbial activity.

3. **SRS A/M Area EZVI**

   A VOC plume persists in SRS's A/M Area as a result of releases from the SRS M-Area Hazardous Waste Management Facility (HWMF). EZVI emulsified vegetable oil in combination with zero valent iron (ZVI) was applied in the Western Sector of A/M Area in 2022 as part of a series of field-scale demonstrations of innovative remediation technologies. This technology was selected to target and degrade residual DNAPL that lingers within the aquifer and continues to act as a long-term source. The targeted aquifer zone being remediated is aerobic with minimal organic content. The results of this study suggest that EZVI treatment could work well for VOC reduction in A/M Area but distributing this product in a silty/sandy aquifer was difficult and the implementation was ineffective and ultimately contributed to remedial underperformance.

4. **SRS TNX**

   Bioremediation (EA) was applied for remediation of chlorinated volatile organic compounds (cVOCs) within the Technical Nuclear Explosives (TNX) facility area at the Savannah River Site (SRS). cVOCs are present within both the soil and groundwater, but the contaminated groundwater plume at the TNX site is confined to the Upper Three Runs Aquifer Unit. Bioremediation was applied to expedite cleanup, promising a quicker completion than other more traditional techniques like pump and treat. Two types of edible oil were deployed: neat oil in the vadose zone and emulsified in the water table aquifer. No solids, supplemental reductants/sorbents, macronutrients, or bioaugmentation were used in the oil application and all oil injections were gravity fed. As a result of an initial treatability (feasibility) study, the existing pump and treat system was discontinued and regulatory record of decision was modified resulting in a significant cost savings and reduced operational footprint. Enhanced Attenuation using edible oil was selected as the official remedy at the TNX Operable Unit. The project is on-going, and the deployment of edible oil was shown to be successful at TNX.

5. **SRS VOC Integrated Demonstration**

Pilot-Field scale bioremediation was applied for remediation of TCE and PCE in M-area at SRS. The contamination resulted from leakage from a former process sewer line that discharged M-Area settling basin at SRS. The primary bioremediation technology in the SRS Integrated Demonstration was a large-scale field pilot study of aerobic cometabolism deployed using horizontal wells and air sparging (biosparging) initiated three decades ago. The sparge air was amended with methane (cometabolite) and nutrients (ammonia and triethylphosphate). The results demonstrated that 1) bacteria capable of degrading TCE/PCE can be stimulated in situ using relatively simple nutrients, 2) biostimulation and biodegradation occurred in situ without production of toxic daughter products, 3) the process is easy to use and can be automated, 4) the cost for adding on methane injection capability is relatively low and easily recovered, 5) that gaseous nutrient injection represents a significant new delivery technique for in situ bioremediation, and 6) combined with in situ air stripping this technology represents a significant improvement in terms of cost and efficiency over conventional baseline technologies used for remediation of chlorinated solvents. Based on the results: 1) this technology was deployed to address elevated concentrations of cVOCs at the SRS sanitary landfill; 2) the gaseous nutrient injection method for phosphorus was patented, licensed and used by industry, 3) the results supported development of ITRC enhanced attenuation technical guidance for cVOCs, and 4) 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 the DOE-LM Mound site).

6. **INL – Test Area North**

An injection well (TSF-05) was operated from the 1950s to 1972 to dispose of all liquid waste streams generated at Test Area North (TAN) at Idaho National Laboratory (INL). The waste injected into TSF-05 mainly consisted of industrial and sanitary wastewater but also included organic, inorganic, and low-level radioactive wastewater. Estimates of total TCE injected range from 350 to 35,000 gallons. The resulting trichloroethene (TCE) plume is nearly 2-miles long (TCE). In addition to contaminants in the wastewater, sludge material containing contaminants, such as TCE and radionuclides (Sr-90, Cs-137, and tritium), accumulated in TSF-05 and in fractures around the injection well. A multi-component remedy was designed to address the varying concentrations of TCE within the plume including active in situ bioremediation (ISB) of the hot spot, pump and treat (P&T) for the medial zone and monitored natural attenuation (MNA) in the distal zone. Active bioremediation operations involved injecting a carbon source (lactate/whey at TSF-05 well, Wilclear Plus/LactOil at TAN-28 well) into the aquifer to create oxidation-reduction (redox) conditions favorable for indigenous anaerobic reductive dechlorination (ARD). Collectively, the three remedy components employed at TAN appear be on track to provide a comprehensive approach to remediating the cVOC (TCE) plume. More time is needed to assess TCE concentrations following rebound testing at TSF-05 and TAN-28. P&T and MNA activities will continue with periodic reassessments to determine if changes in remediation are needed. Major lessons learned from ISB at TAN include understanding the challenges in remediating a fractured basalt subsurface and understanding how injecting amendments may affect other constituents in the subsurface.

7. Hanford Site

Carbon tetrachloride (CT), nitrate (NO_3), and other materials were discharged from 1955 to 1973 to subsurface waste disposal facilities in the 200 Area at Hanford, creating a large plume of CT and NO3. The primary objective for the Hanford groundwater contaminant plume is protection of the Columbian River. A Tri-Party Agreement (DOE, EPA, and the Washington Department of Ecology) governs cleanup under CERCLA and RECRA. The 200-ZP-1 record of decision (ROD) specified 25 years for pump-and-treat, followed by 100 years of monitored natural attenuation (MNA) for CT. More recent assessments indicate that 125 years may not be enough time to achieve CT plume cleanup goals. The presence of other contaminants (uranium, technetium-99, iodine-129, chromium) in the Hanford Central Plateau groundwater complicates the overall remedy. Two independent efforts at Hanford are described below: 1) an ISB pilot and 2) ongoing research to understand and optimize P&T and MNA. An active ISB pilot demonstration took place from 1995-1996 and successfully demonstrated the use of pulsed injections to deliver amendments and stimulate biodegradation of CT in the aquifer while minimizing chloroform accumulation. For Hanford, the biggest issues with deployment of this technology for treatment of the CT plume are the large size of the CT plume (which would require probably thousands of wells), the significant depth to contamination (water table is at a depth of about 250 ft) and thickness of the contamination (up to 60 m), and the associated $1 million cost of drilling a single well. For these reasons, ISB is unlikely to be applied for the entire CT plume. However, ISB may have a role in 1) a permeable reactive barrier (PRB) design of finite extent or 2) in remediation of hot spots. For these reasons, P&T followed by MNA remains the remediation strategy at the Hanford site. Ongoing laboratory studies are being performed to measure abiotic and biotic degradation of CT in site sediment. Site-specific rate results are higher than expected and span each of the relevant hydrogeological units. In addition, ongoing work is targeting optimization of the P&T system to maintain or increase effective mass removal and decrease the duration of the P&T portion of the remedy. These and other efforts will help form a basis for decision making regarding refined remedy approaches.

8. Mound OU1

A full-scale bioremediation treatability study was implemented for TCE and PCE at other cVOCs to remediate groundwater at Mound OU1, a former solid waste landfill area at the former DOE Mound Site in Ohio. Previous remediation steps at OU1 included waste removal, soil vapor extraction and groundwater pump and treat. The EA bioremediation was performed using a combined anaerobic-aerobic structured geochemical zone design. Pump and treat was turned off in 2014 to and the bioremediation was performed as a multiyear CERCLA treatability study from 2014 through 2019 with additional data collection after completion. The starting concentrations for TCE and PCE were low (maximum approximately 50 ug/L). Amendments included commercial emulsified vegetable oil solution in the groundwater plume and neat vegetable oil in the lower vadose zone beneath the former landfill. The system resulted in significant reductions in VOC concentrations, a 12-year reduction in the timeframe for cleanup (2040 to 2028), minimal adverse collateral impacts, and a significant lifecycle cost reduction. The system was monitored using criteria that align with EPA guidance for MNA. Some key monitoring findings include: 1) anaerobic bacteria capable of rapidly degrading TCE/PCE and aerobic bacteria capable of degrading TCE, dichloroethene and vinyl chloride were stimulated in the anaerobic and aerobic zones, respectively, 2) biostimulation and biodegradation occurred in situ, 3) the process was simple to deploy and provided a sustainable shift to an effective attenuation-based remedy, 4) the structured geochemical zone enhanced attenuation provided significant improvement in terms of cost and efficiency over conventional baseline technologies. Based on the results, this enhanced attenuation strategy is currently being implemented as the approved (2023) CERCLA ROD for OU1.

9. **Pinellas**

The Young-Rainey STAR Center is a technology and manufacturing center located on the former site of the Pinellas Plant, a DOE nuclear weapon component manufacturing facility. DOE production, a significant leak from the solvent storage tank resulted in release of significant quantities of TCE and PCE at other cVOCs into the subsurface below the building foundation and subsurface creating multiple near-field zones containing DNAPLs. These long-term sources occur beneath Building 100, a large 4.5 ha (11acre) building. Groundwater contamination, consisting of two dissolved-phase plumes originating from chlorinated solvent source areas has migrated beyond the property boundary, beneath the roadways, and beneath adjacent properties to the south and east. The Pinellas site has had three notable bioremediation activities: 1) a large-scale (1997) pilot study of anaerobic bioremediation using lactate/methanol/benzoate as the electron donor blend, 2) a large-scale (2000) pilot study of aerobic cometabolism biosparging, and 3) a permitted full scale (2014-present) anaerobic bioremediation using emulsified oil reagents/nutrient solutions and bioaugmentation (the full-scale permitted remediation comprises multiple-episodic injections and use of both vertical and horizontal wells to address the dissolved plume and the contamination beneath Building 100). The primary monitoring information collected at Pinellas is cVOC concentrations and trends. At the Pinellas site biogeochemical and hydrogeologic conditions, both anaerobic remediations were successful while the aerobic cometabolism biosparging did not meet project objectives and resulted in spreading the plume. For the anaerobic bioremediation activities, the evidence for significant benefits of biostimulation is strong, while the evidence for significant benefits from bioaugmentation is equivocal. Contamination at the Pinellas site represents one of the most challenging remediation scenarios in the nation and the bioremediation has proven successful. Currently, the permitted bioremediation has mineralized the bulk of the contamination. Current groundwater conditions are generally near or below MCLs for the original parent VOCs and daughter products throughout the area of the plume(s). DOE LM is currently considering options for next steps. Based on the data, plans for transition to an EA strategy and shifting to multiple lines of evidence for monitoring are being developed. These changes in strategy are expected to maintain or accelerate progress toward closure and to reduce costs and potential adverse collateral impacts that might result from additional injection if electron donor.

The information elicited from these case study sites has been organized into an annotated set of tables to support the systematic review. The tables are structured as shown in Figure 5-1. Table 5-1 provides the general information for all the examined DOE bioremediation sites. Tables 5-2 through 5-4 provide key details for bioremediations that were predominantly anaerobic, aerobic and combined anaerobic-aerobic, respectively. The separate tables are structured to provide key details that align with the target biogeochemistry. The overall assessment and lessons learned are also summarized in Tables 5-2 through 5-4.

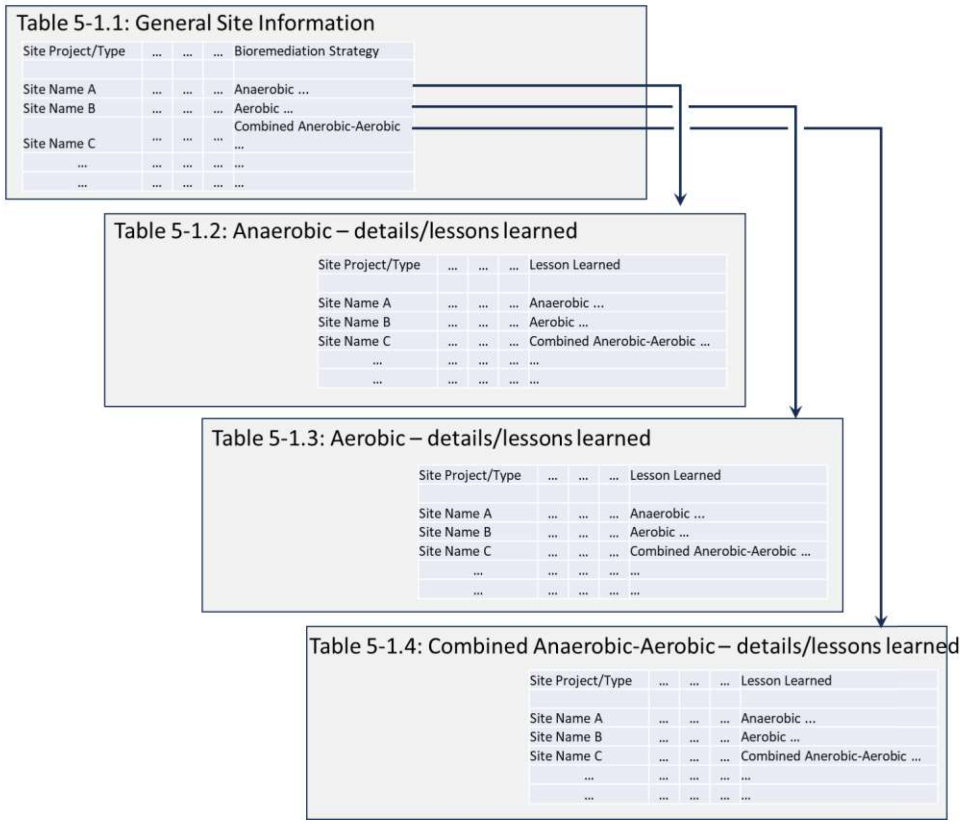

Figure 5-1. Schematic Depiction of Summary Table Structure.

Table 5-1. Case Study Summary Table.

1 – General Information

Site-Project/Type	**Contaminant/Scale**	**Objectives / Overall Assessment**	**Bioremediation Strategy**
INL - In Situ Bioremediation of VOCs at TSF-05 and TAN-28 within Test Area North Active Bioremediation	TCE plume is approximately 2 miles long and exists from 200-400 ft below ground surface: TSF-05 – 19,816 sq. ft. and TAN-28 – 14,000 sq. ft. Co-contaminants: PCE, cis-1,2- DCE, trans-1,2- DCE, VC Cs-137 and Sr-90 TCE concentration before bioremediation TSF-05 – 12,000 - 32,000 µg/L (DNAPL) TAN-28 – 544 - 1,050 µg/L (Medium) Some of contamination is in the vadose zone	CERCLA Compliance Remedial Action Objective is for all cVOCs and radionuclides to be below their maximum contaminant levels by 2095	<u>Anaerobic</u> Notes: to address plume with varying contaminant concentrations, a multi-component remedy was designed. 1)Active bioremediation for remediation of hot spot (focus of this report) 2) P&T for medial zone 3) MNA for distal zone Anaerobic reductive dichlorination of cVOCs in source zone was the primary bioremediation Aerobic cometabolism was quantified for the MNA
PNNL - VOC Arid Integrated Demonstration at Hanford 200 –SP-1 Operable Unit ISB-Pilot P&T/MNA	CT and NO_3 CT plume roughly 5 km by 5.5 km with a thickness of ~60 m at the plume core When bioremediation was initiated, contaminant concentrations were 12.5 ± 0.14 µM CT and 3.87 ± 0.26 µM NO_3	Goal is protection of Columbia River Pilot-project successfully demonstrated the objective of biodegradation of CT while minimizing chloroform production, as well as the efficacy of using pulsed injections to deliver amendments Concern that original ROD of 25 years of P&T with 100 years of MNA may not be adequate Laboratory studies being done to better understand natural attenuation	<u>Anaerobic</u> Primary bioremediation was anaerobic bioremediation pilot study Natural Attenuation -- anaerobic processes (possible supplemental aerobic cometabolism) in bulk aerobic aquifer

Site-Project/Type	Contaminant/Scale	Objectives / Overall Assessment	Bioremediation Strategy
SRS - SRS VOC Integrated Demonstration (full scale pilot test)	TCE/PCE – Test bed area approximately 50,000 cu m of contaminated soil and groundwater	Address technology gaps to meet commitments of RCRA Permit for A/M Area groundwater.	Aerobic Active Bioremediation using cometabolism biosparging
SRS – edible oil injection treatability study at TNX site	TCE, PCE, and CT Largest extent of the TCE plume was 1500 ft long x 300 ft wide x 20 ft thick with a source zone area 200 ft x 280 ft	A multiple lines of evidence approach is used to assess the efficacy of these oils for VOC remediation.	Combined Anaerobic-Aerobic Pump and treat, soil vapor extraction (SVE), excavation, and air lift recirculation have also been applied at TNX as remediation strategies. Edible oil: neat oil in the vadose zone and emulsified in the water table aquifer
SRS – EZVI injections in A/M Area	Prior to treatment, PCE concentrations in the bioremediation area ranged from 23,000 to 15,000 ug/L and TCE concentrations ranged from 6,000 to 4,000 ug/L.	Assessed as hotspot treatment targeting DNAPL. Injected to treat a small area within the large VOC plume in A/M Area, focused on the confined upper LLAZ.	Anaerobic EZVI was injected to treat a small area within the large VOC plume in A/M Area, focused on the confined upper Lost Lake Aquifer Zone.
SRS C-Area Operable Unit –	Tritium and VOCs, primarily TCE Varying degrees of contamination at monitoring wells low levels up to 100 ug/L TCE initially 15 ug/L in the unnamed tributary to Castor Creek and the goal is to reach 5 ug/L	Reduce TCE and tritium concentrations below their MCLs Non-time critical removal action (DOE, EPA, SCDHEC)	Anaerobic Injected Biobarrier design
Mound OU1 Enhanced Attenuation	PCE and TCE Plume extent approximately 2 acres Maximum concentrations approximately 50 ug/L (low)	Maintain and accelerate progress toward remedial objectives (compared to baseline pump and treat) Assure that plume is stable-shrinking Demonstrate that EA remedy is sustainable and durable	Combined anaerobic-aerobic

Site-Project/Type	**Contaminant/Scale**	**Objectives / Overall Assessment**	**Bioremediation Strategy**
Pinellas Star Center Active Bioremediation (three separate projects)	TCE, PCE and related cVOCs Plume extent approximately 30 acres Maximum concentrations high to DNAPL Groundwater background has relatively low dissolved oxygen and contains some fine-grained materials	Accelerate progress toward regulatory objectives Remediate beneath large building	The three bioremediation projects will be documented separately 1) Anaerobic (large scale pilot) 2) Aerobic (large scale pilot cometabolic biosparging) 3) Anaerobic (full scale permit)

2 - Anaerobic

Facility	Electron Donor / Nutrients / Bioaugmentation	Amendment Delivery / Timeframe	Metrics / Performance	Lessons Learned
INL – Test Area North (TAN)	TAN TSF-05 Electron Donor - Whey and sodium lactate Bioaugmentation – No TAN-28 Bioaugmentation - No	TAN TSF-05 Sodium lactate (1999-2004) Whey (2004-2008) Sodium lactate and whey mixture (2008 - 2012) TAN-28 WilClear Plus (Jan. 2016-Oct. 2019) WilClear Plus and LactOil (Oct. 2019-May 2020) WilClear Plus (May 2020 to present) Note – WilClear Plus = “lactate and other fermentable materials and water-soluble nutrients including B-vitamins and yeast factors”	Progress towards regulatory objectives is acceptable but more time needed TAN TSF-05 TCE was degraded below MCLs, currently in rebound test Sr-90 and Cs-137 concentrations increased, but not decreasing TAN-28 TCE decreased to 10 -30 µg/L, when ISB conditions present for 2-5 years a rebound test will be initiated	Top challenge is the hydrogeology of this area Basalt aquifer fracture patterns influenced injection strategies Complex hydrogeology makes well selection for ISB challenging. TAN demonstrates the value of using a multi-faceted approach to remediation Whey powder supported community for ARD, increased dissolution of TCE from source, and was determined to be more cost effective for long-term ISB. Unexpected findings: TAN-29 TCE concentrations increased as TAN-28 decreased, and Sr-90 rose above MCL in wells near TAN-28

Facility	**Electron Donor / Nutrients / Bioaugmentation**	**Amendment Delivery / Timeframe**	**Metrics / Performance**	**Lessons Learned**
Hanford -- VOC Arid Integrated Demonstration at Hanford 200 –SP-1 Operable Unit	ISB Pilot Demonstration Nitrate and acetate No bioaugmentation NA for laboratory study of to assess MNA half-life natural attenuation	ISB Pilot Demonstration Demonstration from 1995-1996 -- Recirculation and offset pulsed injections of acetate and nitrate Laboratory study using sediment from areas of high contamination and from the fringe of the plume designed to understand MNA half-life	Monitoring of CT and NO3 Pilot-project successfully demonstrated the objective of biodegradation of CT while minimizing chloroform production, as well as the efficacy of using pulsed injections to deliver amendments Observed rates of degradation suggested the need for additional studies, faster MNA observed in lab studies than in field	Successful demonstration of the use of offset pulsed injections to degrade CT, to minimize production of CF, and to mix nutrients in aquifer with minimal well biofouling ISB too costly for the entire CT plume, but ISB may have a role in 1) a PRB design of finite extent or 2) in treatment of hot spots Scale may be reasonable for observed discrepancy in degradation rate between laboratory and field MNA half-life P&T optimization with MNA needed to reach cleanup goals
SRS – EZVI injections in A/M Area	Emulsified zero valent iron (EZVI)	EZVI assessed through a field scale corrective action study under a RCRA permit in 2022. Part of larger Corrective Measures Study where several field scale technologies deployed in the Western Sector to identify a final corrective action and develop a corrective action plan.	Only a small percentage of the target area was successfully treated. Reductive conditions and minimal decreases in VOC concentration were observed for one monitoring well where oil was present. Little to no impact seen in the other treatment zone monitoring wells.	Underperformance attributed to the high viscosity of EZVI and the resulting limited contact between oil and sediments. Distributing product in a silty/sandy aquifer not achieved and ultimately contributed to its underperformance. Unlikely that the product was distributed out into the heterogeneous aquifer where contamination resides in both permeable and low permeable sediments; instead, remaining local

Facility	Electron Donor / Nutrients / Bioaugmentation	Amendment Delivery / Timeframe	Metrics / Performance	Lessons Learned
				to the fractures and limiting the aquifer material that came in contact with the EZVI product. Site characterization is key to understanding where the highest concentrations are present
Pinellas Star Center Active Bioremediation Anaerobic large- scale pilot	lactate, methanol & benzoate Bioaugmentation - No	Reagent applied using tranches and water pumping and recirculation system	Concentration changes and trends in wells and in sediments	Short pilot test performed in collaboration with EPA under collaborative demonstration program Significant degradation documented during pilot study that suggested that bioremediation was effective Some collateral impacts associated with anaerobic conditions
Pinellas Star Center Active Bioremediation Anaerobic full scale permitted	Emulsified vegetable oil (commercial blend including nutrients and buffer) Bioaugmentation – Yes using *Dehalococcoides*	Injection in vertical and horizontal wells. Episodic injections made when concentrations trends flattened out	Concentration changes and trends in wells	Long term implementation has resulted in significant progress toward remedial objectives. Parent cVOCs generally near or below MCLs. VC currently the most significant risk. DOE considering transition-ing to EA & expanding metrics to include microbial community structure/function & other attenuation data to transition site toward completion

3 - Aerobic

Facility	**Electron Acceptor / Cometabolite / Nutrients / Bioaugmentation**	**Amendment Delivery / Timeframe**	**Metrics / Performance**	**Lessons Learned**
SRS - SRS VOC Integrated Demonstration (full scale pilot test)	Electron Acceptor - air Cometabolite – methane Nutrients – gas phase ammonia (nitrogen) & triethylphosphate (phosphorus) No bioaugmenation	Parallel horizontal wells were installed; one below and one above water table. Bioremediation was deployed using air sparging into lower well and air extraction from upper well. Methane was added to injection air (continuous and pulsed modalities were tested). Gas-phase nutrients added during second half of pilot study.	Measurements of biostimulation & biodegradation rates, bioremediation performance, cost effectiveness, and ease of use and operation. Effective technology (success for all metrics) for pilot test conditions.	Methanotrohic cometabolism and the associated increases in biodegradation rates were documented, but rates were not as fast as anaerobic bioremediation mechanisms for highly chlorinated VOCs (such as PCE). Data suggest that this technology would not be effective for high (e.g., DNAPL) levels of highly chlorinated VOCs. Pulsed injection of cometabolite was more effective than continuous injection. Addition of N&P limiting nutrients improved biodegradation rates.
SRS – C-Area Operable Unit	Emulsified edible oil Bioaugmentation with *Dehalococcoides mccartyi* and enzyme solution	Creation of subsurface bio-barriers through injection of oil and bioaugmentation Injection of emulsified oil, a buffer, and vitamin C to remove oxygen from the water, with vitamin B injected to promote microbial activity Injections in 2019, ongoing MNA	TCE degradation observed after 3 years Methanogenesis was observed which stimulated co-metabolism Concentrations in 3 surface water stations have dropped below 5 ug/L MNA will be the preferred remedy going forward if water	It was recommended that in the future, oil be injected prior to a second injection of buffer and nutrients to support microbial activity over a longer period Phytoremediation being performed for tritium contamination

Facility	Electron Acceptor / Cometabolite / Nutrients / Bioaugmentation	Amendment Delivery / Timeframe	Metrics / Performance	Lessons Learned
			concentrations for TCE and tritium drop/remain below the MCLs.	
Pinellas Star Center Active Bioremediation Aerobic Large Scale Pilot scale permitted	Cometabolite – methane Nutrients - no	Biosparging into horizontal wells. Operated for 3.5 years.	Degradation of DCE and VC along with cometabolism of residual TCE. Plume stability	System underperformed – minimal degradation was observed, and system operation resulted in plume expansion Underperformance was attributed to poor contact of injected air with plume (preferential flow paths) and limited ability of air to overcome natural low oxygen conditions. The performance did not replicate success of M Area Integrated Demonstration biosparging

4 – Combined Anaerobic & Aerobic

Facility	Electron Donor &-or Acceptor / Nutrients / Bioaugmentation	Amendment Delivery / Timeframe	Metrics / Performance	Lessons Learned
SRS – edible oil injection treatability study at TNX site Enhanced Attenuation	Edible oil: neat oil in the vadose zone and emulsified in the water table aquifer No supplemental reductants/sorbents, macronutrients, or bioaugmentation	Remediation of TNX groundwater began in 1994 with an interim ROD (pump and treat along with airlift recirculation wells)-approved by SCDHEC, EPA, and DOE. SVE and excavation also deployed about 10 years later. Treatability study conducted from 2008 - 2010 to assess oil deployment. After pilot study shown successful, pump and treat system canceled to make edible oil deployment the active remedial action at the TNX Operable Unit (2012).	Pilot study goal was to pull back the plume boundary, while the end state objective was to achieve MCLs for all COCs (not just within the treatment zone but the plume/site as a whole). MLE approach used, where primary LOE focuses on the total mass of the plume and whether it is declining. On-going; very successful at TNX TCE concentrations < MCLs in the treatment zone achieved in 2022, which was much faster than what projected using the pump and treat remedial action.	pH changes from over buffering significantly impacted source zone geochemistry during the injection process. Did not impact the edible oil, but it did cause undesired impacts like mobilization of other contaminants (e.g. uranium). Lessons learned from first application applied during second round (2015).

Facility	Electron Donor &-or Acceptor / Nutrients / Bioaugmentation	Amendment Delivery / Timeframe	Metrics / Performance	Lessons Learned
Mound OU1 Enhanced Attenuation	Structured geochemical Zones Anaerobic areas -- Electron donor – neat vegetable oil in the vadose zone and commercial emulsified vegetable oil in the water table aquifer Aerobic Areas – natural presence of oxygen and recharge from rainwater	Direct push - vertical (geoprobe) for access Gravity feed for neat oil in lower vadose zone Pumping and dosatron blending for groundwater	Multiple lines of evidence including Concentrations trends, plume status, geochemical markers, and microbial ecology	Significant reductions in VOCs Improved estimated timeframe for cleanup Minimal adverse collateral impacts Significant lifecycle cost reduction Discontinued pump and treat and revised ROD to passive EA and final remedy

6.0 Lessons Learned

From the mid-1980s to the present DOE has developed, tested and deployed diverse bioremediation strategies for cVOCs. A systematic review of the projects provides an opportunity to identify crosscutting themes and lessons learned (summarized below). The knowledge gleaned from the DOE bioremediation retrospective is a resource to support current bioremediation operations, and future decisions related to cVOC bioremediation. This retrospective is a tool to support future decision making by DOE managers and project planning and management by scientists/engineers at the various DOE facilities across the nation. The crosscutting themes and lessons learned are organized into four groups – Overarching, Anaerobic Bioremediation, Aerobic Bioremediation, and Combined Anaerobic-Aerobic Structured Geochemical Zones. Themes and lessons learned in which DOE played a leading role are identified where applicable.

<u>Overarching Themes and Lessons Learned (all sites)</u>

1) Underperformance of bioremediation has generally resulted from poor access and delivery of amendments. This has been associated with hydrogeology (e.g., low permeability-layers-heterogeneous aquifer materials), amendment viscosity and injectability, well spacing, surface obstructions such as large building and similar issues. Innovative access techniques matched to site conditions, such as use of directional wells, horizontal wells, fracturing, and trenches were beneficial for sites with specific challenges such as plumes beneath large (multi-acre) buildings.
2) Bioremediation often results in a significant change in the subsurface, and remediation designs should account for potential problems particularly when modifying redox conditions. For example, moving redox conditions from aerobic to anaerobic results in localized adverse collateral impacts on the overall water quality yielding unpalatable water with strong odors due to the generation of reduced gases. Similarly, moving redox conditions from anaerobic to aerobic can result in precipitation of iron, suspension of clay particles, well clogging and generation of turbid water. Balancing the direct beneficial impacts and adverse collateral impacts provides opportunities for right-sizing bioremediation deployments. This balance has been a factor in some recent DOE EA

combined anaerobic-aerobic bioremediation projects and is being considered in future decisions about additional electron donor injections in existing full scale anaerobic bioremediation operations.

3) DOE Bioremediation projects for cVOCs were performed with regulatory oversight under either CERCLA or RCRA. All DOE bioremediations were performed in combination with other protective actions. The combined remedies associated with cVOC bioremediation projects often included pump and treat and sometimes included more aggressive source treatment options such as excavation or chemical oxidation. The combined remedies often included actions that were spatially separated and actions that were temporally separated. An example spatial separation includes pump and treat in a lower concentration area with bioremediation in a higher concentration area. A common example temporal separation would be performing sequential actions such as discontinuing pump and treat coincident with the start of bioremediation. The bioremediation projects typically presumed a follow on MNA but often did not provide detail on how the transition to MNA would be determined. Transition to passive remedies such as MNA was a specific focus of the DOE led EA development efforts that were performed in collaboration with regulators and other federal agencies – EA provides a roadmap and bridge to move from active to passive remediation.
4) In aerobic oligotrophic aquifers (groundwater system with low biomass), MNA rates for the most common cVOCs such as PCE, TCE and CT are relatively slow. Initial studies indicated half-lives in the range of 50 years; addition studies suggest that half lives may be longer (circa 100 years) for some compound such as CT – additional research may be needed to confirm and refine attenuation rates.

<u>For anaerobic bioremediation</u>

- Design Basis and Site Conditions
  - Most anaerobic bioremediation pilot and full-scale projects were designed as active bioremediations to remediate medium to high concentration plumes (near former sources or plume core areas). (INL, Hanford, SRS, Pinellas)
  - There are emerging opportunities in DOE to transition some of the full-scale anaerobic remediation projects – particularly those that have use long lived electron donors. This transition can be performed by restructuring the metrics/monitoring and working with regulators and stakeholders to formally revise the governing record(s) of decision. (Pinellas)
  - Anaerobic remediation projects have proven to be relatively effective under a wide range of baseline hydrogeologic and biogeochemical conditions. This is partly explained by the fact that water is limited in its ability to maintain/deliver electron acceptors (primarily oxygen) into areas with high oxygen demand (due to the low aqueous solubility of oxygen). Thus, addition of electron donors is able to rapidly create the anaerobic conditions that support reductive dechlorination and similar reductive cVOC degradation pathways. (INL, Hanford, SRS, Pinellas)
  - When deployed in an aerobic system with high groundwater quality, anaerobic bioremediation results in a shift in redox status and the associated beneficial degradation of target cVOCs. (INL, Hanford, SRS, Pinellas)

- Electron Donors and Other Amendments (INL, Hanford, SRS, Pinellas)
  - All tested and deployed electron donors have been shown to be effective in supporting bioremediation.

  - Aligning with the scientific literature at the time, the most common electron donors used in pilot and full-scale anaerobic bioremediation in the 1980s through the early 2000s were pure or blended reagents such as lactate, alcohols, benzoate and similar chemicals. Over time, lower cost materials such as industrial byproducts (e.g., whey) were deployed. Various emerging commercial electron donors such as those that incorporated zero valent iron have also been deployed. Recently, most of the anaerobic bioremediation projects have shifted to emulsified vegetable oil reagents.
  - Differences in the performance of electron donors have generally been associated with the injectability of the reagent, and the ability of the reagent to be distributed into the subsurface rather than the specific chemicals included in the amendment. For example, amendments that contained solid zero valent iron were somewhat more difficult to inject and distribute and these provided somewhat lower performance.
  - While both hot and cool amendments have been successfully used to support anaerobic biodegradation, there has been a shift over time toward the cooler amendments such as vegetable oils and whey that provide a longer performance period compared to hotter amendments such as lactate, alcohols or sugars molasses. Note that most commercial vegetable oil amendments contain some lactate and/or similar compounds to support a rapid initiation of bioremediation.
  - Many commercial vegetable oil amendments contain (or have the option for providing supplemental) macro nutrients, micronutrients and buffers to support increased biomass and provide pH control. Vendors have developed processes to reliably supply stable emulsions with droplet sizes < 5 µm or smaller allowing the emulsions, when blended with water, to be readily distributed in the subsurface. Factors that support the observed shift toward commercial vegetable oil-based amendments over the past 30 years include low cost, ease of use, injectability, flexibility and ability to adjust to site needs, effectiveness and longevity.
  - Bioremediation projects that rely on commercial vegetable oil amendments have demonstrated significant robustness over time. In some cases, a single injection has supported effective remediation performance for ten or more years, based on cVOC concentration trends and characterization of the structure and function of the subsurface microbial community.
  - Two of the case studies demonstrated effective deployment of neat (pure) vegetable oil in the lower portion of the vadose zone The pure oil migrated downward and accumulated/spread out at the water table interface. The deployed neat oil supported multiple bioremediation objectives such as reducing mass flux from any residual vadose zone cVOC source (due to partitioning into the oil) as well as creating a nearfield anaerobic treatment zone at the water table. This supplemental electron donor deployment strategy was developed in DOE and represents a cost-effective beneficial action.
- Bioaugmentation (INL, Hanford, SRS, Pinellas)
  - The sites that did not use bioaugmentation demonstrated effective anaerobic cVOC bioremediation. Similarly, the sites that used bioaugmentation also demonstrated effective anaerobic cVOC bioremediation. Thus, the evidence from the systematic review regarding the need for bioaugmentation to support anaerobic designs is equivocal. Currently the cost of bioaugmentation is relatively low so that use of bioaugmentation should be considered if there is data or evidence that the microbial ecology at the site is deficient in some key capability or if testing indicates that the site will experience a significant lag in the spin up for bioremediation to start under field conditions.
  - Modern molecular and biological tools (such as quantitative polymerase chain reaction (qPCR) arrays) were highly useful in many case studies (particularly in the last 20 years) and provided

clear actionable information for a reasonable cost. Collecting baseline and periodic data on the microbial ecology is recommended for current and future bioremediation projects.

For aerobic bioremediation (SRS, Hanford, Pinellas)

- Design Basis and Site Conditions
  - Aerobic bioremediation projects exhibited highly variable levels of success. Compared to anaerobic bioremediation, aerobic remediation for cVOCs was not as robust and resilient and successful projects were limited to a narrower range of environmental conditions. The variable performance for cVOCs is notably different than the literature documented outcomes for bioremediation of nonchlorinated (e.g., "petroleum") hydrocarbons. Most petroleum hydrocarbons degrade efficiently and rapidly under aerobic conditions so that bioventing and biosparging are considered presumptive remedies that work at most sites. However, rated of aerobic degradation of fully (per-) chlorinated cVOCs such as PCE and CT is typically assumed to be near zero and rates for less chlorinated cVOCs increases as the number of chlorines decreases.
  - For aerobic bioremediation of cVOC, cometabolism is a primary mechanism for moderately chlorinated molecules like TCE and chloroform. Cometabolism requires a cometabolite to induce enzyme systems that are able to degrade cVOCs. The cVOC does not induce the enzyme but is the enzyme catalyzes some crossover degradation. Less chlorinated cVOCs such as DCE and VC are subject to cometabolism as well more efficient and more rapid direct metabolism.
  - Most of the DOE aerobic remediation projects were performed using biosparging. One project at SRS (and a nearby follow on deployment at a sanitary landfill) were successful in meeting remedial objectives while a similar deployment at Pinellas resulted in minimal cVOC degradation and spreading of the contaminant plume. Compared to Pinellas, SRS had more conducive baseline biogeochemistry and lithology further emphasizing the somewhat narrow range of conditions needed to support successful biosparging.
  - Due to the variable performance of aerobic cVOC bioremediation, later projects that incorporated aerobic processes in the bioremediation design were performed as combined anaerobic and aerobic structured geochemical zones. These hybrid designs have proven to be more effective, robust, resilient, passive and sustainable compared to active aerobic operations.
- Electron Acceptors, Cometabolites and Other Amendments
  - Air was used as the electron acceptor in all the documented aerobic cVOC bioremediation projects.
  - Methane was used as the cometabolite for all the DOE aerobic cVOC bioremediation projects.
  - Gas phase nutrients (ammonia and triethylphosphte) were used for the SRS aerobic cVOC bioremediation project. The phosphorus addition process was patented by DOE and licensed to industry for commercial use in bioventing, biosparging and bioremediation.

For combined anaerobic-aerobic bioremediation – structured geochemical zones (SRS, Mound)

- Design Basis and Site Conditions
  - DOE exemplars of combined anaerobic-aerobic bioremediation were performed based on an enhanced attenuation design paradigm.
  - Monitoring the numbers, structure and function of the subsurface microbial community has been integral to the success of the combined anaerobic-aerobic remediation projects.
  - Structured geochemical zones provided optimal conditions for rapid degradation of parent cVOCs in the anaerobic areas and less chlorinated daughter cVOCs in the adjacent aerobic areas.
  - Structured geochemical zones minimized the accumulation of DCE and VC and minimized the volume of the aquifer that was impacted by collateral water quality impacts from transitioning to anaerobic conditions. The design strategy also reduced the quantity of reagents and time for deployment and immediately transitioned to a passive attenuation-based monitoring mode. for more cost efficient and safer operation.
  - Molecular tools have demonstrated that site specific adaption of organisms may provide benefits. For example, *Dehalogenimonas* spp. Have been documented in the literature to degrade chloroethanes and chloropropanes, but not TCE. However, the monitoring at DOE EA remedy sites provides compelling data that site specific adaption has resulted in *Dehalogenimonas* spp that are actively degrading TCE. This finding is significant because *Dehalogenimonas* survival does not require conditions that are as deeply reducing compared to *Dehalococcoides*. The additional capabilities of these native bacteria observed in the field data support the structured geochemical zone design. These findings also suggest that bioaugmentation may not be needed at many sites that have microbial communities that have adapted to the contaminants present.
- Electron Donors, Electron Acceptors, Cometabolites and Other Amendments
  - Cool electron donors with higher longevity such as emulsified and neat vegetable oils were used for creating the structured geochemical zones. The commercial emulsified oils contained supporting materials such as lactate, nutrients and buffers.
  - No added electron acceptors were used between the anaerobic treatment zones.

7.0 Recommendations

The portfolio of cVOC bioremediation projects and project experience in DOE has yielded significant success. Two sites have formally transitioned to a passive EA remedy from pump and treat – discontinuing expensive active treatment and now moving toward closure with no further action. All wells and all constituents at both EA sites are near or below MCLs. Some of the full-scale anaerobic bioremediations are also approaching ROD reviews where they may be able to formally transition to an attenuation-based remedy. Aerobic project success for cVOCs was variable but several full-scale projects successfully met their remedial objectives.

Within the retrospective cVOC bioremediation projects, DOE has developed, tested, and deployed several innovative technologies, some of which are patented and licensed. Finally, DOE collaborated with other Federal Agencies (EPA and United States Geological Survey (USGS)), state regulators (through the ITRC), industry and universities to develop technical guidance for implementing EA for cVOCs. This experience as summarized in a systematic review can serve to support DOE managers/decision makers as well as contractors in their project management, project design and field operations responsibilities for current and future cVOC bioremediation.

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Appendix A. Site Interview Questionnaire

Site Interview Questionnaire
Bioremediation Retrospective: Chlorinated Volatile Organic Compounds (cVOCs)

Have you used bioremediation for remediation of cVOCs in groundwater and soil?
If yes:

- Please complete the summary table below and then a site-specific information sheet for each of the project/sites.
- Please try to populate as much of the site-specific information sheet as possible. We will schedule a follow up call to address any gaps or get clarification.
- Please send any reports of supporting documentation that you think would be beneficial to the team as well (e.g. Conceptual Site Model for the area in question)
- The final report summarizing information from different site remediations will be made available to participants through OSTI.

Table A-1. Site Interview Summary Table

Total number of projects/sites	
Titles/identifiers for each project/site (e.g., M Area groundwater, Northwest Plum, etc)	

Site Area:

Site Take Aways	
Key challenges	
Lessons Learned	
Overall assessment of remediation strategy	

Current Status	
Type of project (Pilot Study, RCRA/CERCLA remediation, etc.)	
General information:	
cVOC contaminant(s)	
Maximum source zone concentrations(s)	
Concentration range(s) in bioremediation area when remediation initiated	
Current contamination	
Horizontal and vertical plume size/scale	
Hydrogeology (aquifer type, flow and transport of COCs, water table, etc.)	
Geochemistry (baseline, in source zone, and plume)	
Key modifying factors (co-contaminants, critical receptors, etc.) Summarize conceptual site model (CSM) – please attach with the return of this interview	
What are the interim goals and *end state* objective of the site overall? Are cVOC metrics or criteria considered as part of these objectives?	
List the regulatory statutes/drivers that determine which technologies are assessed/applied (e.g. CERCLA, RCRA, state regs, etc.)	
Is there a targeted/projected *timeframe* for reaching cleanup goals?	

Describe the site *stakeholders*. These may include citizens, community, environmental advocacy members, members of the affected public, or tribal stakeholders.	

Bioremediation Technologies	
What *approaches/technologies* have been applied for cVOC remediation for this plume/site? (e.g., anaerobic, aerobic co-metabolisms, structured geochemical zones, etc.)	
What is the *overall scale* of the pilot or deployed bioremediation technology? (e.g., target zone size and geometry)	
For anaerobic technologies: List the electron donor(s) (e.g., emulsified vegetable oil, molasses, mulch, whey, etc.) and quantities	
Were electron donors or other additives solids, liquids or gases?	
Were supplemental reductants (e.g., ZVI) or supplemental sorbents (e.g., peat/mulch) used in combination with primary electron donor for bioremediation?	
Deployment method (injection, fracking, trenching, soil blending, etc.)	
Deployment description (e.g., number of injection points, spacing, etc.)	
Were macronutrients (N, P) or micronutrients used?	
Did the project use bioaugmentation (e.g., adding *Dehalococcoides* or other specialized organisms)?	
Summary description of deployment strategy	
For aerobic technologies or structured geochemical zones:	

Bioremediation Technologies	
Were any electron acceptors or electron donor(s) used? Please note this survey is not collecting information on in situ oxidation using strong oxidants such as permanganate, persulfate, peroxide or ozone.	
Deployment method (injection, fracking, trenching, soil blending, etc.)	
Deployment description (e.g., number of injection points, spacing, etc.)	
Did project use bioaugmentation (e.g., adding *Dehalococcoides* or other specialized organisms)?	
Summary description of deployment strategy	
When/how long has the technology been deployed?	
Programmatic and regulatory objectives of the bioremediation (what goals was it trying to achieve?)	
How is success defined for the bioremediation technology? (metrics, scales, reporting requirements)	
What monitoring strategies or metrics were used for assessing the performance of the bioremediation:	
Concentration metrics?	
Geochemical metrics? (ORP, pH, etc.)	
Lines of evidence (trends and plume scale parameters)?	
Were molecular and biological tools (e.g., qPCR) used to track the performance?	
Please describe the metrics that were used to document performance to regulators and how these were used to assess progress toward objective.	
Summary assessment of the bioremediation performance: Is the project complete or ongoing?	

Bioremediation Technologies	
Did the project achieve (or is the progress acceptable toward) regulatory/target objectives?	
What are the positives observed in bioremediation performance?	
Were there any negatives (underperformance) issues? If so, were adjustments or contingencies made and how did each work?	
What is the site/regulator/DOE overall assessment of success for the bioremediation?	
Are there any planned/future assessments or deployments of bioremediation technologies? If so, please describe.	
Based on your experiences: What are the top challenges to implementation of these technologies?	
What are the major lessons learned from these activities?	
Are you interested in preparing a short (2 to 3 page) case study to help illustrate DOE's experience with cVOC bioremediation?	

Appendix B. DOE Savannah River Site (SRS)

Bioremediation has been applied at SRS in several areas to target removal of VOCs, though the technologies selected and how they were applied varied. The locations at SRS include the Western Sector of A&M Area, Technical Nuclear Explosives (TNX) facility, Sanitary Landfill, and C-Area Groundwater Operable Unit. Each of these will be discussed separately in the narrative below.

1. **SRS A/M Area**: Emulsified Zero-Valent Iron Field Scale Study in Western Sector of the M-Area Hazardous Waste Management Facility (HWMF)

1.1. Description

SRS's M-Area was historically used for the fabrication of reactor fuel and target assemblies. M-Area operations occurred from 1955 through the end of the Cold War and degreasing solvents containing VOCs were used extensively during those operations. Spent solvent waste resulted in contamination of the soil and groundwater of M-Area primarily through the M-Area Basin and an underground process sewer line. The resulting dissolved solvent plume is composed primarily of TCE and PCE. This plume extends approximately two square miles and is about 200 feet deep. Bioremediation was investigated as a treatment alternative for these VOCs as part of an Integrated Demonstration (ID) Program at the SRS.

At the SRS M-Area Hazardous Waste Management Facility (HWMF), 2.5 million pounds of solvent were released to an earthen basin where it vertically migrated as DNAPL through the vadose zone and into the underlying aquifers. DNAPL continued to travel along local low permeability zones to the north and west of the basin to an area known as Western Sector, where high concentration dissolved VOC plumes are now present. TCE and PCE are the main contaminants of concern in this plume. Though a pump and treat system has been in operation for over 30 years, high concentrations persist in the Western Sector. Due to the size and complexity of the A/M Area VOC plume at SRS, field-scale demonstrations of innovative technologies have long been a part of the overall remedial strategy.

In 2022, EZVI was assessed as one of these technologies through a field scale corrective action study under a RCRA permit. The goal of this pilot study was to evaluate EZVI as a potential hotspot treatment for VOCs. This project was one of several to deploy field scale corrective action technologies in the Western Sector to help conduct a Corrective Measures Study and pick a final corrective action and develop a corrective action plan. This technology was selected to target and degrade residual DNAPL that lingers within the aquifer that would likely provide a long-term source for technologies that focus on cleaning the more permeable portions of the aquifer (i.e., pump and treat chemical oxidation, etc.).

1.2. Approach

EZVI was injected to treat a small area within the large VOC plume in A/M Area, focused on the confined upper Lost Lake Aquifer Zone (LLAZ). The LLAZ is an aerobic aquifer with minimal organic content. Though difficulties were experienced getting material into the ground, over 39,000 gallons of EZVI were successfully injected into the targeted aquifer.

Prior to treatment, PCE concentrations in the bioremediation area ranged from 23,000 to 15,000 ug/L and TCE concentrations ranged from 6,000 to 4,000 ug/L. Emulsified vegetable oil in combination with zero valent iron (ZVI), a supplemental reductant, was deployed at six injection points in a 50-ft by 25-ft area and a 25-ft thick section of the LLAZ using hydraulic fracturing. Five horizons at each injection point were created using 5-ft spacing between fractured horizons during injection. The depth of the injection points allowed for interlocking of the fractured horizons, rather than them being on the same horizon. Four monitoring wells were installed within the treatment zone to monitor changes.

1.3. Level of Success and Major Challenges

Monitoring started at a high frequency and was reduced after six months, continuing for two years. Only a small percentage of the area being targeted was successfully treated. Of the four monitoring wells, changes were seen only in the well where oil was present. Reductive conditions and minimal decreases in VOC concentration were observed in that well only, while little to no impact was seen in the other monitoring wells in the treatment zone. This underperformance is attributed to the high viscosity of the EZVI product and the resulting limiting contact between oil and sediments. It is unlikely that the product was distributed out into the heterogeneous aquifer where contamination resides in both permeable and low permeable sediments; instead, remaining local to the fractures and limiting the aquifer material that came in contact with the EZVI product.

The primary lesson learned from this deployment is that site characterization is key to understanding where the highest concentrations are present. The results of this study suggest that EZVI treatment could work well for VOC reduction in A/M Area, but distributing this product in a silty, sandy aquifer was not done well and ultimately contributed to its underperformance. This study is considered complete and there are no planned future assessments/deployments with this technology. All data has been collected but not all data has been reported the South Carolina Department of Environmental Services (SCDES) yet.

2. **SRS Sanitary Landfill**

The Sanitary Landfill (SLF) began receiving waste in 1974 and the main area of the landfill was filled by 1987. Since then, the SLF was subject to Resource Conservation and Recovery Act (RCRA) requirements because of the presence of RCRA-listed solvents. A RCRA Postclosure Part B Permit Application was applied in 1993 for portions that received the RCRA waste, and a closure plan was submitted and approved in 1995. This plan included installation of the geosynthetic closure cap and was initiated in 1996, where a RCRA style closure was completed over the main and southern portions of the landfill in 1997. Since then, renewal permits were submitted and approved, and a monitoring plan was put in place.

2.2. Approach

Characterization activities at the SLF show the vinyl chloride (VC) plume migrating towards the southern end of the SLF. A full-scale biosparging remedy was initiated in 1999 with trichloroethylene (TCE) and VC as the target of this remediation. Two horizontal wells were installed downgradient of the landfill and perpendicular to groundwater flow. The biosparging remedy included the injection of methane and TDP, with the addition of air, nitrous oxide, and nutrients introduced in 2005. Another well was also installed downgradient for additional monitoring.

2.3. Level of Success and Major Challenges

The original chemistry of the subsurface in this area was primarily aerobic and pH was typical for the SLF. More anaerobic activity was seen outside the landfill with the generation of VC in the transition of the landfill to the water table. Significant geochemical and microbial data has been taken during the remediation period and South Carolina Department of Environmental Services, was pleased with the results.

After the biosparging, consistently lower concentrations of VOCs were observed. The alternate concentration limit (ACL) was reached at POC wells and MCLs were achieved at POE wells. When the biosparging effort was stopped, the constituents remained below MCLs.

This landfill is no longer being used, and a permit renewal is done every 10 years. Tentative information was received that a possible corrective action for 1,4-dioxane will be required, but no corrective actions have been taken at this time.

3. SRS C-Area Operable Unit

3.1. Description

Groundwater in the C-Area Groundwater (CAWG) Operable Unit is contaminated with tritium and VOCs, primarily TCE, due to releases associated with reactor operations. The area is located 1 mile south of C-Reactor near Castor Creek and an unnamed tributary in a very aerobic zone. Contaminant levels are approximately 15 ug/L in the unnamed tributary and the goal is to reach 5 ug/L.

An agreement between the U.S. Department of Energy (DOE), Environmental Protection Agency (EPA), and South Carolina Department of Environmental Services (SCDES) outlined a non time-critical removal action to be conducted at C-Area to ensure human health and environmental protection.

3.2. Approach

The removal action included the injection of an emulsified oil base, a buffer (EOS CoBupHMgTM), baking soda, and vitamin C to remove oxygen from the water. Vitamin B was also included to support microbial activity. The primary species being targeted was *Dehaloccoides mccartyi*. The objective of this removal action was to minimize oxygen and optimize nutrients to stimulate/support microbial degradation. There were two rows of injections and one-time deep push technology injections. Fifteen injection points were spread 15 feet apart in a total of 3 acres in a zone that is 10-15 feet thick. As there was also heating conducted near the source, it is considered a combined remedy.

Figures 3.1 and 3.2 show the TCE plume in the C-Area and the removal action process, respectively.

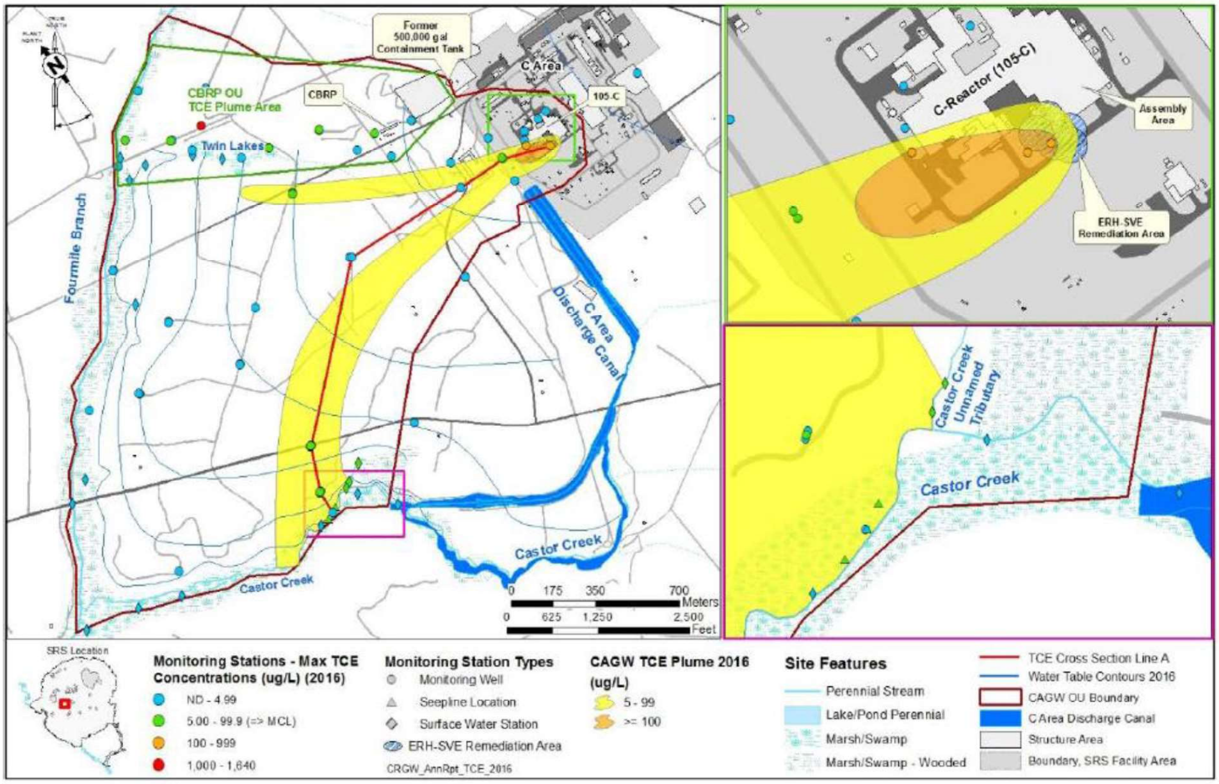

Figure 3.1. C-Area TCE Plume (2016) (SRNS, 2019).

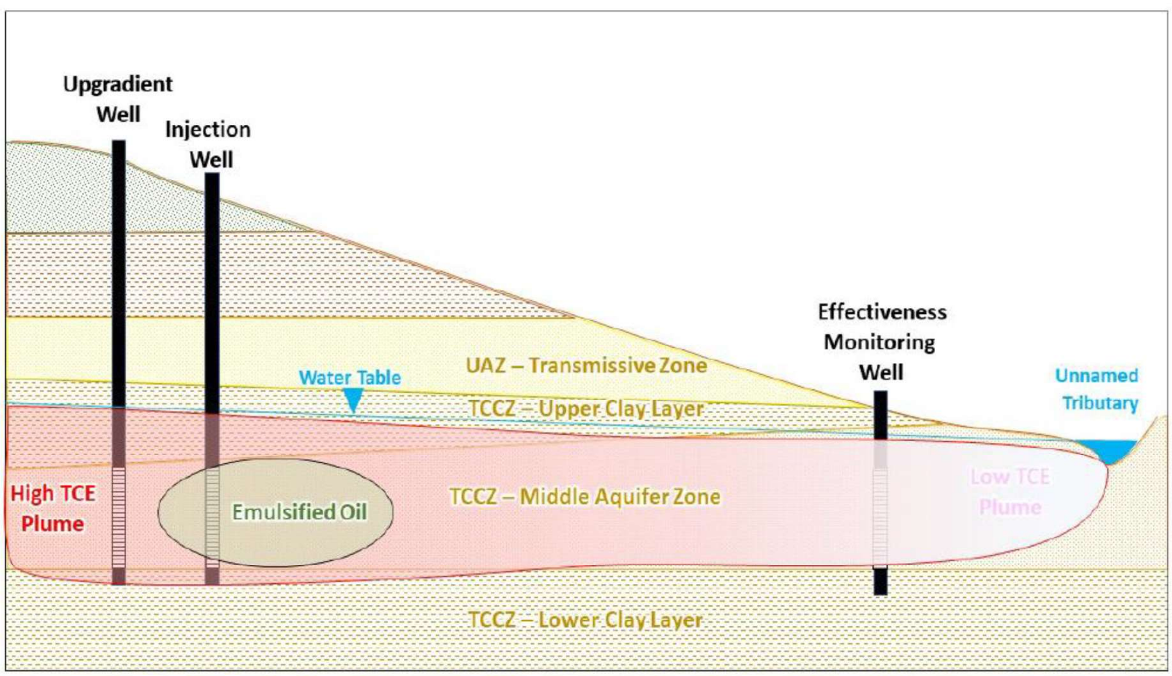

Figure 3.2. Schematic of CAGW Operational Unit Removal Action Process (SRNS, 2019).

SRNS, 2019. Removal Action Report for the C-Area Groundwater Operable Unit Bio-Barrier Non-Time Critical Removal Action, SRNS-RP-2019-00584, Revision 0, Savannah River Nuclear Soutions, LLC, Savannah River Site, Aiken, SC.

3.3. Level of Success and Major Challenges

Remediation is ongoing and is expected to last 3-5 years. It was noticed, however, that TCE started breaking down after 3 years. Between 2020 and 2022, concentrations in three surface water stations have dropped below 5 ug/L. Monitored natural attenuation will be the preferred remedy going forward if the surface water concentrations for TCE and tritium drop/remain below the MCLs.

The microbial community was monitored by using BioTraps from Microbial Insights. Methanogenesis was observed which stimulated co-metabolism.

No additional injections to introduce more reagents are currently planned, but alternative approaches are being considered for future implementation. To help improve the viability of the bioaugmentation conducted, it is recommended that oil be injected prior to (and separate from) a second injection to support microbial activity using additional buffer/nutrients.

Appendix C. DOE Hanford

1. VOC Arid Integrated Demonstration and Monitored Natural Attenuation

1.1. Description

Carbon tetrachloride (CT), nitrate (NO_3), and other contaminants were discharged from 1955 to 1973 to subsurface waste disposal facilities in the 200 Area at Hanford, creating a large plume of CT and NO_3. A Tri-Party Agreement (DOE, EPA, and the Washington Department of Ecology) governs cleanup under CERCLA and RECRA. The Hanford Advisory Board, that includes community and Tribal stakeholders, tracks remediation progress and provides feedback. The primary objective for Hanford groundwater environmental cleanup work is protection of the Columbia River. The 200-ZP-1 record of decision (ROD) specified 25 years of pump-and-treat (P&T) remediation, followed by 100 years of monitored natural attenuation (MNA) for CT. More recent assessments indicate that 125 years may not be enough time to achieve CT plume cleanup goals. The presence of other contaminants (uranium, technetium-99, iodine-129, chromium) in the Hanford Central Plateau groundwater complicates the overall remedy.

1.2. Approach

The CT contaminant plume has a spatial extent of roughly 5 km by 5.5 km, with an approximate thickness of 60 m at the plume core, which originated within the Hanford 200-ZP-1 operable unit. The hydrogeology of this area consists of unconfined and confined aquifers. Two independent efforts at Hanford are described below: 1) an ISB pilot and 2) ongoing research to understand and optimize P&T and MNA. An in situ bioremediation (ISB) pilot demonstration took place from 1995- 1996 with two main objectives: 1) to demonstrate the successful degradation of CT without significant accumulation of chloroform and 2) to demonstrate an effective design strategy using pulsed amendment injections (Hooker et al., 1988). The other approach, as prescribed in the ROD, remains the primary remedial strategy for the plume and consist of P&T followed by MNA.

1.2.1. ISB-Demonstration

The active bioremediation demonstration took place within the CT plume at a location away from the source zone area. The ISB system used recirculation and offset pulsed injections of amendment species to distribute the amendments in the subsurface. This approach was intended to minimize injection well biofouling and stimulate anaerobic denitrifying conditions for degradation of CT, while also minimizing (or eliminating) accumulation of chloroform (Hooker et al., 1998). The degradation of CT is influenced by nitrate/nitrite concentrations, so finding the right conditions for degradation was important. Injection wells, in a dipole recirculation system with a 12 m spacing, were used. Acetate (~ 18 mM) and nitrate (~ 5-8 mM) were introduced into the aquifer via the recirculation stream in pulses offset by a few hours.

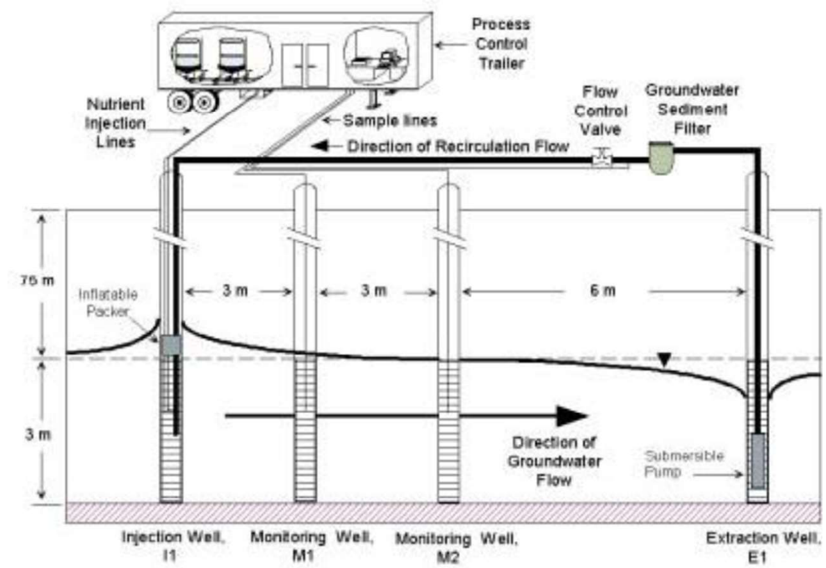

Figure 1. Recirculating/monitoring well system used for bioremediation operations.

Two sampling wells were located between the recirculation wells, as shown in Figure 1. The wells were drilled to a 320-foot depth. Two separate tests were performed in non-interacting aquifer layers. There was an upper and a lower biostimulation zone. Concentrations of CT and chloroform were measured every two weeks. Temperature, pH, and oxidation/reduction were monitored continuously.

1.3.2. Monitored Natural Attenuation

As stated previously, the 200-ZP-1 record of decision (ROD) specified 25 years of (P&T) remediation, followed by 100 years of MNA for CT. This ROD was based on a MNA half-life determined from the literature. Later, it was determined that the MNA half-life is likely longer than the value previously obtained from the literature, leading to laboratory studies to investigate MNA half-life.

These recent laboratory bioremediation studies used Ringold sediment from areas of high contamination and from the fringe of the plume (see Figure 2). Finer sediments from the cores were selected and spiked with CT for laboratory studies designed to gain insight into MNA. Complete biotic degradation was observed in 2-4 months across the full range of site-specific CT concentrations. Abiotic degradation was effective but only at low concentrations of CT. For the biotic tests they used a heat-killed control and observed first-order degradation of CT, while abiotic tests had a much lower capacity for CT degradation. There is potential for significant heterogeneity of biotic processes. Understanding the potential impact of heterogeneity on MNA will help inform future demonstrations and remediation strategies.

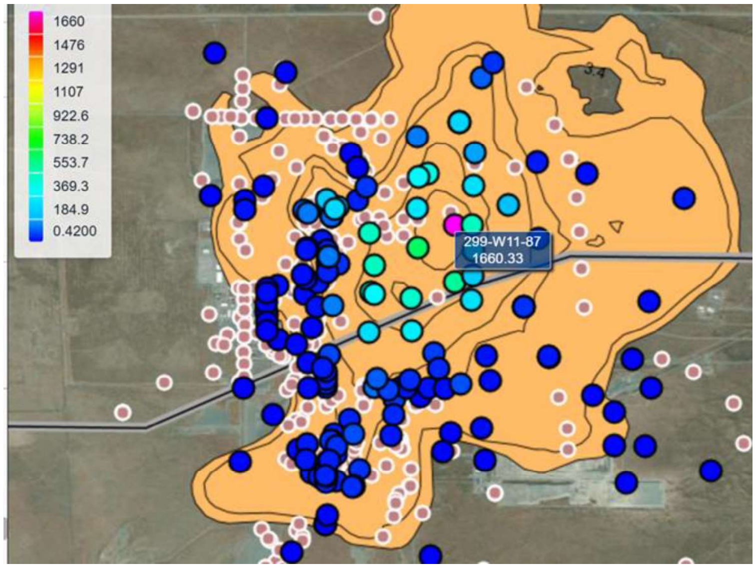

Figure 2. CT contamination (µg/L) across the plume (2023).

Recent CT concentrations were 8.06 µg/L (2022) and 18.50 µg/L (2023) near the source. Across the entire plume, a maximum concentration of 2000 µg/L was observed with an annual average maximum of 1660 µg/L based on 6 samples (Figure 2).

1.4. Level of Success and Major Challenges

The pilot-scale demonstration was completed in the late 1990s and successfully demonstrated the objective of biodegradation of CT while minimizing chloroform accumulation, as well as the efficacy of using pulsed injections to deliver amendments. When bioremediation was initiated, the contaminant concentrations were 12.5 ± 0.14 µM CT and 3.87 ± 0.26 µM NO_3. During the 7-month ISB test, the CT concentration dropped by 3.71 µM, representing an estimated total of 1.42 kg of CT destroyed (Hooker et al., 1988).

The pilot-scale demonstration of ISB successfully demonstrated the objective of biodegradation of CT while minimizing chloroform accumulation, as well as the efficacy of using pulsed injections to deliver amendments. For Hanford, the biggest issues with deployment of this technology for treatment of the CT plume are the large size of the CT plume (which would probably require thousands of wells), the significant depth to contamination (water table is at a depth of about 250 ft) and thickness of the contamination (up to 60 m). Treatment of the entire CT plume would likely be infeasible and cost prohibitive at a cost of $1 million to install a single well. However, ISB may have a role in 1) a permeable reactive barrier (PRB) design in targeted areas or 2) in remediation of hot spots.

Ongoing laboratory studies designed to understand MNA half-life (as described above) are being performed to measure abiotic and biotic degradation of CT in site sediment. Degradation rates are higher than expected based on field observations and span each of the relevant hydrogeological units. The preliminary half-life determined through these studies is not firm yet. It is hypothesized that scale may influence the higher degradation rate observed in the laboratory studies versus MNA observed in the plume. In addition, ongoing work is targeting optimization of the P&T system to maintain or increase effective mass removal and decrease the duration of the P&T portion of the remedy. These and other efforts will help form a basis for decision making regarding refined remedy approaches.

References:

Hooker, B.S., R.S. Skeen, M.J. Truex, C.C. Johnson, B.M. Peyton, and D.B. Anderson. 1998. "In Situ Bioremediation of Carbon Tetrachloride: Field Test Results." *Bioremed. J.*, 1:181-193.

Appendix D. DOE Paducah

1. DOE Paducah Gaseous Diffusion Plant

1.1. Description

The former Paducah Gaseous Diffusion Plant was operated until 2013. As a result of the over 60 years of enriched uranium production at this location, several environmental contaminants have been identified onsite that require remediation. DOE is actively planning and implementing environmental restoration and waste management approaches, in addition to deactivation and decommissioning activities, to address the impacted soil, surface water, groundwater, and other contaminated media onsite. TCE in the groundwater specifically is a concern at this site. There are two four-mile-long dissolved phase TCE plumes (the Northeast and Northwest Plumes) that are believed to originate from one main source location- the C-400 building/complex (and possible nearby, upgradient sources).

1.2. Approach

- High pressure water to create horizontal openings/zones, then inject iron and sand into openings to allow for emulsified vegetable oil and microbe (TCE-reducing bacteria) injections
- Vegetable oil provides nourishment for bacteria and generates subsurface conditions to degrade the TCE
- Northeast plume area treated with pump and treat with air stripper to target high concentration portion of TCE plume
- Pump and treat in northwest plume area to contain/treat high concentration portion of plume
- Deep soil mixing remedial action using a large (8-ft) diameter auger, followed by steam with vapor extraction/treatment and zero-valent iron injection completed in 2015 to target Southwest plume sources
- DOE recommended implementing bioremediation and long-term monitoring at solid waste management unit (SWMU) 211-A in the upper Regional Gravel Aquifer (RGA)
    - the C-400 Complex OU and the area directly north of the C-720 "Machine Shop" Building are referred to as SWMU 211-A
    - Enhanced bioremediation should be effective even against higher VOC concentrations associated with DNAPL-like concentrations known to exist in the upper RGA (EPA, DOE, and Kentucky Department of Environmental Protection (KDEP)) all agreed on this approach in December 2021)

1.3. Level of Success and Major Challenges

- Southwest treatment approach designed to remove organic solvents (primarily TCE) from 258 soil columns to a depth of approximately 60 feet bgs. The remedial action recovered 24 +/-12 gallons of VOCs during operation. Passive treatment using zero-valent iron (ZVI) may still be occurring.

Appendix E. DOE Idaho National Laboratory

1. Test Area North

1.1. Description

From about 1953 to 1972, liquid waste generated at Test Area North (TAN) at Idaho National Laboratory (INL) was disposed of in the TSF-05 injection well, dispersing contaminants into the Snake River Plain Aquifer beneath TAN. The waste injected into TSF-05 mainly consisted of industrial and sanitary wastewater but also included organic, inorganic, and low-level radioactive wastewater. Records provide little information on the types and volumes of organic wastes injected into the groundwater. Estimates of the amount of TCE that may have been injected in the well range from as little as 350 gal (1,325 L) to 35,000 gal (132,489 L) (ICP, 2006). As a result of the waste injected into TSF-05, VOC and radionuclide groundwater plumes emanate from this well. The resulting trichloroethene (TCE) plume is nearly 2-miles long (TCE). In addition to contaminants in the wastewater, sludge material containing contaminants, such as TCE and radionuclides (Sr-90, Cs-137, and tritium), accumulated in TSF-05 and in fractures around the injection well. Contaminants trapped in sludge are released to the groundwater as it flows through and by TSF-05. An initial remediation effort removed sludge from the bottom 55 ft of TSF-05 in 1990 (approximately 255-310 ft below ground surface (bgs)) (ICP, 2006).

The site of the plume was identified as Operable Unit (OU) l-07B and consists of groundwater contamination located in the northern portion of the Idaho National Laboratory (INL) Site (Figure 1). A Record of Decision (ROD) was signed in 1995 selecting groundwater pump and treat as the remedy for the site (U.S. Department of Energy Idaho Operations Office [DOE-ID] 1995). The plume was divided into three zones based on TCE concentration and as allowed by the ROD, additional technologies were evaluated and ultimately some were implemented as described below (see Figure 2). Contaminants of concern identified were trichloroethylene (TCE), cis-1, 2-dichloroethene (cis-DCE), trans-I, 2-DCE (trans-DCE), and tetrachloroethene (PCE), as well as several radionuclides.

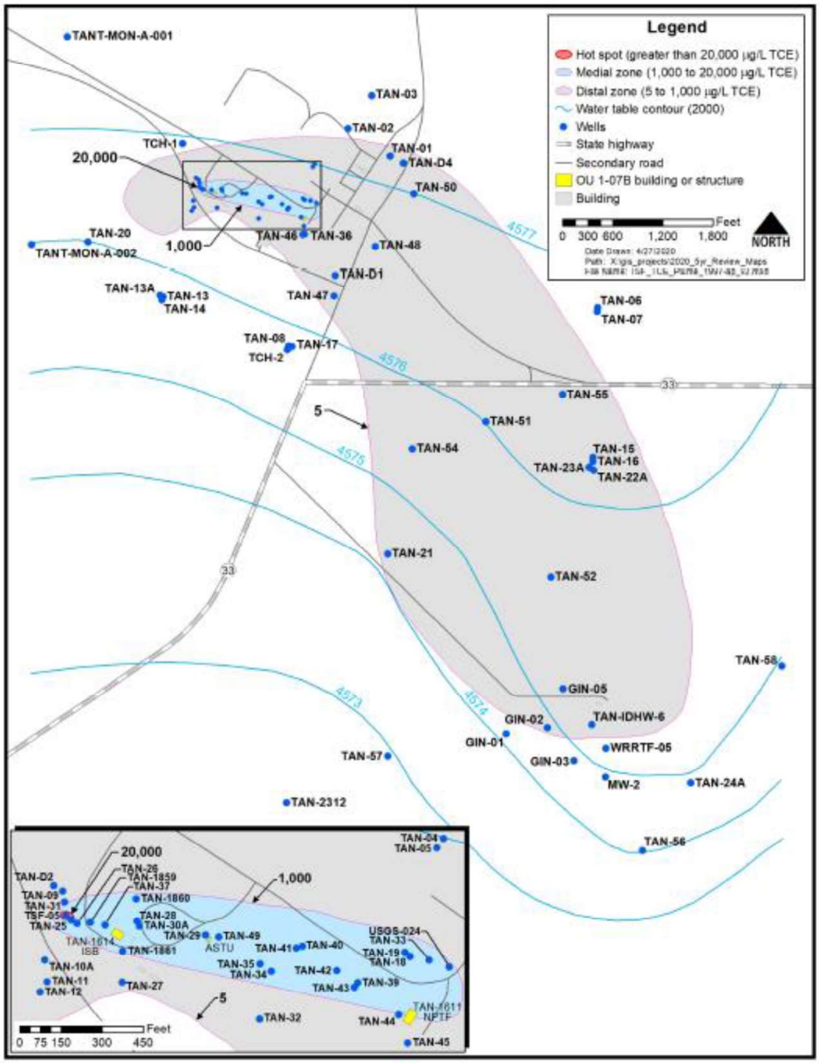

Figure 1. Operable Unit 1-07B wells and geographical locations of hot spot, medial, and distal zones based on 1997 TCE concentrations (DOE-ID, 2021).

The subsurface geology of TAN is similar to most of the INL site and is characterized by basalt flows with sedimentary interbeds, overlain by fine-grained sediments. There are two main interbeds at TAN, the P-Q and Q-R interbeds, which both consist of clay or silt that retard contaminant transport. The P-Q interbed, located in the vadose zone approximately 200 ft below ground surface near the injection well TSF-05. This interbed appears to be laterally discontinuous, with the thickness ranging from 3 to 14 ft. The Q-R interbed, is in the same vicinity, and is in the saturated zone approximately 410 ft below ground (DOE-ID, 2021). Groundwater flow is generally through fractures and other permeability enhancing features such as rubble zones, flow boundaries, and cavities in the basalt.

The bottom of the plume is bounded by the Q-R interbed which acts as a confining layer. The approximate extent of the residual source contamination (see Figures 1 and 2) has an area of 19,816 sq. ft and extends vertically from 200 ft bls to the Q-R interbed located at approximately 410 ft bls. Currently, the water level at TSF-05 is approximately 233.95 ft bls; therefore, some of the contamination is within the vadose zone (Traub interview, 2025).

The regulatory driver for remediation at TAN is compliance with the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). A 2001 ROD Amendment applied the following remedial action objectives (RAOs) to the full plume (DOE-ID, 2021).

- Restore contaminated groundwater by 2095 by reducing all contaminants of concern (COC) to below their maximum contaminant levels (See Table 1) and a 1E-04 total cumulative carcinogenic risk-based level for future residential use, and for noncarcinogens, until the cumulative hazard index is less than 1.
- Reduce the concentrations of VOCs to below MCLs and a 1E-05 total risk-based level for above ground treatment processes in which treated effluent will be reinjected into the aquifer.
- Implement ICs to protect current and future users from health risks associated with (1) ingestion or inhalation of, or dermal contact with, contaminants in concentrations greater than the MCLs; (2) contaminants with greater than a 1E-04 cumulative carcinogenic risk-based concentration; or (3) a cumulative hazard index of greater than 1, whichever is more restrictive.

Table 1. MCLs for TAN VOCs

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

The stakeholders of the site include citizens, environmental advocacy members, regulatory agencies, and the citizens advisory board (CAB). The Shoshone-Bannock Tribes of the Fort Hall Indian Reservation, an independent sovereign entity, comprise another key member of the community.

1.2. Approach

A multi-component remedy was designed to address the varying concentrations of TCE within the plume at TAN (See Figures 2 and 3). These include in situ bioremediation (ISB) of the hot spot, pump and treat (P&T) for the medial zone and monitored natural attenuation (MNA) in the distal zone. ISB is the focus of this review, but the other complementary remediation approaches are important to meeting the objective of the ROD of having all cVOC levels below their MCLs by 2095. A conceptual site model of the TCE source and plume is shown in Figure 3.

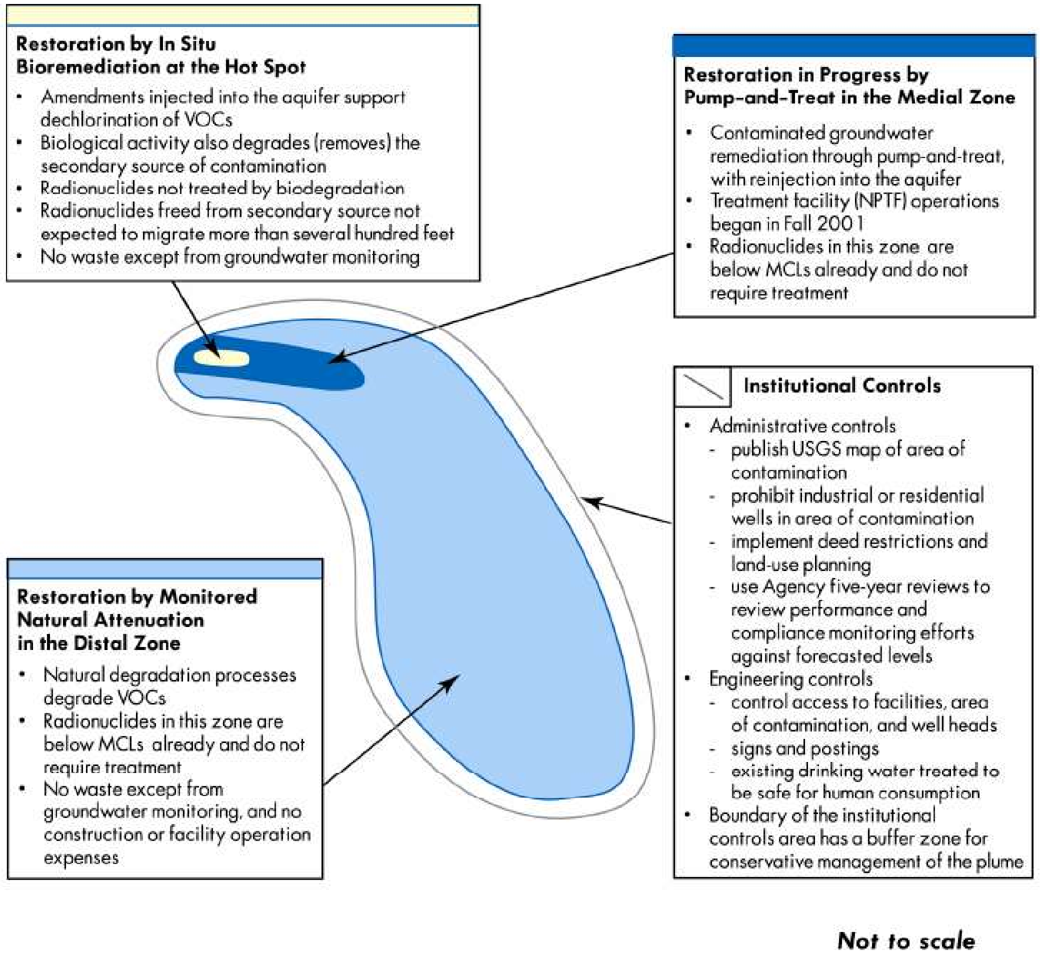

Figure 2. Illustration of the three zones of the TCE plume at TAN (INL ICP, 2007).

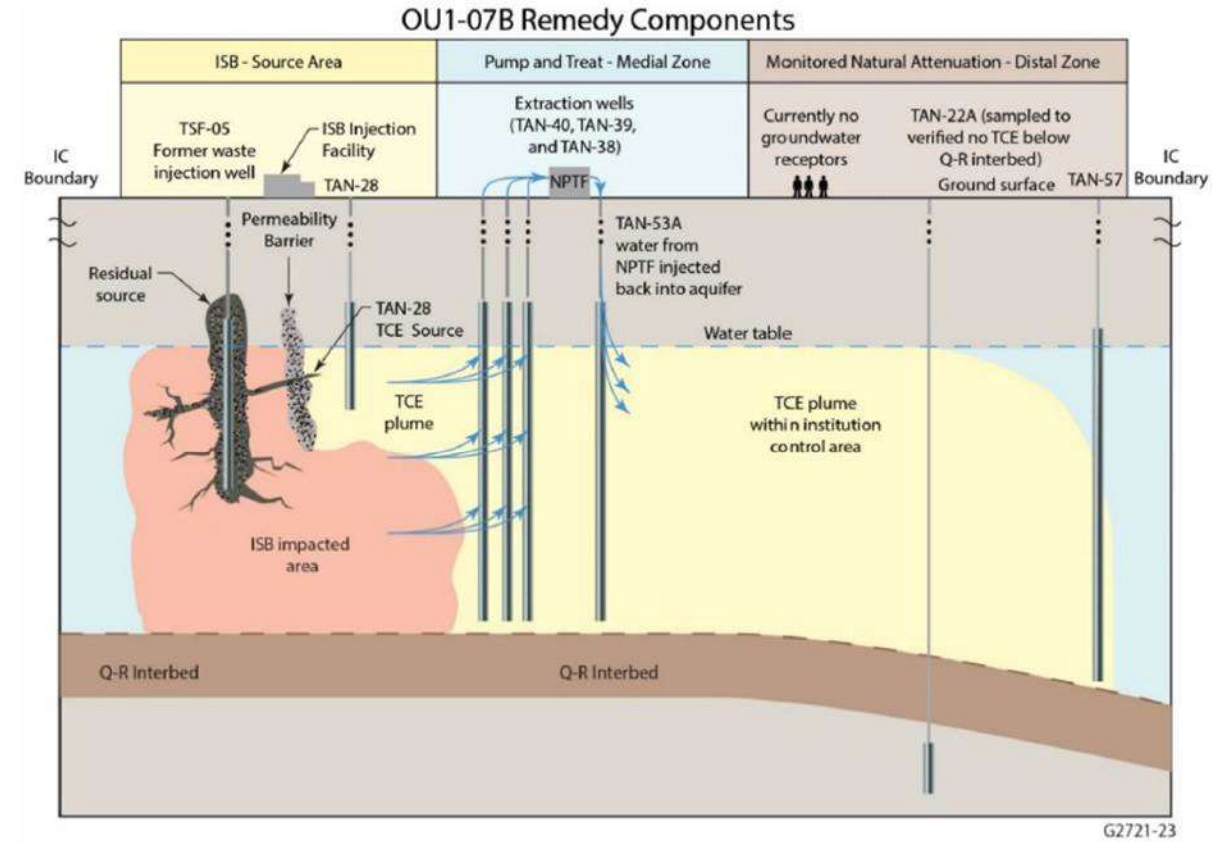

Figure 3. Conceptual site model of the trichloroethylene source and plume (DOE-ID, 2020).

ISB has been the main remedy the TAN TCE source zone, also referred to as the hot spot. This remedy was selected for the source zone in the 2001 Record of Decision (ROD) amendment. ISB amendment has been injected into and near source areas to encourage the growth of indigenous bacterial populations to metabolize the TCE and other accompanying volatile organic compounds (VOCs). ISB operations involved injecting a carbon source (electron donor) into the aquifer to create oxidation-reduction (redox) conditions favorable for indigenous anaerobic microbes to degrade chlorinated ethenes (PCE, TCE, cis-1,2-DCE, trans-1,2- DCE and VC) to innocuous end products (ethene, methane, and carbon dioxide) through anaerobic reductive dechlorination (ARD).

1.2.1. ISB

The ISB component of the remedy consists of two parts: 1) an ISB electron donor optimization and rebound test near former injection (source) well TSF-05 and 2) ISB operations to treat the source of TCE in well TAN-28. An amendment (e.g., sodium lactate or whey) was injected into the source area through the former waste injection well, TSF-05, or through other injection wells (e.g., TAN-25, TAN-31, and TAN-1859) near TSF-05. These tests ran from January 1999 until April 2012. In July 2012, ISB transitioned to a rebound test. In 2016, the ISB component of the remedy was split into two parts: 1) an ISB rebound test for the TSF-05 vicinity and 2) the TAN-28 TCE source action to address the TCE source impacting Wells TAN-28, TAN-29, and TAN-1860A. Implementation of the injection strategy to enhance electron donor distribution will work toward achieving the goals to effectively distribute electron donor to the entire source area, sustain efficient ARD conditions, and cut off flux of VOCs from the residual source.

1.2.1.1. TSF-05

The former waste injection well, TSF-05, was identified as the source of the groundwater contamination. An amendment (e.g., sodium lactate or whey) was injected into the source area through the former waste injection well, TSF-05, or through other injection wells (e.g., TAN-25, TAN-31, and TAN-1859) in the immediate vicinity of TSF-05. ISB operations took place from the start of the ISB field test in January 1999 until the last injection in April 2012. In July 2012, ISB transitioned to a rebound test (DOE-ID, 2021).

ISB at TAN included an enhanced electron donor distribution strategy designed to increase the distribution of electron donor to include the entire residual source area to cut off flux to downgradient and cross-gradient locations and to maintain efficient degradation of residual source material (ICP, 2007). The strategy included iterative monthly injections as well as multi-well injections. Some wells were modified to handle increased flux and a packer was installed in TAN-1859 to facilitate radial distribution of electron donors to the portions of the aquifer with higher TCE. Both the iterative and multi-well injections were success at expanding extent of electron donor distribution. Different electron donors were also investigated to see if one resulted in greater distribution.

ISB targeted the TSF-05 injection well and other nearby wells. These injections used whey powder and sodium lactate injections. From 1999 to 2004, only sodium lactate was injected. From 2004 to 2008, only whey was injected. From 2008 to 2012, a mixture of whey and sodium lactate was injected. Mixture of whey to sodium lactate ranged from 10 lb of whey to 1 gal of lactate to 5 lb of whey to 1 gal of lactate. The amount of sodium lactate injected into the well depended on the well as the lactate was used to reduce the pH drop that occurred following injections. All injections were performed with a potable water; the percent of whey to potable water was approximately 10% on average (Traub interview, 2025).

Amendment was injected into wells TSF-05, TAN-1859A, TAN-31, and TAN-25. The injections focused on performing two simultaneous well injections (i.e., into TAN-31 and TSF-05A) and then in the same day performing another two simultaneous well injections (i.e., into TAN-1859B and TSF-05A). Another injection event would typically follow 2 months later. Typically, 6,000 lb of whey would be used in each simultaneous injection and between 550 and 1,100 gal of sodium lactate, depending on the well. Injections into the TAN residual source area were performed from 1999 to 2012. In 2012, the TAN residual source area transitioned into a rebound test (Traub interview, 2025).

No bioaugmentation was needed as historical sampling showed populations of *Dehalococcoides*. During the period of injections from 1999 to 2012, monthly samples of VOCs, chemical oxygen demand (COD), alkalinity, tritium, ferrous iron, sulfate, methane, and ethene were collected from nearby wells. Ferrous iron, methane, and sulfate were used to determine the redox conditions within the wells. VOCs were also analyzed to determine how redox conditions were affecting the TCE source. Lastly, ethene was monitored to determine if complete reduction of TCE to ethene was occurring (Traub interview, 2025).

The effects of different injection strategies on the efficiency of the ARD reactions were assessed by examining changes in the relative concentrations of TCE and reductive daughter products cis-dichlorethene (cis-DCE), VC, and ethene. High concentrations of ethene relative to TCE, cis-DCE, and VC indicate that ARD reactions are efficiently dechlorinating TCE to non-toxic daughter products.

One rate-limiting step affecting the ISB remedial timeframe at TAN is the mass transfer of TCE from the residual source material to the aqueous phase where it is available for microbial biodegradation. This rate was found to be a function of whey powder concentration (ICP, 2007). Multi-well injection strategy was found to not only increase the biologically active area, it appeared to also contribute to degradation of residual source material along the periphery of the residual source area (ICP, 2007).

Background geochemistry for the TAN residual source area is nitrate concentrations between 1 and 4 mg/L-N, iron concentrations of less than 0.05 mg/L, sulfate concentrations between 33 and 50 mg/L, and methane concentrations between 0 and 20 µg/L. Current concentrations of these analytes within and near the residual source zone can be seen in Table 1. Background dissolved oxygen (DO) conditions were aerobic, and pH was circumneutral in TSF-05A and neutral to slightly acidic in TSF-05B. Currently, water quality parameters are not collected at TAN (Traub interview, 2025). Along with VOC contamination, Cs-137 and Sr-90 are also COCs. The strategy for Cs-137 and Sr-90 is monitored natural attenuation. Institutional controls are in place to control exposure pathways that could result in unacceptable risks to potential receptors (Traub interview, 2025).

The project is currently ongoing as the system is still showing reduced conditions and has not rebounded to background. Once the system has rebounded to background continued sampling will determine if TCE concentrations rebound and if more ISB injections must be performed to treat the residual source area. To date the project has demonstrated success at creating redox conditions and lowering the concentration of TCE in the residual source zone. As previously stated, once conditions return to pretreatment background conditions signaling the end of the rebound test initiated in 2012, the success of the ISB remediation can be determined. The assessment of success will be if conditions within the residual source area return to background and VOC concentrations remain below their MCLs. On the other hand, If the rebound test is completed and VOC concentrations are above the MCL ISB injections into the TAN residual source wells will likely resume.

1.2.1.2. TAN-28

Following ISB injections into the TAN residual source zone (TSF-05 area), TCE concentrations remained high within TAN-28; concentrations in 2012 ranged from 544 to 1,050 µg/L. The TAN-28 TCE source could be sludge and waste materials from former waste injection well TSF-05 that flowed into open cracks or fissures in the vicinity of the well and later filled (or partially filled) the formerly open spaces. The area of the TAN-28 Source Action is approximately 14,000 sq. ft. Like the TAN residual source area, the verticality of the TAN-28 source area goes from approximately 200 ft bls to the Q-R interbed located at approximately 410 ft bls. Currently, the water level at TAN-28 is approximately 232 ft bls; therefore, some of the contamination is within the vadose zone (Traub interview, 2025).

ISB has also been the main remediation strategy for the TAN-28 source area, a secondary contamination area that is affecting a different area of the TAN facility has been occurring from 2016 to present (Traub interview, 2025). ISB amendment injections included Wilclear Plus and LactOil and occurred in Wells TAN-2272A and TAN-37A (DOE-ID, 2021). Additional injections occurred at TNA-1860A and TAN-2336 (Traub interview, 2025).

From January 2016 to October 2019, WilClear Plus was used in injections. From October 2019 to May 2020, WilClear Plus and LactOil were used in injections. From May 2020 to present Wilclear Plus has been used in injections (https://jrwbioremediation.com/our-products/wilclearplus/). The injections are performed with a potable water; the amendment percentage to potable water is approximately 10%. No other supplemental reductants or supplemental sorbents are used in the injection (Traub interview 2024).

Injections occurred every 2 to 3 months. From 2016 to 2023, injections were performed into a single well. From 2023 to present, most injections are performed into two wells over the course of 2 days. Injections use between 250 and 1,000 gal of WilClear Plus. No bioaugmentation was needed as historical sampling showed populations of *Dehalococcoides* (Traub interview, 2025).

Background geochemistry for the TAN-28 source area is nitrate concentrations between 1 and 4 mg/L-N, iron concentrations of less than 0.05 mg/L, sulfate concentrations between 33 and 50 mg/L, and methane concentrations between 0 and 20 µg/L. Background DO conditions were aerobic and pH was circumneutral. Current concentrations of these analytes within and near the TAN-28 source zone can be seen in Table 2. Along with VOC contamination, Sr-90 is also a COC. The strategy for Sr-90 is monitored natural attenuation. Currently, water quality parameters are not collected at TAN (Traub interview, 2025).

Testing within the TAN-28 source area mostly looks at concentrations of redox analytes, such as methane, sulfate, iron, and nitrate. COD and alkalinity are also analyzed within TAN-28 and the injection wells. VOCs are also analyzed to determine how redox conditions are affecting the TCE source. Lastly, ethene is also monitored to determine if complete reduction of TCE to ethene is occurring.

Regulators are shown Table 3, which provides redox conditions within the wells near the TAN-28 source area and provides TCE concentrations within TAN-28. The two lines of evidence provide data on how injections are affecting the TAN-28 source area (Traub interview, 2025). TCE concentrations within TAN-28 have fallen from 1,280 µg/L in 2013 to typically having concentrations less than 30 µg/L. Concentrations are mostly below 10 µg/L within TAN-28 but tend to show a rise in TCE during the summer, which is likely due to snow melt and water levels rising mobilizing more of the TAN-28 TCE source. The decrease in TCE within TAN-28 is favorable and is likely indicative of an overall decrease of the TAN-28 source (Traub interview, 2025).

Since this system is heavily influenced by basalt fracture patterns, and unlike the previous injection strategies where the injections were performed in an around the injection well, determining injection strategies to affect the TAN-28 source can be an issue. To improve success in affecting the TAN-28 source, several wells were drilled to act as injection wells, including TAN-2271, TAN-2272, and TAN-2336. The timeframe of amendment moving through the basalt system can also be a negative as sometimes it will take the amendment months to travel to the TAN-28 source zone (Traub interview, 2025).

Lastly, it was expected that as the TAN-28 source was remediated, concentrations within TAN-29 (see Figure 1) would decrease. However, currently, TAN-29 has the highest concentration of TCE at the TAN facility, ranging from 200 to 400 µg/L. More time is needed to determine if concentrations within TAN-29 will decrease as concentrations have within TAN-28. If concentrations do not decrease, this may indicate another source zone which and will likely require drilling a new injection well to remediate the source (Traub interview, 2025).

The top challenge is the hydrogeology of this location with, and attempting to pinpoint, which injection well will provide the best treatment of the TAN-28 source area. The other challenge is cost, drilling more injection wells in the location of TAN-28 would likely provide a more efficient remediation strategy; however, drilling injection wells 250 ft bls is very costly and adds a focus on using current infrastructure to solve issues. Determining the placement of new wells is also difficult due to the complex hydrogeology (Traub interview, 2025).

Table 2 Concentrations of redox analytes in and near the TAN residual source area from

Well	Methane (µg/L) Apr-24	Sulfate (mg/L) Apr-24	Iron (mg/L) Apr-24	Nitrate (mg/L) Apr-24
Wells in the Residual Source				
TSF-05A	15,100	73.3	3.57	NS
TSF-05B	26,600	0.442	4.68	NS
TAN-25	30,400	5.67	2.170	NS
TAN-31	23,600	19.2	0.276	NS
TAN-1859B	27,100	37.2	4.79	NS
Near Source Wells				
TAN-D2	NS	NS	3.56	ND
Non-ISB conditions	<1,000	>33	<0.05	>0.5
Changing Redox conditions	1,000 -5,000	10 - 33	0.05 - 0.3	0.1 - 0.5
ISB conditions	>5,000	<10	>0.3	<0.1
Background ranges are as follows:				
Nitrate - 1 to 4 mg/L-N		NS - Not Sampled		
Iron - less than 0.05 mg/L				
Sulfate - 33 to 50 mg/L				
Methane - 0 to 20 ug/L				

October 2023 (Traub interview, 2025).

Table 3. Concentrations of analytes near the TAN-28 source area from October 2023 (Traub interview, 2025).

Well	Methane (µg/L) Q1 2023	Sulfate (mg/L) Q1 2023	Iron (mg/L) Q1 2023	TCE (µg/L) Q1 2023	Alkalinity (mg/L) Q1 2023	COD (mg/L) Q1 2023
Wells in the Residual Source						
TAN-28	16,100	29.5	1.19	2.55	488	25.4
TAN-29	56	34.6	ND	354	199	NS
TAN-1860A	12,700	ND	17.2	0.47	4960	1,590
TAN-37A	11,800	5.04	8.47	0.49	2,610	614
TAN-2336	2,440	2.22	7.45	1.23	7,790	1,990
Non-ISB conditions	<1,000	>33	<0.05			
Changing Redox conditions	1,000 -5,0(	10 - 33	0.05 - 0.3			
ISB conditions	>5,000	<10	>0.3			
Background ranges are as follows:						
Nitrate - 1 to 4 mg/L-N						
Iron - less than 0.05 mg/L						
Sulfate - 33 to 50 mg/L						
Methane - 0 to 20 ug/L						

1.2.2. Pump and Treat

Pump and treat operations at TAN involve extraction of contaminated groundwater, treatment through air strippers, and injection of treated groundwater back into the Snake River Plain Aquifer (SRPA). Air stripping is a process that removes VOCs from contaminated groundwater. To treat VOCs in the medial zone, the New Pump and Treat Facility NPTF (completed in January 2001) was put into operation in October of 2001 and continues to be operated voluntarily, approximately 4 days per week (DOE-ID, 2021). The goal of the voluntary operation of the NPTF is to reduce the amount of TCE migrating into the distal (MNA) portion of the plume to aid the MNA part of the remedy. TCE concentrations in most of the medial zone wells are significantly lower than the historically defined concentration range of 1,000 to 20,000 µg/L. TCE concentrations range from 24.2 to 40.7 µg/L in wells TAN-33, TAN-36, and TAN-44 at the downgradient edge of the New Pump and Treat Facility extraction wells. It has been recommended by DOE-ID that the NPTF continue to be operated long-term on a voluntary basis (DOE/ID-12028).

1.2.3. Monitored Natural Attenuation

Natural attenuation occurs through physical, chemical, and biological processes that act without human intervention to reduce the mass, toxicity, mobility, volume, and concentration the VOCs in the groundwater. Groundwater in the distal/MNA zone is monitored to compare actual measured VOCs to groundwater-model-predicted concentration curves to evaluate ongoing natural attenuation as well as any plume expansion. Data collected in support of MNA for the distal part of the TCE plume indicate that VOC concentrations in most wells are trending consistent with the MNA groundwater flow model predictions. Additional time is needed to confirm that the TCE concentrations in all Operable Unit 1-07B wells will be below the maximum contaminant level by 2095 as defined in the remedial action objective. While there has been some plume expansion, it is less than the 30% limit allowed in the Record of Decision Amendment (DOE-ID 2001).

1.3. Level of Success and Major Challenges

Performance is assessed by examining whether each component is (1) achieving individual component remedial objectives and (2) working together to remediate the entire contaminant plume. The observation of complete reductive dechlorination of trichloroethene to ethene, together with evidence of accelerated mass transfer of trichloroethene from the residual source, provides the technical basis for implementing in situ bioremediation as the final hot spot remedy in place of pump and treat.

The ISB rebound test at TSF-05 is still ongoing as the hot spot still has ARD conditions, more time is needed to dissipate ARD conditions before the residual source in the aquifer can be evaluated. TSF-05 is currently split into two depths, TSF-05A (the shallow sampling location) and TSF-05B (the deep sampling location). The pump for TSF-05A is located at approximately 239 ft bls and the pump for TSF-05B is located at approximately 271 ft bls. The highest concentrations within these locations in Fiscal Year (FY) 2024 was non-detect and 3.94 µg/L, respectively. TSF-05B was non-detect in the other sampling event in FY 2024. The TAN residual source area is currently in a rebound test. Currently conditions within TSF-05 have not returned to pretreatment background conditions. Once conditions return to pretreatment background conditions, the success of the ISB remediation can be determined. The highest TCE concentration prior to performing remediation was approximately 32,000 µg/L (Traub interview, 2025).

Injections to address the secondary source material contributing cVOCs to TAN-28 appear to be on track. TCE concentrations have decreased within TAN-28 and the redox analytes within the well indicate ISB conditions (Table 3). Injections will stop once complete ISB conditions are present within TAN-28 for a period of 2 to 5 years, and then a rebound test will be performed to see how TCE concentrations respond. At the conclusion of the rebound test, a determination will be made if further ISB is warranted. The effectiveness of ISB in treating the TCE in the aquifer will be assessed when redox conditions reach background levels in each of the source area wells, indicating the end of the rebound test. The NPTF will continue to operate voluntarily for the long-term in the medial zone. While there has been some plume expansion, it is within the limit allowed in the Record of Decision Amendment. Monitored natural attenuation of VOCs in the distal zone as well as Sr-90 and Cs-137 will continue to be evaluated. Sr-90 and Cs- 137 concentrations need to decline at a rate to be below MCLs by 2095 for trends to be acceptable. Institutional controls (ICs) are in place to control exposure pathways that could result in unacceptable risks for human exposure and will remain in place until all COCs are below MCLs. Currently, there are no human receptors for the TAN TCE (VOC) and radionuclide groundwater plumes because there are no wells within or near the plume that are used as a water supply. Additional time and data are needed to confirm MNA is on track to meet RAOs. The remedy timeframe is roughly 25% complete. IF MNA is determined to not adequately remediating this zone P&T will need to be implemented (DOE-ID, 2021). The three remedy components employed at TAN appear be on track to provide a comprehensive approach to remediating the VOC (TCE) plume.

The work at TAN not only demonstrates the value of using a multi-faceted approach to remediation, but also identified a few challenges and unexpected outcomes that may be of value for future remediation undertakings. Major lessons learned from ISB at TAN include understanding the challenges in remediating a fractured basalt subsurface and understanding how injecting amendments may affect other constituents. Due to the complex subsurface geology, material injected into the TSF-05 injection has been transported to different areas of the subsurface, such as the TAN-28 source area. Therefore, a more complex injection strategy has evolved due to the creation of secondary source areas. Secondly, following injections of whey into TSF-05 and other wells near the residual source zone, concentrations of Cs-137 and Sr-90 increased due to cation displacement. Cs-137 rose to a peak of 6,690 pCi/L in 2016 within TSF-05B, above the Cs-137 MCL of 200 pCi/L. Sr-90 rose to a peak of 3,470 pCi/L in 2011 within TSF-05A, above the Sr-90 MCL of 8 pCi/L. Concentrations of the radionuclides are decreasing since injections have been stopped for the rebound test. Wilclear Plus has been used in the TAN-28 source area remediation and the Cs-137 plume has not spread to the wells near the TAN-28 source injections. While Sr-90 has risen above the MCL in wells near the TAN-28 source injections, it has peaked at a lower concentration of 660 pCi/L within TAN-37A in 2018 when injections were being performed into TAN-37A (Traub interview, 2025). Installation of new wells was needed to target residual TCE inaccessible using existing bioremediation wells, a strategy that may be needed at other sites.

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Appendix F. DOE Mound

1. Mound OUI Enhanced Attenuation

1.1. Summary

A full-scale bioremediation treatability study was implemented for volatile organic compounds (VOCs), TCE and PCE, to remediate groundwater at Mound OU1, a former solid waste landfill area at the former DOE Mound Site in Ohio. Leaching/leakage of VOCs from OU1 contaminated the underlying Buried Valley Aquifer (BVA), an important water resource servicing the region. The landfill was used from 1948 to 1974 for the disposal of trash, debris, and liquid waste. In 1977, much of the waste was relocated and encapsulated onsite ("cocooned"). Since that time, various actions were implemented to remove VOC mass and reduce the source mass flux to the BVA; these included: soil vapor extraction (SVE) operated from 1996 until 2003, miscellaneous spot removal of VOC contaminated soils in 1996, and large-scale removal and offsite relocation of bulk contaminated soil and waste materials (including cocooned materials) from 2007 to 2010.

Remediation of OU1 contaminated groundwater is managed under CERCLA with Ohio EPA and US EPA serving as lead regulatory authorities. Groundwater pump and treat (P&T) was initiated in 1996 to control contaminated groundwater beneath and downgradient of the former landfill and to reduce contaminant concentrations with a regulatory target level set at drinking water standards (MCLs). Based on measured P&T removal rates and concentration responses, the projected timeframe to achieve remedial action goals was estimated to be 2040.

To accelerate the timeframe, DOE LM, OEPA and USEPA agreed to evaluate bioremediation. P&T was discontinued in 2014 -- from 2014 to 2019, a multiyear CERCLA treatability study of full-scale bioremediation – enhanced attenuation using a combined anaerobic-aerobic strategy based on "structured geochemical zones." The system resulted in significant reductions in VOCs, an improved estimated timeframe for cleanup (2027), minimal adverse collateral impacts, and a significant lifecycle cost reduction.

The results of the treatability study demonstrated that 1) anaerobic bacteria capable of rapidly degrading TCE/PCE and aerobic bacteria capable of degrading TCE, dichloroethene and vinyl chloride were stimulated in the anaerobic and aerobic zones, respectively, 2) biostimulation and biodegradation occurred in situ, 3) the process was simple to deploy and provided a sustainable shift to an effective attenuation-based remedy, 4) the structured geochemical zone enhanced attenuation represents a significant improvement in terms of cost and efficiency over conventional baseline technologies. Based on the results: 1) this enhanced attenuation strategy is currently being implemented as the approved (2023) CERCLA remedy record of decision for OU1.

Deployment of the structured geochemical zones relied on multiple defenses to reduce source mass flux to groundwater and to degrade VOC contamination, including:

1) pure ("neat") soybean oil deployed in the lower section of the vadose zone beneath former landfill footprint. The neat oil spreads out at the water table interface to intercept/reduce future VOC flux from vadose zone. Migrating VOCs partition into oil "shield" while slow degradation of the oil uses up oxygen and preconditions groundwater for an anaerobic treatment zone.
2) Several sequential treatment zones are set up by injecting emulsified vegetable oil and nutrients downgradient – see Figures 1 and 2.

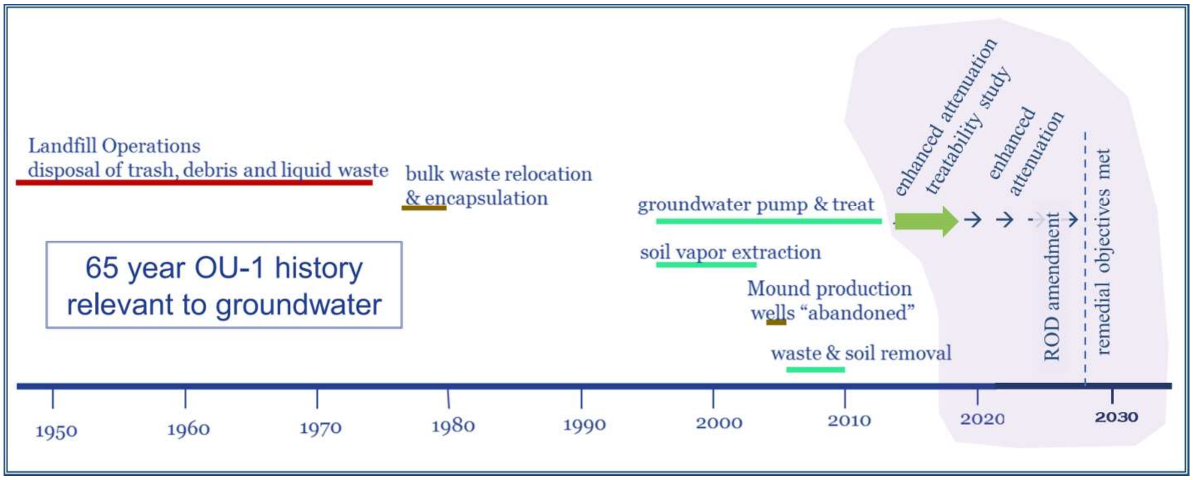

Figure 1. Simplified groundwater timeline for Mound OU1 – bioremediation activities are in shaded cloud -- right portion of figure.

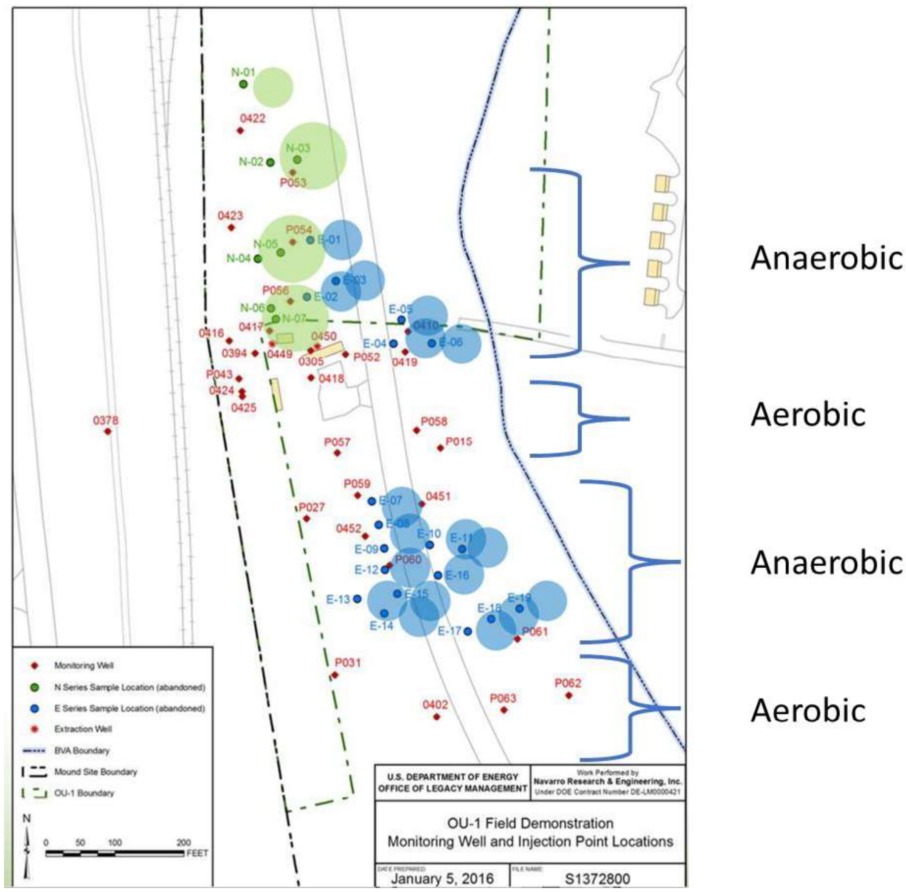

Figure 2. Location of amendment injections – N locations (green) represent locations where neat oil was injected into the lower vadose zone and E locations (blue) represent locations where emulsified vegetable oil were injected below the water table. Remediation relied on existing microbial community – no bioaugmentation was used at this site.

1.2. Notes

1.2.1. Conceptual Site Model (CSM) Overview

- Buried valley aquifer.
- Full-Scale CERCLA Treatability Study
  - 2014-2018 with ongoing monitoring
  - Deployment based on previous successful demonstration at SRS TNX site.
- Mainly TCE and PCE and trichloroethane present
  - Nominal max concentrations of 50 ug/L at beginning of deployment
- Plume size and scale: largest extent – 2 acres
- Type of Bioremediation – Combined anaerobic-aerobic structured geochemical zones
- Bioaugmentation – No
- Electron Donor – emulsified vegetable oil (commercial reagent blend)
- Electron Acceptor – none added.
- Nutrients – included in emulsified reagent blend.

1.2.2. Site challenges

- High value aquifer

1.2.3. Regulatory and Stakeholder

- CERCLA Treatability Study.
- Metrics – multiple lines of evidence.
- Regulatory drivers → CERCLA

1.2.4. Performance

- Used multiple lines of evidence approach that was developed and aligned with the EPA guidance on Monitored Natural Attenuation. These metrics were intended to: document trends and changes in VOC concentration in wells and related groundwater quality plume metrics (mass, center of mass, and plume spread), document that the geochemical conditions are present that will support known attenuation mechanisms, document that specific biological attenuation mechanisms are present and stable at the site, and to estimate attenuation rates, and the progress toward and remediation timeframe to meet remedial goals.
  - 1. First Line of Evidence—Trends in cVOC Mass and Concentration,
  - 2. Second Line of Evidence – Geochemical Footprint
  - 3. Third Line of Evidence—Enhanced Attenuation Microbial Community Data
  - 4. Degradation Rates and Remediation Timeframe

1. The objective of the first line of evidence is to document trends in concentration and mass of cVOCs and daughter products over time and in individual wells. MAROS Version 3.0 was used to evaluate the first line of evidence based on individual well concentration trends and the overall dissolved plume mass trends. Individual well trends rely on linear regression and Mann-Kendall statistics to determine the concentration trend category (increasing, probably increasing, stable, no trend, probably decreasing, or decreasing). For the overall plume, MAROS uses a method of moments analysis to estimate total dissolved mass for each sampling event (zeroth moment), center of mass location (first moment), and plume spread (second moment). The following were key results for the first line of evidence:

- PCE—For the 10 wells that had concentrations exceeding the MCL (5 µg/L) at any time during the period from August 2014 to October 2022, all 10 of these wells were classified as having decreasing or probably decreasing trends. At the end of the monitoring period (October 2022), only one OU-1 monitoring well exceeded the MCL for PCE (Well P053 at 6.04 µg/L).
- TCE—For the 10 wells that had concentrations exceeding the MCL (5 µg/L) at any time during the period from August 2014 to October 2022, all 10 of these wells were classified as having decreasing or probably decreasing trends. At the end of the monitoring period (October 2022), none of the OU-1 monitoring wells exceeded the MCL for TCE.
- cDCE—Only well P054 had concentrations of DCE greater than the MCL (70 µg/L) at any time during the period from August 2014 to October 2022. In October 2022, the cDCE trend in P054 was classified as decreasing and the concentration at the end of the monitoring period (October 2022) was below the MCL. At the end of the monitoring period (October 2022), none of the OU-1 monitoring wells exceeded the MCL for cDCE.
- VC—For the 8 wells that had concentrations of VC greater than the MCL (2 µg/L) at any time during the period from August 2014 to October 2022, 6 of these wells was classified as stable or having no trend and 2 were classified as having increasing or probably increasing trends. Of these wells that were classified as increasing (P057 and P060) both were below the MCL in October 2022. At the end of the monitoring period (October 2022), One well was above the MCL for VC (well 0417 with a VC concentrations of 2.12 µg/L).
- The concentrations of PCE and TCE in all wells on the western plume boundary have remained below the respective MCLs for the entire monitoring period.
- The OU-1 monitoring well network had 16 exceedances of MCLs in the baseline condition in 2014. The deployment of the EA has reduced the number of MCL exceedances to 2 (through October 2022). Several wells have concentrations just above or just below the MCLs; therefore, the number of exceedances is expected to vary as the site progresses toward remediation goals. Nonetheless, the EA has resulted in a significant observed reduction in cVOCs in the groundwater. In 2018 (the end of the CERCLA Treatability Study), the estimated timeframe to achieve MCLs for all constituents in all wells was projected to be 2028. The site continues to track toward that timeframe.

2. The objective of the second line of evidence is to evaluate geochemical data that can be used to demonstrate indirectly the type(s) of attenuation processes in different areas as the structured geochemical zones are developed at the site – i.e., Are geochemical conditions conducive to attenuation? Example analytes include bulk conditions (e.g. oxygen, ORP, pH, TOC), competing electron acceptors in anaerobic zones (e.g., oxygen, sulfate and nitrate), and diagnostic indicators (e.g., methane and iron). The geochemistry was manipulated by the addition of the emulsified soybean oil amendment. The product has both fast and slow-release electron donors. Lactate (fast release) increases microbial growth while rapidly creating anaerobic conditions in the treatment zones. Soybean oil droplets are retained on the aquifer materials and slowly ferment to provide electron donor to maintain reducing conditions. Overall, the geochemistry data documented that structural geochemical zones were created and maintained and are favorable for both anaerobic and aerobic degradation processes. The zones exhibit persistence (i.e., have been sustained for over 8 years) and continue to support the design basis enhanced attenuation (passive bioremediation).

3. The objective of the third line of evidence is to document that one or more recognized attenuation mechanisms are occurring at the site. In the case of OU-1, the site-specific documentation of attenuation mechanisms focused on the design basis of structured geochemical zones and how the attenuation manifested in observable patterns of contaminant profiles and changes in the microbial community. Thus, the two subtopics supporting the third line of evidence were (1) the presence and pattern of

daughter products and (2) the subsurface response of the microbial community/ecology to the EA deployment. In both subtopics, the data demonstrated that the expected anaerobic and aerobic attenuation mechanisms are operating in the target areas. In the anaerobic treatment zones, the EA strategy was validated by the presence and sequential appearance or disappearance of daughter products via reductive dechlorination and the presence of significant populations of organisms that have been documented to attenuate cVOCs under anaerobic conditions. In the surrounding near-field aerobic zones, the EA strategy was validated by low concentrations of reductive dechlorination daughters (due to aerobic degradation of these compounds) and by the presence of significant populations of organisms that have genetic markers for known cometabolic enzymes. Collection of the supporting data for the third line of evidence, from both anaerobic and aerobic areas, provided a robust approach to that recognized attenuation mechanisms are in place in the OU-1 groundwater. The following are excerpted from the 2022 data interpretation:

- In all anaerobic treatment zone wells, the pattern of daughter products over time was a reasonable variant of the patterns observed in the scientific literature for anaerobic bioremediation or biostimulation sites -- the wells exhibit the "classic" progression in which the daughter products form and degrade in sequence. Several wells exhibit some degree of cDCE stall, a condition in which cDCE degradation is relatively slow compared to the parent cVOCs, causing cDCE levels to build up above parent levels. In general, the cDCE concentrations increased after the initial EA deployment in the anaerobic treatment zones. The cDCE generally peaked in the 2016 to 2017 period and have decreased over time through the remainder of the EA monitoring period (through October 2022). All cDCE concentrations in treatment zone wells were below the MCL (70 ug/L) at the end of monitoring period. VC was detected in all the anaerobic treatment zone wells during the monitoring period for the EA Field Demonstration. At the end of the monitoring period (October 2022), the VC concentration was below the MCL (2 ug/L) for all treatment zone wells.
- In the aerobic plume interior wells, the maximum levels of daughter products were relatively low compared to those of the anaerobic treatment zone wells. This pattern is consistent with attenuation of the cVOC daughter products in aerobic conditions. The daughter products would be expected to degrade as water flows out of anaerobic treatment zones then into and through the plume interior, where the DO levels are above 1 to 2 mg/L. Similarly, parent and daughter products in the aerobic sentinel wells (downgradient) were below MCLs at the end of the monitoring period (October 2022).
- To assess the biological response of the subsurface in representative areas of the OU-1 aquifer, qPCR measurements of microbial community DNA isolated from groundwater samples were performed annually. For the microbial monitoring, six wells that represent different biogeochemical settings were sampled to provide information on changes within and downgradient of the reductive treatment zones, as well as provide insights on changes near the original source and in the distal portion of the plume. The microbial data collected was extensive and definitive. The data documented increases in total eubacteria throughout the site (typical increases were 2 to 4 orders of magnitude). In the anaerobic treatment zones, known degraders (e.g., *dehalococcoides* spp.) increased to significant levels. Importantly, native organisms (e.g., *dehalogenimonas* spp.) were documented to be significantly effective in contributing to the degradation. Cometabolic organisms and organisms that Are known to degrade DCE and VC were stimulated in the aerobic environments surrounding the treatment zones. The data indicated significant and sustainable changes in the microbial community (i.e., enhanced attenuation) and that there was no need for bioaugmentation at the Mound OU1 site.

4. Estimation of attenuation and degradation rates assists in evaluating progress toward remediation goals and projecting the remediation time frame and was specified as a metric in the Field Demonstration Work Plan (DOE, 2014a). The overall objective of the attenuation rate evaluation is 1) to examine the trends in individual wells and changes in overall contaminant mass in the plume to determine reasonable quantitative estimates of degradation rate constants for the real-world field conditions in the plume, 2) to examine the patterns of concentration on long-sects to develop quantitative rate estimates, and 3) to examine concentrations in downgradient sentinel wells to provide field confirmation that the plume is not expanding. All these metrics indicated significant progress toward remediation goals and meeting goals in the 2028 timeframe. However, continued calculation of these metrics over the final years has become difficult because there are only a few detected concentrations in the dataset. Thus, a close out metric based on simply tracking numbers of exceedances of MCLs was recently proposed. This metric is also consistent with meeting remedial goals approximately within the calculated 2028 timeframe (Figure 3).

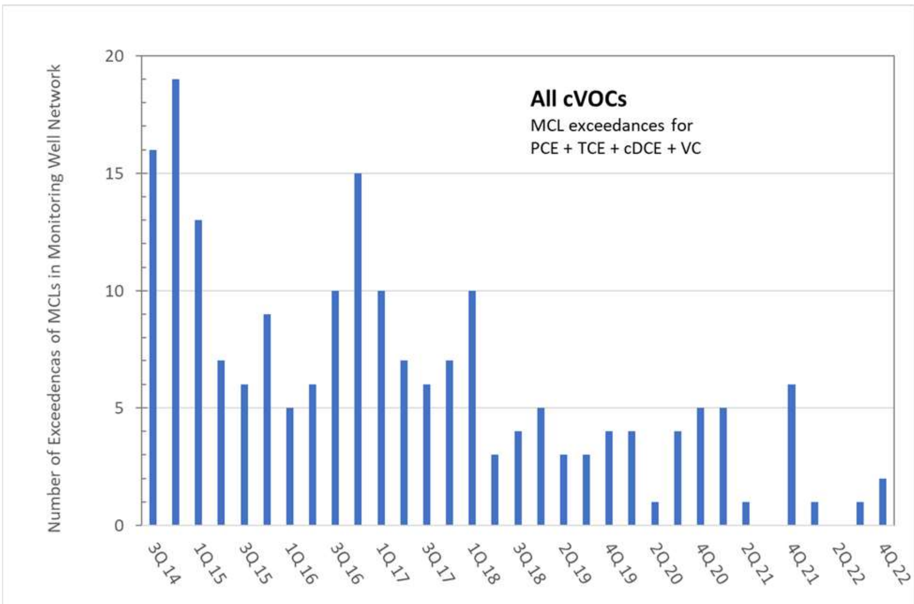

Figure 3. Number of MCL exceedances in monitoring well network.

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Appendix G. DOE Pinellas

1. Pinellas Groundwater Bioremediation Activities

1.1. Summary

The Young-Rainey Science Technology and Research Center, or Young-Rainey STAR Center is a technology and manufacturing center located in Largo (Pinellas County) Florida, United States. The STAR Center is the former site of the Pinellas Plant, a nuclear weapon component manufacturing facility operated by the United States Department of Energy (DOE). It produced radioisotope-powered electronic components for the United States nuclear weapons program. DOE production activities resulted in release of significant quantities of VOCs into the subsurface and the presence of multiple near-field zones containing separate phase dense nonaqueous phase liquids (DNAPLs). These long-term sources occur beneath Building 100, a large 4.5 ha (11acre) building that housed manufacturing facilities during US DOE operations. Groundwater contamination, consisting of two dissolved-phase plumes originating from chlorinated solvent source areas has migrated beyond the property boundary, beneath the roadways, and beneath adjacent properties to the south and east. Groundwater contamination will persist as long as the onsite contaminant source remains. The site is now owned by Pinellas County, and most of the space inside the building is leased to private companies. The remediation was overseen by DOE LM. To the extent practicable, DOE has opted to minimize characterization or remediation through the floor of the building, instead choosing to conduct work from outside the building. Plans for future decommissioning the building and restructuring the site are currently being developed. The Pinellas site has had three notable bioremediation activities: 1) an early large-scale pilot study of anaerobic bioremediation using lactate/methanol/benzoate as the electron donor blend, 2) a large-scale pilot aerobic cometabolism biosparging, and 3) a permitted full scale anaerobic bioremediation using emulsified oil reagents/nutrient solutions and bioaugmentation (the full-scale permitted remediation comprises multiple-episodic injections and use of both vertical and horizontal wells to address the dissolved plume and the contamination beneath Building 100). The Pinellas site is one of the most challenging remediation scenarios in the Nation and the bioremediation has been successful. Throughout the monitoring network, the bioremediation has mineralized the bulk of the contamination and current groundwater conditions are generally near or below MCLs for the original parent VOCs and daughter products throughout the area of the plume(s).

1.2. **Notes**

1.2.1. Conceptual Site Model (CSM) Overview

- CSM – Releases into the subsurface beneath a large (4.5 acre) building and from nearby facilities
- Fine grained sand and silt materials
- Geochemistry – generally low DO and two large scale plume(s) with nearfield DNAPLs
- (1) Large Scale Field Demonstration for anaerobic bioremediation (pilot study) – 1997
- (2) Large Scale Field Demonstration for aerobic cometabolic biosparging (pilot study) – circa 2000
- (3) Full scale permitted bioremediation using anaerobic bioremediation – multiple injections of electron donor and *dehalococcoides*
- Mainly TCE and PCE and trichloroethane present
    - Nominal max concentrations -- DNAPL – residual solvent and water at solubility
    - Deployment area: < 100 ug/L to >10,000 ug/L
- Plume size and scale: largest extent – approximately 30 acres
- Type of Bioremediation – (1) Anaerobic dechlorination / (2) Aerobic cometabolism / (3) Anaerobic Dechlorination

- Bioaugmentation – (1) No, (2) No, (3) Yes
- Electron Acceptor – (1) NA, (2) air, (3) NA
- Electron Donor – (1) lactate, methanol & benzoate, 2) NA, (3) emulsified soybean oil
- Cometabolic carbon Source) – (1) NA, (2) methane, (3) NA
- Nutrients – (1) , (2) NA, (3) included in commercial emulsified oil concentrate.
- Site challenges
- Large-Complex Plume
- Early adopter of technology.

1.2.2. Regulatory and Stakeholder

- (1) 1997 pilot study performed in partnership with EPA under the Innovative Treatment Remediation Demonstration (ITRD) Program.
- Metrics – (1) & (2) VOC concentration in water and sediment, operation, performance and cost information, (3) VOC concentrations and distribution in groundwater
- Regulatory drivers à CERCLA

1.2.3. Performance

The following narratives provide a performance synopsis for each of the major bioremediation activities.

1. Large-scale pilot study of anaerobic bioremediation using lactate/methanol/benzoate as the electron donor blend – February 7, 1997 to June 30, 1997 -- 1997 pilot study of anaerobic bioremediation -- In early 1997, the Innovative Treatment Remediation Demonstration (ITRD) Program conducted a pilot study at the Pinellas STAR Center's Northeast Site to treat cVOCs using in situ anaerobic bioremediation in the surficial aquifer. Pretest monitoring data indicated that some biodegradation of these contaminants is already occurring at the site. The primary objectives of this pilot study were to 1) evaluate the use of amendment injection to enhance in situ anaerobic biological degradation rates of chlorinated VOCs in areas of moderate contaminant concentrations and 2) obtain operating and performance data to optimize the design and operation of a full-scale system. During the short operational period of this pilot study, there was no emphasis on reducing any contaminants to a specific regulatory level.

The pilot system was in an area of the site that had total chlorinated contaminant concentrations in ground water generally ranging from 10-400 ppm, with one monitoring well having concentrations more than 2900 ppm. The bioremediation pilot system consisted of three 8-ft deep, gravel-filled, surface infiltration trenches and two 240-ft long horizontal wells with 30-ft screened intervals. The horizontal wells, directly underlying and parallel to the middle surface trench, were at 16- and 26-ft depths. The study area was about 45 feet by 45 feet and extended from the surface down thirty feet to a thick, clay confining layer 30 feet below the surface. Ground water was extracted from the upper horizontal well and recirculated via the surface trenches and lower horizontal well while benzoate, lactate, and methanol were added to the recirculated water to serve as a carbon source (electron donor) for the dechlorinating bacteria. The nutrient concentrations were selected based on an earlier laboratory treatment study conducted through the ITRD Program. To assess hydraulic flow characteristics and nutrient delivery, a bromide tracer was added to the water reinjected through the deep horizontal well and an iodide tracer was added to the water fed to the surface trenches. VOC, tracer, and nutrient concentrations were monitored bi-weekly at 16 well clusters (each with 4 vertically discrete sampling intervals) spaced throughout the treatment area. VOC concentrations of the extracted ground water were also continuously monitored. The system operated from February 7, 1997 to June 30, 1997. During this period, ground water was extracted and recirculated at a rate of about 1.5 gpm. Approximately 250,000 gallons of water, based on soil porosity of about two pore volumes, were circulated during the pilot study. Tracer and nutrient monitoring data indicated that nutrients were delivered to 90% of the central treatment area during operations. Wells not showing breakthrough were generally in the areas of lower conductivity and perimeter wells. Where nutrient breakthrough was observed, significant declines in total chlorinated VOC concentrations (70-99%) were generally observed. These values correlated well with the results observed from the extraction. For those wells where nutrient arrival was not observed, generally in areas of lower permeability or perimeter wells, only modest contaminant reductions were recorded. Degradation rates of as high as 1-2 ppm per day were observed in the higher concentration areas, greater than 100 ppm, while in areas with lower concentrations, degradation rates of 0.05 to 0.10 ppm per day were observed. There was no evidence of significant degradation product build up in any monitoring well, and many wells with contaminant concentrations below 10 ppm showed contaminant reductions to regulatory allowable levels.

The cost of the pilot system totaled approximately $400,000 with over half the costs associated with sampling and analyses. Most of the sampling and analyses were discretionary and were used to verify the system concept and design. This level of sampling would not be needed during a full-scale bioremediation project. System construction costs were about $90,000 while operating costs were about $30,000 or $0.12 per gallon of water treated. The modeling, hydrogeologic, nutrient transport, and operating cost data developed during this pilot operation suggest that the Northeast Site could be remediated using nutrient injection in approximately 2-3 years at a cost of about $4-6M. From the results of the pilot study, nutrient addition to stimulate existing in situ anaerobic biological degradation of chlorinated solvent contaminated soil and ground water appears to be a feasible and cost-effective remediation approach at the Pinellas Northeast Site for areas of moderate contaminant levels.

2. Large-scale pilot aerobic cometabolism biosparging

Following the anaerobic pilot test, a large-scale biosparging pilot was implemented (in 1999) at the Young – Rainey STAR Center. The goal of the aerobic cometabolic biosparging project was to convert from the natural anaerobic, reducing conditions to aerobic, oxidizing conditions to facilitate the aerobic biodegradation of dichloroethene and vinyl chloride and the aerobic cometabolism of trichloroethene. The biosparging system consisted of three horizontal wells 480 feet (146 m) long with a 180 foot (55-m) screened interval 24 feet (7.3 m) below land surface; each well connected to a blower system that injected atmospheric air into the wells. After 3 years of operation, the system had little if any effect on decreasing contaminant concentrations via aerobic processes. The lack of effectiveness was due mainly to high oxygen demand caused by the naturally reducing conditions, the presence of nearby DNAPL that recontaminated the area, and the heterogeneity and presence of small particle size sediments in the aquifer matrix that led to formation of preferential airflow pathways. Additionally, the sparging) and associated subsurface pressurization during operation of the system appeared to facilitate plume movement and resulted in plume expansion. The biosparging system was stopped on May 13, 2003 after 3.5 years of operations because of the apparent inability of the biosparging system to convert the subsurface to aerobic conditions.

Several lines of evidence from these evaluations indicate that the biosparging system did not meet performance objectives and the system had not converted subsurface conditions from anaerobic to aerobic after nearly 2 years of continuous operations. The following conclusions were drawn from these investigations:

- High chemical oxygen demand of the aquifer was not met within a reasonable time frame, thus preventing biosparging operations from changing the aquifer from anaerobic to aerobic conditions. Soil chemical oxygen demand averaged 38,000 mg/kg, and groundwater chemical oxygen demand averaged 197 mg/L.

- Phospholipid fatty acid and respiratory quinone analyses conducted by the University of Tennessee Center for Biomarker Analysis showed that aquifer conditions were reducing and anaerobic.
- Mann-Kendall trend analysis indicated that reductive dechlorination is the primary remedial process ongoing at the site.
- Air exiting from monitor wells at distances of 200 ft (61 m) from the biosparge wells demonstrated that air was being released through preferential flow pathways and, therefore, was contacting only very limited areas in the subsurface.
- The lateral extent of the contaminant plume expanded during implementation of biosparging operations.
- Because of the existence of strongly anaerobic conditions and preferential airflow pathways, it is unlikely that the biosparging system would be able to decrease contaminant concentrations to below the remediation goals in a reasonable time frame at this site.

3. Permitted full scale anaerobic bioremediation using emulsified oil reagents/nutrient solutions and bioaugmentation -

Based on the previous bioremediation pilot tests, a permitted anaerobic bioremediation based was developed, permitted and implemented. The objective of this work was to enhance the biodegradation of contaminants in contaminant source areas beneath the building and in the downgradient contaminant plumes that extend to the south and east of Building 100. The system relied on emulsified vegetable oil (EVO, electron donor) and nutrients along with bioaugmentation to accelerate the naturally occurring biodegradation. Treatment was applied via three access scenarios: (1) onsite vertical bioinjection, (2) offsite vertical bioinjection, and (3) horizontal bioinjection beneath Building 100. Injection of emulsified vegetable oil (EVO) and the microorganism *Dehalococcoides mccartyi* (DHM) took place in three phases from October 2014 to November 2015 at the Building 100 Area on the Pinellas County, Florida, Site. The technical approach consisted of installing horizontal wells from outside the building footprint, extending through and around the identified subsurface treatment areas, and terminating beneath the building. Two 107 m (350 ft) long wells, two 122 m (400 ft) long wells, and four 137 m (450 ft) long wells were installed to intersect the inferred source areas and known contaminant plumes beneath the building. The horizontal wells were oriented along the long axis (centerline) of the plumes (longsects) to maximize the concordance of the bioremediation process with the highest concentration areas.

Onsite vertical bioinjection was performed using direct push for access. A commercially available electron donor EVO amendment was used for the biostimulation (Terra System's SRS-SD small droplet EVO). A commercially available culture of *Dehalococcoides* was used for the bioaugmentation (DHM, TSI DC culture). of DHM were used for injection. EVO and DHM were injected at 62 injection points at the Building 100 Area starting on October 20 and ending on November 21, 2014. Additional EVO and DHM was injected into former groundwater recovery wells. The project used 12,320 L (3,255 gallons [gal]) of concentrated (60%) EVO and 22 L of concentrated TSI DC. The concentrated EVO amendment oil was diluted (9:1 water/amendment) ratio for distribution in the subsurface, resulting in a total injected volume of approximately 123,200 L (32,550 gal). Offsite vertical injections were performed in a similar manner using the same materials. EVO and DHM were injected at 33 injection points at three offsite properties starting on February 2 and ending on February 18, 2015. The project used 7,950 L (2,100 gal) of concentrated EVO and 14 L of concentrated DHM. The total injected volume of SRS diluted at a 9:1 ratio was 13,250 L (3,500 gal), and the total volume of SRS injected at a 6.4:1 ratio was 49,200 L (13,000 gal), for a total injected volume of 62,500 L (16,500 gal) of diluted SRS.

For treatment under the large building footprint, horizontal well installation began on July 6, 2015, and was completed on September 28, 2015. The horizontal wells are constructed of fiberglass-reinforced epoxy. The wells have an inside diameter of 7.6 cm (3 inches) and an outside diameter of 8.9 cm (3.5 inches). The slots were designed to distribute the reagent injection along the entire target length of the well – the slots are 0.33 mm (0.013 inch) wide and 3.8 cm (1.5 inches) long, with one slot per 61 cm (2 ft) section of well. The same EVO and DHM materials were used for biostimulation and bioaugmentation in the horizontal wells. This initial injection into the horizontal wells started on November 2, 2015, and ended on November 18, 2015. The oil was diluted with municipal tap water at a 9:1 water/oil ratio. The project used 16,850 L (4,450 gal) of concentrated EVO and 30 L of concentrated DHM. Injection flow rates ranged from about 64 to 95 L (17 to 25 gal) per minute.

Additional injections of EVO and DHM (with similar materials and quantities) were performed in October 2016 and July 2019. Time trends and patterns in VOC concentrations – e.g., things like downward concentration trends stalling were the basis for performing these "contingency" injections. There has been little characterization of the field microbial ecology (i.e., limited data on the structure and numbers in the microbial community in the subsurface). Notably, the bioremediation has performed well and concentrations throughout this challenging plume have declined from levels indicative of DNAPL down to relatively low concentration (typically below 10 ppb for parent and daughter VOCs with some areas in the 10s to 100s of ppb range. This final permitted project has been highly successful and is on a path toward meeting remedial objectives.

See appended Figures 1-3 below for some example performance:

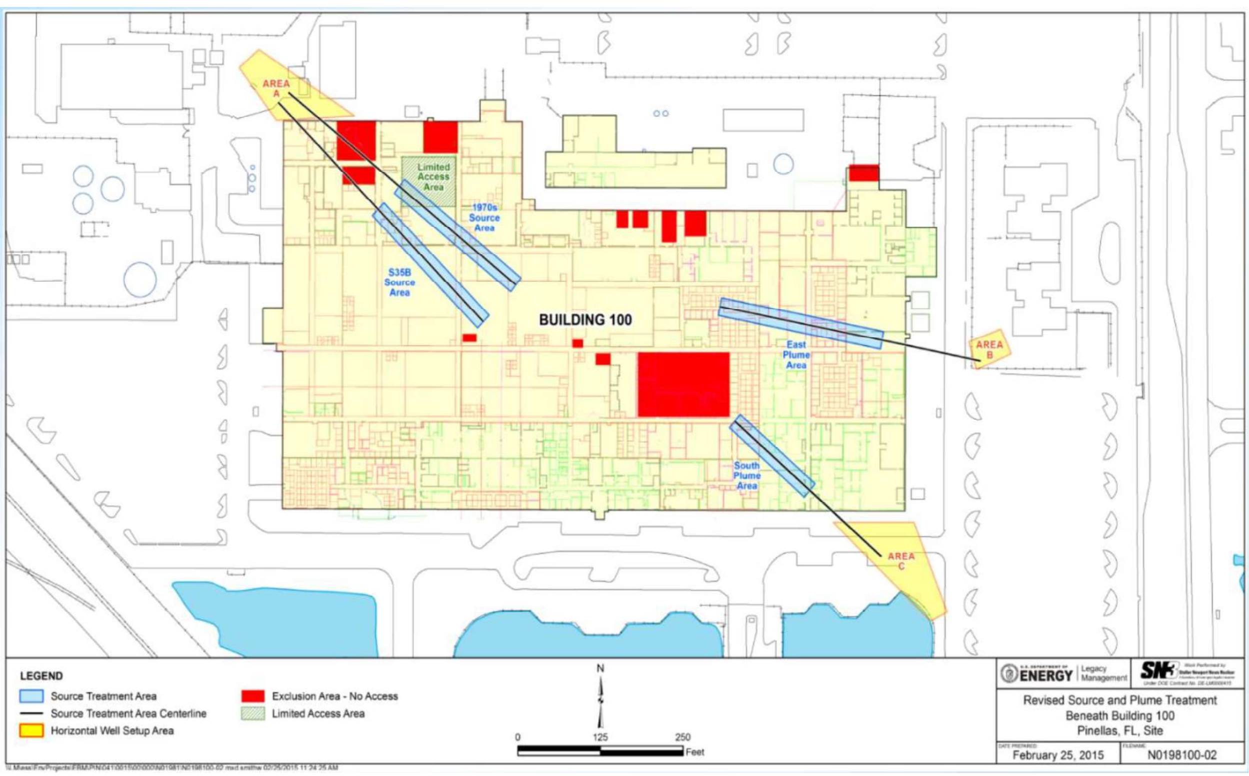

Figure 1. Horizontal well locations beneath Building 100

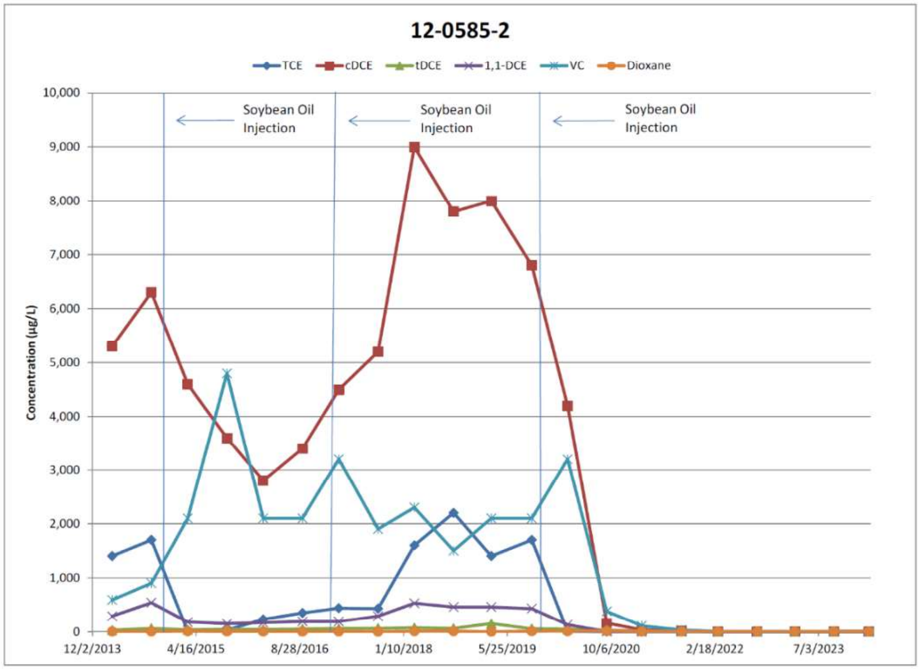

Figure 2. Example monitoring well (12-0585-2) concentration trends in response to oil injections in horizontal wells.

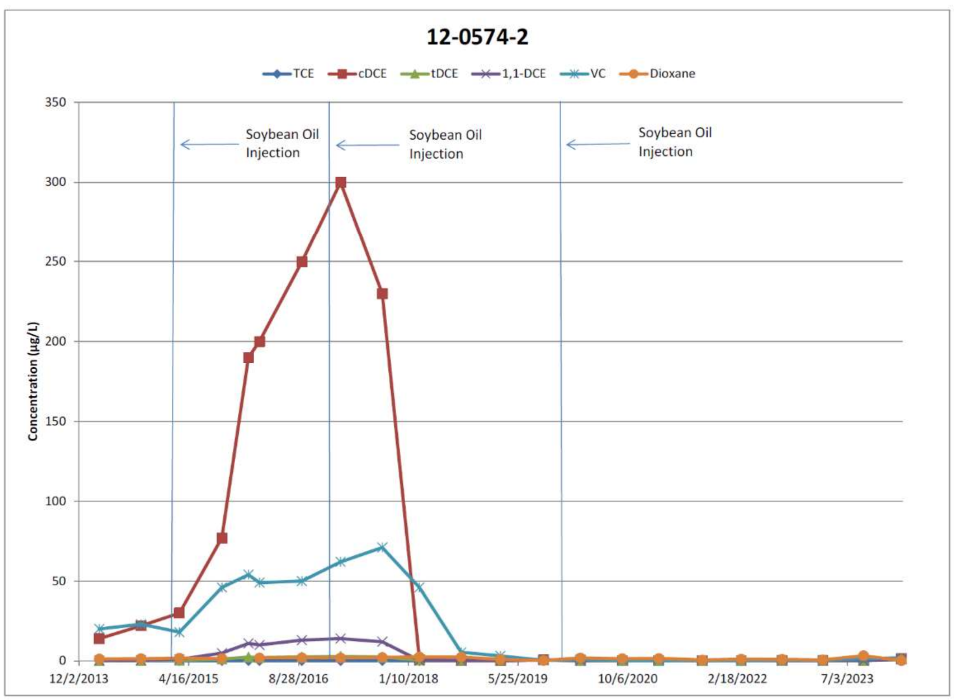

Figure 3. Example monitoring well (12-0574-2) concentration trends in response to oil injections in horizontal wells.

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