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Enhanced Bioremediation of a Consortium of Contaminants at a Historic Chemical-Production Facility

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Site Introduction

- Historic, former manufacturing plant for specialty chemicals and adhesives
- Operations began in 1952, discontinued in 2001
- Currently decommissioned and dismantled, undergoing active remediation in some target areas

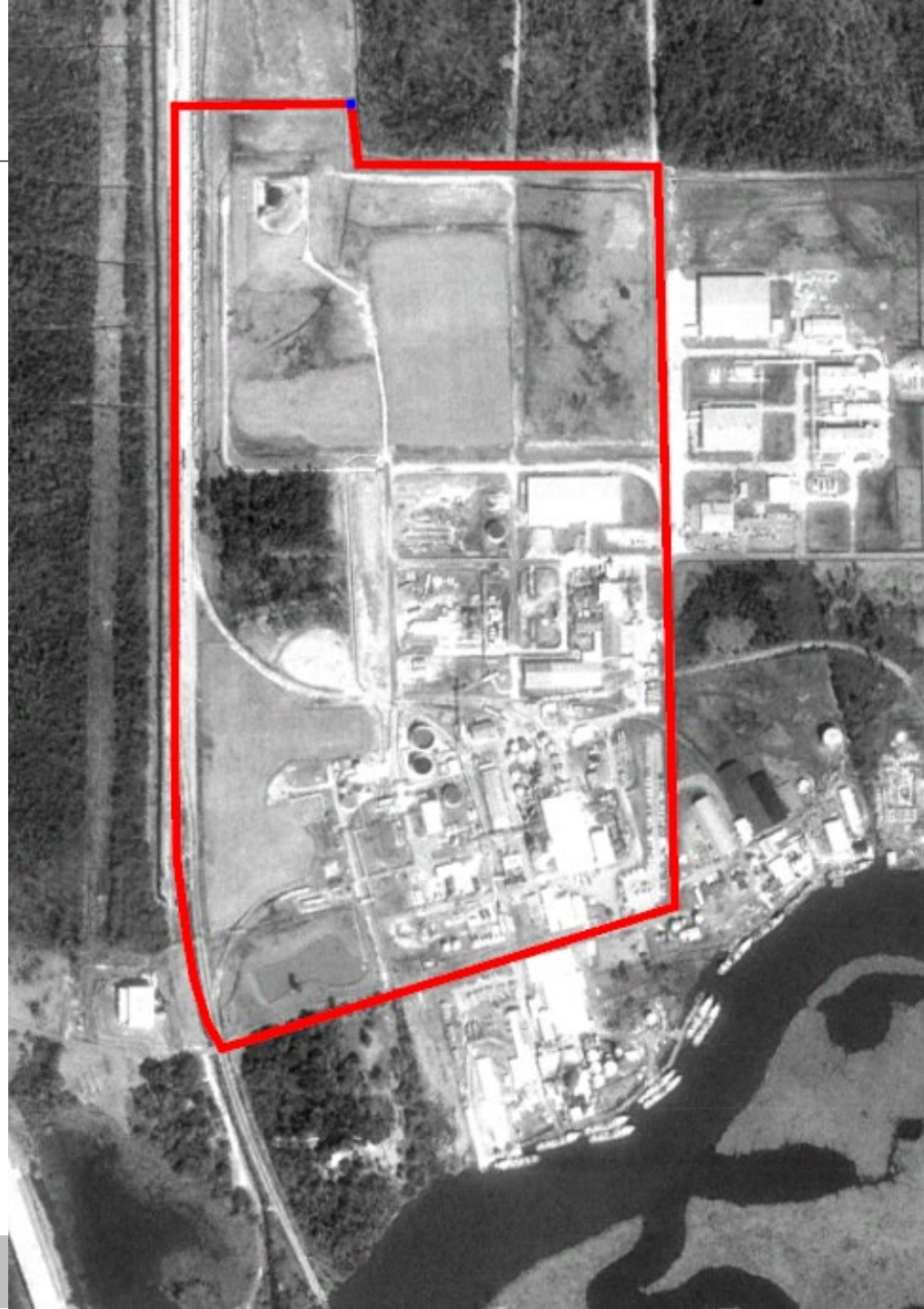


Image curtesy of USGS, 1992

Area of Concern

- One of the more significant sources of remaining COCs at the Facility

- *Bis*-(2-chloroethoxy)methane (BCEM) manufacturing from 1955 until decommissioning, used for synthetic rubber production

Area of Concern, from Google Earth (Feb 2017)

Area of Concern

- Current primary COCs include:
  - 2-chloroethanol
  - *bis*(2-chloroethyl)ether (BCEE)
  - *bis*(2-chloroethoxy)methane (BCEM)
  - 1,2-dichlorethane
  - 1,4-dioxane
  - 1,2,3-trichloropropane

Area of Concern, from Google Earth (Feb 2017)

Additional Site COCs

Chlorinated Ethenes

- 1,1-Dichloroethene
- cis-1,2,-Dichloroethene
- trans-1,2-Dichloroethene
- Vinyl Chloride

Chlorinated Ethanes

- Chloroethane
- 1,1-Dichloroethane
- 1,1,1-Trichloroethane
- 1,1,2-Trichloroethane

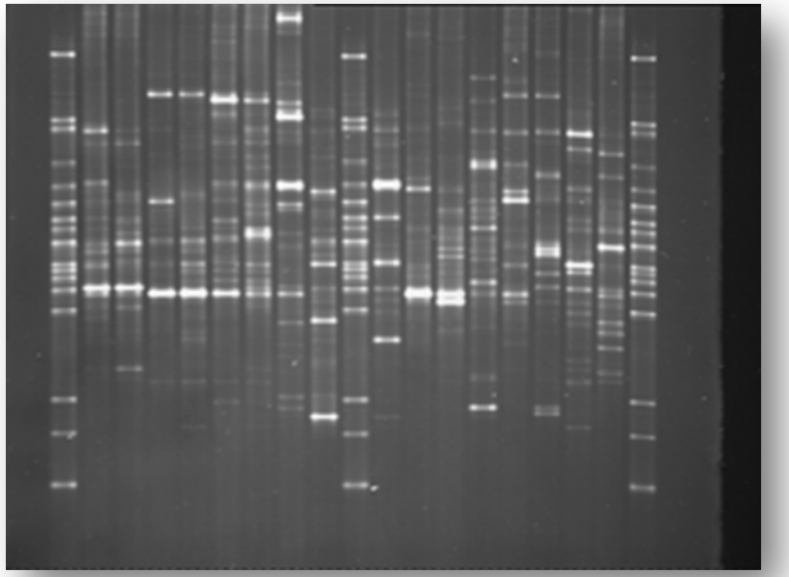
2014-2018: Indigenous Microbial Community Assessments

- 2014: qPCR analyses
  - 20 Genes: reductive dechlorination populations, functions
  - 9 Genes: metabolisms, co-metabolisms
  - 3 Genes: general population groups

- 2015: Lab-based, in-situ bioremediation treatability studies performed
  - Biostimulation via two different electron donors
  - Bioaugmentation with commercially-available dechlorinating microbes
  - All primary COCs evaluated

- 2018: Repeated above qPCR analyses, new locations

Results of 2014 and 2018 qPCR Analyses



- Similar results for both years, generally between sample locations
- Total microbial population size approximately 10^5 cells/mL
- Genes quantifying dechlorination metabolic pathways and populations generally between 10^0 – 10^3 cells/mL

Results of 2015 Lab-Based Groundwater Treatability Studies

- 1,4-Dioxane, BCEE
  - Not reduced by either biostimulation or bioaugmentation
- BCEM
  - Was reduced at 40 ºC and pH 3
  - 1,000 mg/L to BDL in 200 days
  - Not further reduced by either biostimulation or bioaugmentation
  - Hydrolysis?
- 2-Chloroethanol:
  - Reduced by both biostimulation and bioaugmentation
- 1,2-DCA:
  - Was reduced by both biostimulation and bioaugmentation
  - Effectiveness possibly reduced by 1,4-dioxane and BCEM concentrations

Overall Results of 2014 - 2018 Microbial Analyses

- Overall, initial prospects of indigenous biodegraders uncertain
- qPCR limited – targeted only those genes that were previously identified
  - Population and functional genes commonly associated chlorinated ethenes, ethanes
  - Common metabolisms, co-metabolisms
- Not likely to capture more exotic microbes, biodegradation pathways
- Biostimulation / bioaugmentation studies run under anaerobic conditions standard for common chlorinated ethenes, ethanes

Simultaneous Remedial Efforts

- Interim-measure, groundwater treatment system to treat recovered groundwater
- Needed more efficient remediation method to better target variety of compounds found, under actual site conditions
- Plan: perform multiple test studies to discern best treatment option
  - Primary focus: 1,4-dioxane removal

SEE- Aerobic Bioreactor Study

- Steam enhanced extraction (SEE) was evaluated as base for in-situ source treatment
- Proven remedial technology for DNAPL and VOC's
- Concern: concentrate COCs, require additional steps for GW treatment
- Solution: combine SEE with other technologies such as aerobic bioreactor seeded with sewage sludge
  - Bunton et al., 2018

Part of Parsons' SEE-Bioreactor

Aerobic Bioreactor Study – Unexpected Results

- Potential biological attenuation of key site COCs

Percent of Influent Mass Attributable to Removal by Biological Reaction

Compound	1,2-DCA	1,4-Dioxane	2-Chloroethanol	BCEM	BCEE
Percent	77	8.0	> 99	> 99	> 87

- Significantly changed our focus going forward

- Suggested bioremediation as possible in-situ treatment method

New Questions to Answer

- Primary COC biodegraders
  - From on-site groundwater?
  - From sewage sludge used to seed the SEE study?
- Bioaugmentation more efficient study path?
- What environmental conditions would maximize overall COC biodegradation in-situ?

New Questions to Answer

- Primary COC biodegraders
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- Bioaugmentation more efficient study path?

- What environmental conditions would maximize overall COC biodegradation in-situ?

The Current Plan…

- Genetic investigation into indigenous microbial community
- Biostimulation, bioaugmentation studies
- Bioreactor study data

- Laboratory bioaugmentation studies

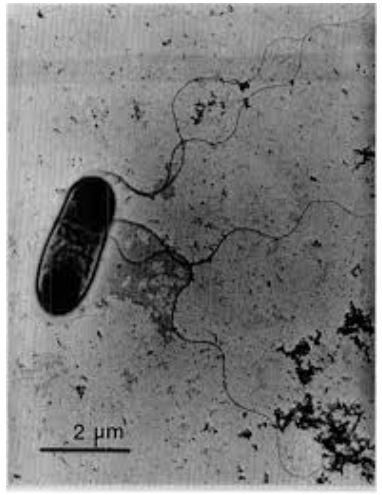
- In situ pilot study

- Full scale implementation

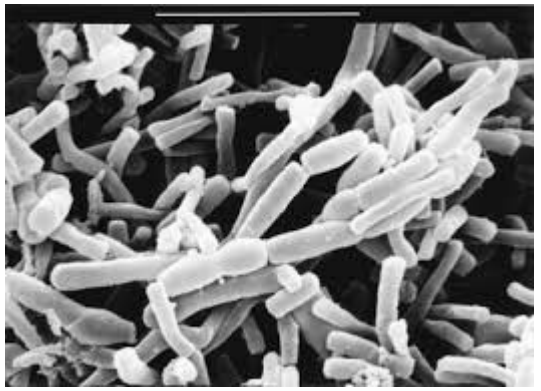
Phase 2: Lab Studies Using Bioaugmentation Cultures

- Multiple rounds of bioaugmentation lab studies conducted by the Facility with Parsons assistance

- Using two cultures:
  - *Pseudonocardia* ENV 478
  - *Xanthobacter* ENV 481



Xantho. image: Reding et al., 1992



Pseudo. image: Lee et al., 2001

- Both come from taxonomic phyla known for capabilities to degrade complex contaminants, including chlorinated compounds

Pseudonocardia ENV 478

- Known biodegradation of:
  - BCEE, co-metabolic
  - 1,4-Dioxane, co-metabolic
- No literature precedent for biodegradation of:
  - BCEM
  - 2-chloroethanol
  - 1,2 DCA

Xanthobacter ENV 481

- Known biodegradation of:
  - BCEE
- No literature precedent for biodegradation of
  - BCEM
  - 1,4-Dioxane
  - 2-chloroethanol
  - 1,2 DCA

Lab Studies Using Bioaugmentation Cultures: What We Need to Understand

- Microbial biodegradation potential of COC's
  - Individually, co-mingled

- Required environmental conditions for maximum effectiveness
  - Oxygen requirements
  - Additional carbon source to support co-metabolism (?)
  - Salinity, pH tolerance
  - Cytotoxicity assessments in presence of various COC concentrations
  - Cell density needed to maintain efficient biodegradation

Pseudonocardia ENV 478

- Known biodegradation of:
  - BCEE, co-metabolic
  - 1,4-DX, co-metabolic
- Biodegradation observations:
  - BCEM
  - 2-chloroethanol
  - 1,2-DCA

Xanthobacter ENV 481

- Known biodegradation of:
  - BCEE
- Biodegradation observations:
  - BCEM
  - 1,4-DX
  - 2-chloroethanol
  - 1,2 DCA

Walecka-Hutchison et al., 2019 and Whaley et al., 2019

Translating Lab Results Into Phase 3: Pilot Study

- How to maximize overall COC biodegradation under in-situ conditions?
- What happens when ENV 478 and ENV 481 are together?
- Biodegradation mechanisms?
- Difference between aquifers?

Significance

- Captured biodegradation of complex chlorinated contaminants
- Part of much larger, multi-phase project demonstrating enhanced bioremediation of unique, complex set of contaminants
- Setting stage for upcoming in-situ pilot study
- Highlights key information, important questions that need to be answered to ensure success

Questions?

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