Idaho National Engineering and Environmental Laboratory

Long-term Stewardship of Mixed Wastes: Passive Reactive Barriers for Simultaneous In Situ Remediation of Chlorinated Solvent, Heavy Metal, and Radionuclide Contaminants

William A. Apel and Frank Roberto, INEEL
Robin Gerlach and Al Cunningham, MSU
Brent Peyton, WSU

May 7, 2003

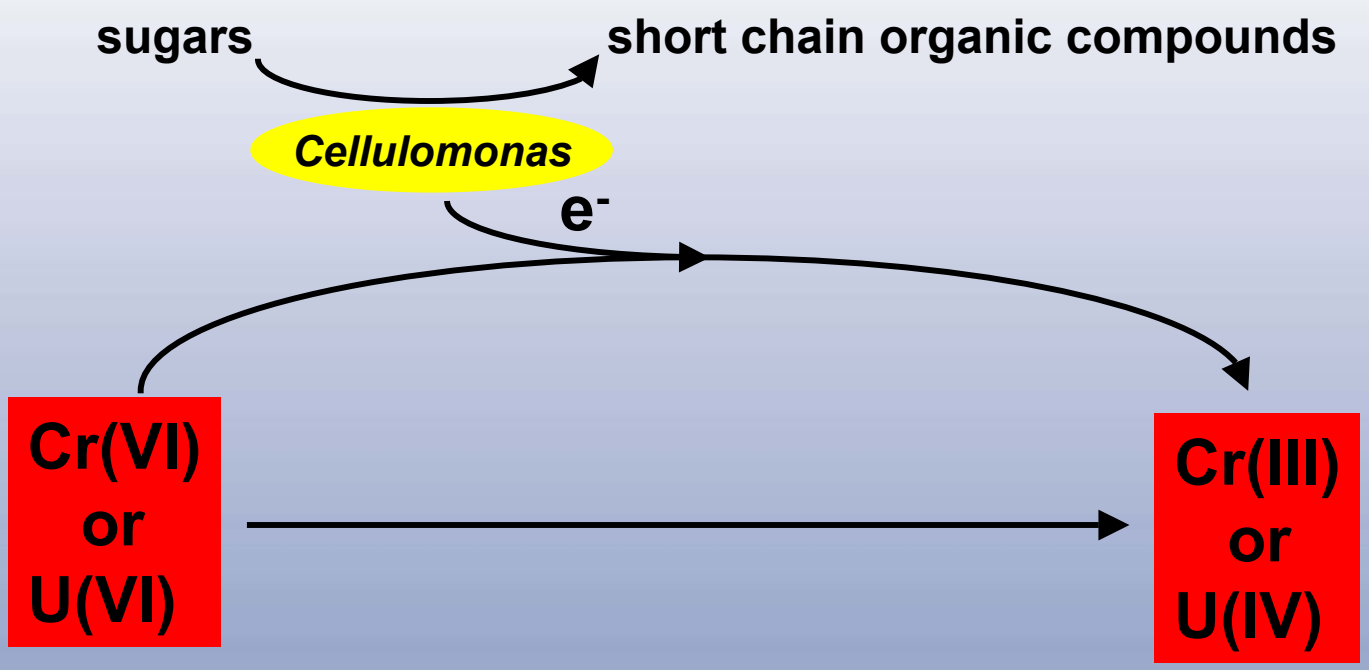
Goal

Conduct the research necessary to develop a passive reactive barrier (PRB) technology for the in situ degradation of halocarbons and simultaneous immobilization of heavy metals and radionuclides in groundwater. Indigenous Cellulomonas spp. will be targeted as the active biological portion of the barrier.

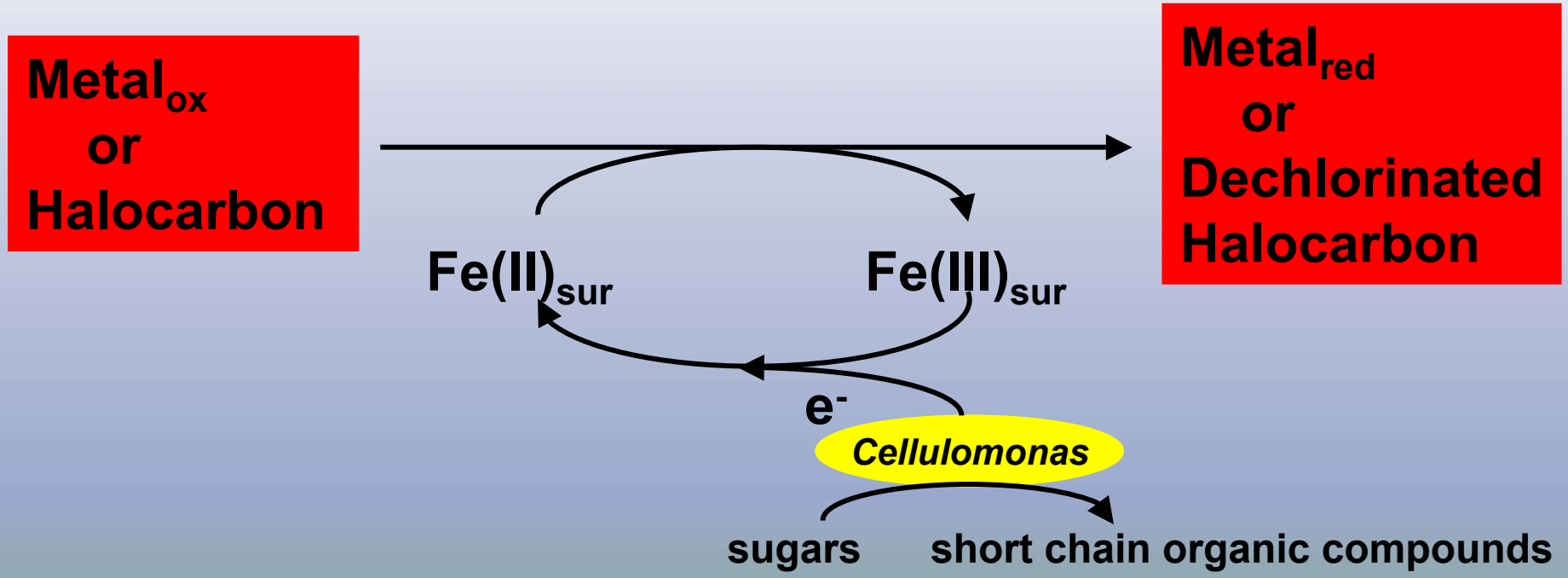
Why Cellulomonas?

- A previous NABIR project examined Cr(VI)-reducing microbial communities in the Hanford 100 Area subsurface. 16s rDNA analysis showed that Cellulomonas spp were preeminent members of these communities even from samples taken 5 km and several years apart.
- These Cellulomonas spp. can grow on a variety of carbon sources including fatty acids, sugars, and complex compounds such as cellulose.
- The Hanford 100 Area Cellulomonas isolates can reduce U(VI), Cr(VI), and Fe(III).
- This reduction can occur in the absence of supplemental carbon sources.

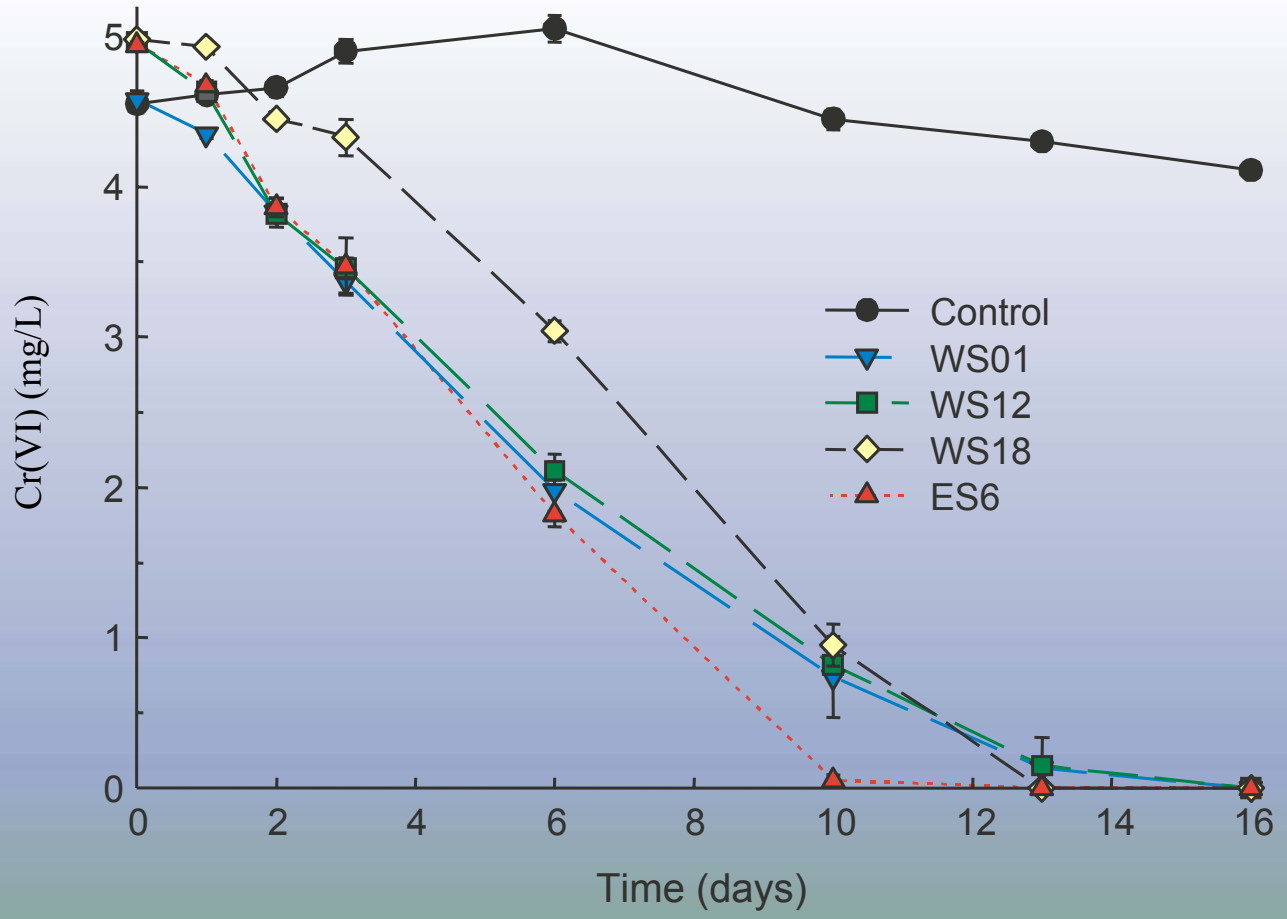
Direct Metal Reduction by Cellulomonas



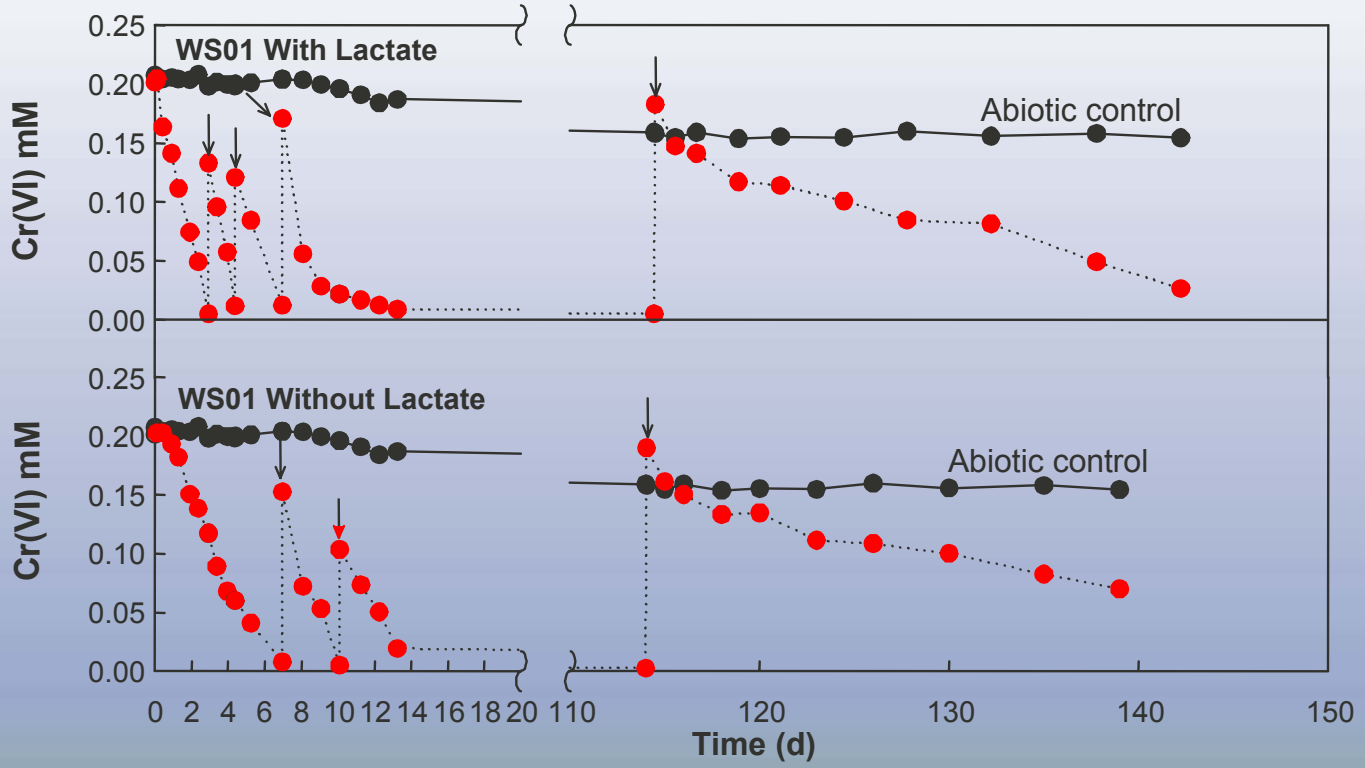
Indirect Metal Reduction and Halocarbon Dechlorination by Cellulomonas



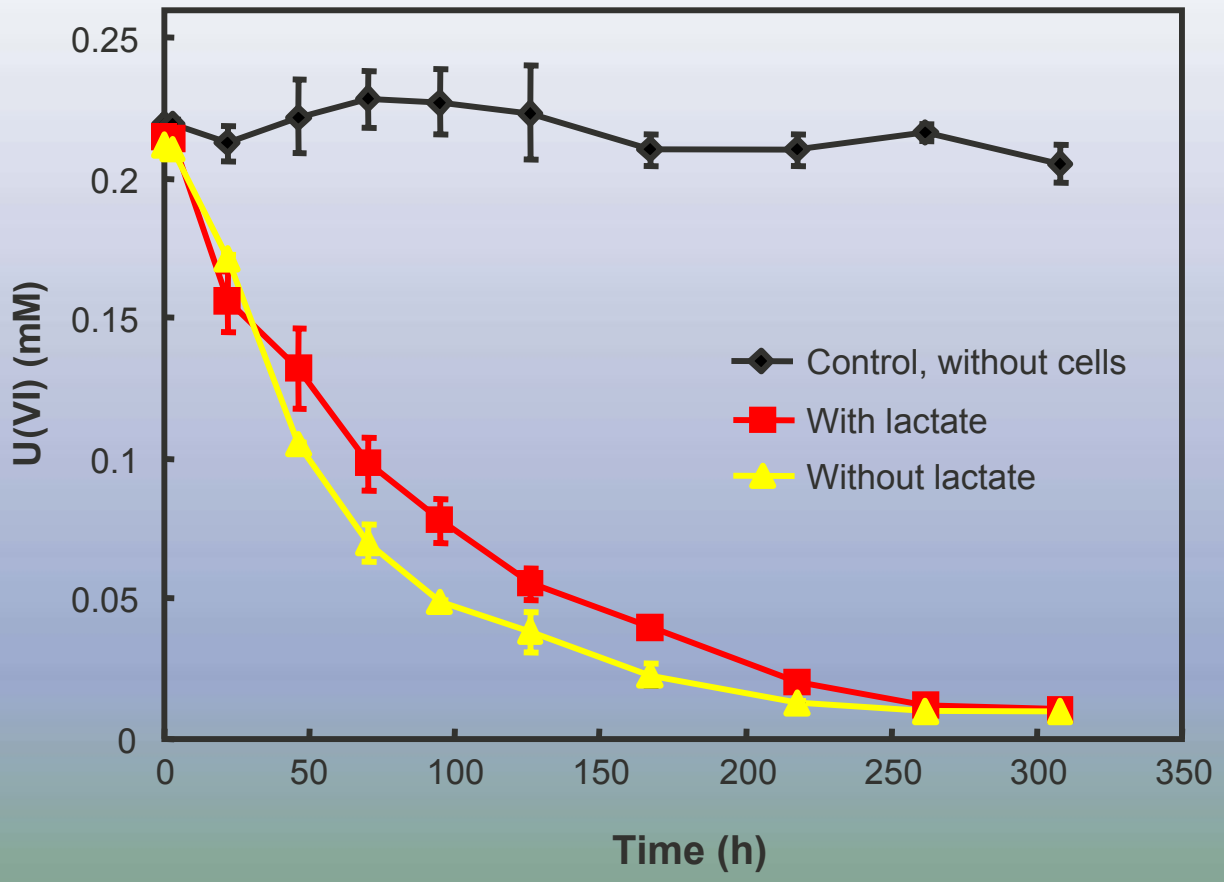
Cr(VI) Reduction by Hanford Cellulomonas Isolates



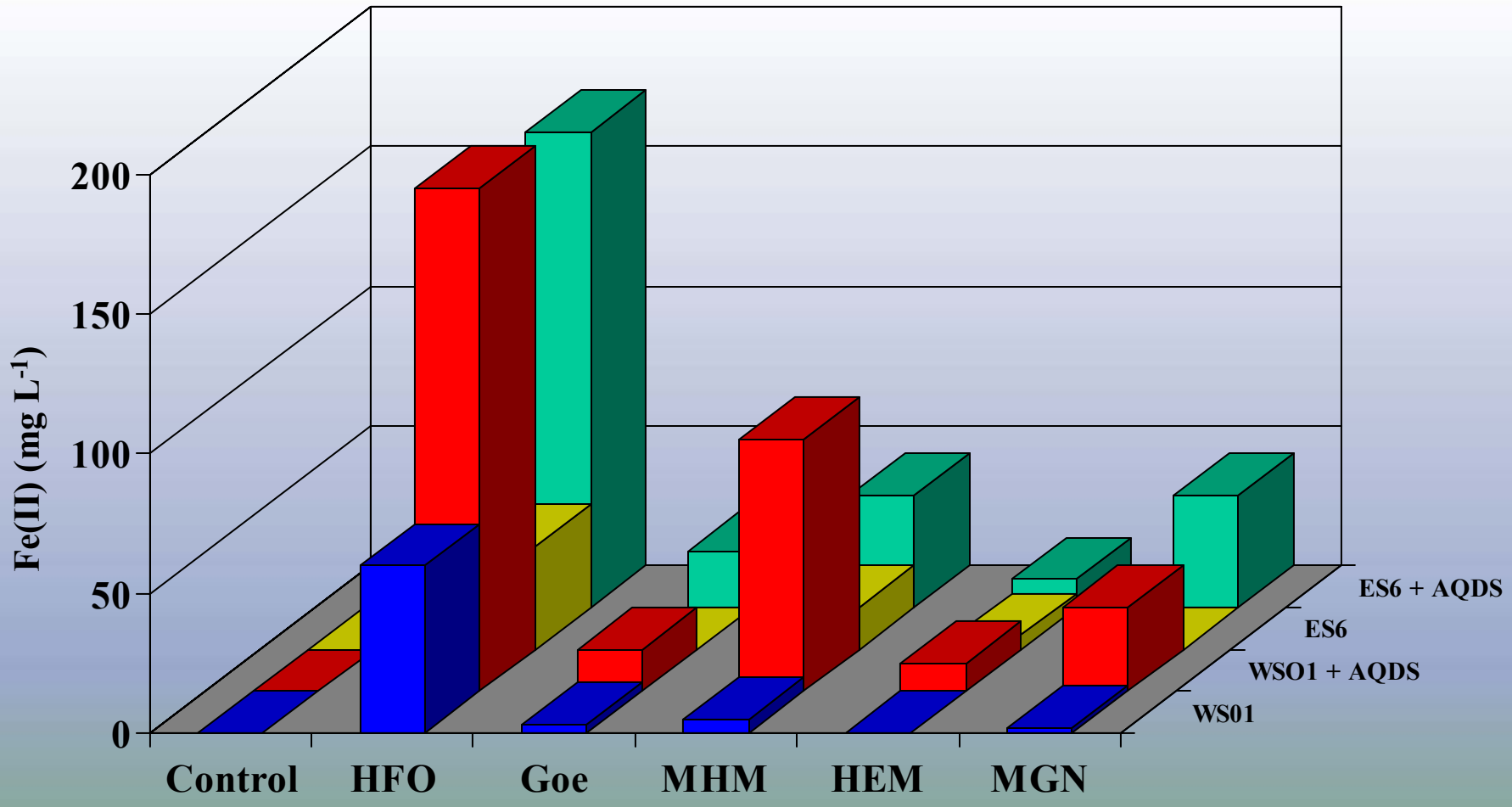
Cr(VI) Reduction by Cellulomonas WS01 Suspensions With and Without Lactate



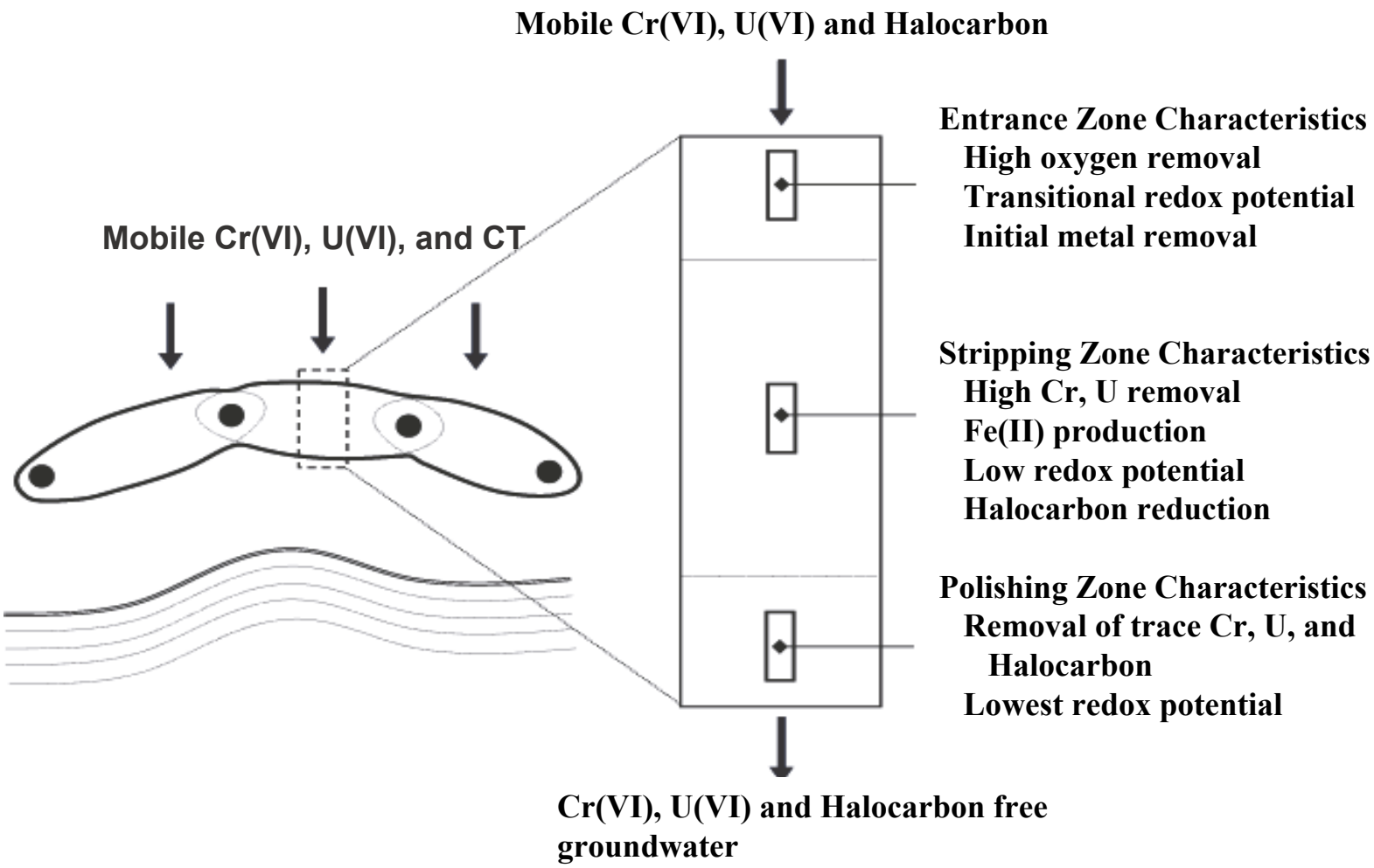
U(VI) Reduction by Cellulomonas WS01 Suspensions With and Without Lactate



Iron Reduction by Cellulomonas



Schematic of Cellulomonas PRB



Hypotheses To Be Tested

- *A wide spectrum of electron donors and acceptors will allow simultaneous treatment of groundwater contaminated with mixtures of halocarbons, radionuclides and heavy metals using a Cellulomonas PRB.*
- *Indigenous Cellulomonas spp. can provide long-term activity as the active component of PRBs even in the absence of carbon sources.*
- *Cellulomonas spp. are present at numerous DOE subsurface sites and represent a metabolically important group influencing the fate of reducible groundwater contaminants.*
- *The choice of electron donor and development of effective substrate addition strategies will allow establishment and control of Cellulomonas communities to form effective PRBs that will degrade halocarbons and immobilize heavy metals and radionuclides.*

Tasks

- *Task 1 (W. A. Apel) – Identification of electron donors and acceptors to maximize halocarbon degradation and reduction of radionuclides and heavy metals.*
  - *Testing the ability of Cellulomonas strains to degrade/reduce combinations of carbon tetrachloride (CT), U(VI), and Cr(VI) with various electron donors (e.g. molasses, sucrose, glycerol, lactate, etc.) and electron acceptors (fermentative, HFO, goethite, etc.)*
  - *Expect to determine kinetics of degradation/reduction of mixtures of CT/Cr(VI)/U(VI) with various electron donors and acceptors.*

Tasks (cont.)

- *Task 2 (R. Gerlach)- Evaluation of long-term metal reduction activity of Cellulomonas spp.*

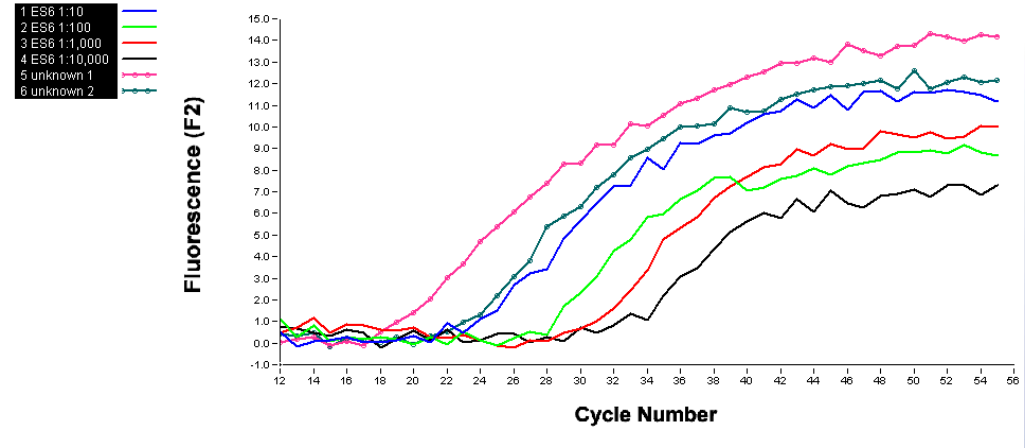
  - *Will investigate long term metal/radionuclide reduction capabilities under carbon-starved conditions. This includes elucidation of metal/ radionuclide reduction mechanisms under these conditions as well as determining, once metal/ radionuclide reduction has ceased, whether activity can be restored through nutrient additions.*

Tasks (cont.)

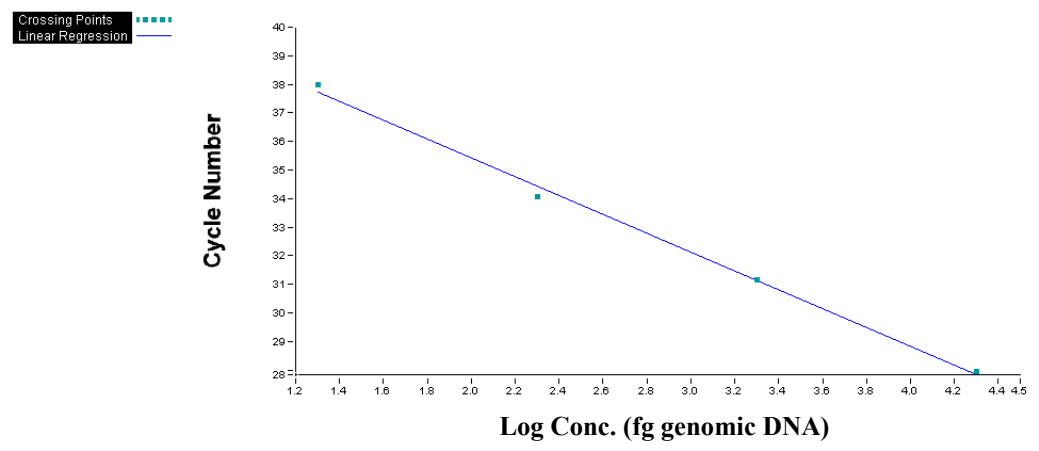
- *Task 2 (cont.)*
  - *Information gained will lead to understanding reduction kinetics under carbon-starved conditions as well as helping estimate nutrient addition cycles necessary to maintain activity under field conditions.*

Tasks (cont.)

- *Task 3 (F. Roberto) – Evaluation of Cellulomonas spp. distribution at DOE subsurface sites and its importance in influencing the fate of reducible groundwater contaminants.*
  - *Will use molecular techniques (real-time PCR) to determine abundance of Cellulomonas spp. in subsurface environments at DOE sites.*
  - *Deliverables will include methodologies for surveying Cellulomonas spp. in subsurface communities and determination of its abundance in subsurface soils and sediments at various DOE sites.*

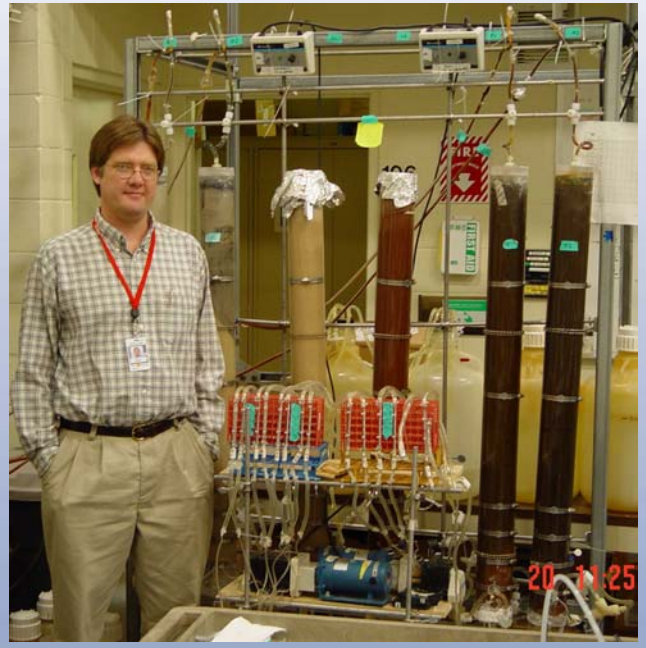


Based on the standard curve generated using a dilution series of ES6 genomic DNA (20 to 20,000 fg) and an estimated concentration of 5 fg DNA per cell, we can detect as few as 4 cells.



Tasks (cont.)

- *Task 4 (B. M. Peyton) - Development of nutrient injection strategies for PRBs.*



- *Column studies will be conducted and data from those studies together with information from Tasks 1 and 2 will be incorporated into a model to predict operational parameters for field deployment of a Cellulomonas-based PRB. This model will enhance our ability to predict plume migration, reduction/degradation rates, and remediation endpoints.*

Tasks (cont.)

- *Task 4 (Cont.)*
  - *Deliverables include descriptions of surface-associated bacterial distribution and solid-phase reductive capacity, as well as laboratory tested substrate addition strategies.*

Summary

- *This is a new, three year EMSP project to begin development of PRBs based on indigenous Cellulomonas spp.*
- *Project consists of four tasks:*
  - *Understand effects of electron donors and acceptors on radionuclide/metal reduction by Cellulomonas spp.*
  - *Study radionuclide/metal reduction by Cellulomonas under long term, carbon starved conditions.*
  - *Use molecular techniques to assess presence of Cellulomonas spp at various DOE sites.*
  - *Develop a nutrient injection strategy for Cellulomonas-based PRBs.*