VIMSS

ESPP
Environmental Stress Pathway Project

PCAP
Protein Complex Analysis Project

IPTAI
INDIANA-PRINCETON-TENNESSEE ASTROBIOLOGY INITIATIVE

BERKELEY LAB
75 Years of World-Class Science 1931-2006

Bioremediation: the Hope and the Hype

biological processes for environmental clean-up (good, bad, and ugly)

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The Problem

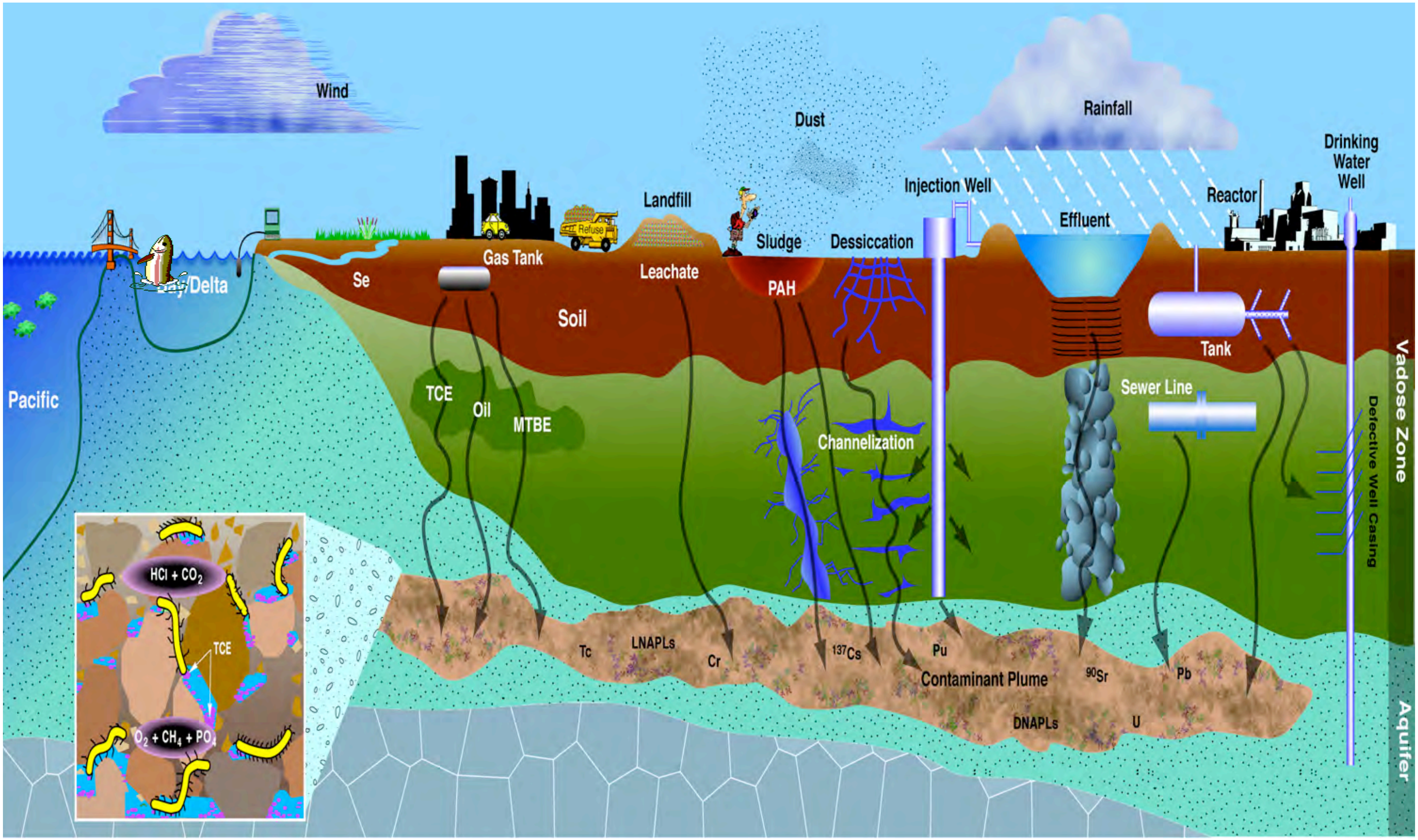
73,000,000 U.S. Citizens
live within 4 miles of
Superfund Site

4,000,000 U.S. Citizens
live within 1 mile of
a Superfund Site

The Sources

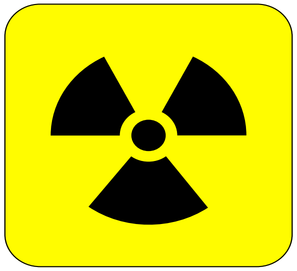
Understanding, monitoring and controlling the environment

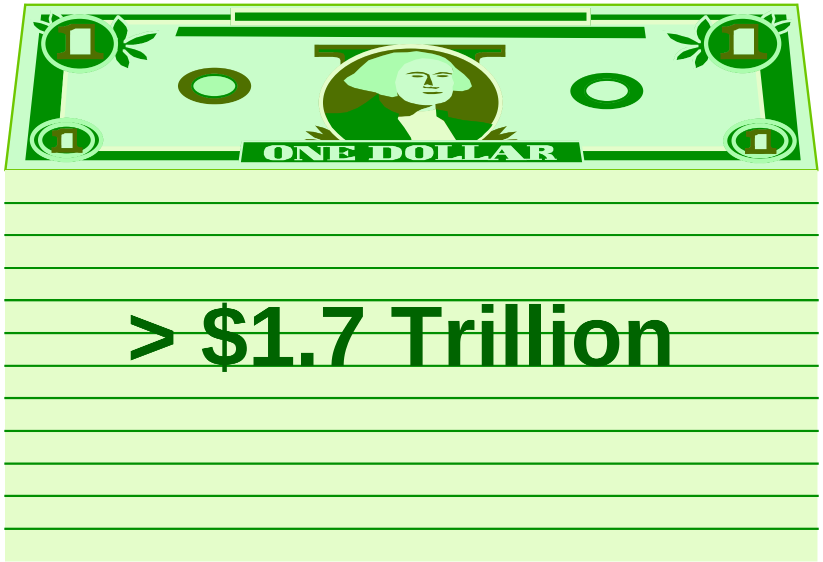
(the need for biosustainability everywhere)



The Cost

Hazardous Waste Remediation
in the United States
could cost





> $1.7 Trillion

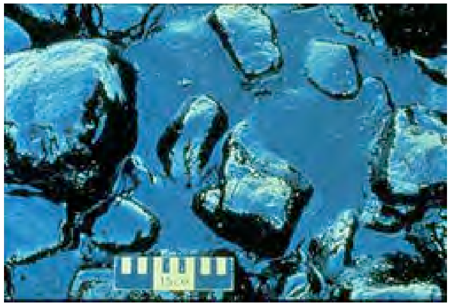
Amoco Cadiz Spill 1978

- 227,000 tons heavy crude oil
- Entire Normandy coast impacted
- Spill was so large they only treated areas that impacted economy the most
- Large areas in remote parts of coast abandoned
- Best available treatment - detergents (dispersants)

1. **Spill was impetus for variety of international cleanup and tanker regulations.**
2. **Ecological studies done 10 and 20 yrs after the spill have demonstrated that**
    - **the areas that were *'treated'* have not recovered yet**
    - **the areas that were *untreated* recovered in < 5 yr!!!!!!**

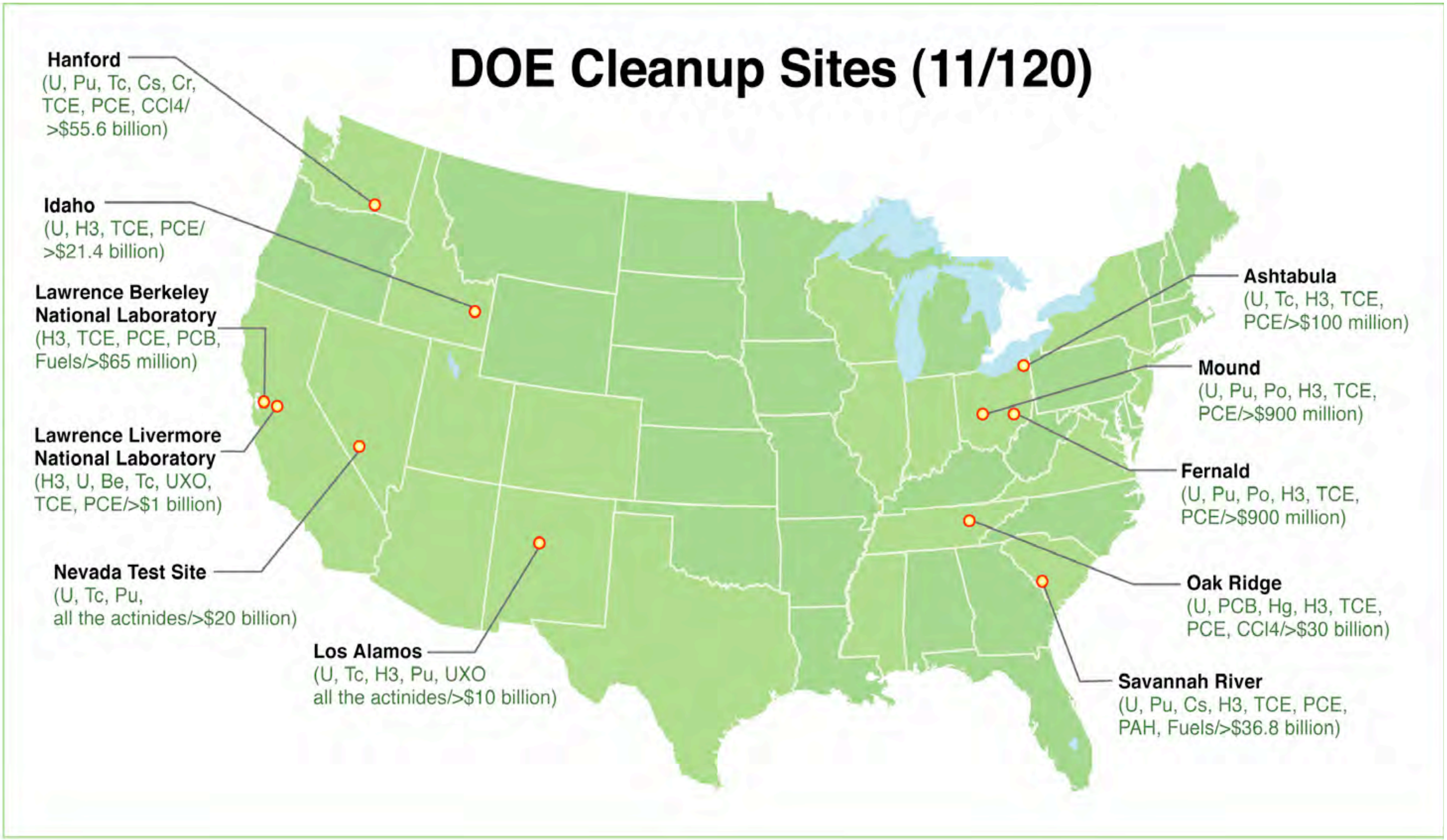
18 yrs later Exxon Valdez spill

- 3/24/89 Supertanker spills 11 million gallons of crude into Prince William Sound, 1,300 miles of coastline impacted (largest spill in US history)
- Cleanup involved: burning, mechanical, dispersants, and bioremediation
- Litigation is still going after 18 years, cost so far >$7 billion



- Congress passed the 1990 Oil Pollution Act
- Fertilized areas were dramatically cleaner the first year, but no difference after the second winter
- Long term impact of treatments (dispersants and bioremediation) will severely impact ecology of sound for many decades

The DOE Problem



DOE Cleanup Sites (11/120)

Hanford
(U, Pu, Tc, Cs, Cr,
TCE, PCE, CCl4/
>$55.6 billion)

Idaho
(U, H3, TCE, PCE/
>$21.4 billion)

Lawrence Berkeley National Laboratory
(H3, TCE, PCE, PCB,
Fuels/>$65 million)

Lawrence Livermore National Laboratory
(H3, U, Be, Tc, UXO,
TCE, PCE/>$1 billion)

Nevada Test Site
(U, Tc, Pu,
all the actinides/>$20 billion)

Los Alamos
(U, Tc, H3, Pu, UXO
all the actinides/>$10 billion)

Ashtabula
(U, Tc, H3, TCE,
PCE/>$100 million)

Mound
(U, Pu, Po, H3, TCE,
PCE/>$900 million)

Fernald
(U, Pu, Po, H3, TCE,
PCE/>$900 million)

Oak Ridge
(U, PCB, Hg, H3, TCE,
PCE, CCl4/>$30 billion)

Savannah River
(U, Pu, Cs, H3, TCE, PCE,
PAH, Fuels/>$36.8 billion)

Fernald, Ohio

Original Mission: Uranium purification 1952-1989, 500 million pounds, 1,050 acres near Cincinnati

31 million pounds of nuclear product, 2.5 billion pounds of low-level radioactive, hazardous and mixed waste, and 2.5 million yd^3 contaminated soil and debris.

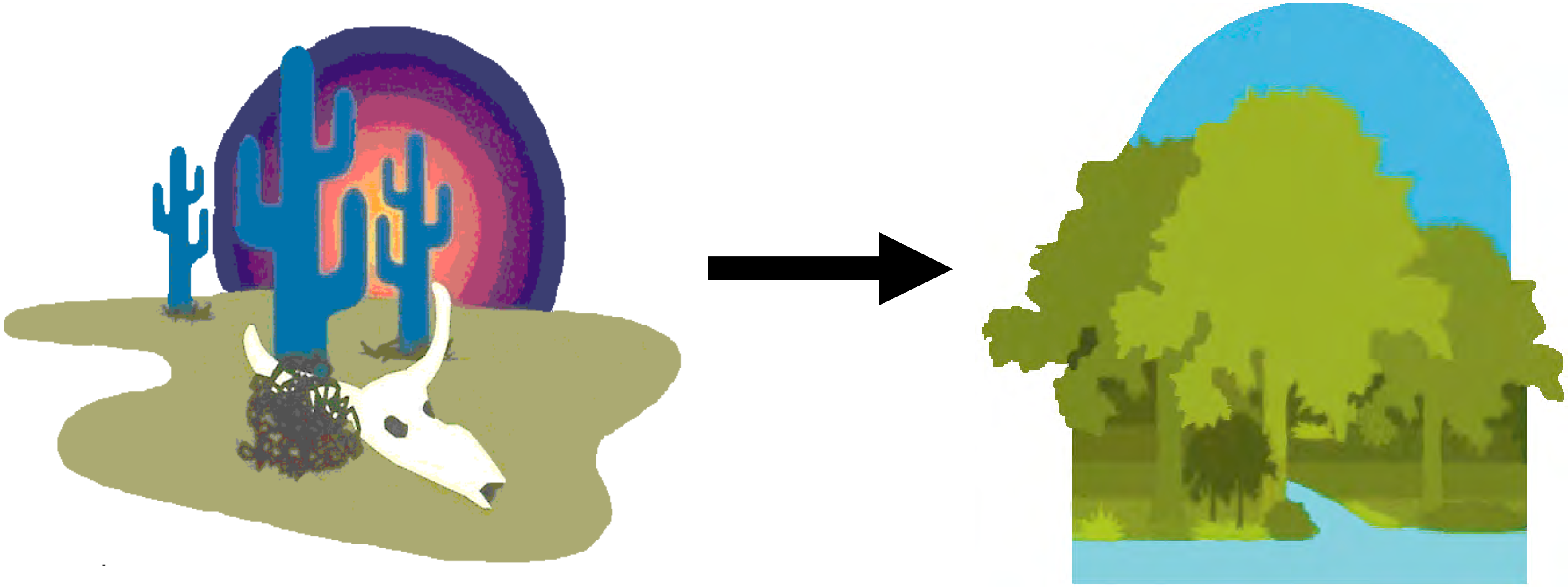
Closed in 2006, ***"Weapons to Wetlands"!!!!!!!!***

On-site Disposal Facility - 2.88 million yd^3

3 trains with 60 cars operating 24/7 hauling contaminated soil to EnviroCare, UT and Nevada Test Site for 5 years.

Bioremediation

Understanding, monitoring and controlling the environment for remediation with biological processes

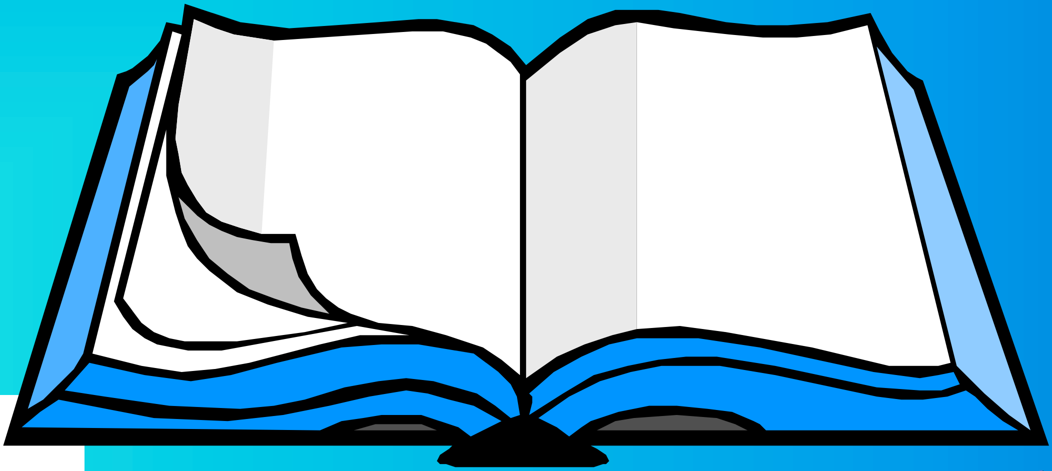


Benefits of Bioremediation

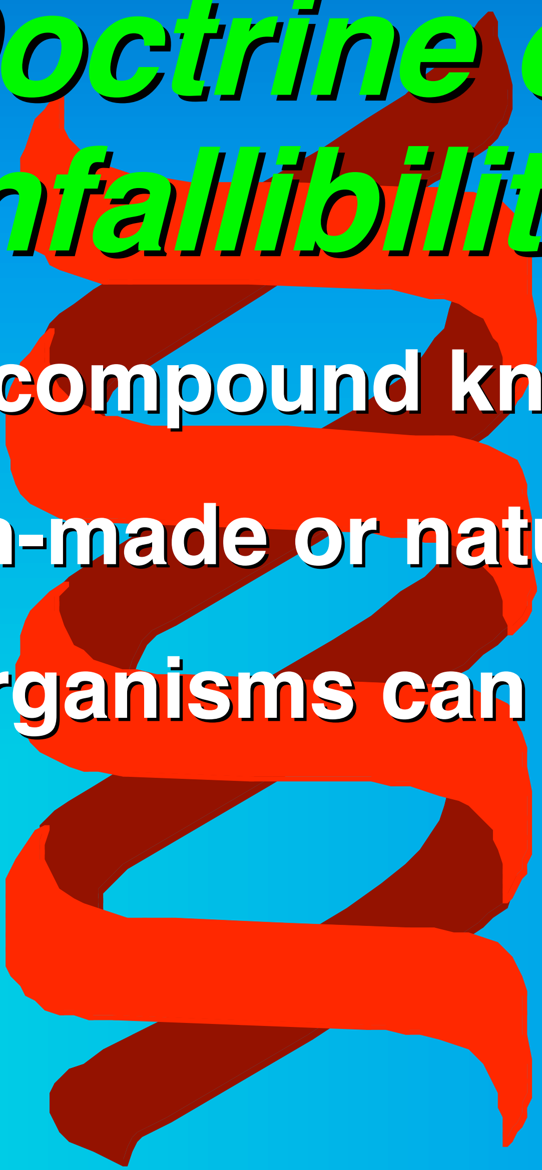
- Terminal Destruction
- On Site
- Environmentally Sound
- Cost Effective

Bioremediation

First mention of "bioremediation" in Scientific Citation Index 1974–1996 in abstract, title or keywords is in 1987.



Doctrine of Infallibility



There is no compound known to man,

man-made or natural,

that microorganisms can not degrade

Microbial* Life on Earth

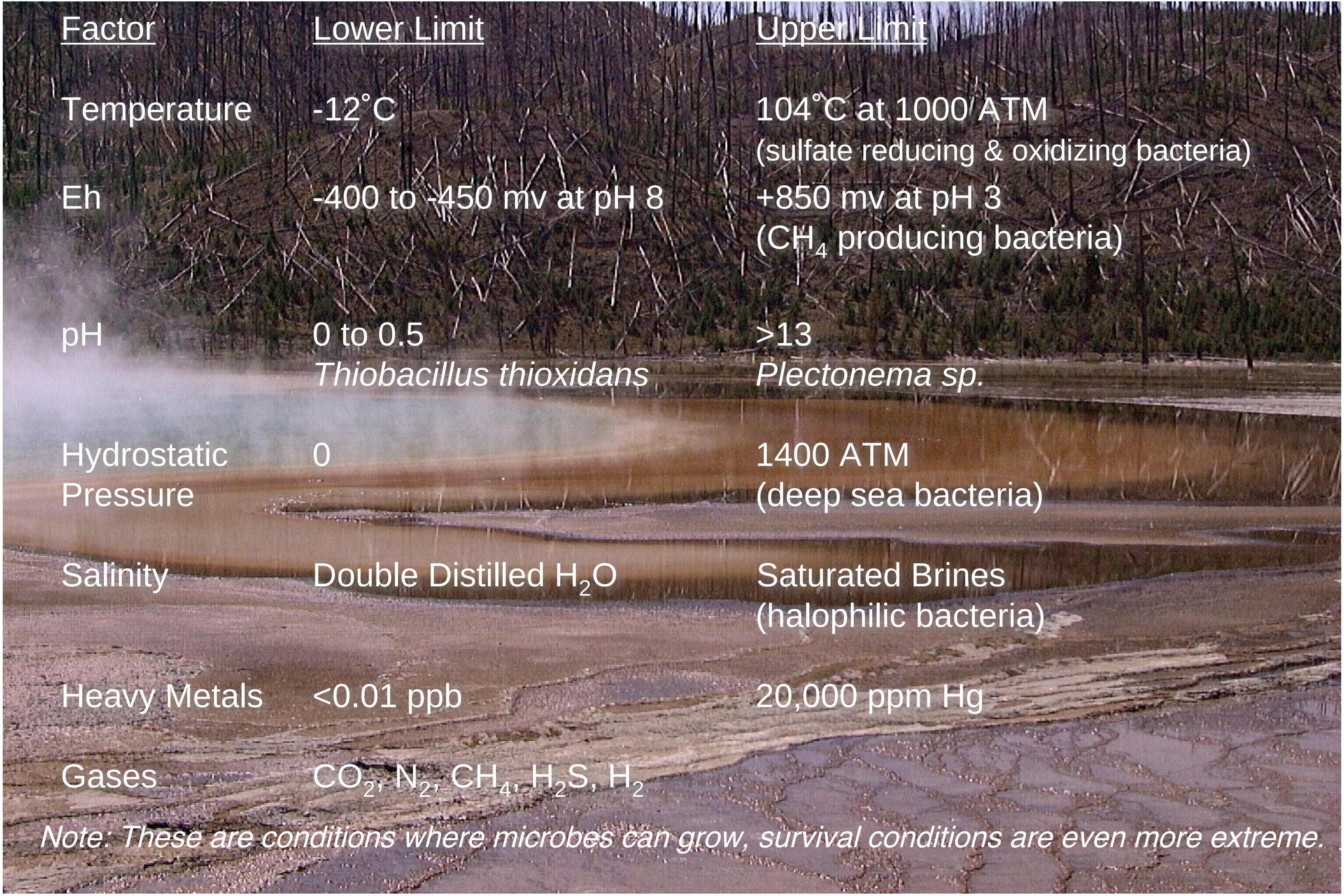
	Cells
Open Ocean	1.2×10^{29}
Soil	2.6×10^{29}
Oceanic Subsurface	3.5×10^{30}
Terrestrial Subsurface	$0.25\text{-}2.5 \times 10^{30}$
All sources	$4\text{-}6 \times 10^{30}$

- 60% of all biomass on earth
- 350-550 Pg of Carbon (60-100% more C then all plants)
- 85-130 Pg of N and 9-14 Pg of P (10 times more than all plants)
- 10^5-10^7 species
- Capable of 4 simultaneous mutations in every gene in 0.4 h
- Capable of dividing every 20 minutes
- Human Body 10^{14} cells with 10^{15} bacteria, 5K-10K species
- > 3.7 billion years of microbial evolution on earth

**** Prokaryotes only, Pg = 10^{15} g***

(in part Whitman et al., 1998)

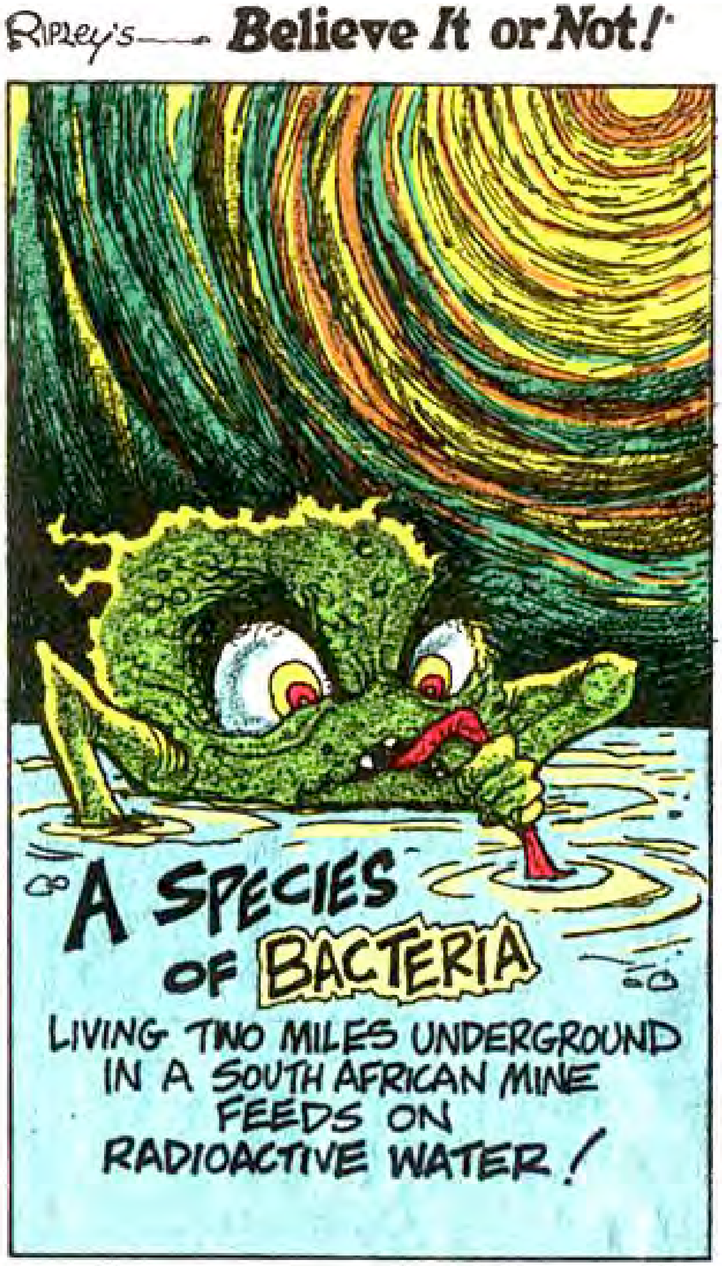
Microbial Growth Capabilities



Factor	Lower Limit	Upper Limit
Temperature	-12°C	104°C at 1000 ATM (sulfate reducing & oxidizing bacteria)
Eh	-400 to -450 mv at pH 8	+850 mv at pH 3 (CH_4 producing bacteria)
pH	0 to 0.5 *Thiobacillus thioxidans*	>13 *Plectonema sp.*
Hydrostatic Pressure	0	1400 ATM (deep sea bacteria)
Salinity	Double Distilled H_2O	Saturated Brines (halophilic bacteria)
Heavy Metals	<0.01 ppb	20,000 ppm Hg
Gases	CO_2, N_2, CH_4, H_2S, H_2	

Note: These are conditions where microbes can grow, survival conditions are even more extreme.

IPTA Astrobiology (NASA)
Detection of bio-sustainable energy and nutrient cycling in the deep subsurface of Earth and Mars



Lin et al. Science 10/06

Life in the slow lane.

- DNA was extracted from:
  - 3 Myr old fracture water
  - 8,000 ft. South Africa Gold Mine
  - Analogs to Mars?

Homestake Mine
8,000 ft Lead, SD

Normal Microbial Requirements

Parameters	Optimum Levels
Available soil water	25-85% holding capacity
Oxygen	Aerobic >0.2 mg/l DO Anaerobic: O_2 < 1%
Redox potential	Aerobes & Facultatives: > 50mv Anaerobes: < 50mv
pH	5.5-8.5
Nutrients	C:N:P of 120:10:1
Temperature	15-45° C (Mesophiles)

“…1 g of soil typically contains 1 million to 10 billion microbial cells representing about 4,000-10,000 species…” (Torsvik et al., 1990)

Factors that Affect Biodegradation

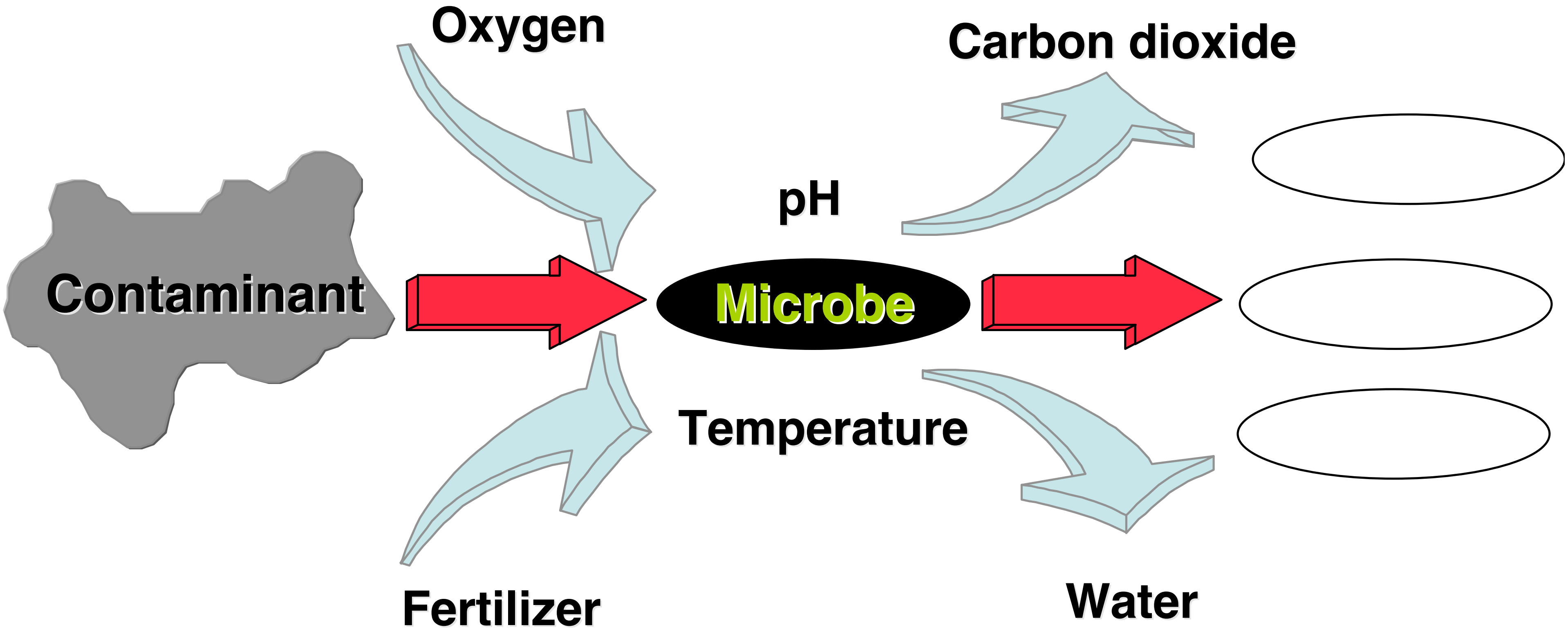
The Contaminant

- molecular size, shape, charge and functional groups, concentration
- solubility in water; lipid / water partition coefficient
- solid / liquid / gas; volatilization
- toxicity
- possibility of spontaneous nonenzymatic reactions

The Environment

- mechanical accessibility
- pH, pO_2, temperature, redox potential
- presence of interfaces
- ionic composition and concentration
- water and wind speed, light quality and intensity
- presence of co-metabolites, essential nutrients, reactive radicals, etc.
- presence of appropriate organisms or plasmids

Bioremediation explained



Oxygen

Carbon dioxide

pH

Contaminant

Microbe

Temperature

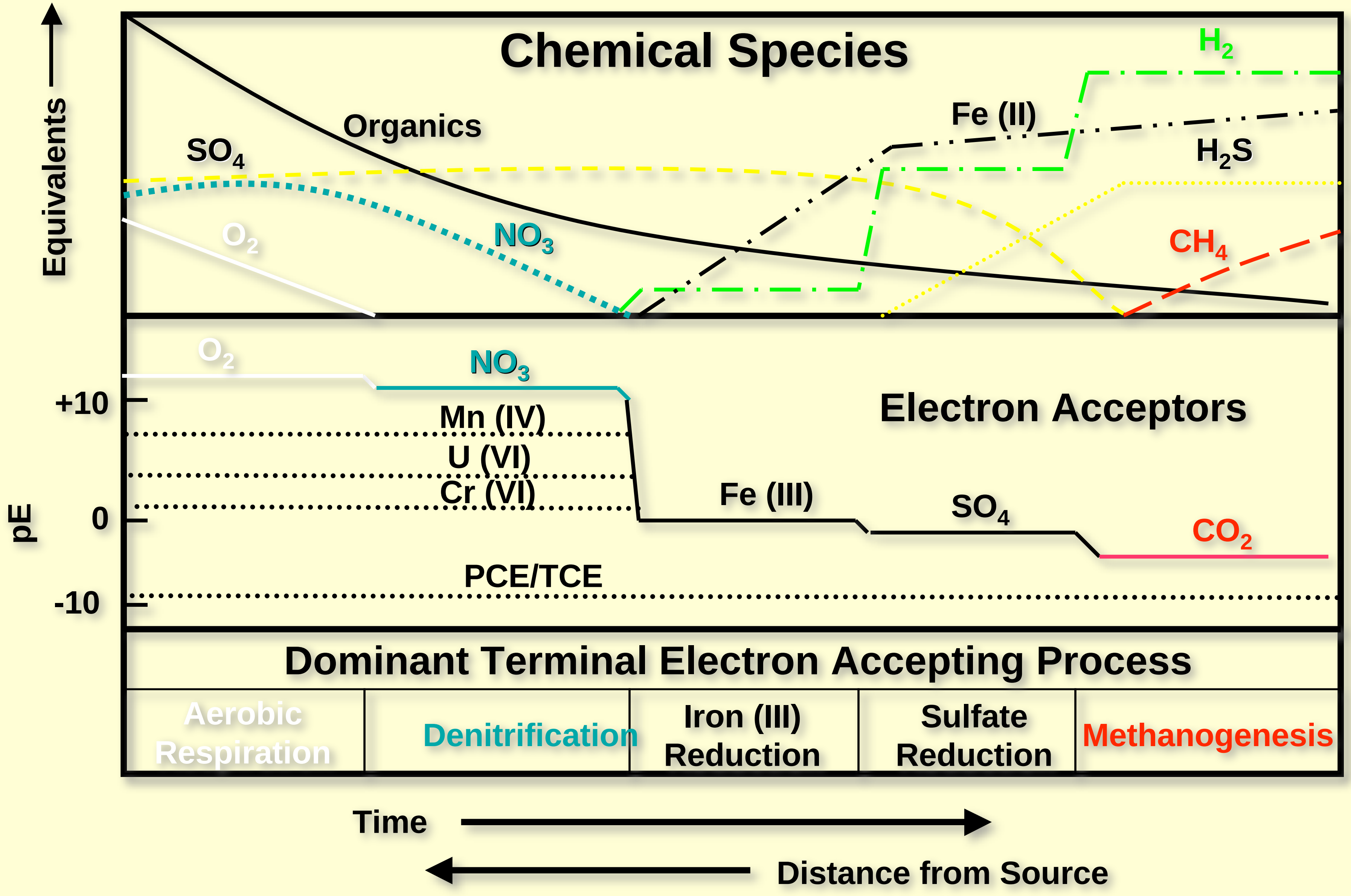
Fertilizer

Water

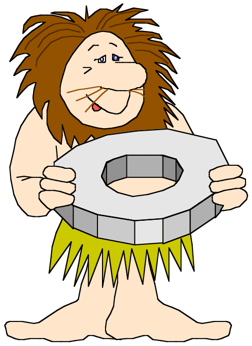
TEA and ED?

- Terminal Electron Acceptors (TEA)
  - Oxidizing agent in cellular respiration
  - O_2, NO_3, Fe(III), SO_4, CO_2
- Electron Donors (ED)
  - Reducing agents
  - Energy sources: usually carbon sources also e.g. sugars, etc.

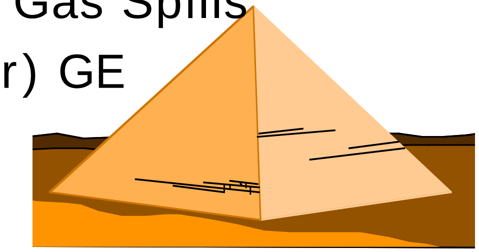
Critical Biogeochemistry

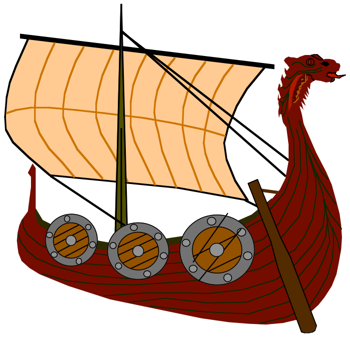


Bioremediation Historical Perspective

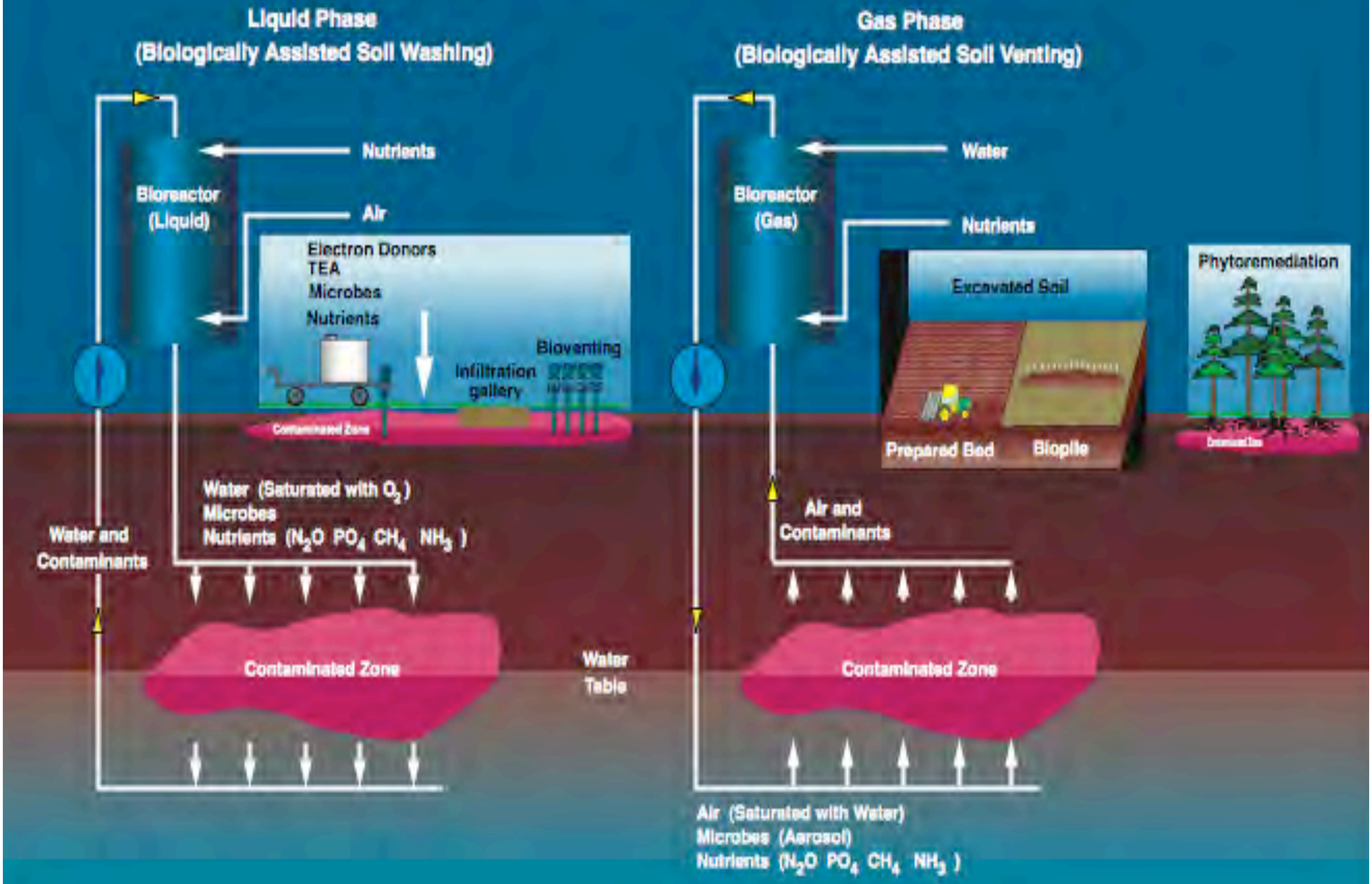


prehistoric	Fermentation (Second Oldest Profession?)
6000 BC	Kitchen middens, compost piles
1900 BC	Greeks walled refuse bioreactors
1891	First Waste Water Treatment Plant (Sussex, UK)
1946	Zobell Demonstrates Oil Biodegradation
1950	Petroleum Land-Farming Widely Used
1968	Bilge Water of Queen Mary Biotreated (Bioaugmentation)
1974	Raymond Patent for In Situ Biotreatment of Gas Spills
1981	First U.S. Patent on life (petroleum degrader) GE
1988	French Limited Superfund Site Test
1989	Exxon Valdez Spill Demonstration by EPA
1992	SRS Integrated Demonstration for TCE/PCE
1993	GE Hudson River Caisson Demonstration for PCB
1997	UT/ORNL lysimeter tests of GMO
1999	Oyster Site release of Adhesion-less strain



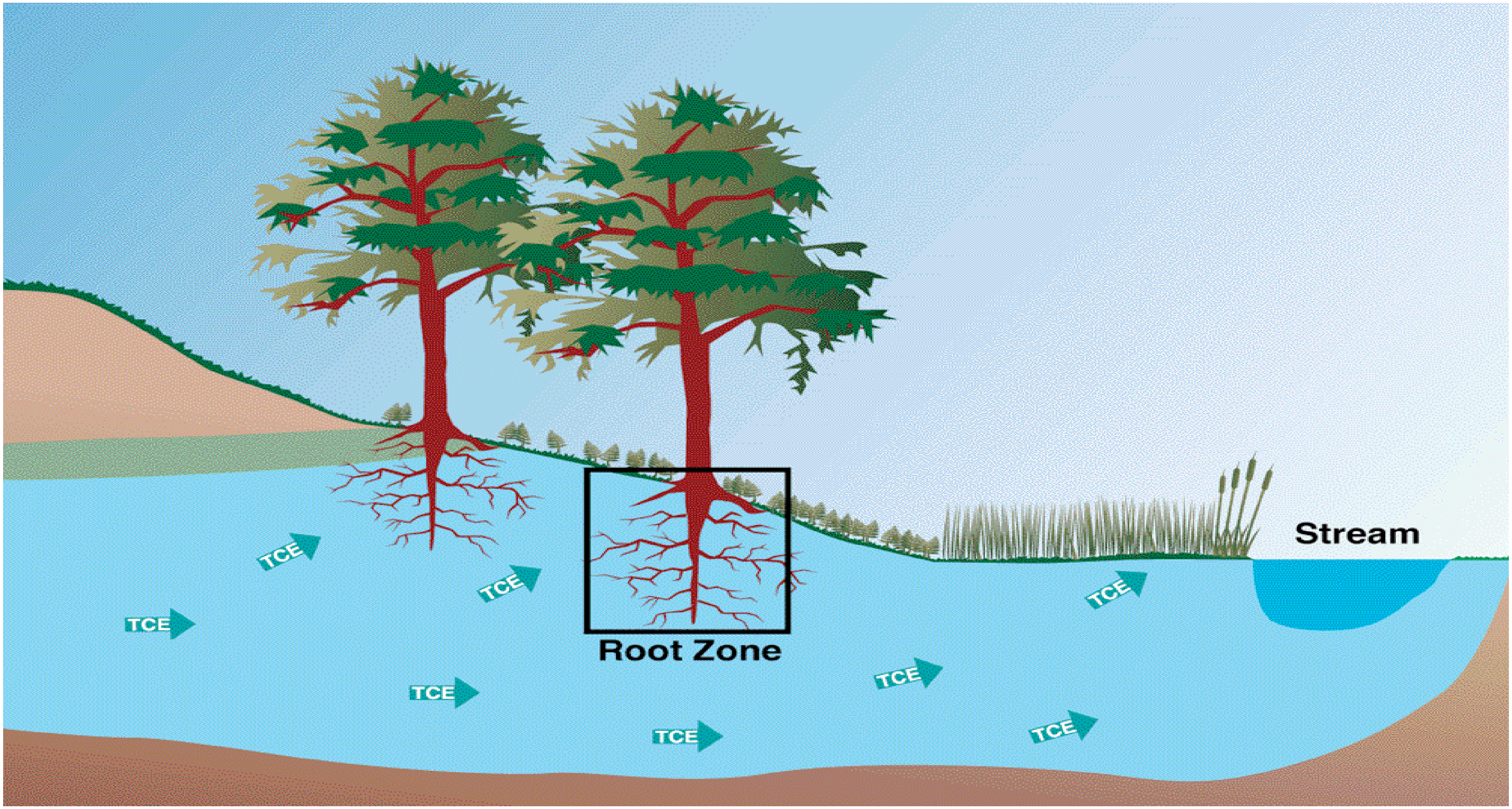


Bioremediation Technologies



Intrinsic Bioremediation

Unmanipulated, unstimulated, unenhanced biological remediation of an environment; i.e. biological *natural attenuation* of contaminants in the environment.



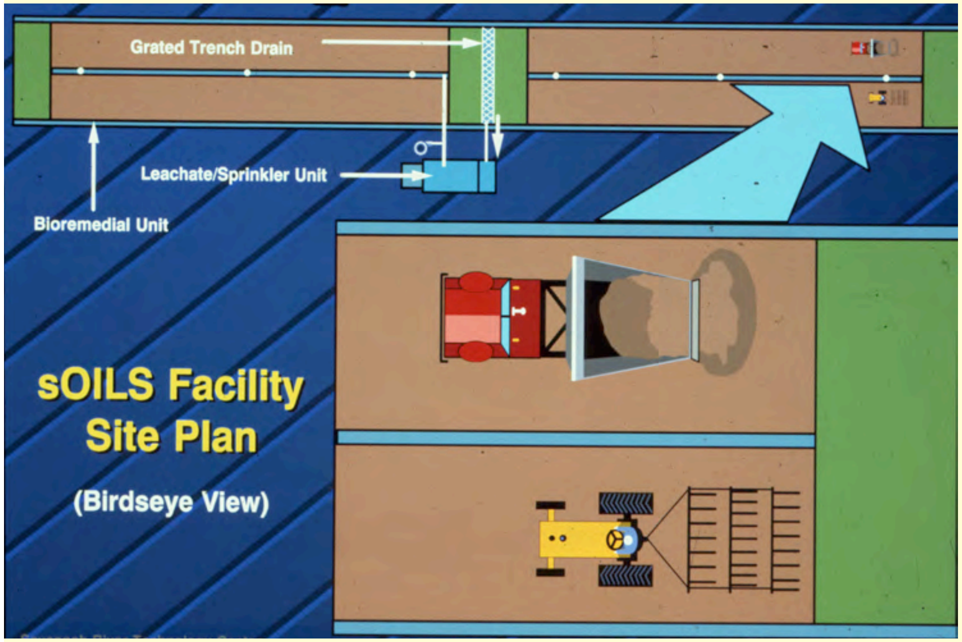
Land Farming

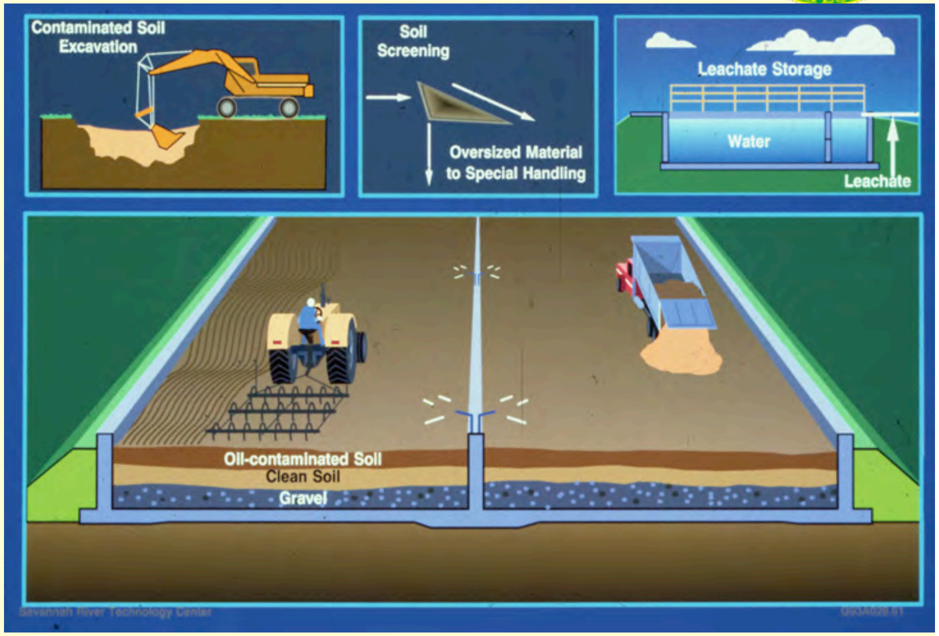
Biologically treating uncontained surface soil, usually by aeration of the soil (tilling) and addition of fertilizer or organisms, hence farming.

	Prepared Bed	Bioventing
Various (Bartha, 1986)	52–641	
SRS	10–107	10–65
Poland (refinery biopile)	33–121	
Italy (biopile – crude)	60	
Hill AFB, Utah		10
Tyndall AFB, Florida		2–20
The Netherlands		2–5
The Netherlands		8
Patuxent River NAS, Maryland		3
Fallon NAS, Nevada		5
Eicklson AFB, Alaska		1–10
Kenai, Alaska		21
Tinker AFB, Oklahoma		2.7–18

*all values in mg TPH/kg soil/day

sOILS Facility





Polish Refinery

Before

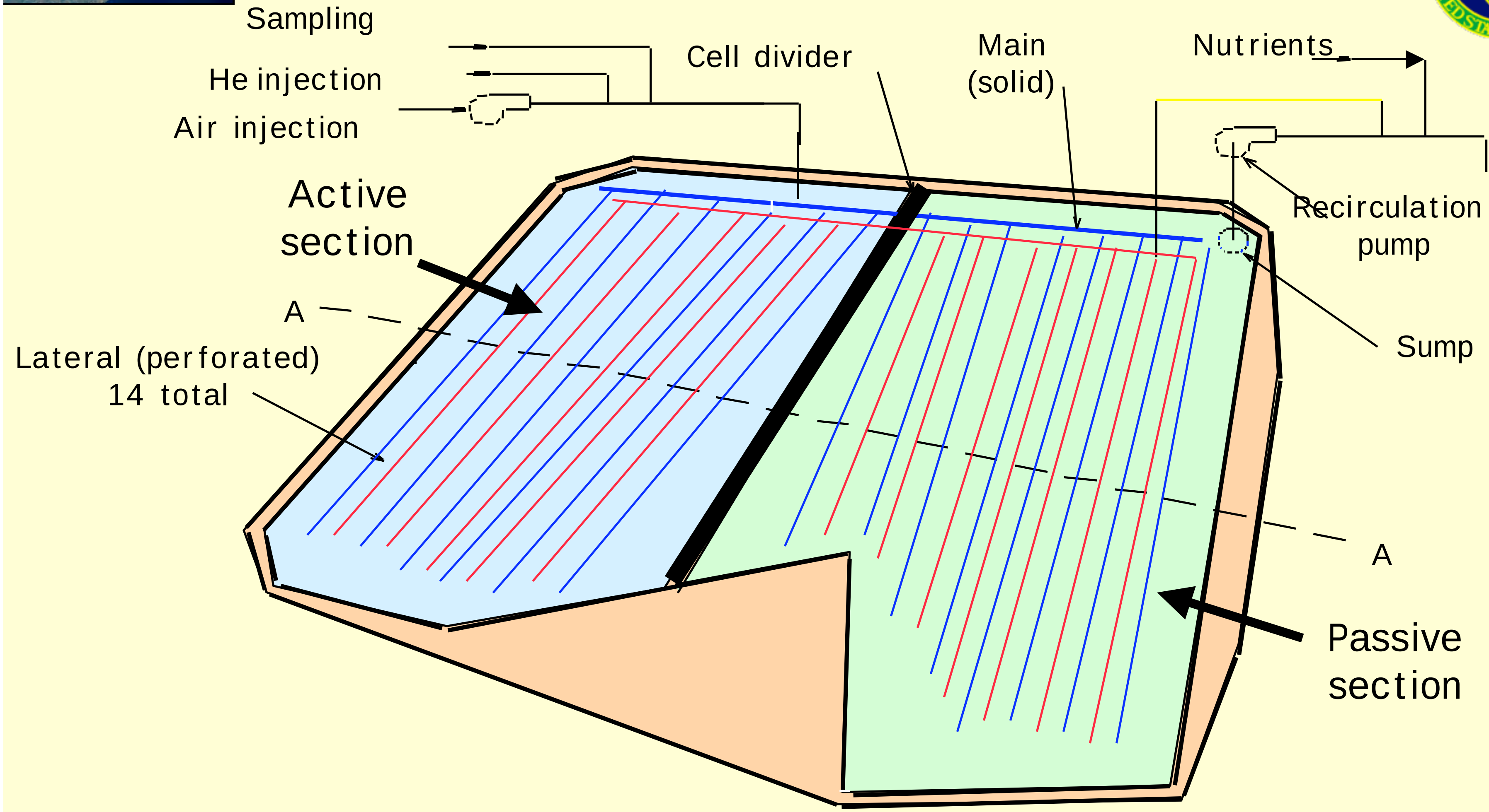
4167 yd^3 of sludge contaminated soil

After

18 Months

120 metric tons destroyed (81%)

Green Zone



Sampling

He injection

Air injection

Cell divider

Main (solid)

Nutrients

Recirculation pump

Active section

A

Lateral (perforated) 14 total

Sump

A

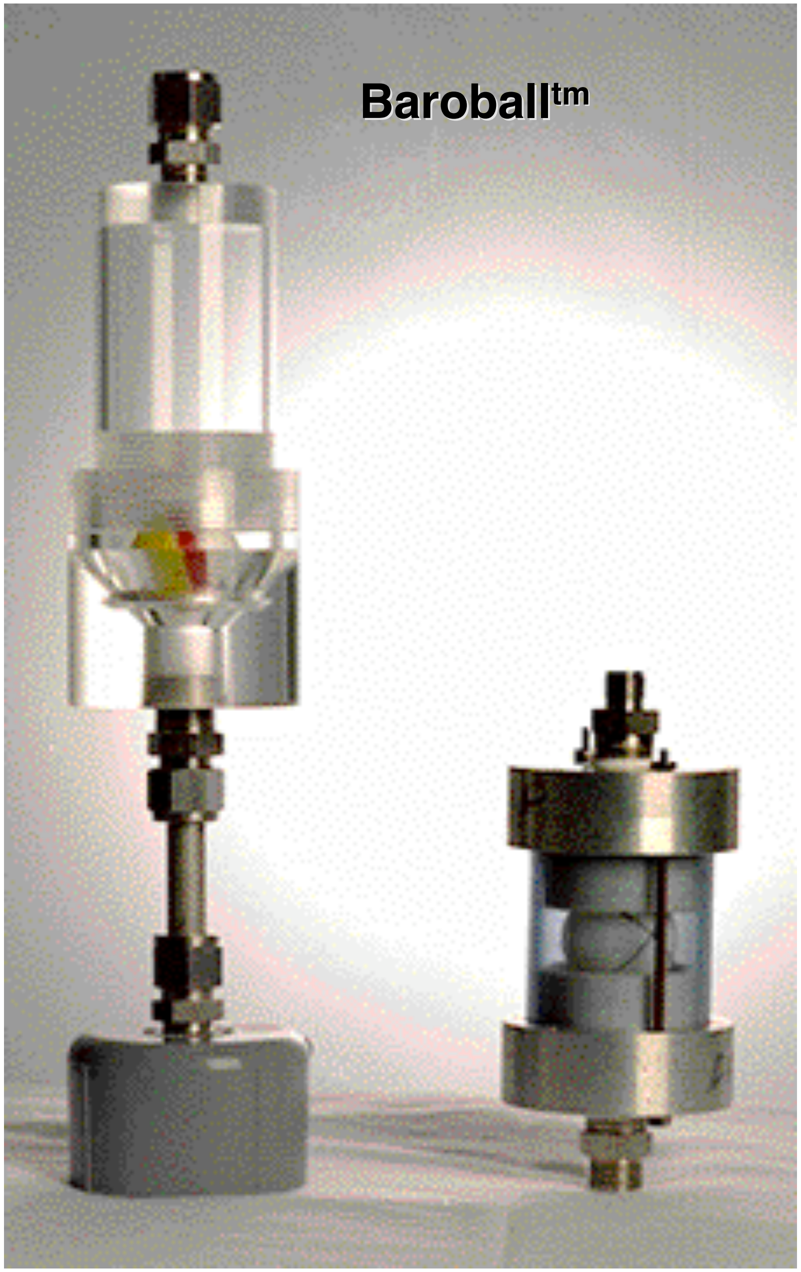
Passive section

Passive Bioremediation

Using natural processes for biostimulation, e.g. barometric pumping, natural infiltration, to deliver nutrients or manipulate the environment, i.e. engineering controls

Campaign	Passive	Active
OC-1	44*	119
OC-2	82	94
OC-3	33	0
OC-4	0	37
OC-5	60	121

*mg TPH/kg Soil/day



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Model Assumptions

$$m(t) = M/R^3(R^2-2a\Delta ct/\gamma)^{3/2}$$

NAPL (**fraction A**) content	~ 40% of total TPH inventory in soil
Readily available fraction content	~ 45% of total TPH inventory in soil
Sorbed fraction content	~ 15% of total TPH inventory in soil
Soil porosity: =	~ 0.3

Characteristics of NAPL fraction (**Fraction A**):

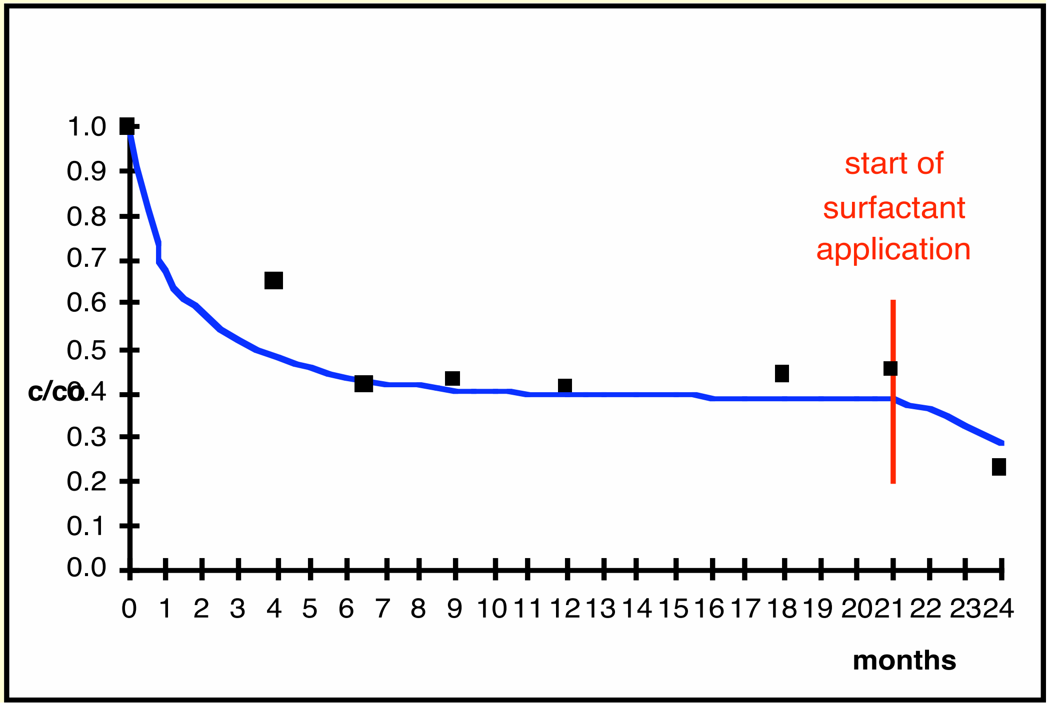
Average radius of aggregates (droplets)	R= 1.0 cm
Solubility in water	c= 10 mg/l before the surfactant was added c= 100 mg/l after the surfactant was added

Characteristics of readily available fraction (**Fraction B**):

Average radius of soil aggregates:	r_0 =	1.0 cm
Desorption coefficient	K_d =	100
Pore diffusivity of contaminant	D_{eff} =	5×10^{-11} cm^2/s
Liquid mass transfer coefficient	k_l =	1×10^{-5} cm/s

Characteristics of sorbed fraction (**Fraction C**):

Average radius of soil aggregates:	r_0 =	30m
Desorption coefficient	K_d =	1×10^5
Pore diffusivity of contaminant	D_{eff} =	5×10^{-13} cm^2/s
Liquid mass transfer coefficient	k_l =	1×10^{-5} cm/s



Biostimulation - The addition of organic or inorganic compounds to cause indigenous organisms to effect remediation of the environment, e.g. fertilizer, surfactants.

Bioaugmentation - The addition of organisms to effect remediation of the environment, e.g. contaminant-degrading bacteria injection into an aquifer, GMO.

Biostimulation Requirements

1. correct microbes must be present
2. ability to stimulate target microbes
3. ability to deliver nutrients
4. C:N:P - 30:5:1 for balanced growth (Paul and Clark, 1989) 100:10:2 in field practice (Litchfield, 1993)

Gases: air, oxygen, nitrous oxide, propane, methane, triethyl phosphate, etc.

Liquids: lactic acid, molasses, vegetable oil, acetate, Chitin, hydrogen release compound (HRC®), MRC®, etc.

Solids: bulking agents (saw dust, agricultural byproducts), oxygen release compound (ORC®), etc.

D Area Oil Seepage Basin

2 trenched horizontal wells at 3 m

1 blower (200 scfm)

Methane, N_2O, TEP

In less than 6 months

Methylene Chloride: 2300 ppb to < 2 ppb
Vinyl Chloride: 300 ppb to < 5 ppb
Dichloroethylene: 100 ppb to < 2 ppb
Trichloroethylene: 100 ppb to < 5 ppb
Tetrachloroethylene: 50 ppb to <10 ppb
BTEX: 50 ppm to < 1 ppm
No Action ROD filed 6/98, granted 3/99

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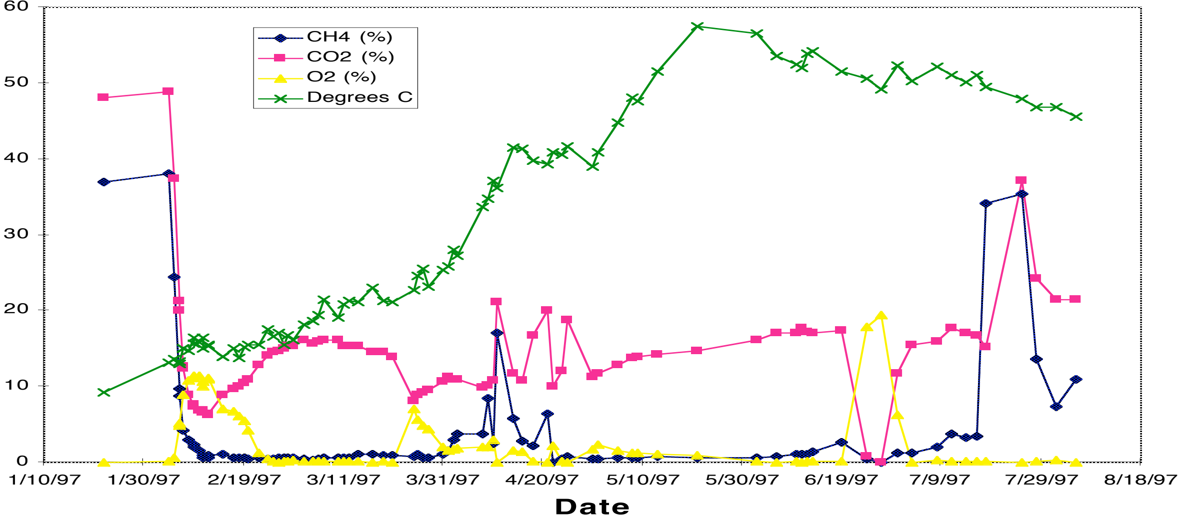
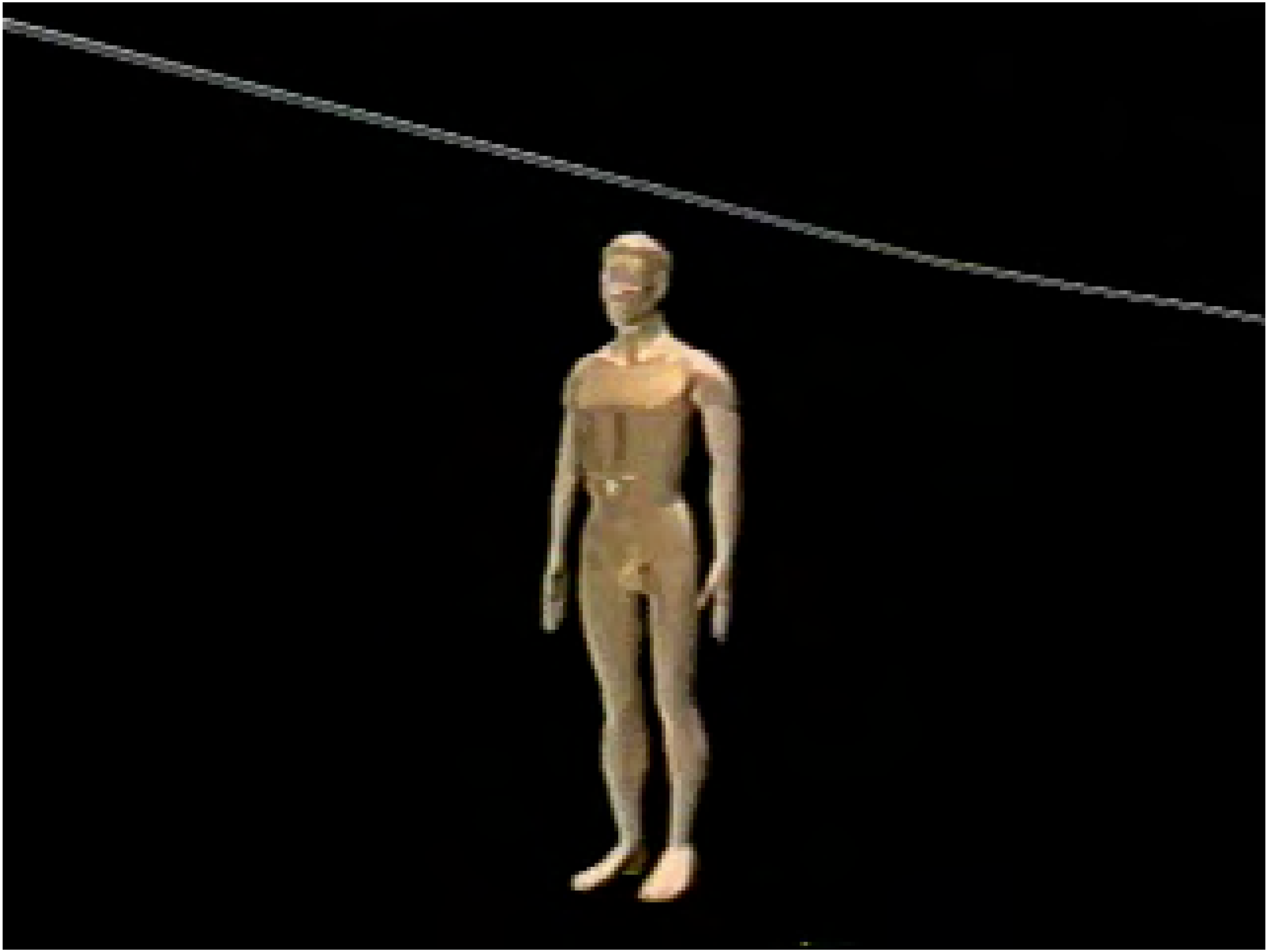
Aerobic Landfill Bioremediation

- increased biodegradation rate
- increased subsidence
- eliminated need for leachate treatment
- stabilized refuse mass sooner
- decreased long-term liability and monitoring costs
- decreased leaching of metals and organic contaminants
- decreased methane generation
- Reduced life cycle costs





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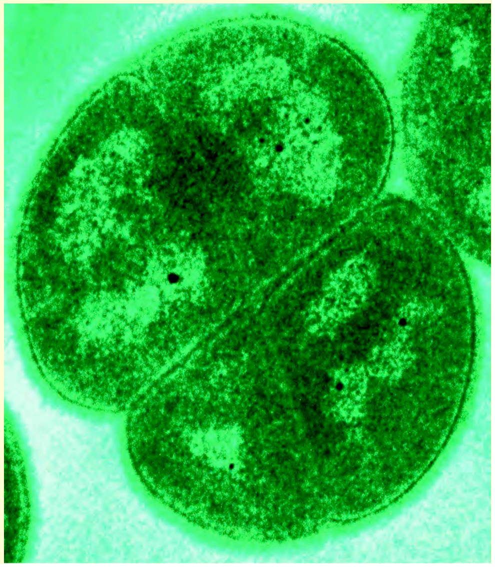
Bioaugmentation Advantages

1. “new” spills where microflora has not had time to adapt or grow (vector)
2. recalcitrant contaminants (GMO)
3. biomass can not establish or maintain itself (GMO)
4. biobarrier (ultramicrobacteria, GMO)
5. controlled environment (GMO)

Pseudomonads (oil spills) - several commercial products

Dehalococcoides ethenogenes (chlorinated solvents) new products from Regenesis and GeoSyntec



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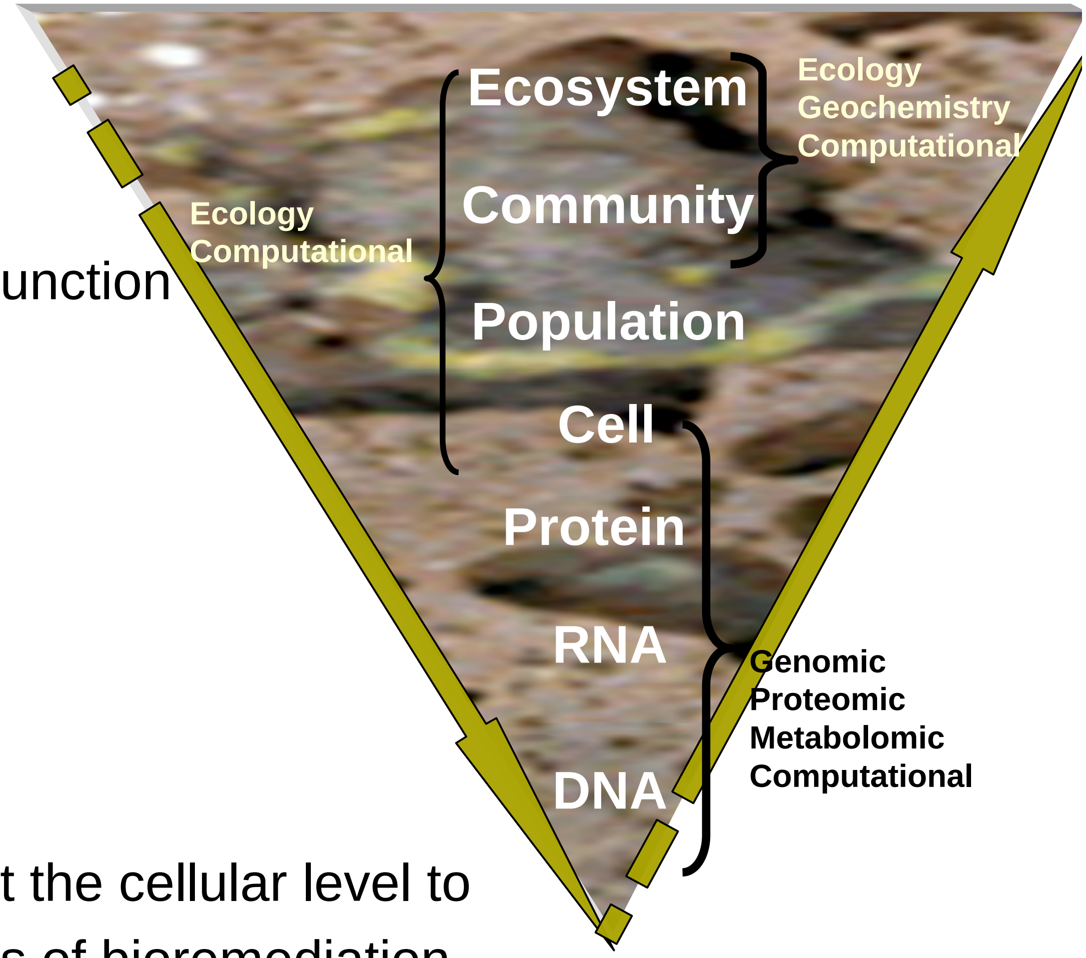
Systems Biology Approach

Ecosystem

Identify key factors (i.e., stresses) that drive community structure and composition and impact the survival and efficacy of heavy metal-reducers



Ecosystem
Community
Population
Cell
Protein
RNA
DNA

Ecology
Geochemistry
Computational

Ecology
Computational

Genomic
Proteomic
Metabolomic
Computational

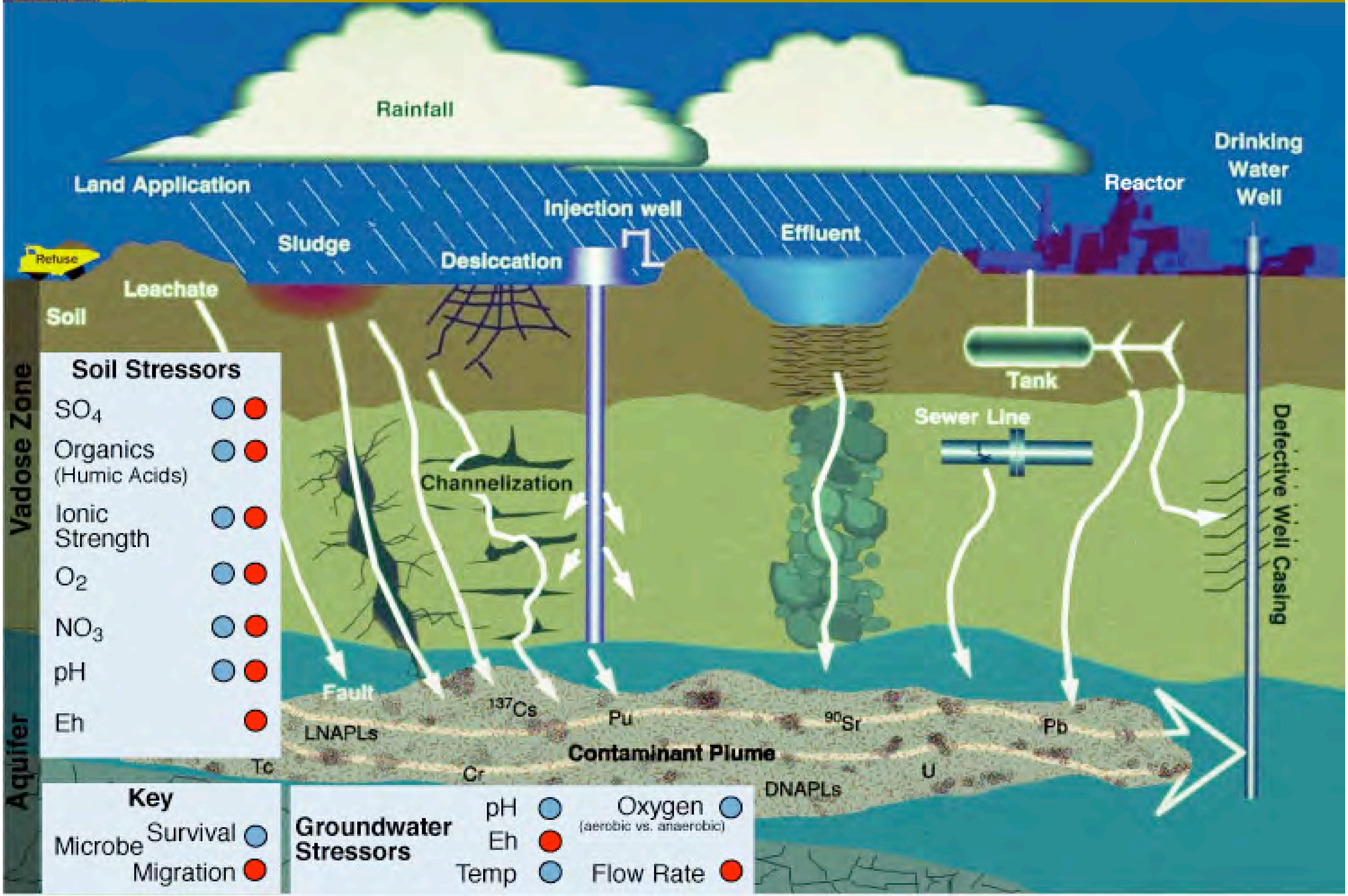
Community and Population

Understand impacts on structure/function relationships

Cell

Analyze DNA, RNA, and protein at the cellular level to understand cellular effects in terms of bioremediation

Stress Response Pathways in Biogeochemistry



Soil Stressors

SO_4 🔵 🔴

Organics (Humic Acids) 🔵 🔴

Ionic Strength 🔵 🔴

O_2 🔵 🔴

NO_3 🔵 🔴

pH 🔵 🔴

Eh 🔴

Key

Microbe Survival 🔵

Microbe Migration 🔴

Groundwater Stressors

pH 🔵

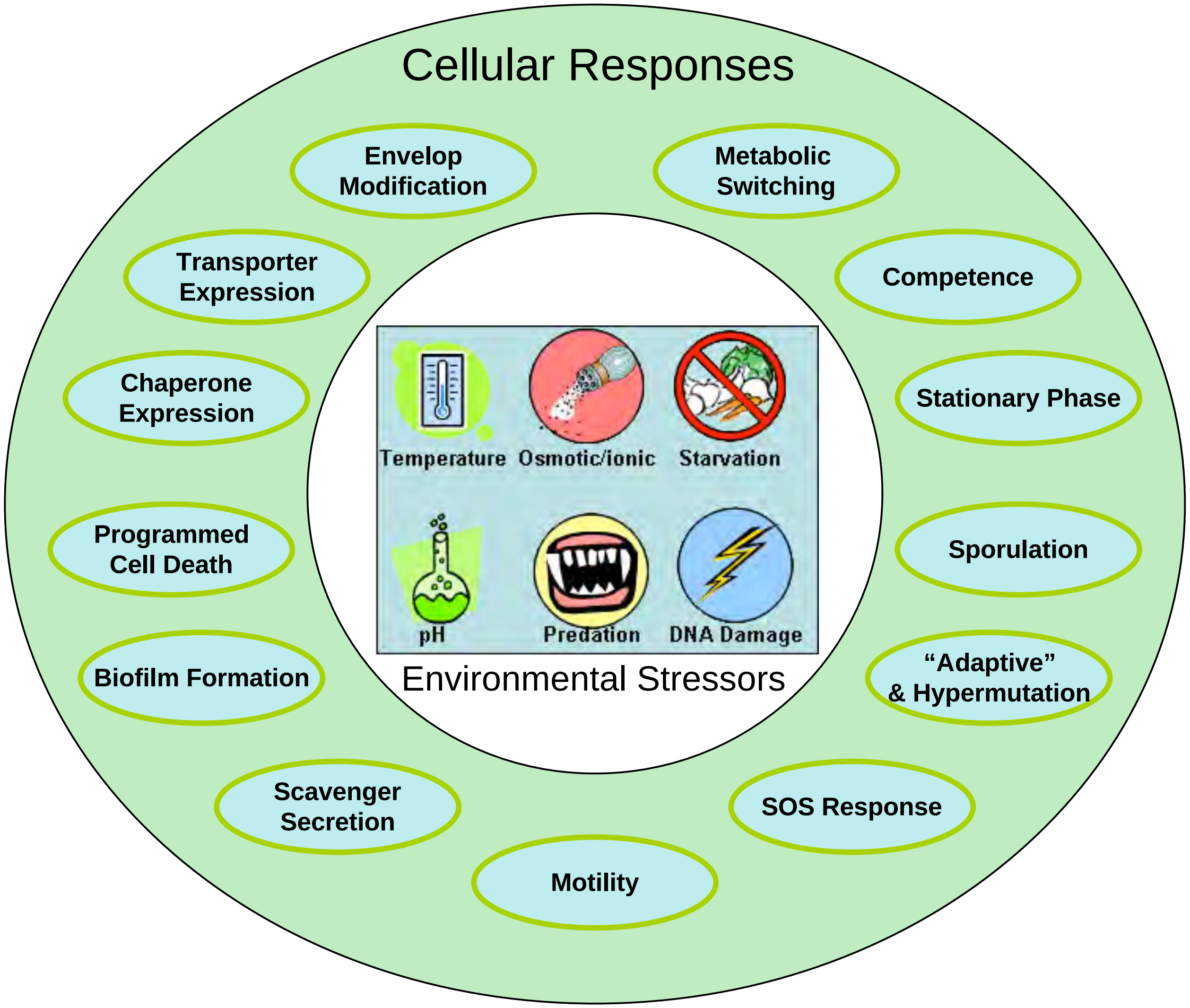
Eh 🔴

Temp 🔵

Oxygen 🔵
(aerobic vs. anaerobic)

Flow Rate 🔴

Relevant Stress Responses



Cellular Responses

Envelop Modification

Metabolic Switching

Transporter Expression

Competence

Chaperone Expression

Stationary Phase

Programmed Cell Death

Sporulation

Biofilm Formation

“Adaptive” & Hypermutation

Scavenger Secretion

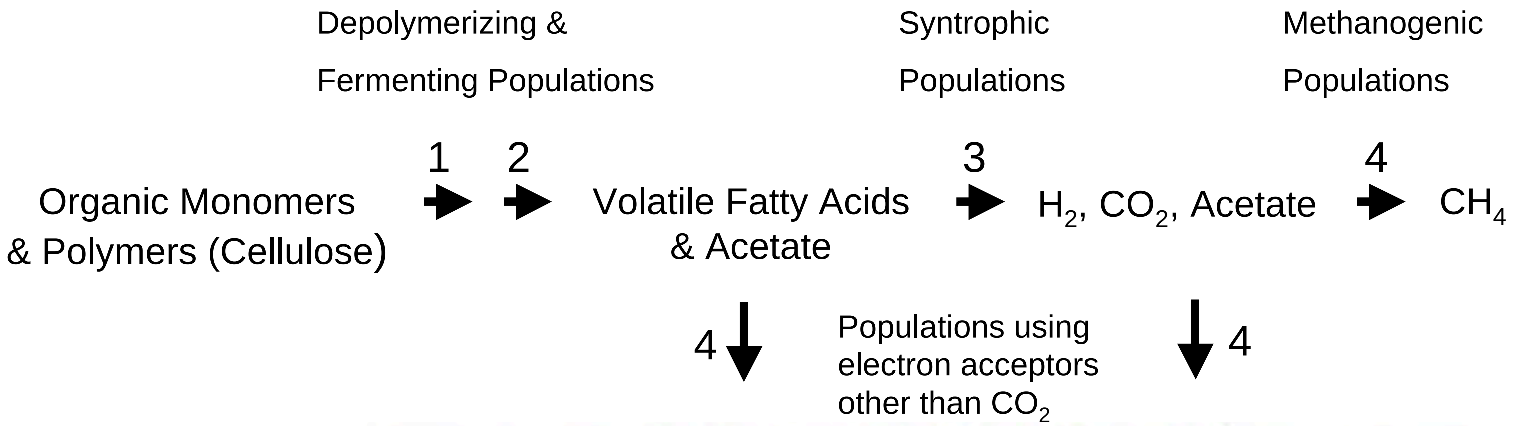
SOS Response

Motility

Environmental Stressors

(Hazen & Stahl, 2006 - Current Opinions in Biotechnology)

Multiple Trophic Interactions Determine Delivery of Electron Donors to Terminal Electron Accepting Species



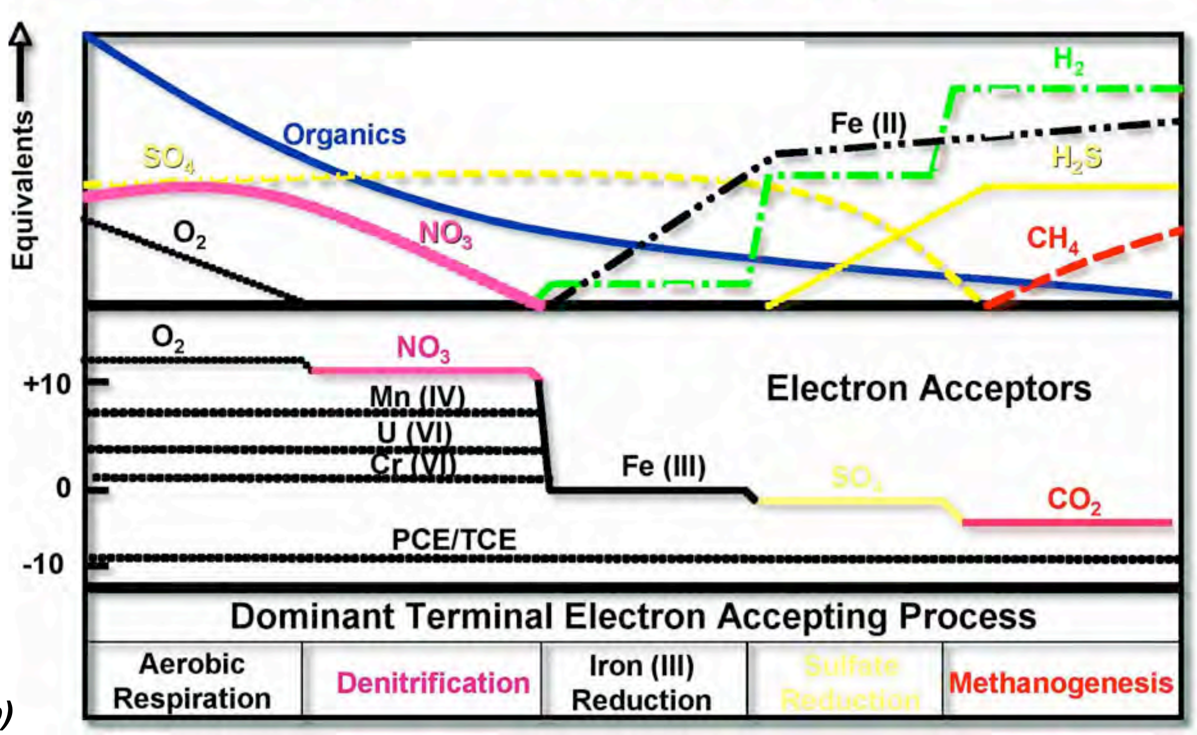
Depolymerizing & Fermenting Populations

Syntrophic Populations

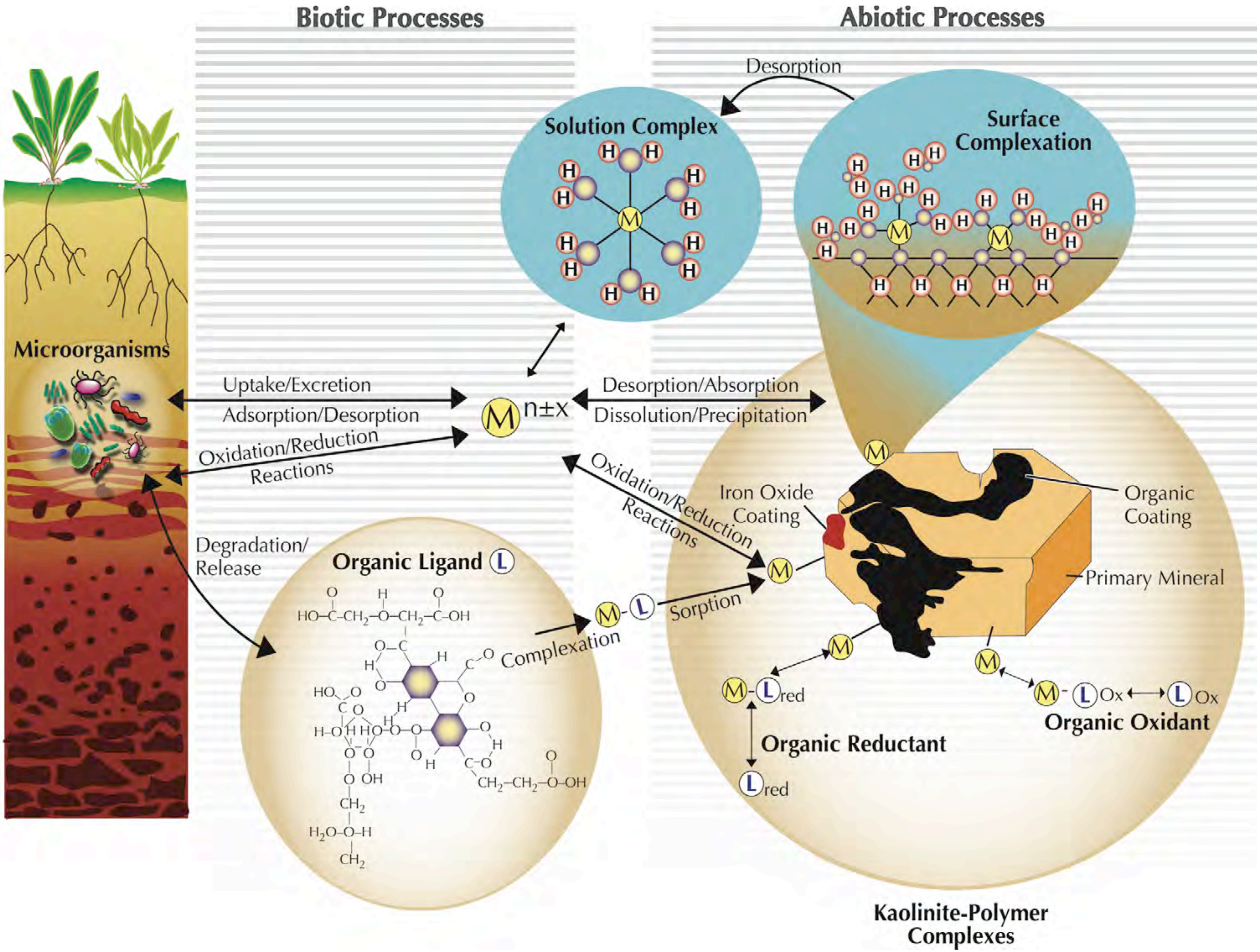
Methanogenic Populations

Organic Monomers & Polymers (Cellulose) 1 → 2 → Volatile Fatty Acids & Acetate 3 → H_2, CO_2, Acetate 4 → CH_4

4 ↓ Populations using electron acceptors other than CO_2 ↓ 4



(Hazen and Stahl, in prep)


Biotic Processes
Abiotic Processes
Desorption
Solution Complex
Surface Complexation
Microorganisms
Uptake/Excretion
Adsorption/Desorption
Oxidation/Reduction
Reactions
M n±x
Desorption/Absorption
Dissolution/Precipitation
Oxidation/Reduction
Reactions
Iron Oxide
Coating
Organic
Coating
Primary Mineral
Degradation/
Release
Organic Ligand L
Complexation
M-L
Sorption
M-L red
Organic Reductant
L red
M-L Ox
L Ox
Organic Oxidant
Kaolinite-Polymer
Complexes

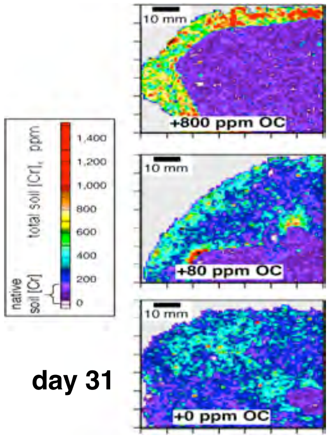
Mesoscale Studies on Cr(VI) Bioreduction Lab Studies

Jiamin Wan, Tetsu Tokunaga, Mary Firestone, Eoin Brodie and Terry Hazen (ERSP/NABIR supported 1998-2004)

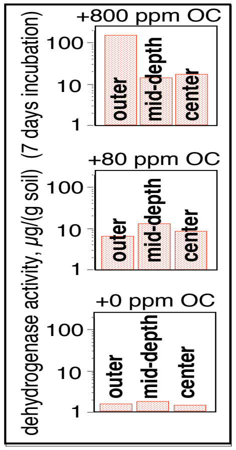
Tokunaga, T. K. J. Wan, M. K. Firestone, T. C. Hazen, K. R. Olson, D. J. Herman, S. R. Sutton, and A. Lanzirotti. 2003. *In-situ* reduction of Cr(VI) in heavily contaminated soils through organic carbon amendment. J. Environ. Qual. 32:1641-1649.

Tokunaga, T. K., J. Wan, T. C. Hazen, E. Schwartz, M. K. Firestone, S. R. Sutton, M. Newville, K. R. Olson, A. Lanzirotti, and W. Rao. 2003. Distribution of chromium contamination and microbial activity in soil aggregates. J. Environ. Qual. 32:541-549.

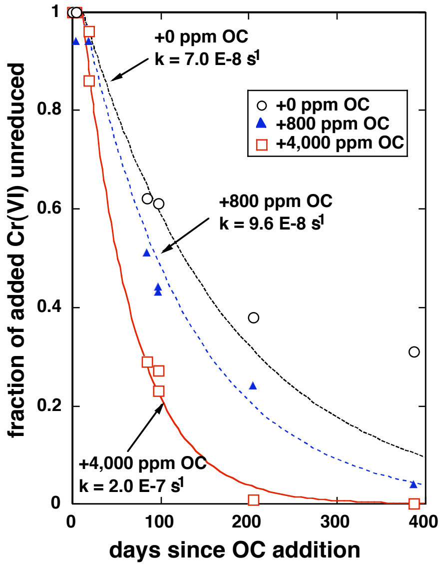
Tokunaga, T. K., J. Wan, M. K. Firestone, T. C. Hazen, E. Schwartz, S. R. Sutton, and M. Newville. 2001. Chromium diffusion and reduction in soil aggregates. Environmental Science & Technology 35:3169-3174.



day 31



initially 1,000 ppm Cr(VI) in pore waters

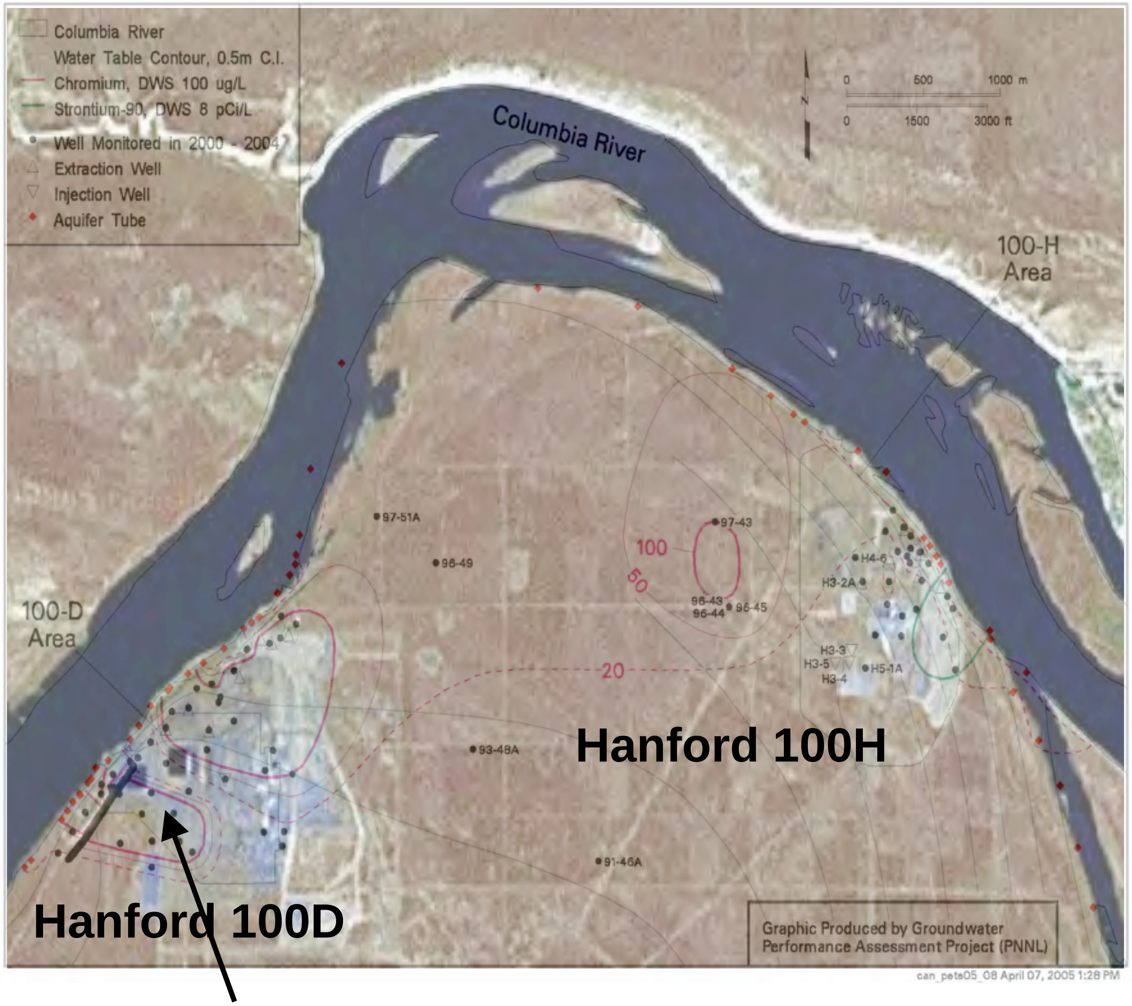


Fe(III) + Microbial reduction → **Fe(II)**

Fe(II) + **Cr(VI)** → **Cr(III) Precipitation**

Hanford 100H Site Characterization

Cr Concentration Map

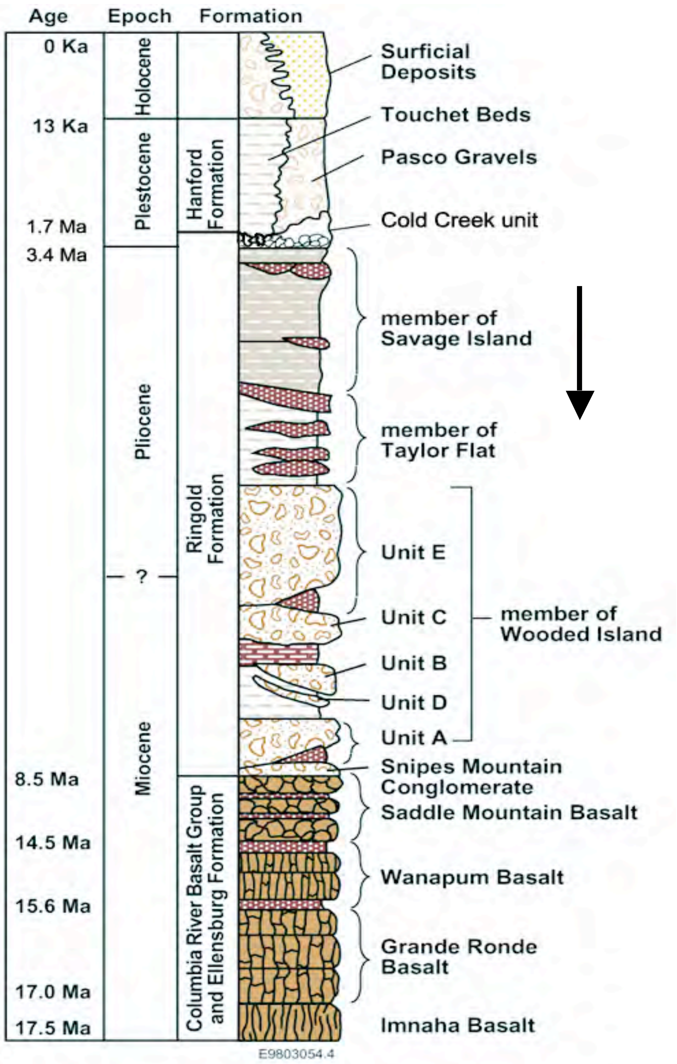


Hanford 100H

Hanford 100D

The Cr source is believed to be sodium dichromate ($Na_2Cr_2O_7.2H_2O$)

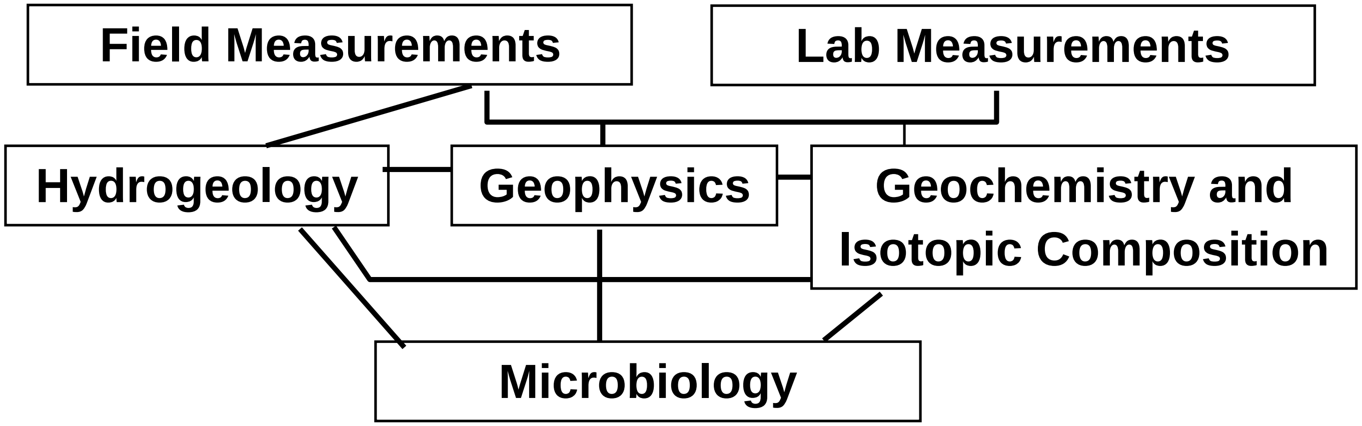
Lithological Column

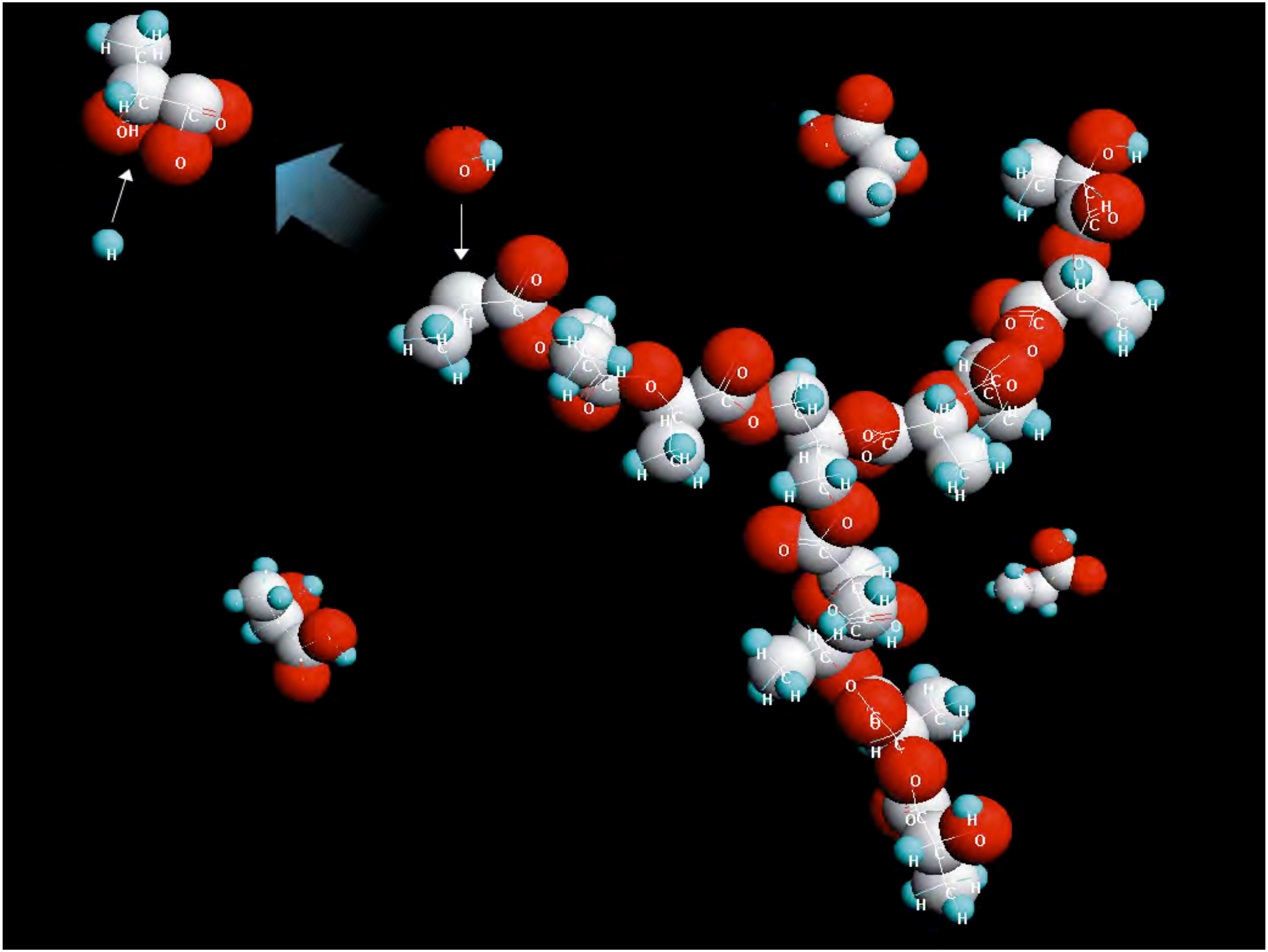


Overall Objective

To carry out field investigations to assess the potential for immobilizing Cr(VI) in groundwater using lactate-stimulated bioreduction of Cr(VI) to Cr(III) at the Hanford 100H site, and to determine critical community structure changes and stressors that would enable control and predictions of fundamental biogeochemistry that enables this bioremediation strategy for Cr(VI)

Integrated Approach

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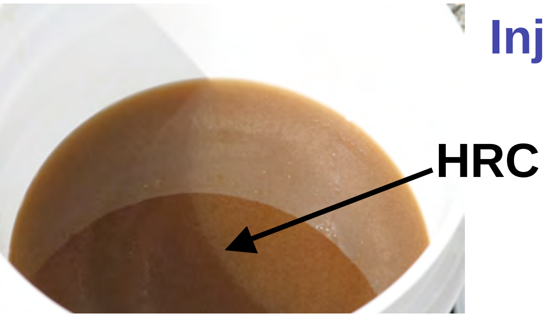
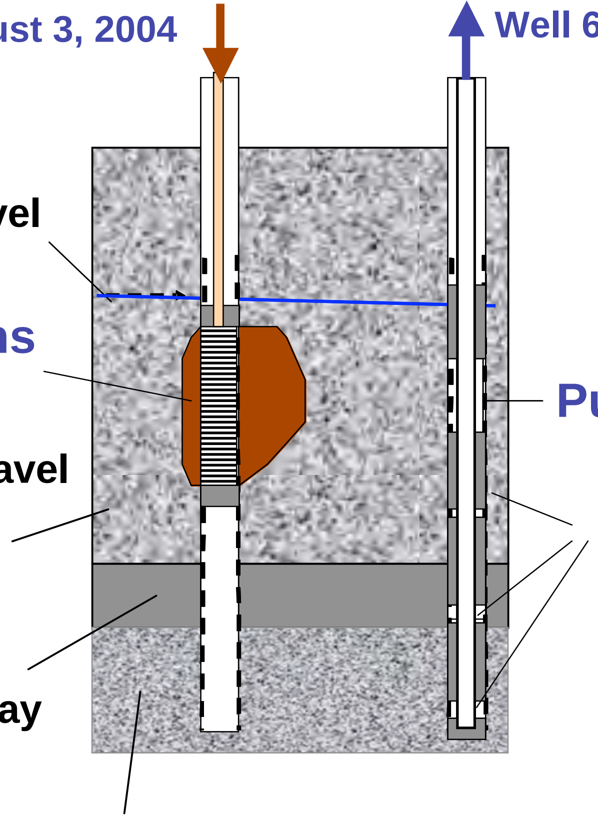
Field HRC Injection Test





Injection of 40 lbs of ^{13}C-labeled HRC

Well 699-96-45, August 3, 2004

Pumping - 27 days

Well 699-96-44

Groundwater level

Injection at depths of 44 ft to 50 ft

Hanford sandy gravel and gravelly sand

Ringold clay

Ringold silt

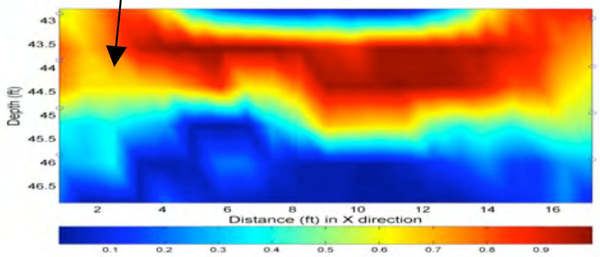
Pumping

Water samplers

Post-HRC Injection Changes in Electrical Conductivity

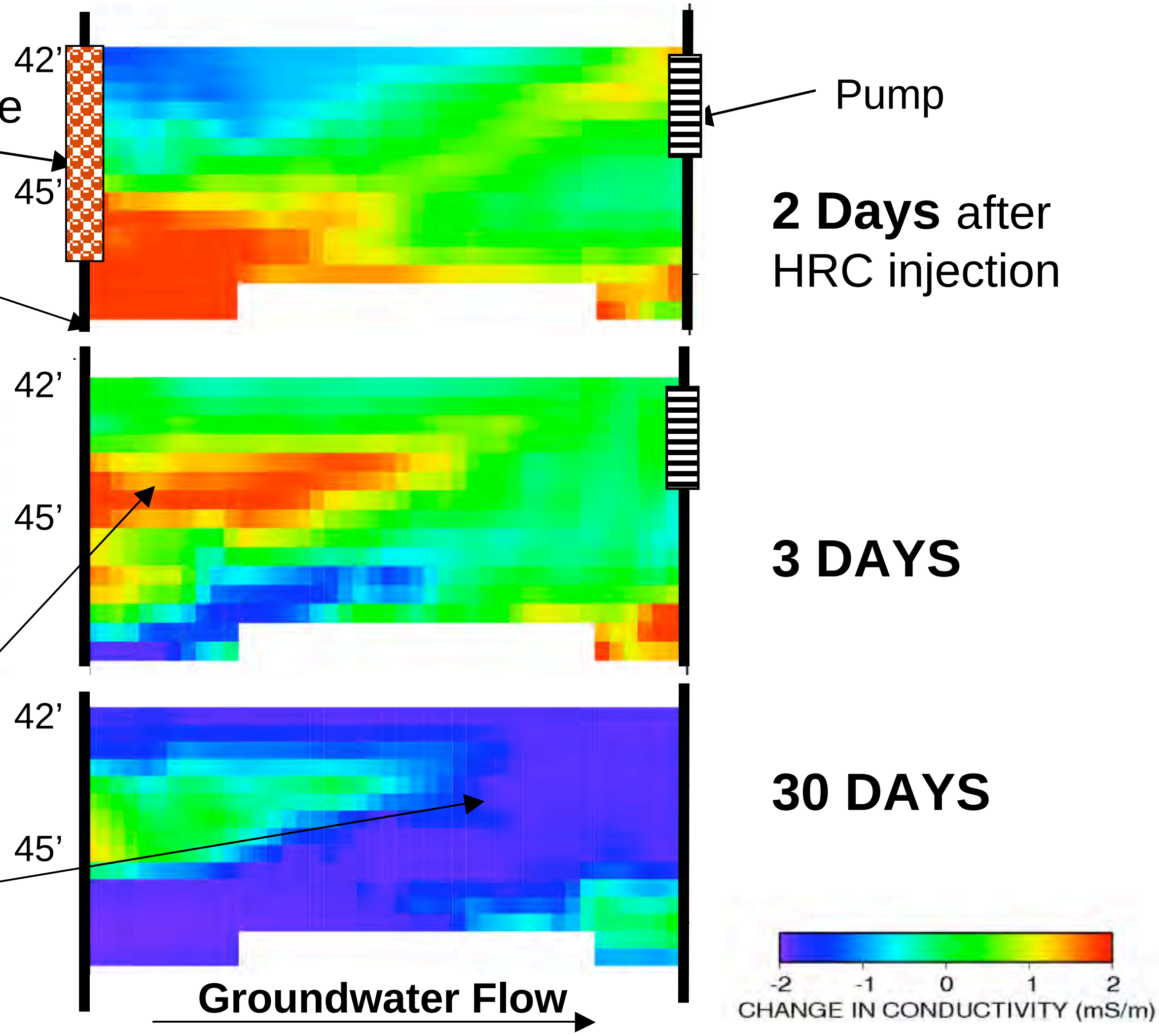
HRC Injection Zone

High K_{sat}



Hypothesis: Lactic acid

Hypothesis: Reaction halo due to formation of precipitates



Pump

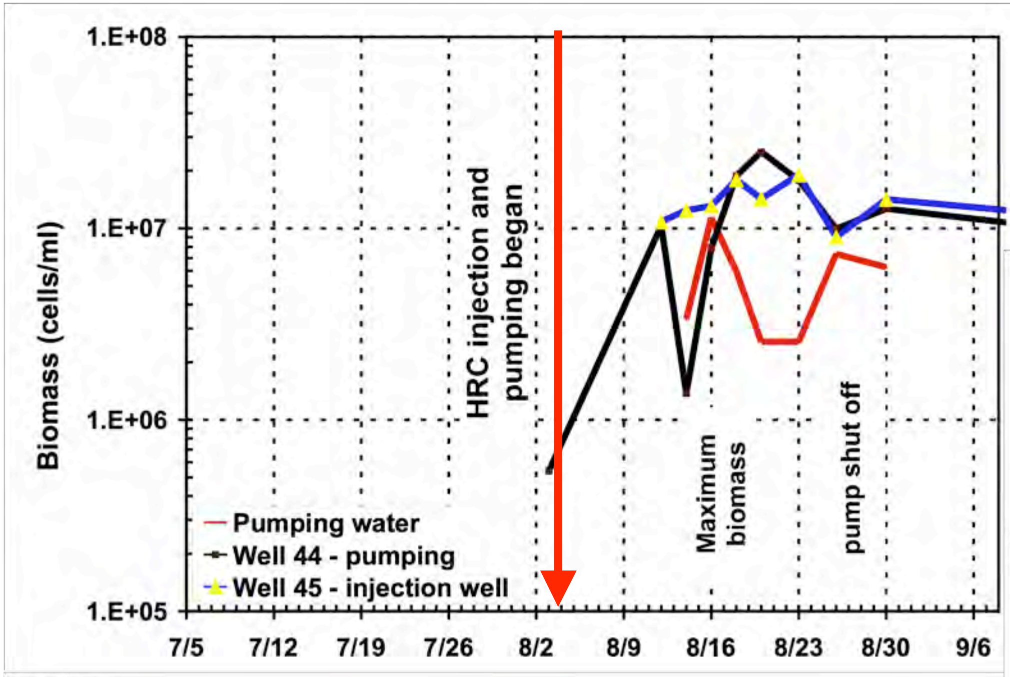
2 Days after HRC injection

3 DAYS

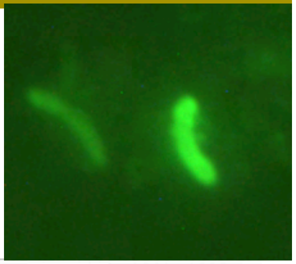
30 DAYS

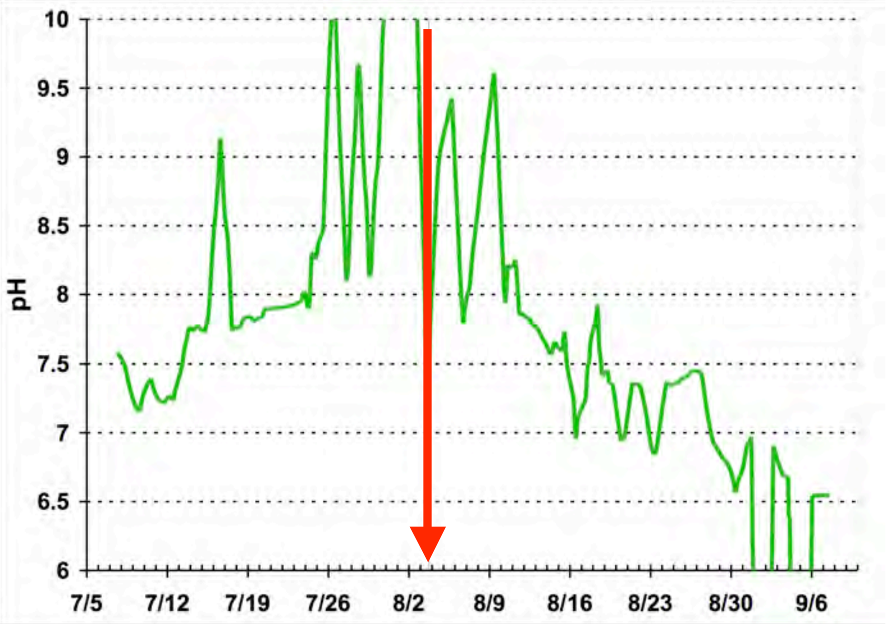
Groundwater Flow

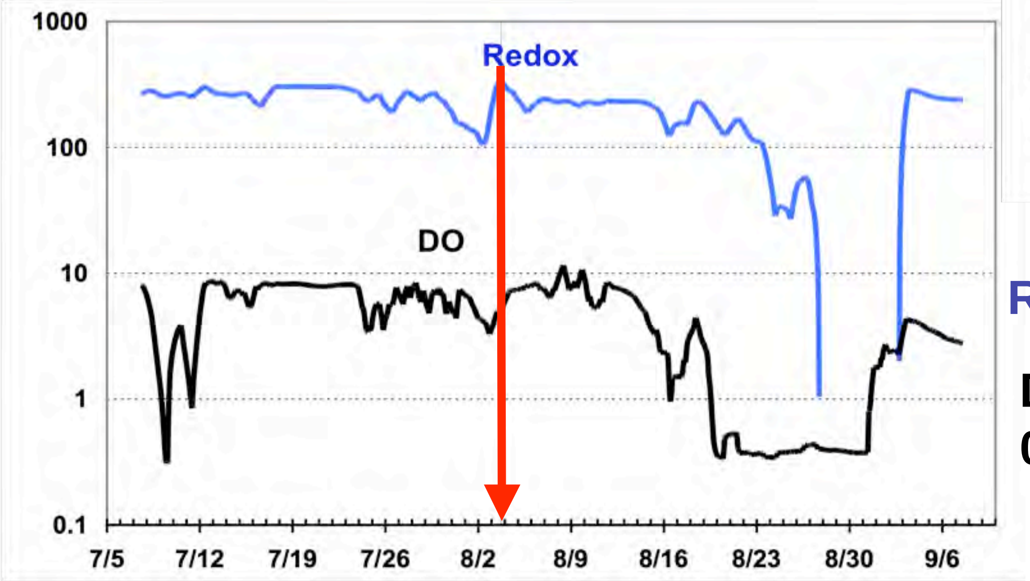
Results of HRC Biostimulation



D. vulgaris **(direct fluorescent antibody)**





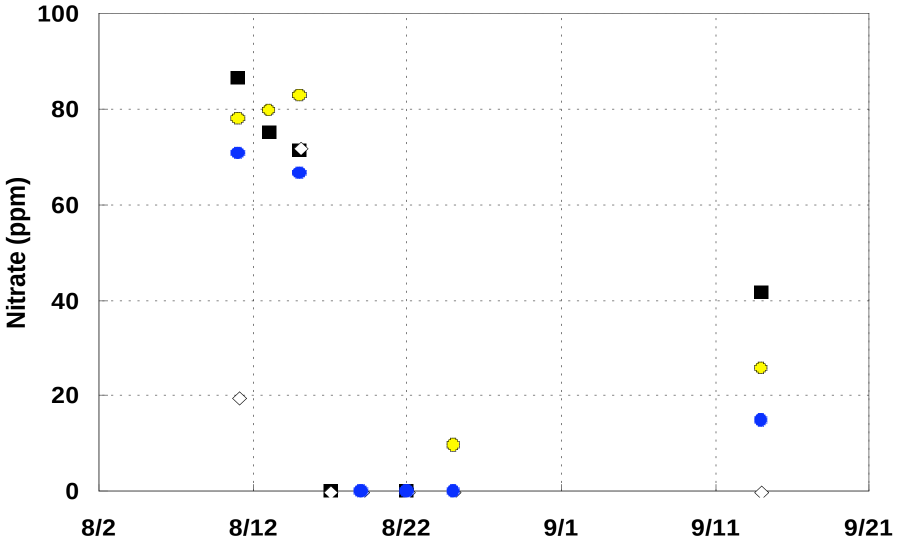


Redox dropped from 240 to -130 mV

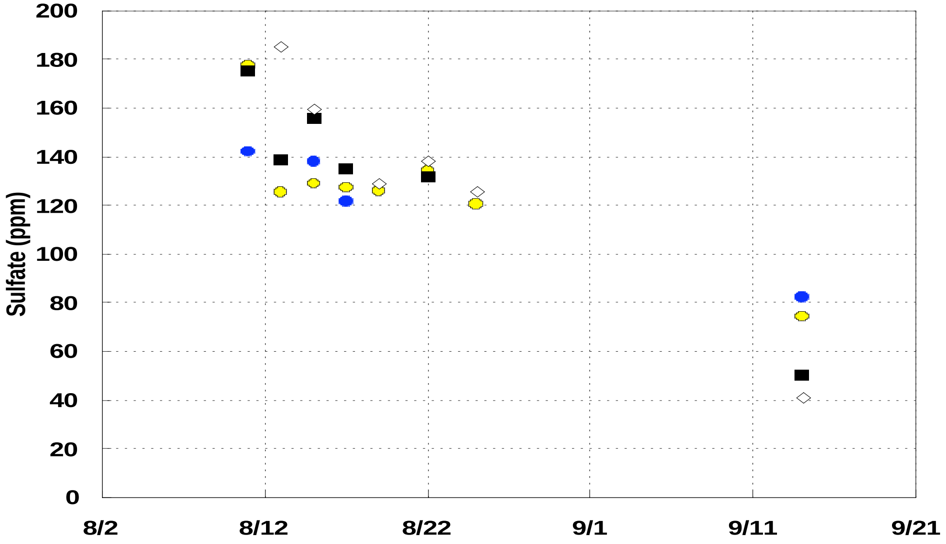
DO dropped from 9 mg/l (~100%) to 0.35 mg/l (4.5%)

Biogeochemical Evidence of Microbial Metabolism in Groundwater

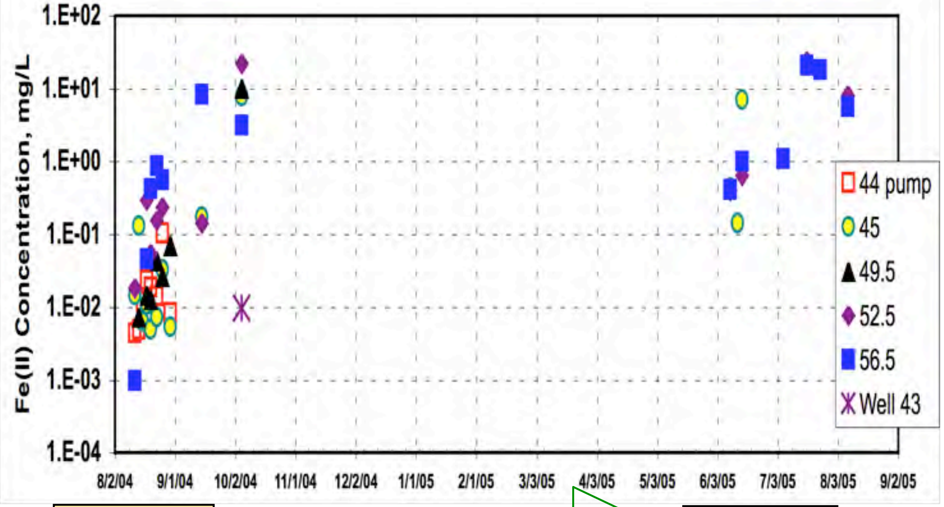
Denitrification



Sulfate reduction

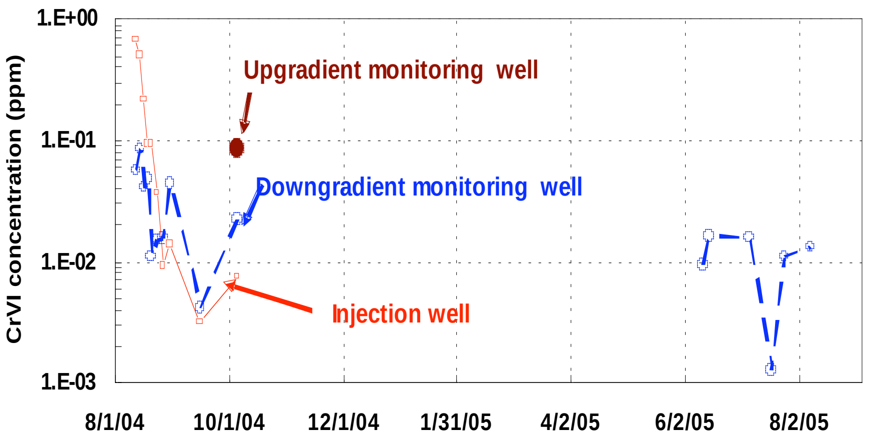


Iron Reduction



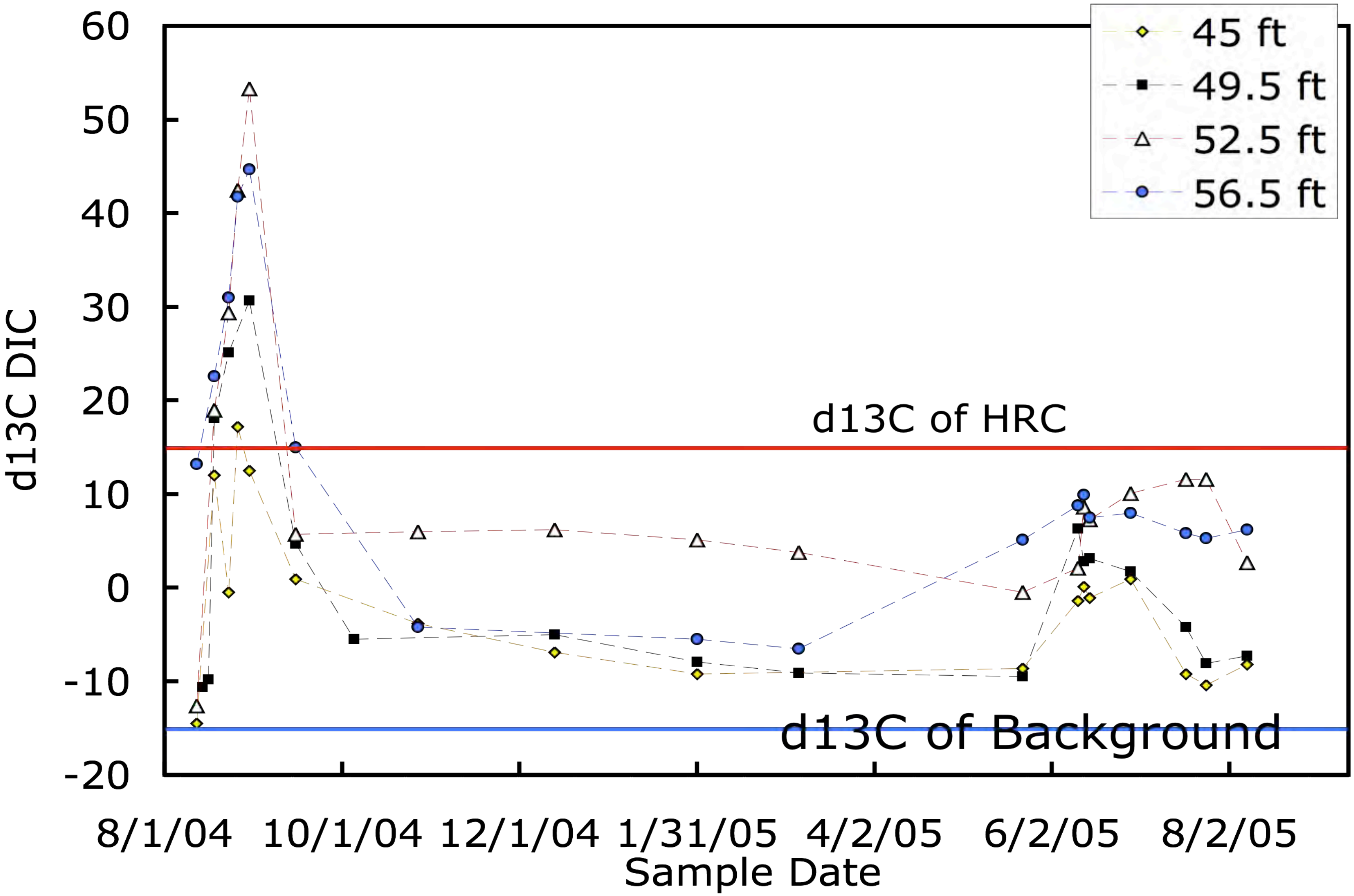
Fe(III) + Microbial reduction → **Fe(II)**

Average Soluble Cr(VI) Concentration



Fe(II) + Cr(VI) → **Cr(III) Precipitation**

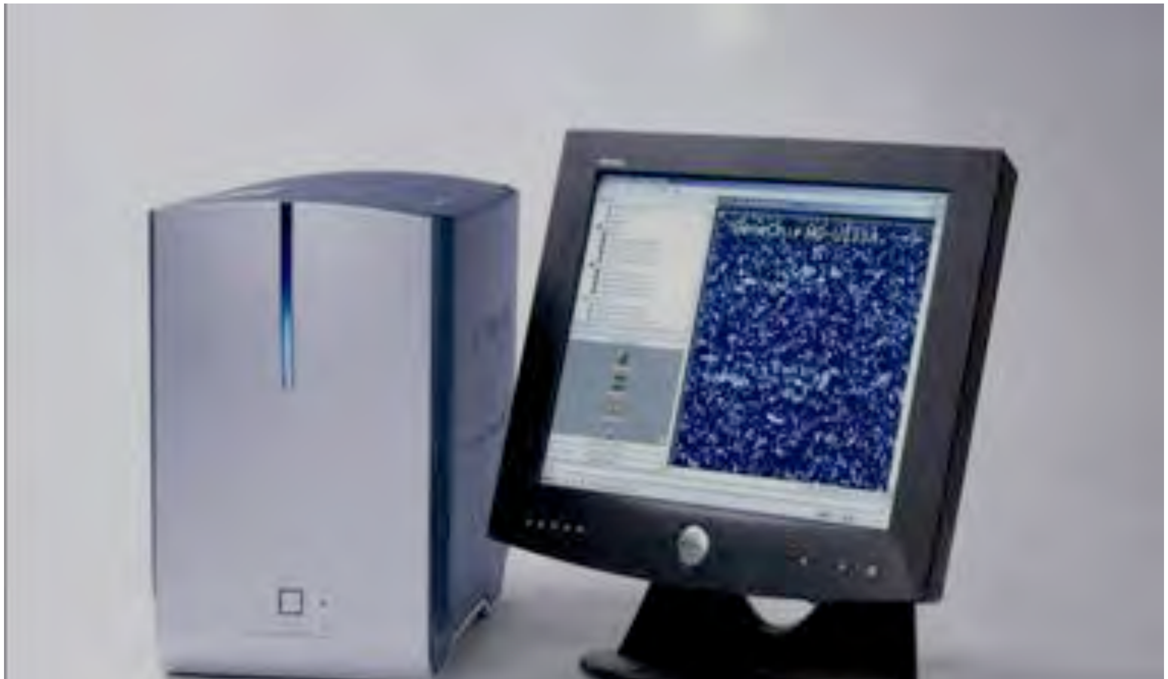
Biogeochemical Evidence of Microbial Metabolism in Groundwater

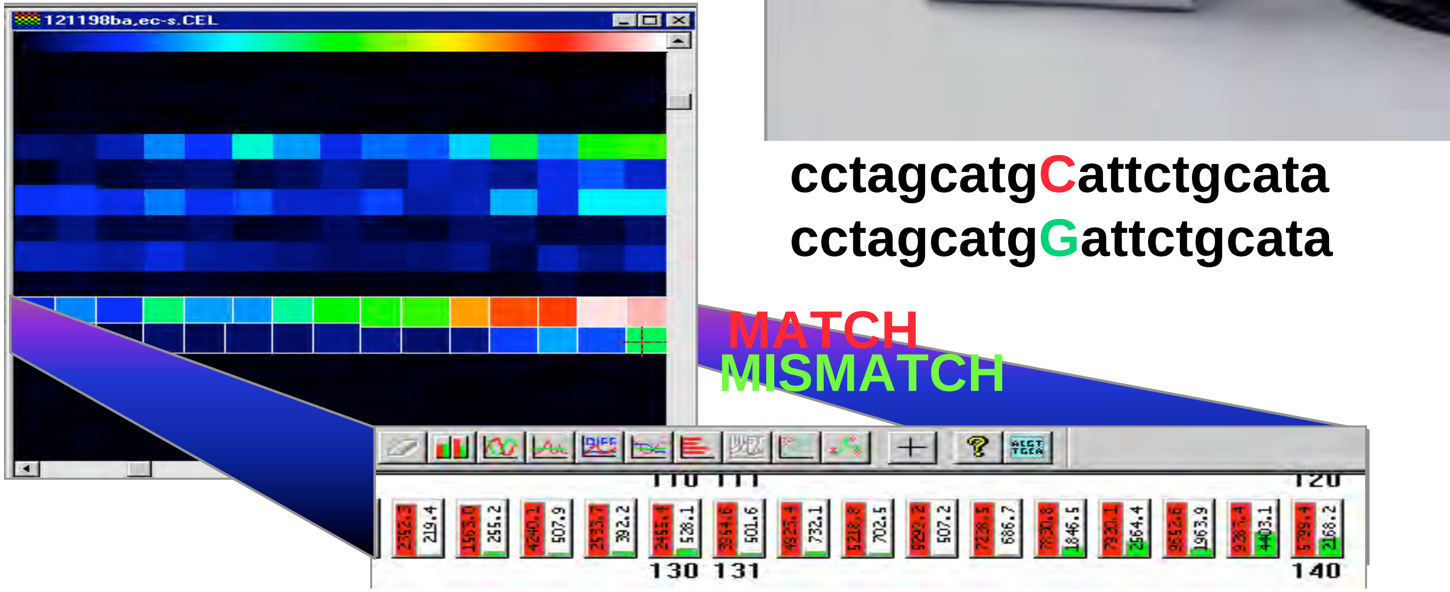


d^{13}C of Dissolved Inorganic Carbon is Byproduct of HRC Metabolism

DOE 16s rDNA microarray

- Rapidly detect the composition and diversity of microbes in an environmental sample
- Massive parallelism - 550,000 probes in a 1.28 cm^2 array
- all 9,900 species in 16S rDNA database
- Single nucleotide mismatch resolution



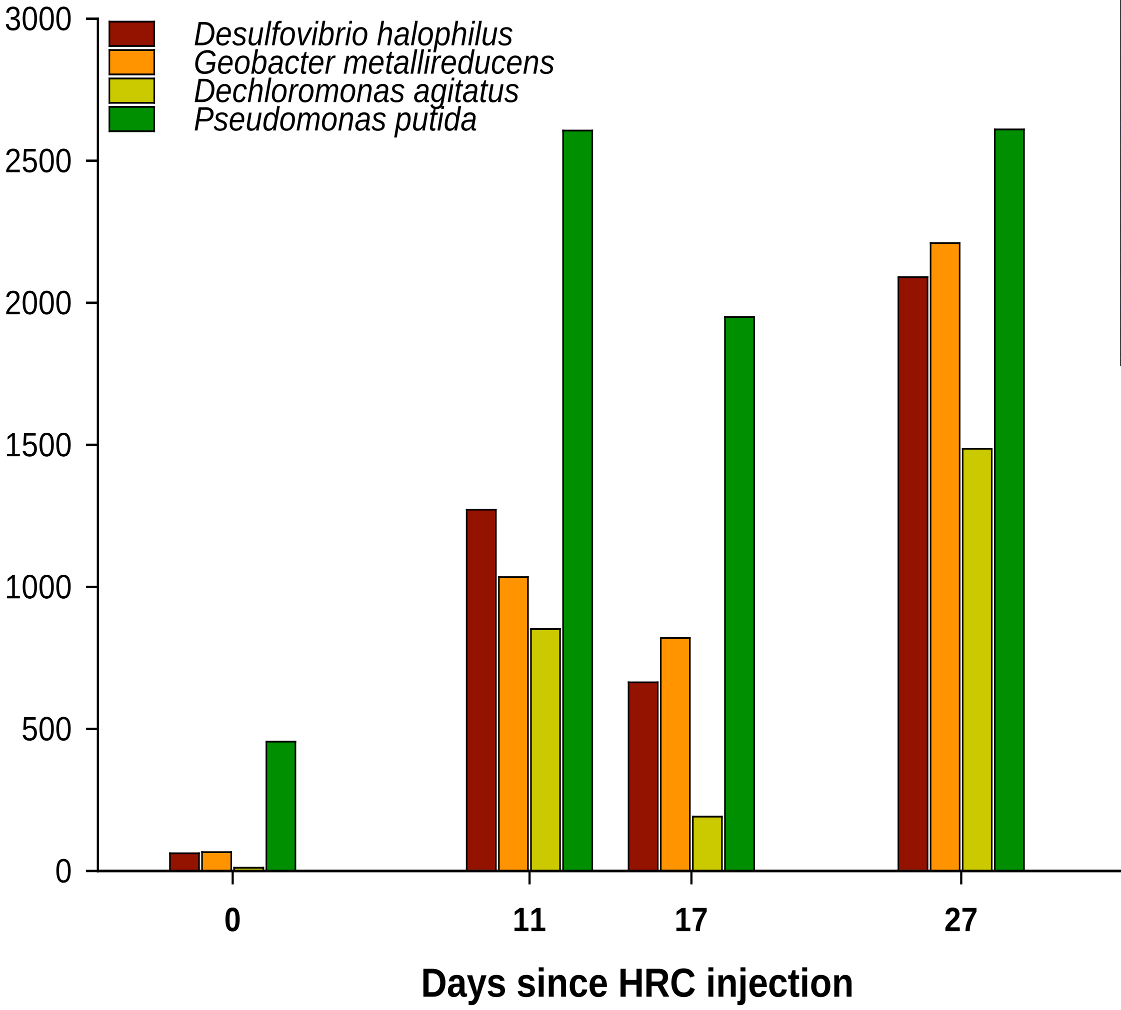


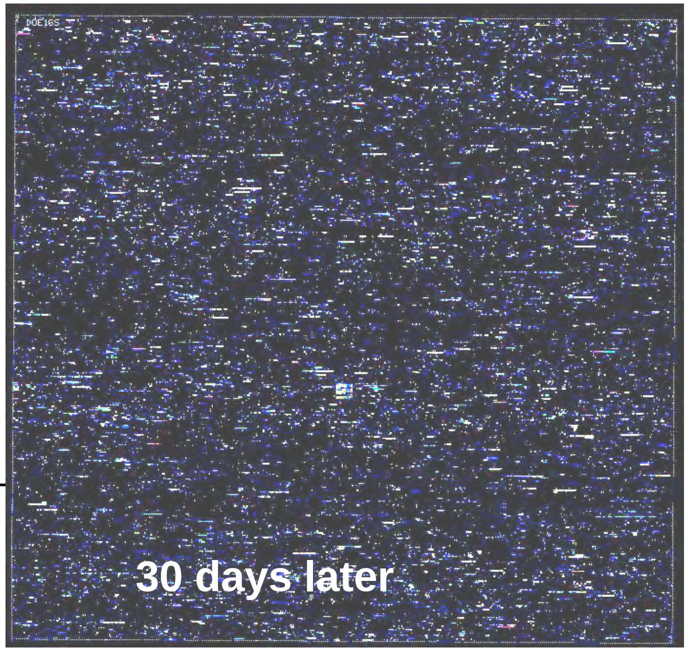
cctagcatgCattctgcata
cctagcatgGattctgcata

MATCH
MISMATCH

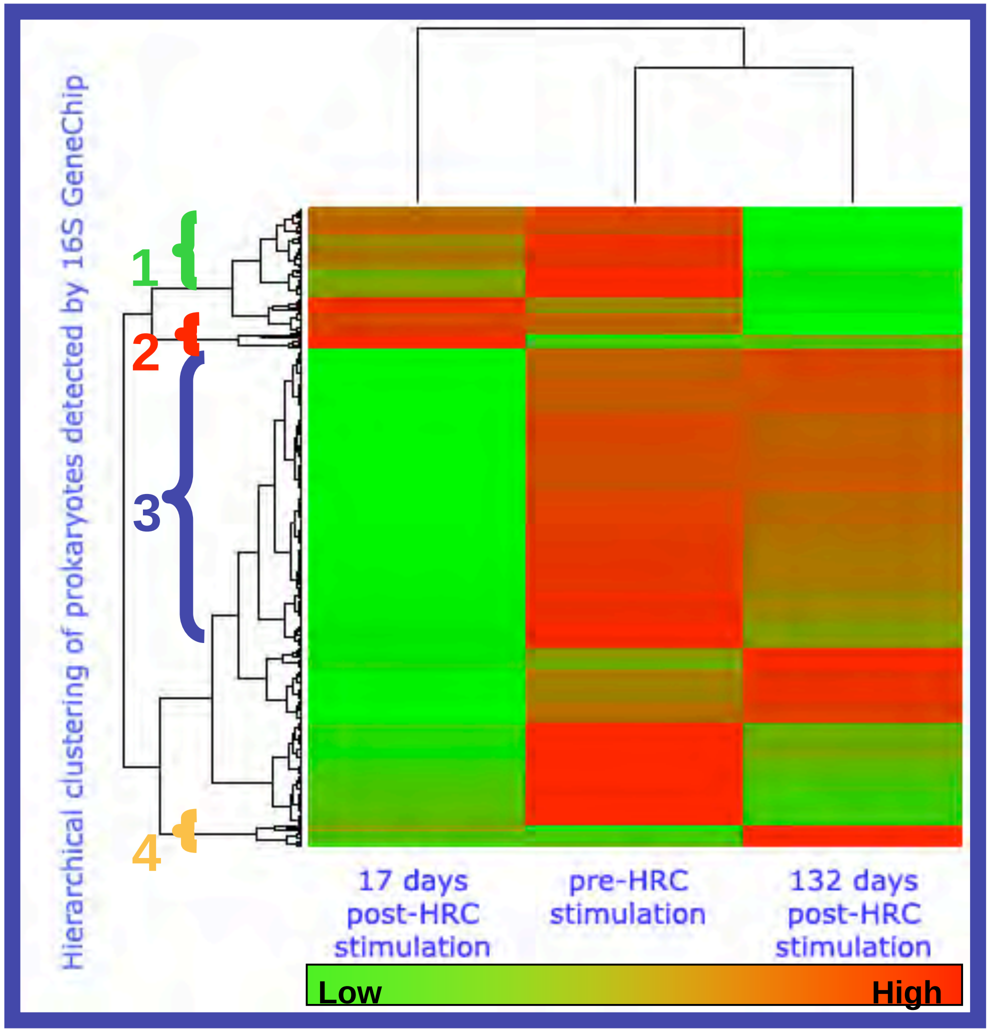
Microarray analysis of bacterial community changes during Cr(VI) remediation at Hanford 100H site:

Dynamics of some significant organisms.





High Density Microarray Analysis



Hierarchical clustering and heatmap plot of 16S GeneChip analysis of microbial community sub-families detected during chromate bioremediation. PCA groups are indicated by brackets.

Bacteria and Archaea Detected

Grouped according to response to HRC during chromate remediation

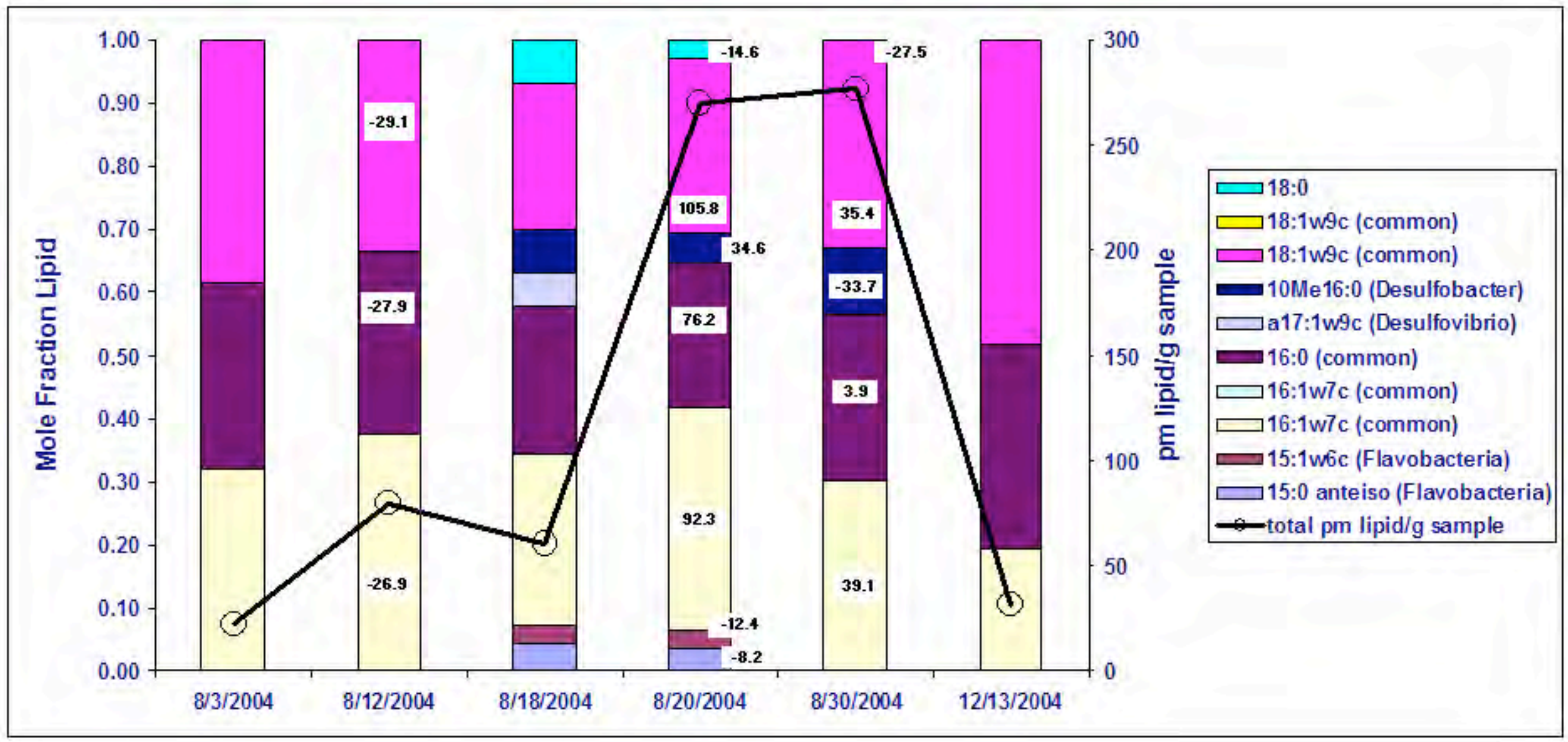
Group1 organisms decline
Pseudomonas, Burkholderia (Denitrifiers)
Acidithiobacillus, Thiothrix (Sulfur oxidizers)
Leptothrix (Iron oxidizer)

Group2 organisms increase then decline
Acidovorax, Thauera (denitrifiers)
Flavobacteria (aerobes, use glycerol)

Group3 organisms decline then return
Mainly oligotrophic bacteria

Group 4 organisms increase in late stages
Legionella, Chlamydophila, Flectobacillus.

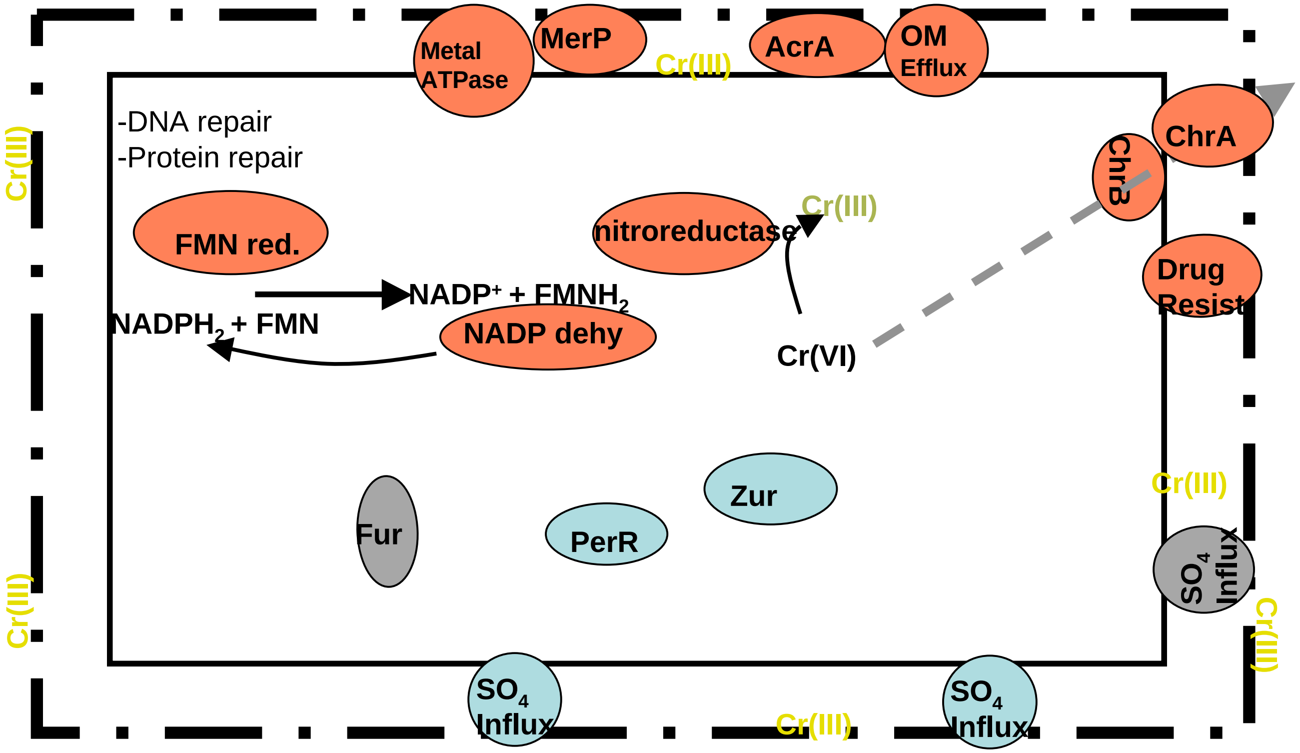
^{13}C Phospholipid Analysis



- General bacterial biomarkers indicate rapid enrichment in ^{13}C
- ^{13}C ratio is greater than expected (overall spiked HRC ratio was 15 per mil)
  - ^{13}C polylactate used as spike it is not esterified to glycerol backbone
  - it is released and consumed more rapidly
- Biomarkers for *Flavobacteriaceae* increased following injection but showed minimal enrichment with ^{13}C.
  - *Flavobacteria* do NOT typically utilize lactate, but may use glycerol (backbone, unlabeled)

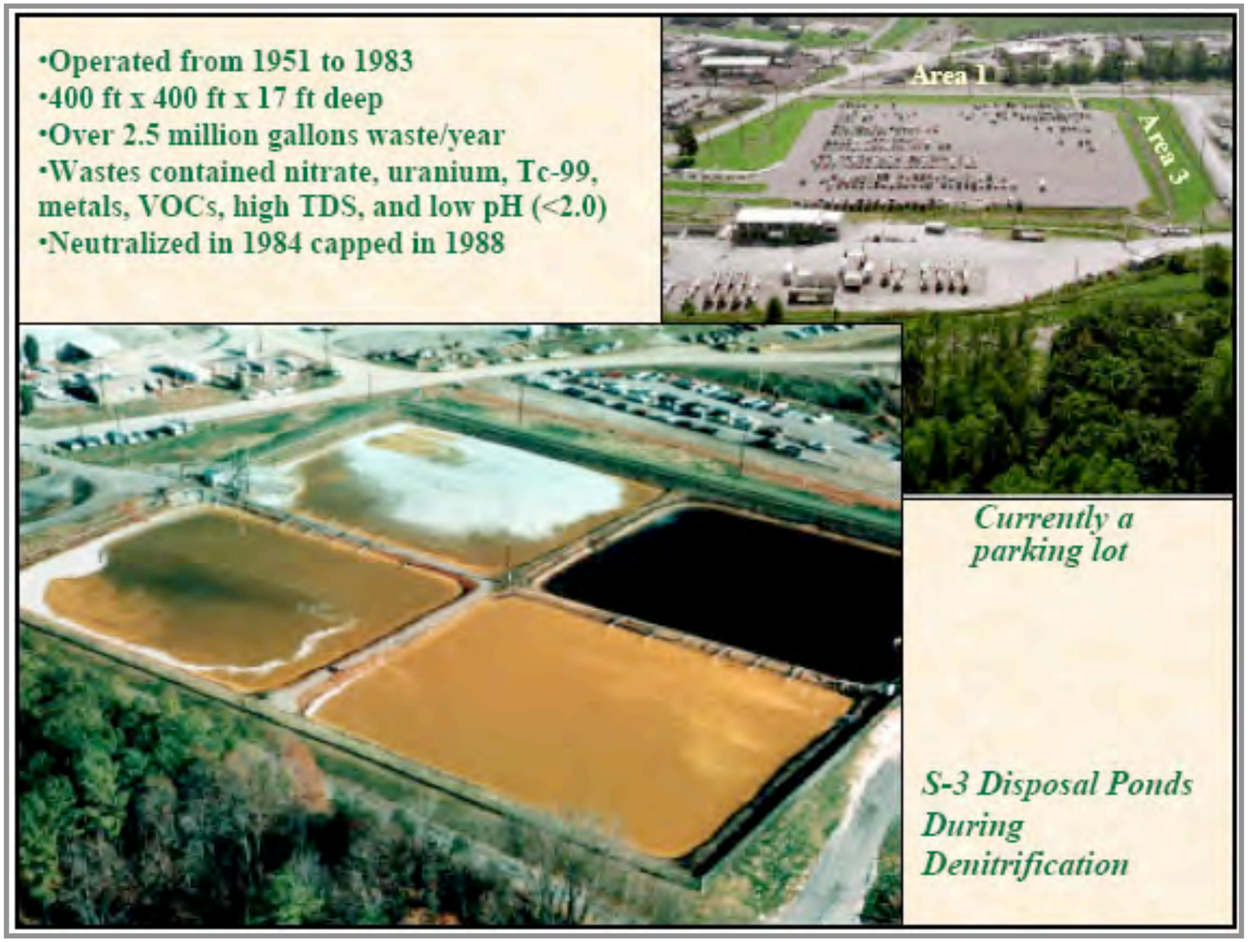
What Cellular Systems are Involved in Cr(VI) Responses in *Desulfovibrio vulgaris* Hildenborough?

- Sulfate influx down-expressed
- Metal efflux up-expressed
- *chr*AB up-expressed
- FMN dependent nitroreductase, NADH dehydrogenase, and FMN reductase up-expressed



Klonowska, A., He*, Z., He, Q., Hazen*, T.C., Thieman, S.B., Alm*, E.J., Arkin*, A.P., Wall*,J.D., Zhou*, J. and Fields*, M.W. Global Transcriptomic Analysis of Chromium(VI) Exposure of *Desulfovibrio vulgaris* Hildenborough Under Sulfate-Reducing Conditions. (in preparation)

Field Research Center, Oak Ridge TN



- Operated from 1951 to 1983
- 400 ft x 400 ft x 17 ft deep
- Over 2.5 million gallons waste/year
- Wastes contained nitrate, uranium, Tc-99, metals, VOCs, high TDS, and low pH (<2.0)
- Neutralized in 1984 capped in 1988

Currently a parking lot

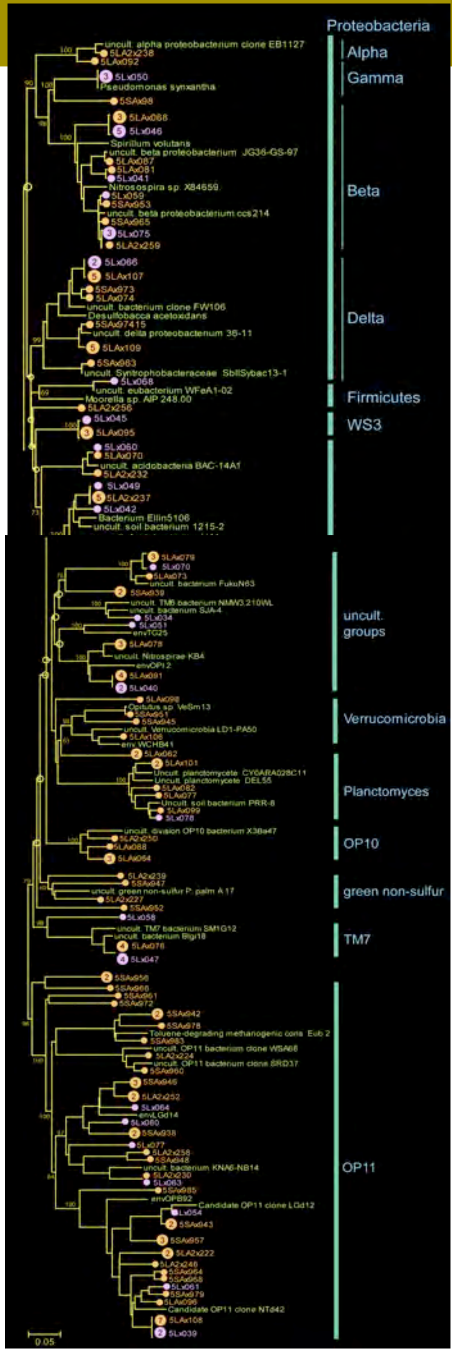
S-3 Disposal Ponds During Denitrification

S-3 Waste Disposal Ponds

- Unlined
- Received liquid nitric acid and uranium-bearing wastes

~320 million liters 1951-1983

Environmental Whole-Genome Amplification To Access Microbial Populations in Contaminated Sediments



- Recovery of adequate amounts of DNA for molecular analyses can often be challenging in stressed microbial environments.
- Developed multiple displacement amplification (MDA) methods for unbiased, isothermal, amplification of DNA
- Subsequently applied these technologies to understand stressed, low biomass, populations in multiple sediments contaminated with Uranium on the Oak Ridge Reservation
- Over 4000 clones were end sequenced. 5% of all clones were identified as belonging to Deltaproteobacteria (primarily, Geobacter and Desulfovibrio-like)
- Significant overabundance of proteins (COGs) associated with: 1) Carbohydrate transport & metabol. 2) Energy production & conversion, 3) Postranslational modification, protein turnover, & chaperones. --- All of which may be important in adaptation to environmental stressors such as low pH, high contaminate loads, and oligotrophic nature of the subsurface environment

Statistics on amplified metagenome library end-sequences

Library	Area 3, Deep	%	Area 3, Shallow	%	Area 2	%	Total	%
Number of clones sequenced	960		864		864			
Sequences generated	1,920		1,728		1,728			
Quality sequences [a]	1,394	100	1,118	100	1,509	100	4,021	100
Sequences that form contigs	370	26.5	152	13.6	141	9.3	663	16.5
Number of contigs assembled	101		53		54		208	
Sequences with similarities to known proteins [b]	928	66.6	692	61.9	990	65.6	2,610	64.9
Highest similarity to bacterial proteins	901	64.6	629	56.3	890	59.0	2,420	60.2
Highest similarity to Deltaproteobacteria proteins	35	2.5	23	2.1	155	10.3	213	5.3
Highest similarity to archaeal proteins	12	0.9	43	3.8	79	5.2	134	3.3
Highest similarity to eukaryotic proteins	12	0.9	18	1.6	21	1.4	51	1.3

a. Sequences >400nt in length

b. e-values <1e-10 from BLASTX searches against the NCBI protein database

Abulencia, C.B., Wyborski, D.L., Garcia, J., Podar, M., Chen, W., Chang, S. H., Chang, H.W., Watson, D., Brodie, E.L., Hazen, T.C. and Keller, M. (2006) Environmental Whole-Genome Amplification to Access Microbial Populations in Contaminated Sediments. *Appl. Environ. Microbiol*. **72**(5):3291-3301 [download pdf]

Metagenomic Analysis of NABIR FRC Groundwater Community

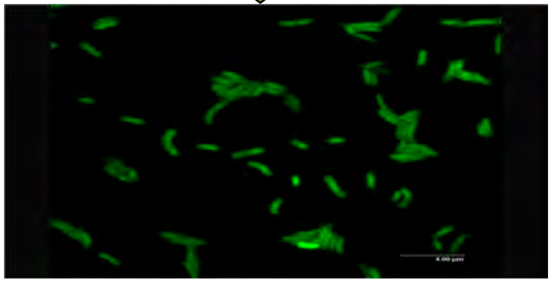
Data: Jizhong Zhou et al.

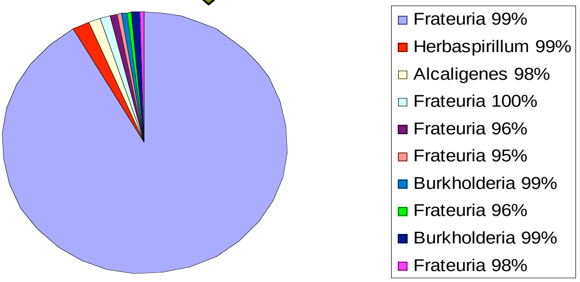
Metagenomic sequencing:

- Almost like a mono-culture
- 52.44 Mb raw data assembled into contigs totaling ~5.5 Mb
- 224 scaffolds (largest 2.4 Mb)
- Genes important to the survival and life style in such environment were found

Extremely low diversity:

- Dominated by *Frateuria*-like organism
- At least 2 *Frateuria* phylotypes
- Azoarcus species: less abundant

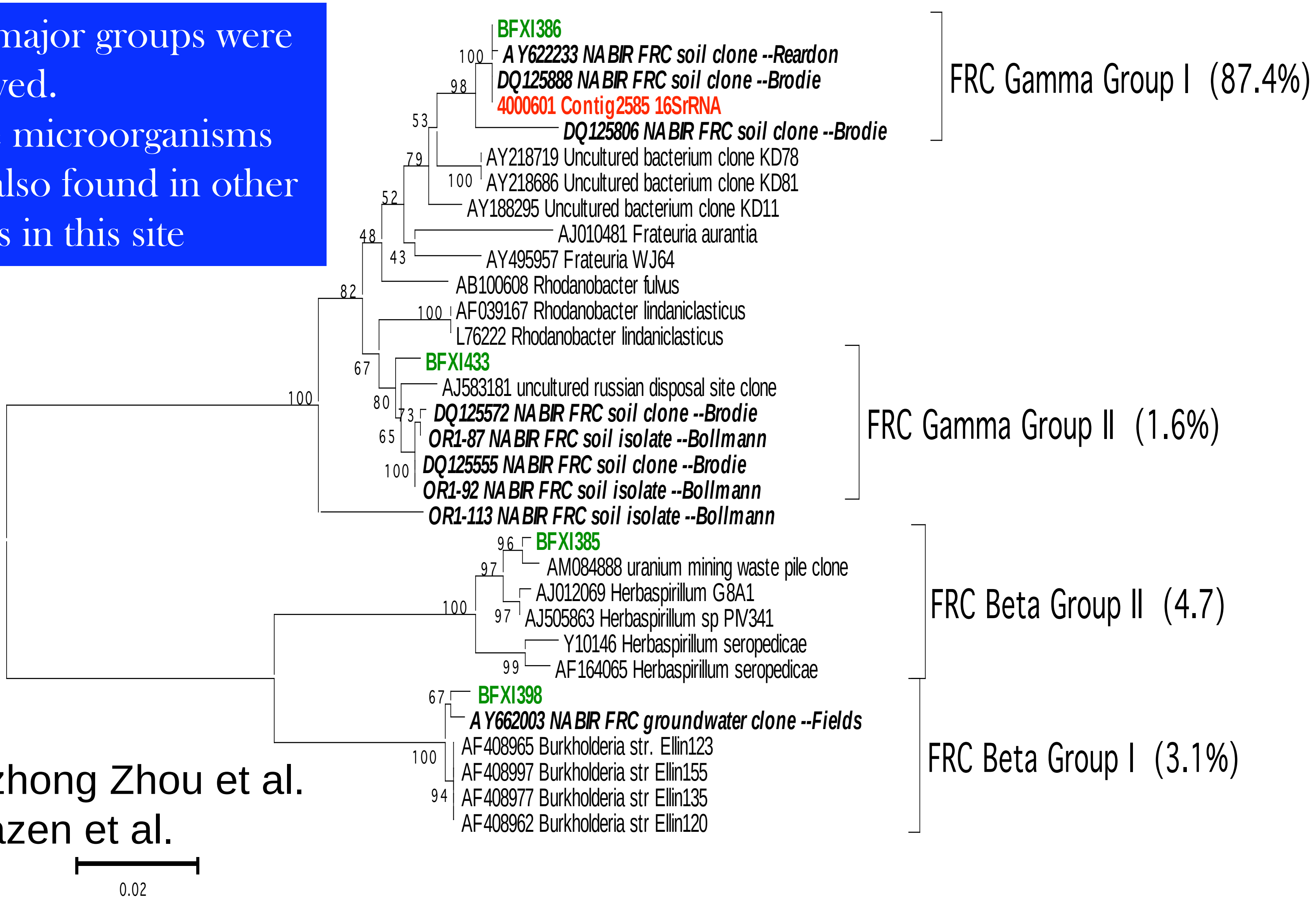




These results suggest that contaminants have dramatic effects on the groundwater microbial communities, and these populations are well adapted to such environments.

Phylogenetic Tree of SSU rRNA Genes

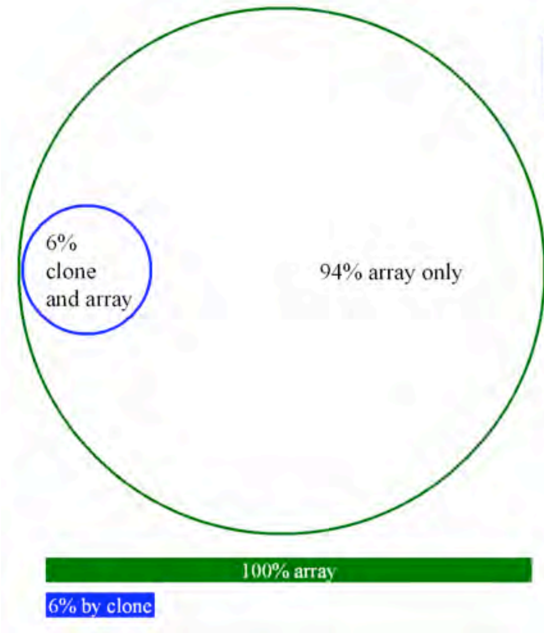
- Four major groups were observed.
- These microorganisms were also found in other studies in this site



Data: Jizhong Zhou et al.
Terry Hazen et al.

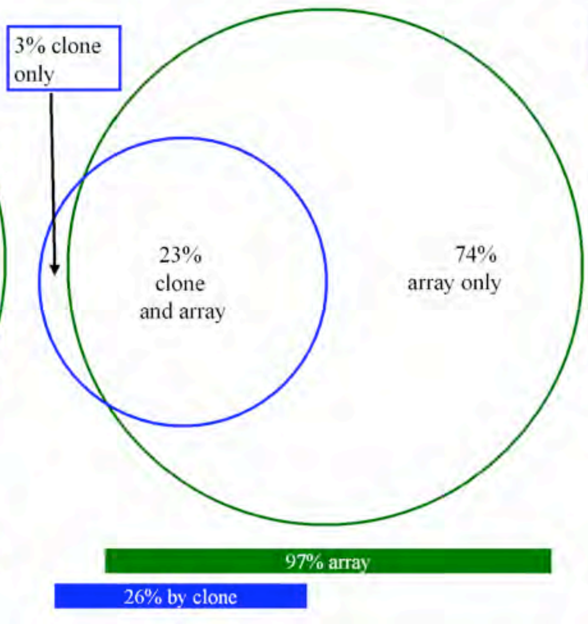
Accuracy V Clone libraries

Cr(VI) groundwater



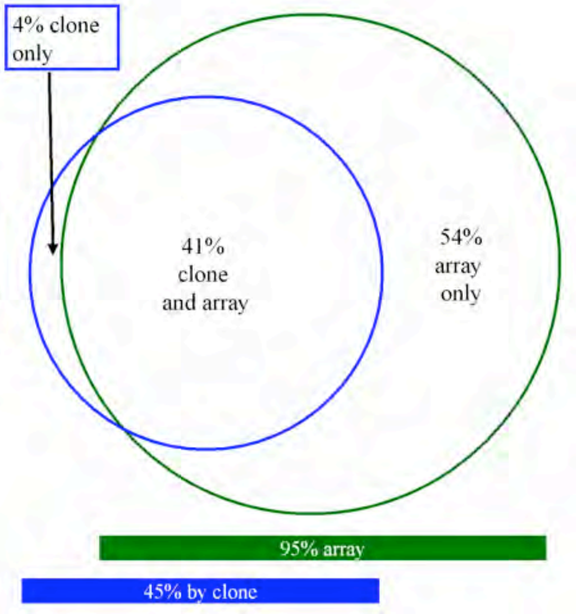
384 clones

U(VI) subsurface soil



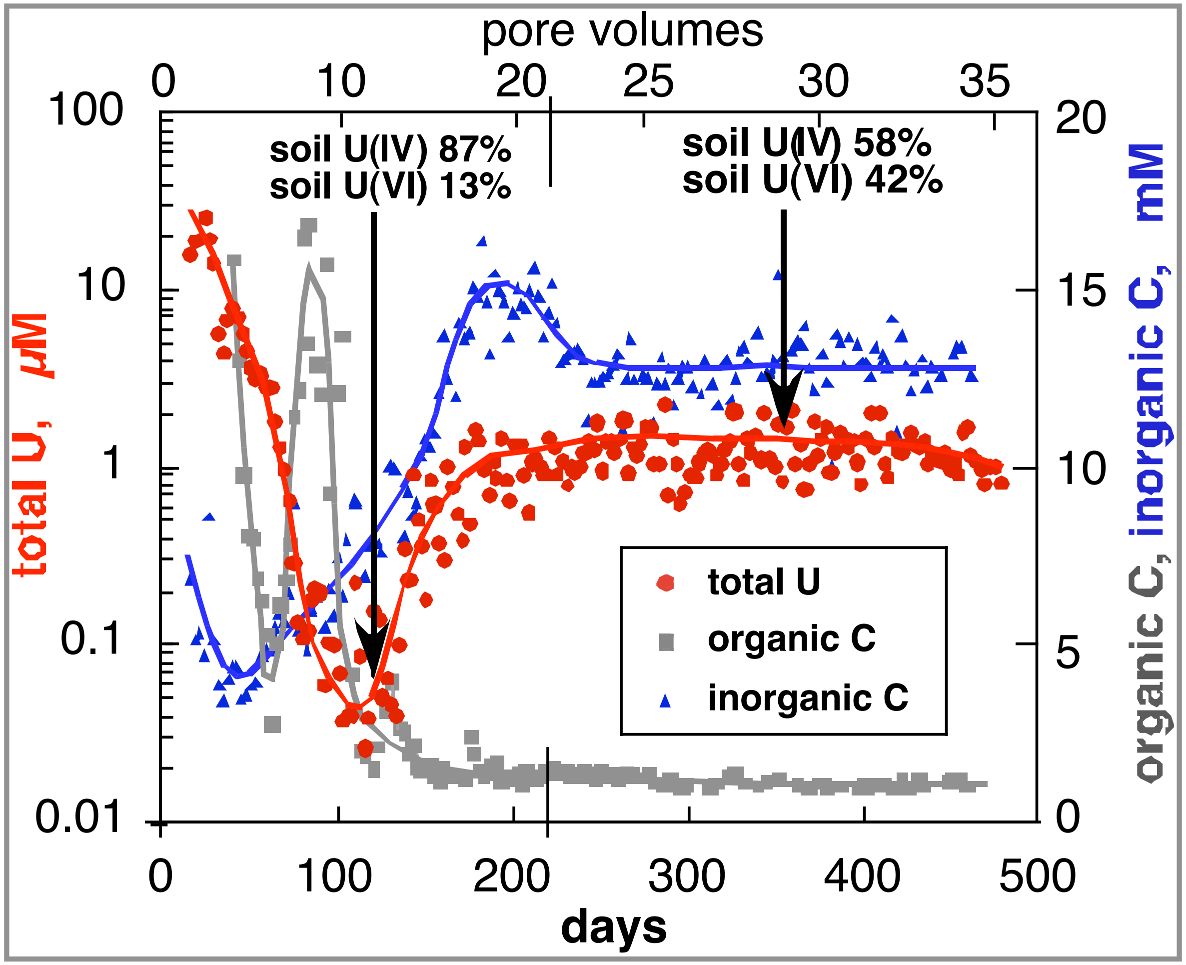
768 clones

Urban aerosol



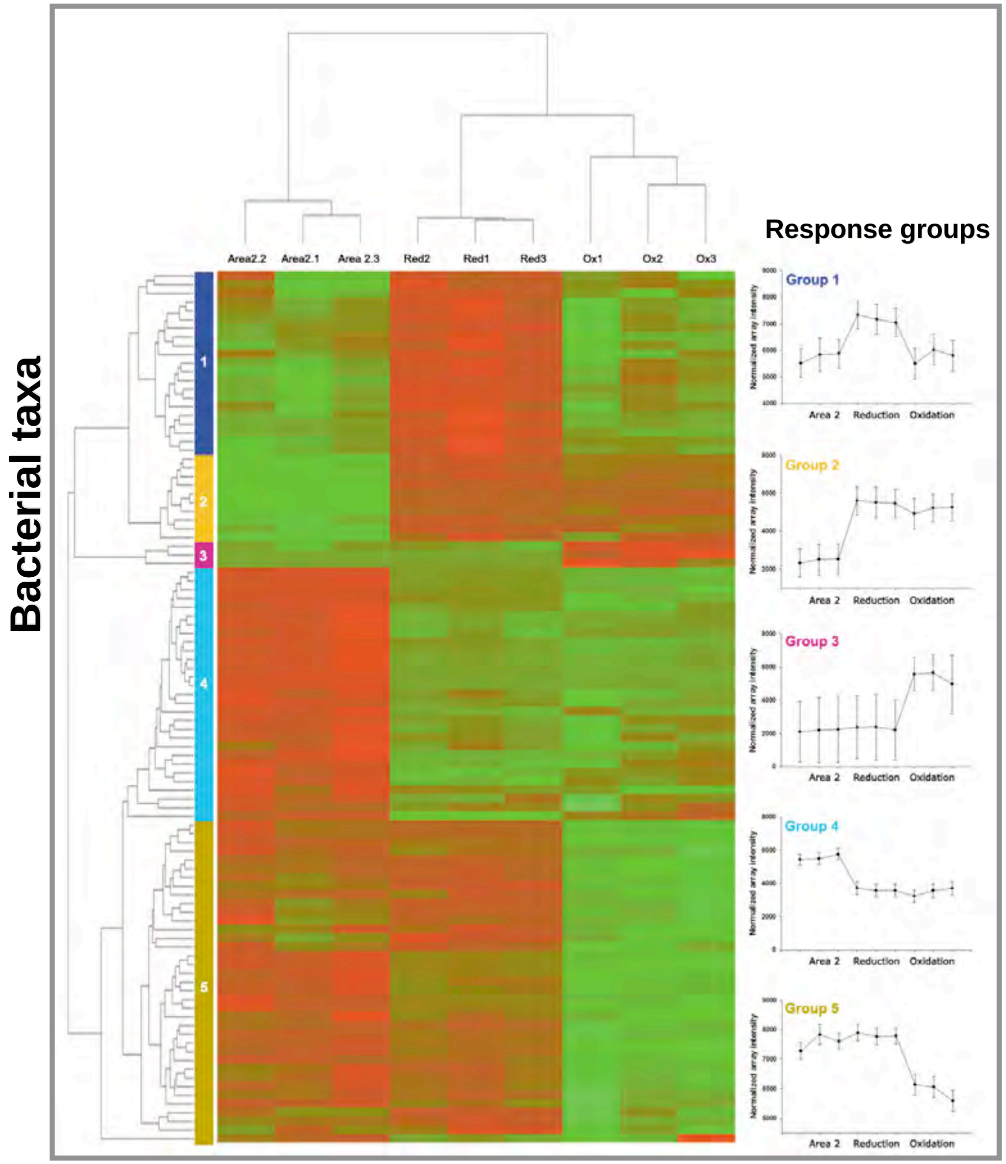
768 clones

Uranium Anaerobic Reoxidation



Microbial metabolism - CO_2 produced increasing dissolved IC

Bi-directional clustering of array data



Column sediment samples

Major components of response groups

- ***Arthrobacter sp.***
- **Alpha-proteobacteria**
- ***Azoarcus sp.***

- ***Geothrix***
- ***Geobacter***
- ***Anaeromyxobacter***

Uranium reducers

- ***Acidobacteria***
- ***Desulfovibrio***

Syntrophy?

- ***Actinobacteria***
- ***Firmicutes***
- ***Alpha-proteobacteria***

- ***Actinobacteria***
- ***Alpha-proteobacteria***

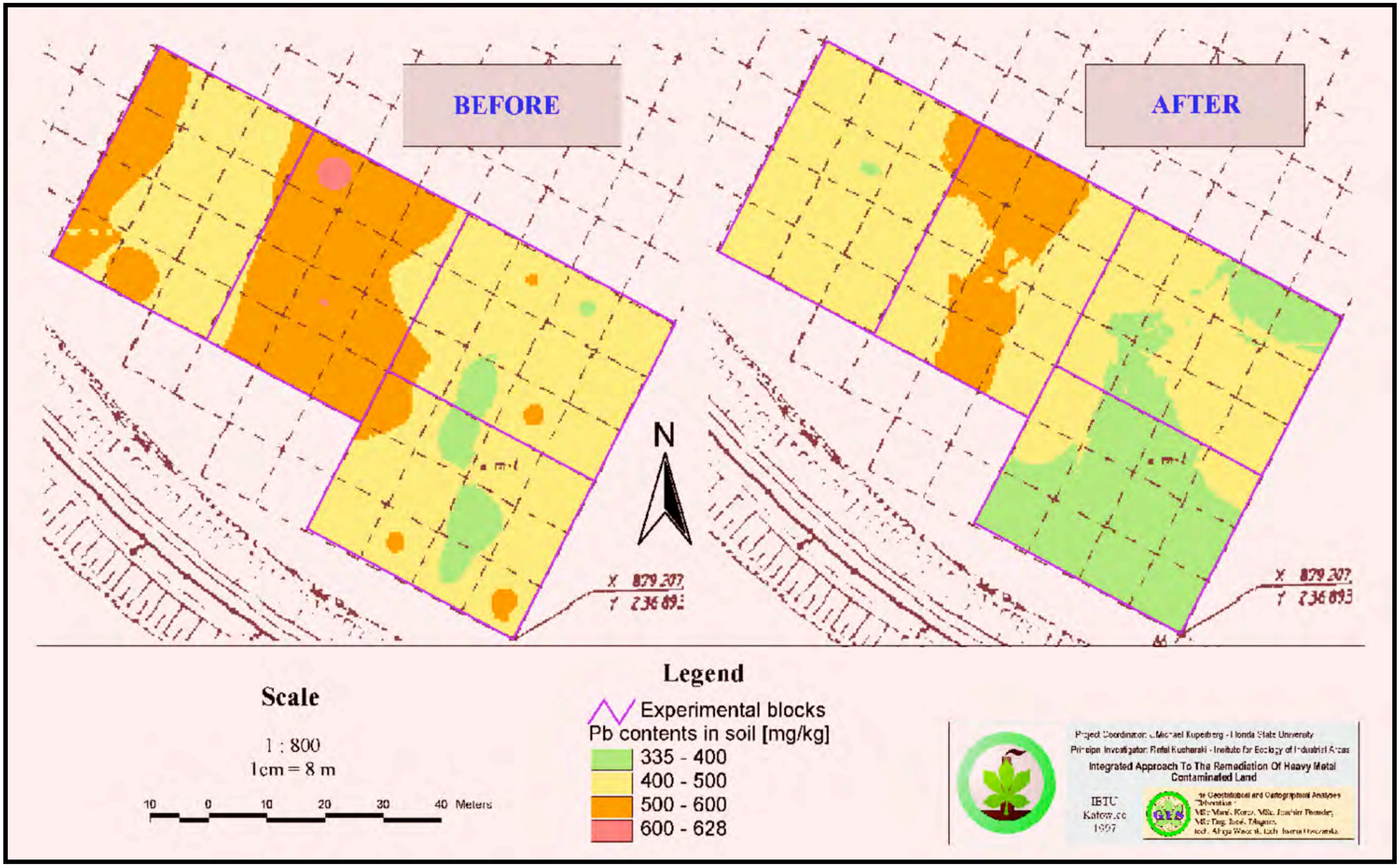
Brodie, E. L., T. Z. DeSantis, D. C. Joyner, S. M. Baek, J. T. Larsen, G. L. Andersen, T. C. Hazen, P. M. Richardson, D. J. Herman, T. K. Tokunaga, J. M. Wan, and M. K. Firestone. 2006. Bacterial population dynamics during uranium reduction and re-oxidation: Application of a novel high density oligonucleotide microarray approach. Appl. Environ. Microbiol. 72:6288-6298

Integrated approach to the Phytoremediation of Lead-Contaminated Lands Katowice, Poland

- Site selection & characterization
- Screening for best heavy metal accumulators
- Selection of amendments
- Amendment application technique
- Harvest and biomass disposal
- Ecological risk assessment
- Economic evaluation

U.S. DOE Office of Environmental Management
Institute for Ecology of Industrial Areas (Katowice, Poland)
Florida State University, Central European Advanced Technologies
Edenspace (Phytotech)

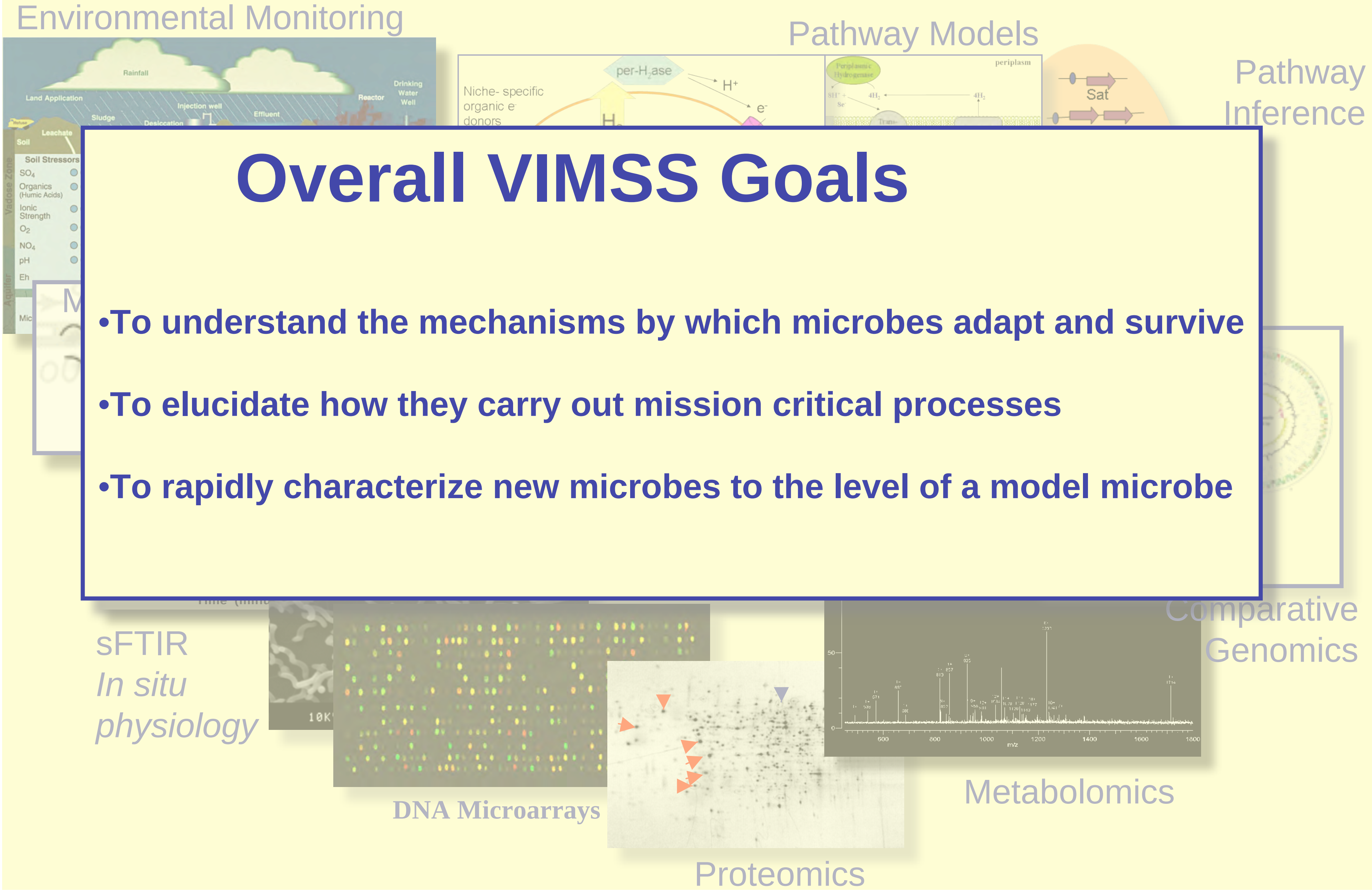
Results after one year



Summary

- Bioremediation of holds great promise for remediating some of our most recalcitrant contaminated sites (oil, chlorinated solvents, Pb, U, Cr). Biostimulation and natural attenuation are the most dominant field applications to date. Good
- Critical biogeochemical characteristics and monitoring need to be considered for selection of the most appropriate strategy and monitoring (factors: sensitivity, specificity, quantification, sorption, reoxidation, mobilization, toxicity, bystander effects), bioaugmentation has been very limited and may always be). Bad
- Phytoremediation and bioaccumulation/biosorption strategies also show promise but may have fatal flaws (life cycle analyses that include all risks and cost are critical). Ugly
- Biomobilization and treatment trains that end in natural attenuation maybe the best long-term solutions especially for mixed waste.
- A Systems Biology approach may be one of the only ways that we that we can enable sustainable environmental biotechnology applications.

Virtual Institute of Microbial Stress and Survival



Overall VIMSS Goals

- **To understand the mechanisms by which microbes adapt and survive**
- **To elucidate how they carry out mission critical processes**
- **To rapidly characterize new microbes to the level of a model microbe**

Acknowledgments

BERKELEY LAB

Adam Arkin, Eric Alm, Kat Huang, Dylan Chivian, Janet Jacobson, Jay Keasling, Aindrila Mukhopadhyay, Eoin Brodie, Sharon Borglin, Hoi-Ying Holman, Jil Geller, Elenor Woezi, Jenny Lin, Dominique Joyner, Rick Huang, Romy Chakraborty, Boris Faybishenko, Mark Conrad, Joern Larsen, Zouping Zheng, Gary Andersen, Todd DeSantis, Tetsu Tokunaga, Jiamin Wan, Susan Hubbard, Ken Williams, John Peterson, Natalie Katz, Jill Banfield, Tamas Torok, Seung Baek, Don Herman, Mary Firestone

Pacific Northwest National Laboratory ...delivering breakthrough science and technology — Phil Long, et al.

REGENESIS — Steve Koenigsberg, Ana Willet

JGI DOE JOINT GENOME INSTITUTE — Paul Richardson, Phil Hugenholtz

MISSOURI — Judy Wall, et. al.

MONTANA STATE UNIVERSITY — Mathew Fields, et. al.

THE UNIVERSITY OF CHICAGO — Stephen Sutton, Matthew Newville

Sandia National Laboratories — Anup Singh, et. al.

OAK RIDGE NATIONAL LABORATORY — Jizhong Zhou, et. al.

Massachusetts Institute of Technology — Eric Alm

U Washington — David Stahl, et. al.

DIVERSA — Martin Keller, et. al.

Princeton — T. C. Onstott, et. al.

Indiana University — Lisa Pratt, et. al.

DOE GENOMICS:GTL ACCELERATING DISCOVERY FOR ENERGY AND ENVIRONMENT OFFICE OF SCIENCE U.S. DEPARTMENT OF ENERGY

Environmental Remediation Sciences

NASA

Center for Environmental Biotechnology

Contacts

Terry C. Hazen tchazen@lbl.gov

Center for Environmental Biotechnology

- http://www-esd.lbl.gov/CEB

Virtual Institute for Microbial Stress and Survival

- http://vimss.lbl.gov
- http://www.microbesonline.org - Comparative Genomics Database, Genome Browser, Operon Browser, Regulon Browser, Metabolic Maps of >430 sequenced bacteria and archaea
- http://greengenes.lbl.gov - 16S rRNA gene database and workbench

Environmental Remediation Technology Program

- http://www-esd.lbl.gov/ERT

Ecology Department

- http://www-esd.lbl.gov/ECO

DOE Environmental Remediation Sciences Program

- http://www.lbl.gov/ERSP

DOE Genomics:GTL Program

- http://doegenomestolife.org

NASA Indiana Princeton Tennessee Astrobiology Initiative (IPTAI)

- http://www.indiana.edu/%7Edeeplife/homepg.html

Ernest Orlando Lawrence
Berkeley National Laboratory