

Integration of Ecogenomics, Phenomics, Transcriptomics, Proteomics, Lipidomics, Metabolomics, Fluxomics, Bioinformatics, and Biogeochemistry: The New Frontier of Environmental Biotechnology



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<http://vimss.lbl.gov>



Presentation Outline

- I. **Why we need this approach ?**
 - A. **Microbial Genomics (State of the Science)**
 - B. **Environmental Biotechnology? (State of the Science)**
- II. **Biogeochemistry**
 - A. **Case Studies: Diffusion limited environments**
 - B. **Field to Lab Case Studies: Gaseous Nutrient Injection Modeling, Aerobic Landfill Bioreactors “Smart Storage”**
- III. **Ecogenomics & Transcriptomics**
 - A. **Bioremediation Case Studies lab and field: phenotype arrays, 16S array, PLFA, lab and field**
- IV. **Phenomics, Proteomics & Lipidomics**
- V. **Metabolomics & Fluxomics**
- VI. **Bioinformatics**
- VII. **Omics Integration Example: Virtual Institute for Microbial Stress and Survival - Rapid deduction of stress response pathways in metal-reducing bacteria**
- VIII. **Summary**

**Science fiction and reality are becoming more and more blurred.
Ecogenomics at Berkeley Lab!**



Genomics - How far we have come!

VIMSS

- **Human Genome Project started in 1990**
- **Scientific project of the millennia**
- **Great advances in sequencing throughput**
- **Human genome sequence completed in April 2003**
- **Since 1995 >150 microorganisms have been sequenced, >100 in the last 2 years**
- **TIGR discovers 1.2 million new bacteria/archaea genes in the Sargasso Sea March 2, 2004**





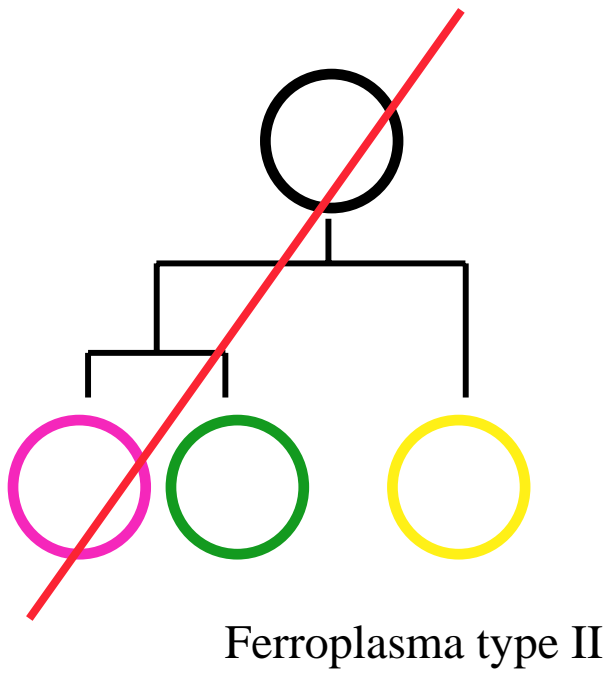
VIMSS

- The current Joint Genome Institute throughput is ~2.0-2.5 billion bases per month
 - In theory, JGI could sequence >400 microbes per year*
 - In practice, this would be very difficult to achieve
 - JGI could reasonably sequence ~ 100-200 microbes per year
 - This throughput depends on receiving high-quality DNA from the collaborators
- *Note: This is the capacity for single isolates they have started doing whole microbial communities***



Community structure and metabolism through reconstruction of genomes from the environment

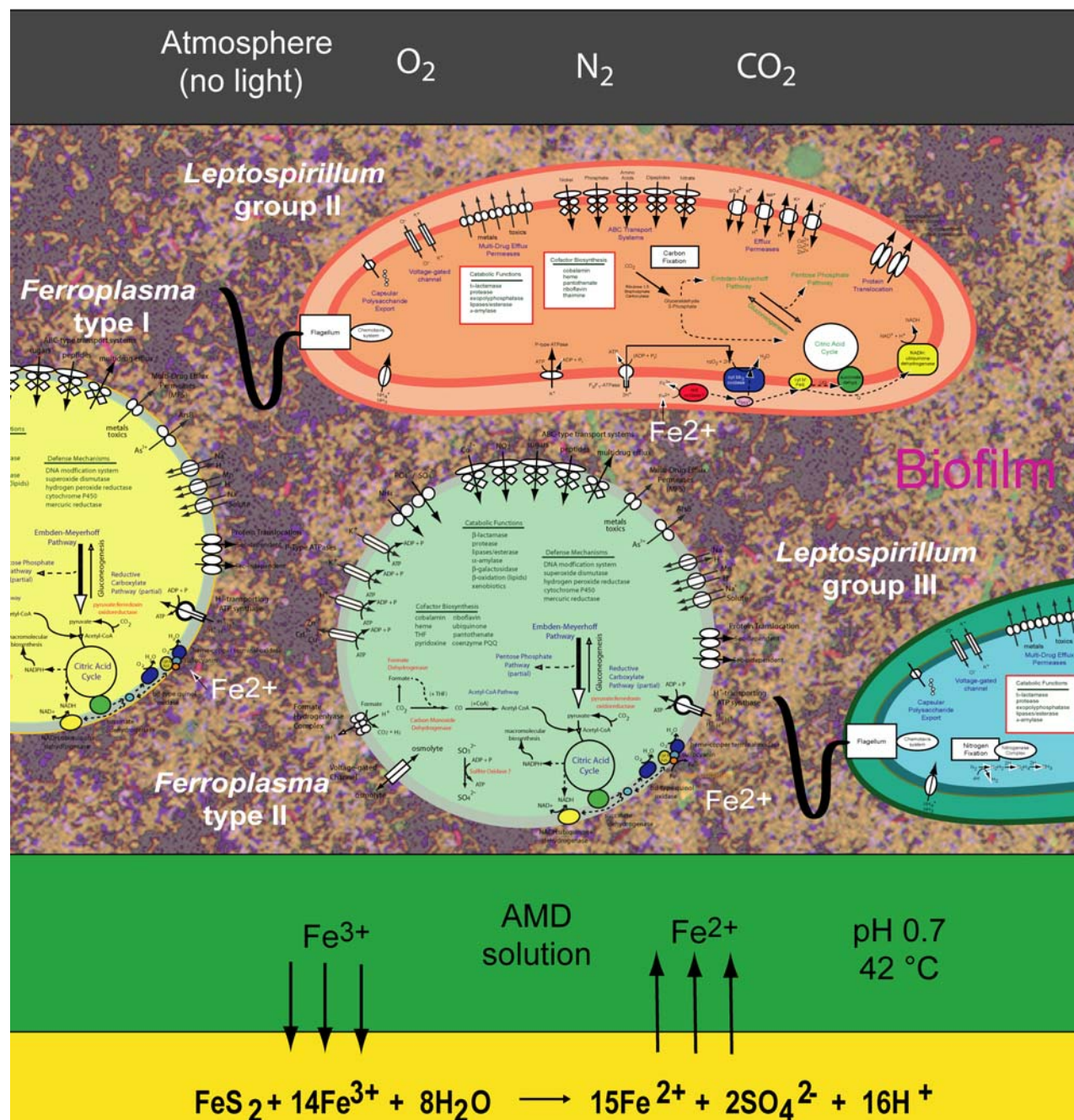
Tyson et al., *Nature* (2004)



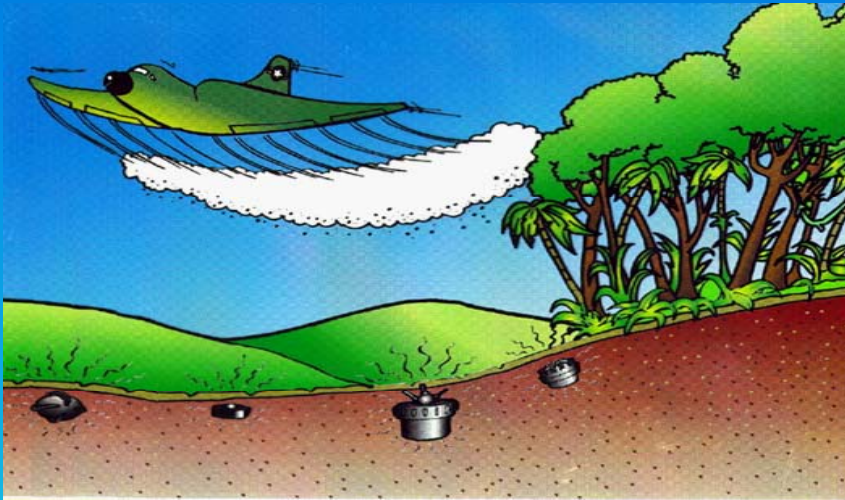
Ferroplasma type II



Mosaic genome types



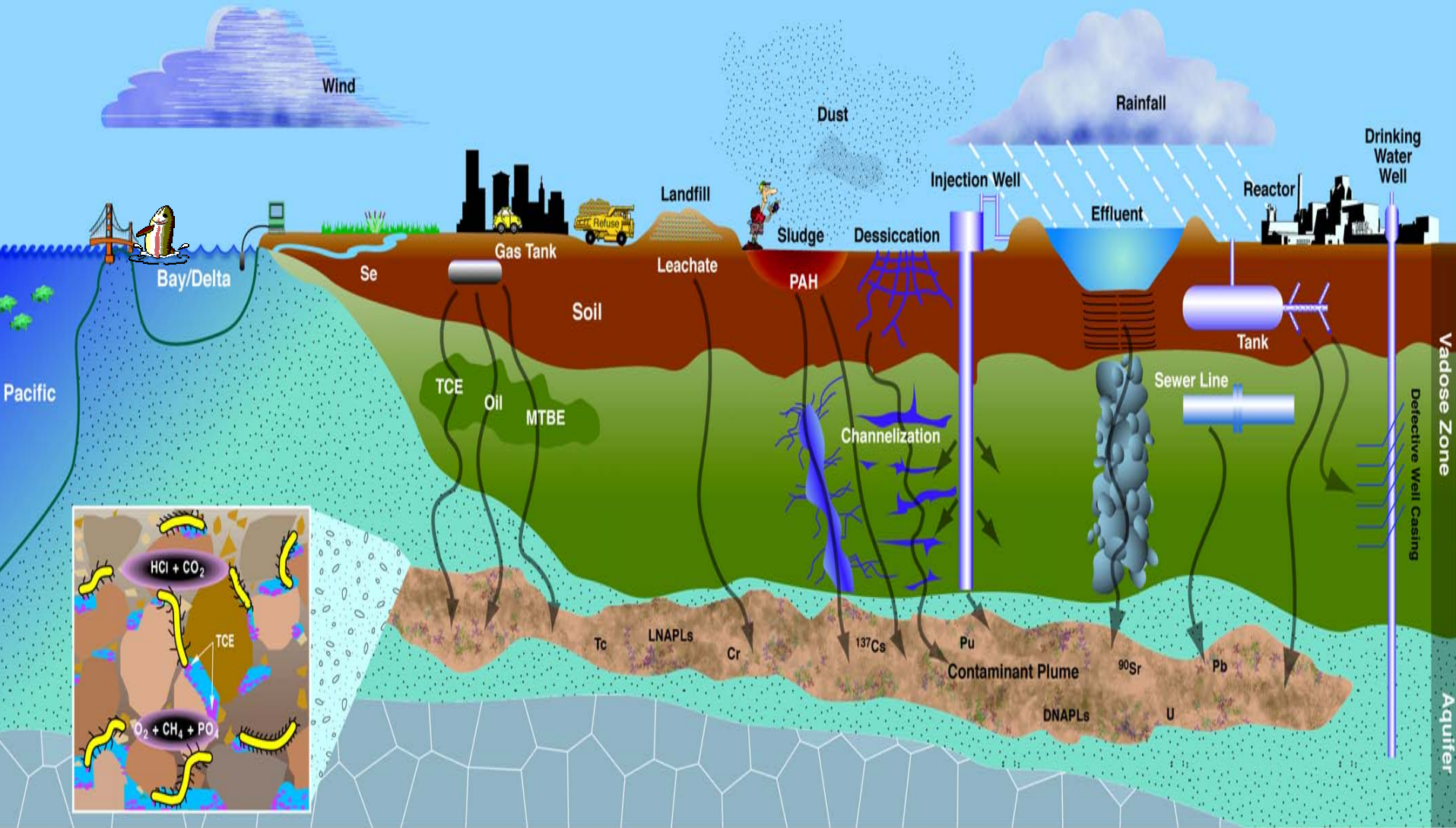
Microbial Mine Detection System (MMDS)



Humanitarian Demining
In Situ Detection
Bioluminescence

Environmental Biotechnology

Understanding, monitoring and controlling the environment with biological processes (the need is everywhere)

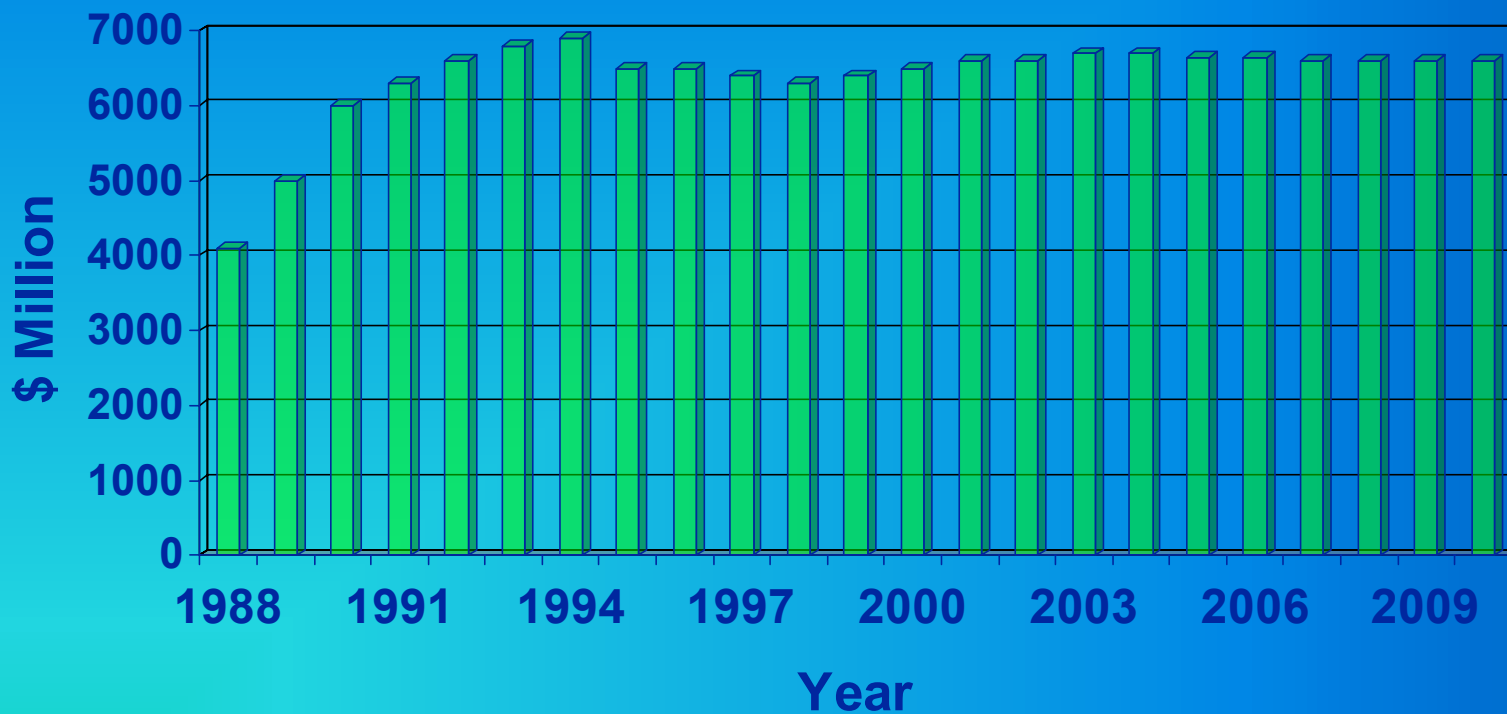


Market Created by RCRA and CERCLA

- 21,000 RCRA hazardous waste generators,
- 6000 RCRA hazardous waste treatment, storage, and disposal facilities,
- 1,500-3,500 RCRA corrective action sites,
- 1,500 to 2,100 Superfund NPL sites,
- 19,000 state nonSuperfund sites,
- 231,000-295,000 underground storage tanks that are leaking (90% petroleum),
- 1,800 Department of Defense installations with 7,300 sites,
- 110 Department of Energy facilities with up to 4,000 contaminated areas/facilities.
- Total ~333,000 sites

(US EPA. 2004)

US Remediation Market



Site Characterization 2003



- Drill & Sample 70%
- Portable GCs & Field Instrumentation 21%
- On-Site Mobile Labs 13%
- Soil/Gas Surveys 11%
- Non-intrusive Scanning 9%





- Carbon Adsorption 22%
- Air Stripping 27%
- Air Sparging 12%
- Biological Treatment 13%
- Advanced Oxidation 8%
- Others 15%

3968 applications



Soil Remediation 2003



■ Excavation/Dispose off-site	37%
■ Soil Vapor Extration	19%
■ Cap & Containment	24%
■ Solidification/Stabilization	10%
■ In Situ Bioremediaiton	11%
■ Ex Situ Bioremediation	10%
■ Monitored Natural Attenuation	4%
■ Thermal Desorption	6%
■ Soil Washing	1%
■ On-site incineration	1%

6,706 applications



Hazardous Waste Remediation in the United States could cost

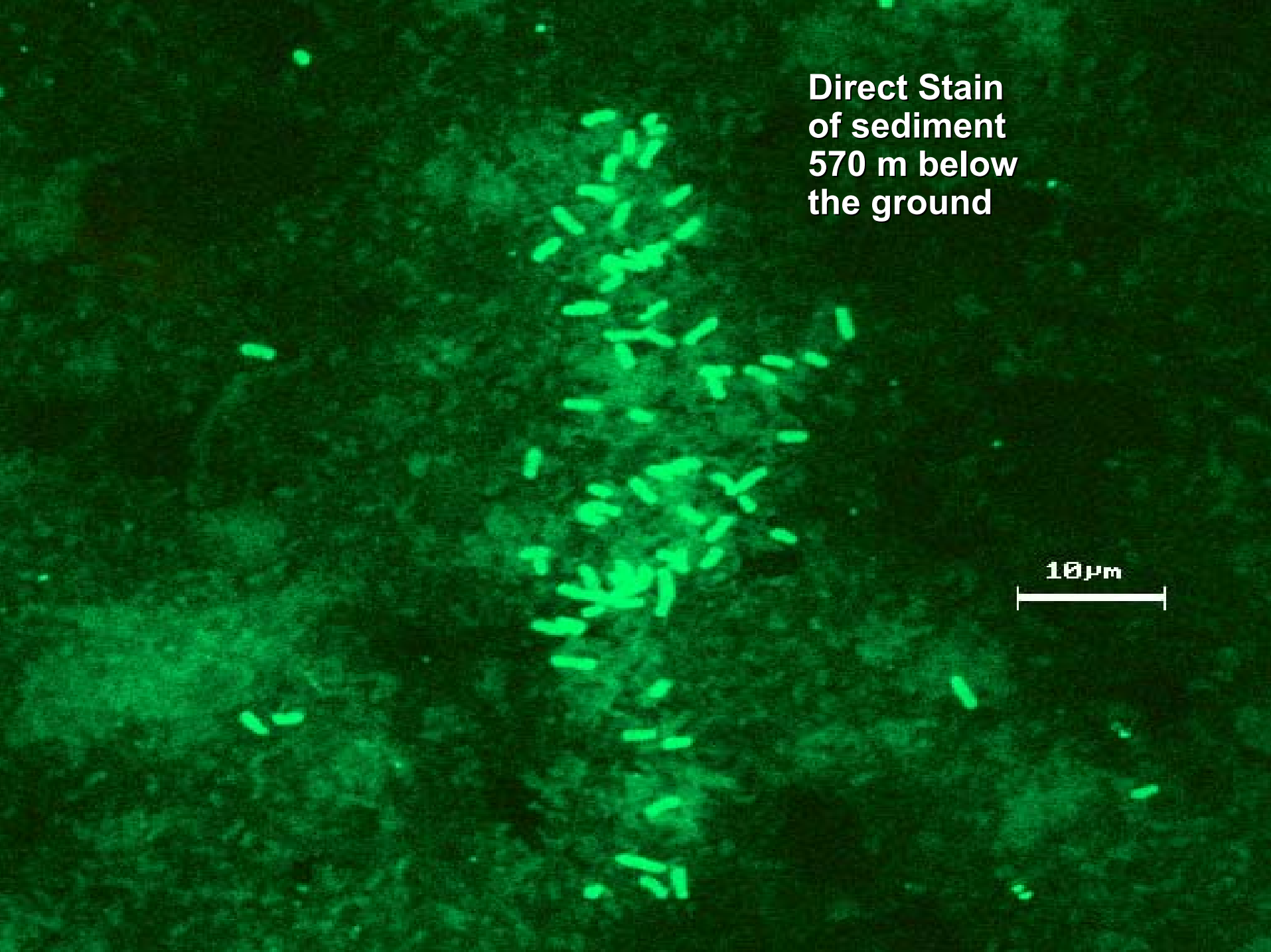
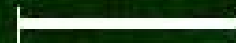


> \$1.7 Trillion



**Direct Stain
of sediment
570 m below
the ground**

10 μ m



Microbial* Life on Earth

- | | Cells |
|--------------------------|---------------------------|
| ↵ Open Ocean | 1.2×10^{29} |
| ↵ Soil | 2.6×10^{29} |
| ↵ Oceanic Subsurface | 3.5×10^{30} |
| ↵ Terrestrial Subsurface | $0.25-2.5 \times 10^{30}$ |
| ↵ All sources | $4-6 \times 10^{30}$ |
- ↵ 60% of all biomass on earth
 - ↵ 350-550 Pg of Carbon (60-100% more C than 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
 - ↵ > 3.7 billion years of microbial evolution on earth



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

(in part Whitman et al., 1998)

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

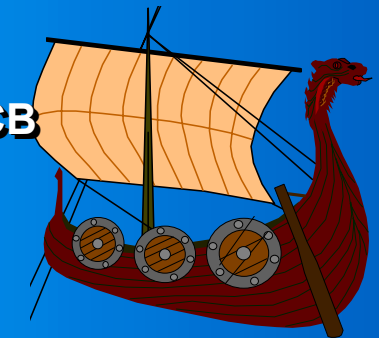
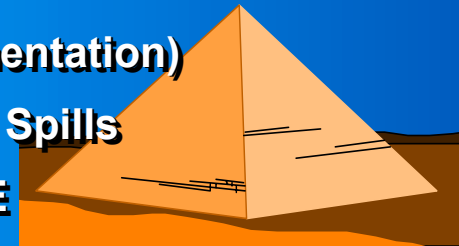
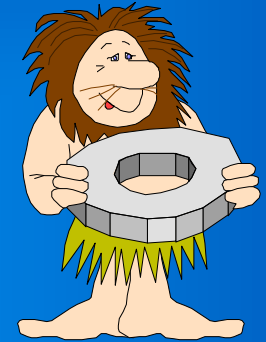
GE Hudson River Caisson Demonstration for PCB

1997

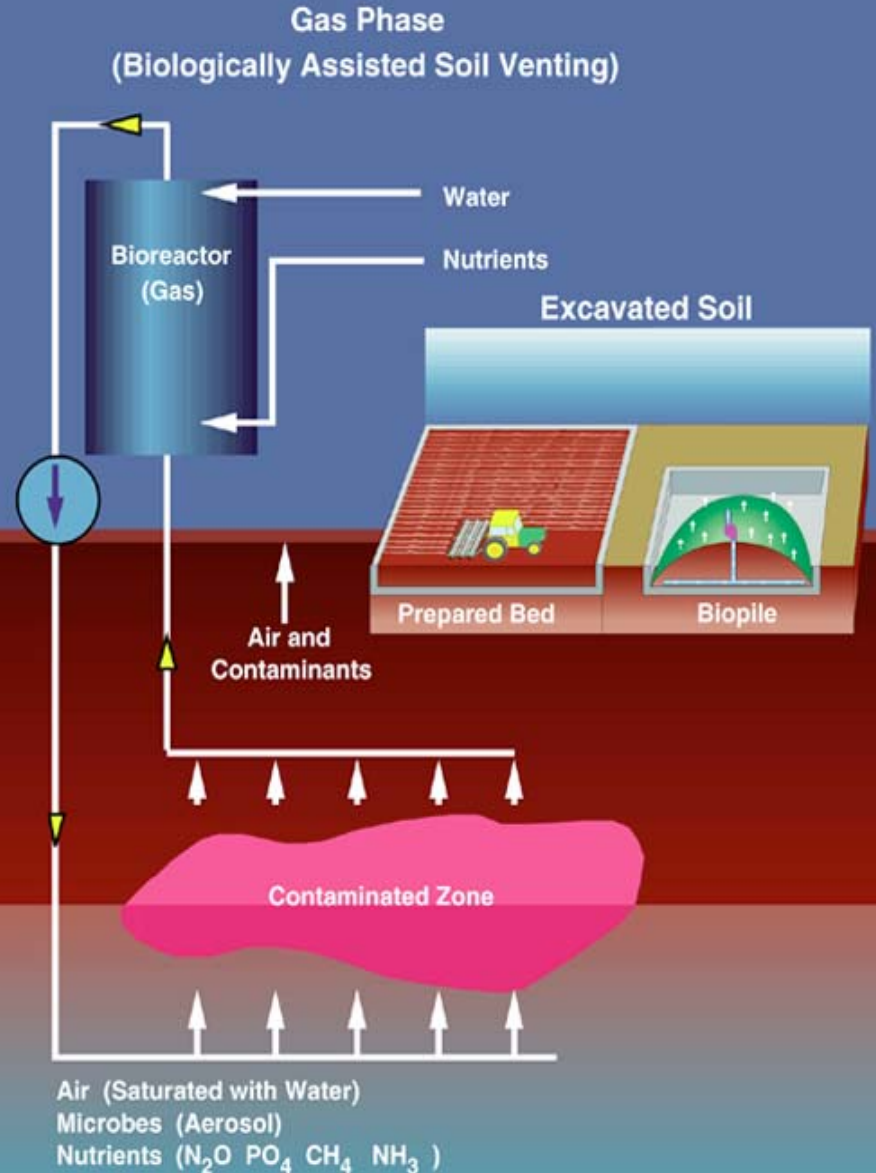
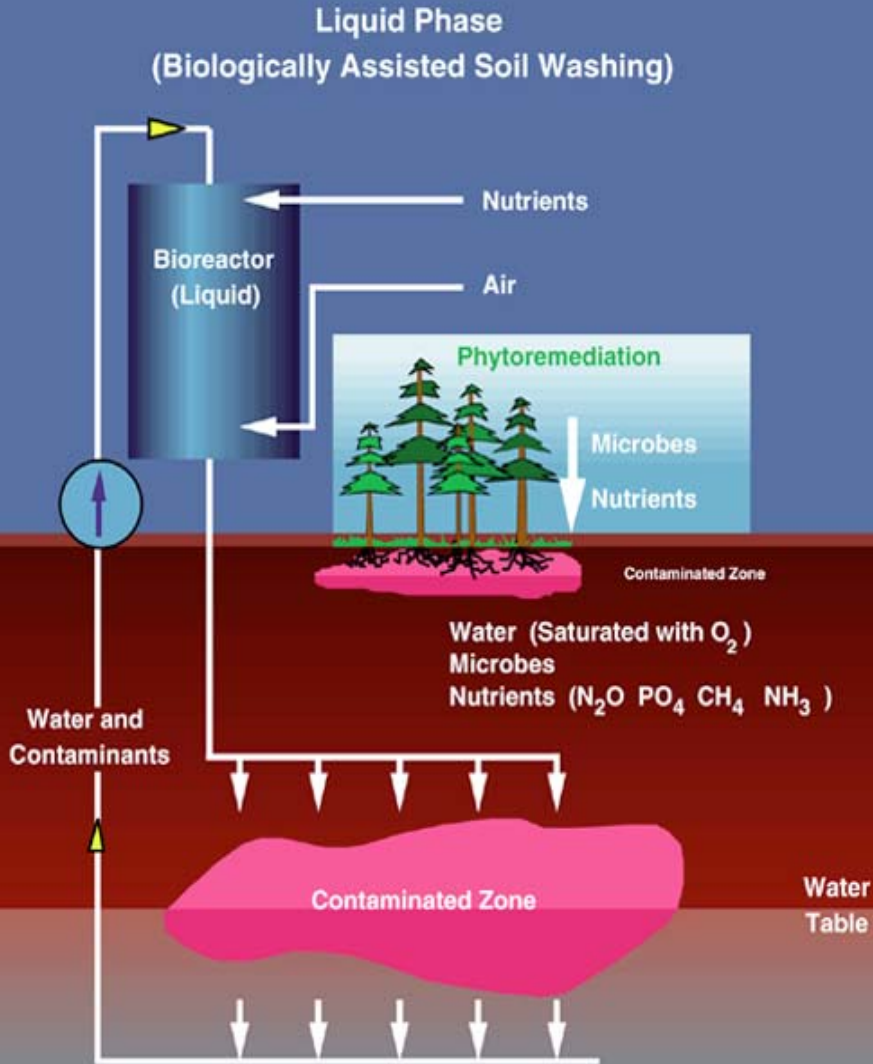
UT/ORNL lysimeter tests of GMO

1999

Oyster Site release of Adhesion-less strain

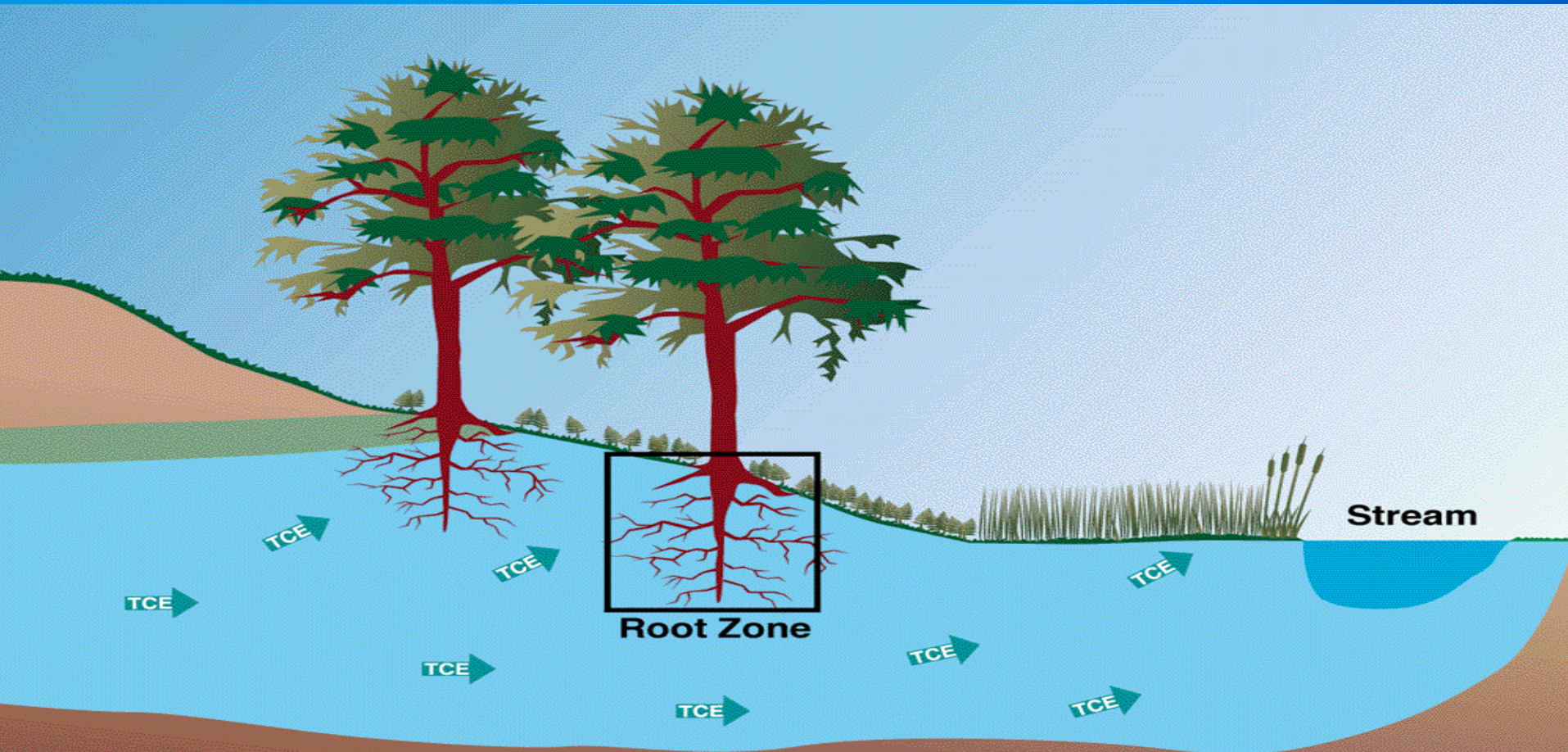


Bioremediation Technologies



Intrinsic Bioremediation

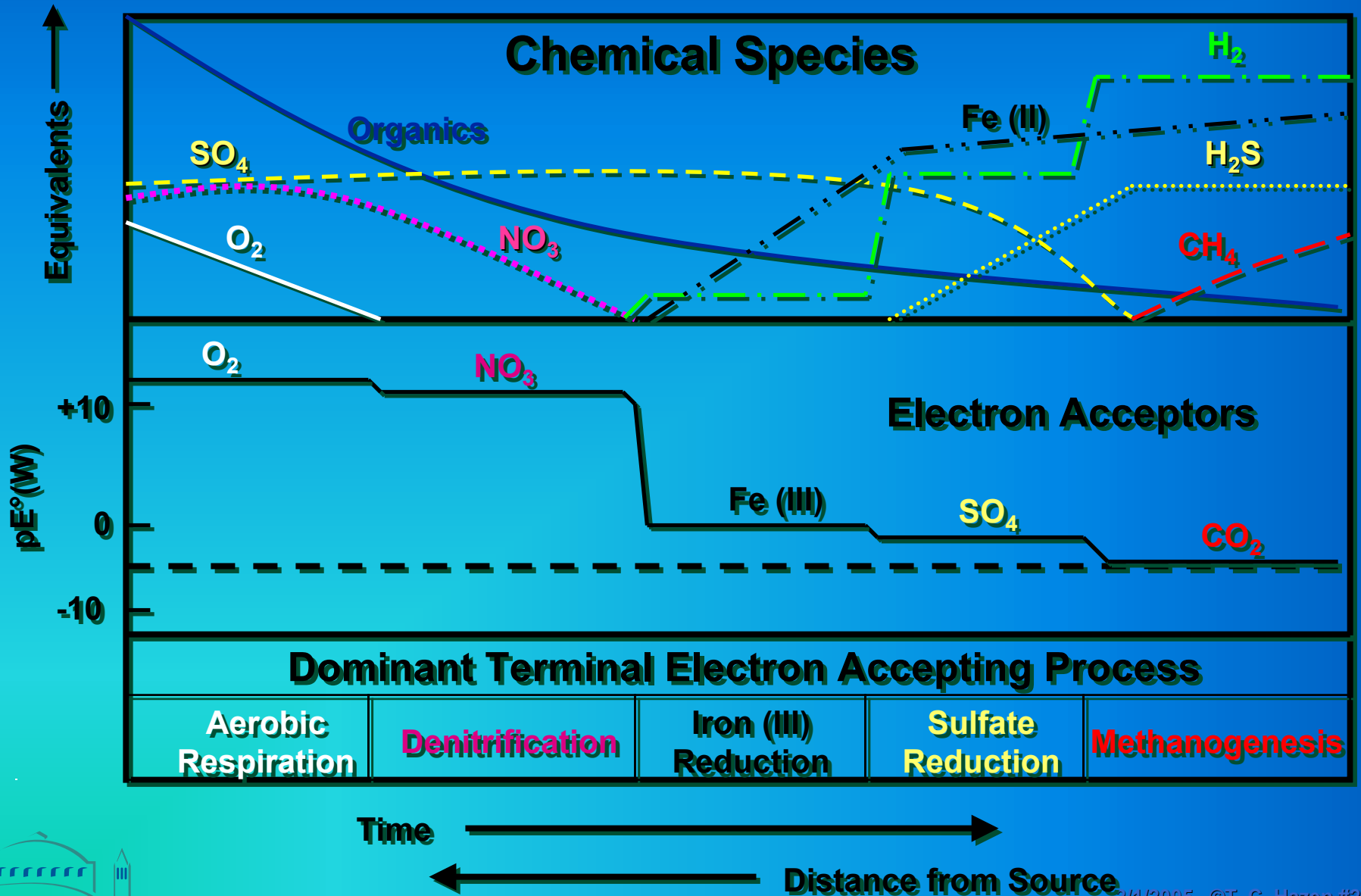
Unmanipulated, unstimulated, unenhanced biological remediation of an environment; i.e. biological natural attenuation of contaminants in the environment. NRC Lines of Evidence for Natural Attenuation 1) Reduction in concentration along the flow path downgradient, 2) Documented loss of contaminant mass by a) chemical and geochemical data, b) biological decay rate data, and 3) Microbiological laboratory data supporting degradation and decay rates.



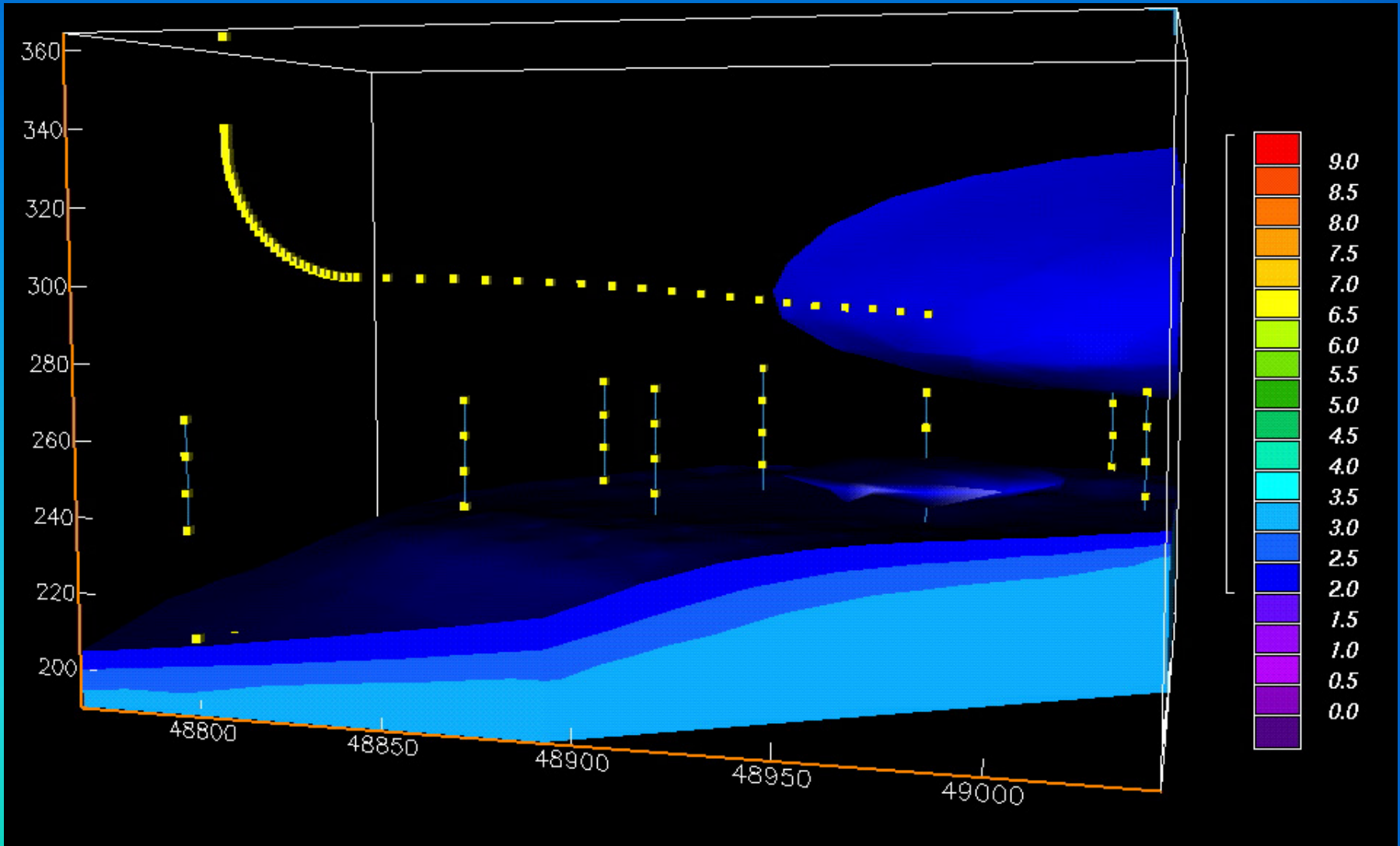
Biogeochemistry

- ☞ Interactions between microbes and the geology, hydrology, and chemistry of the environment**
- ☞ Stable isotope analyses for abiotic/biotic analyses**
- ☞ Issues of scale from molecular to cells to mesoscale to field (pilot and deployment)**
- ☞ Models with fundamental basis that can predict risk from weeks to years to millennia**
- ☞ New basis for understanding all of the possibilities and consequences of environmental control and for building more realistic treatment trains that end in natural attenuation**

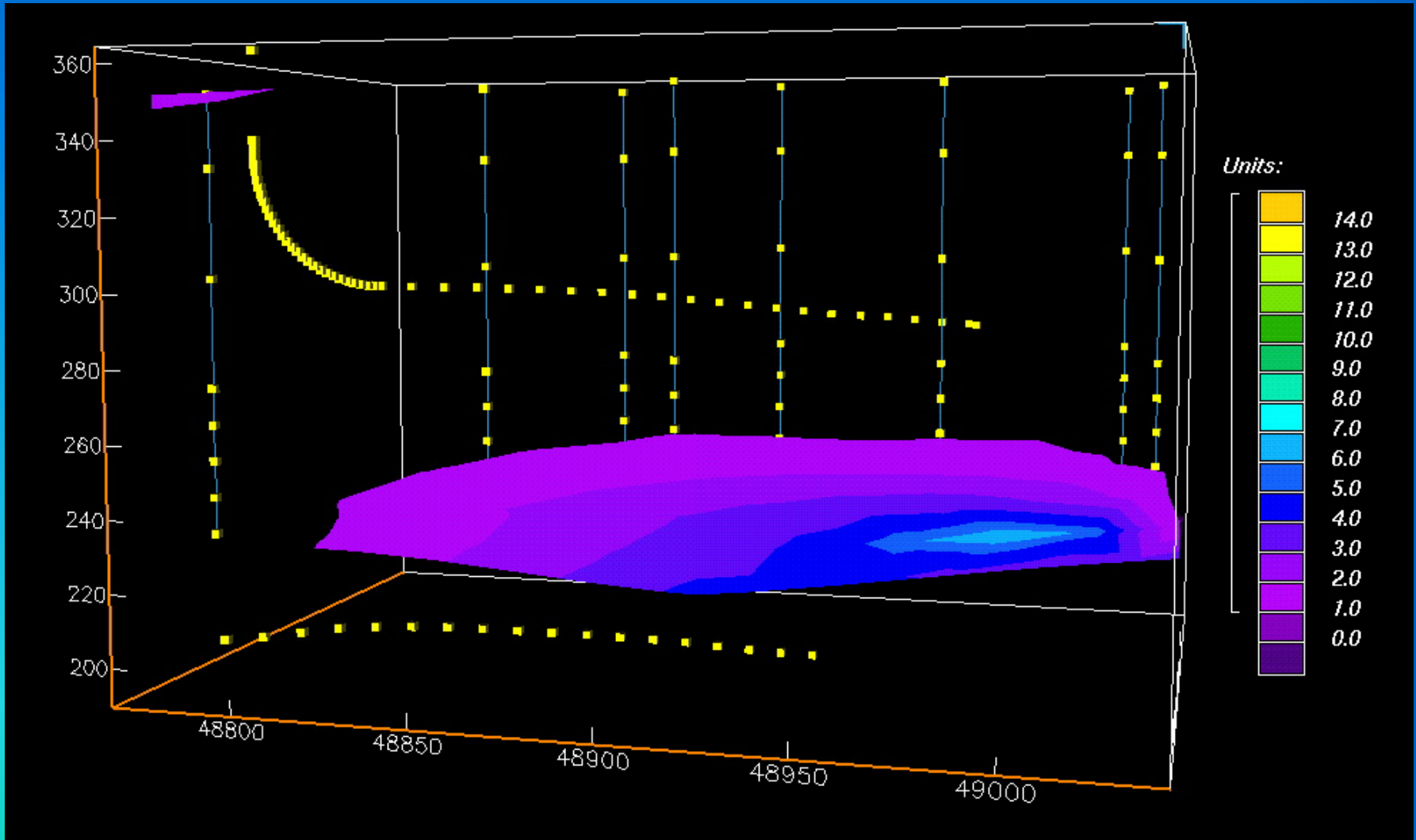
Critical Biogeochemistry



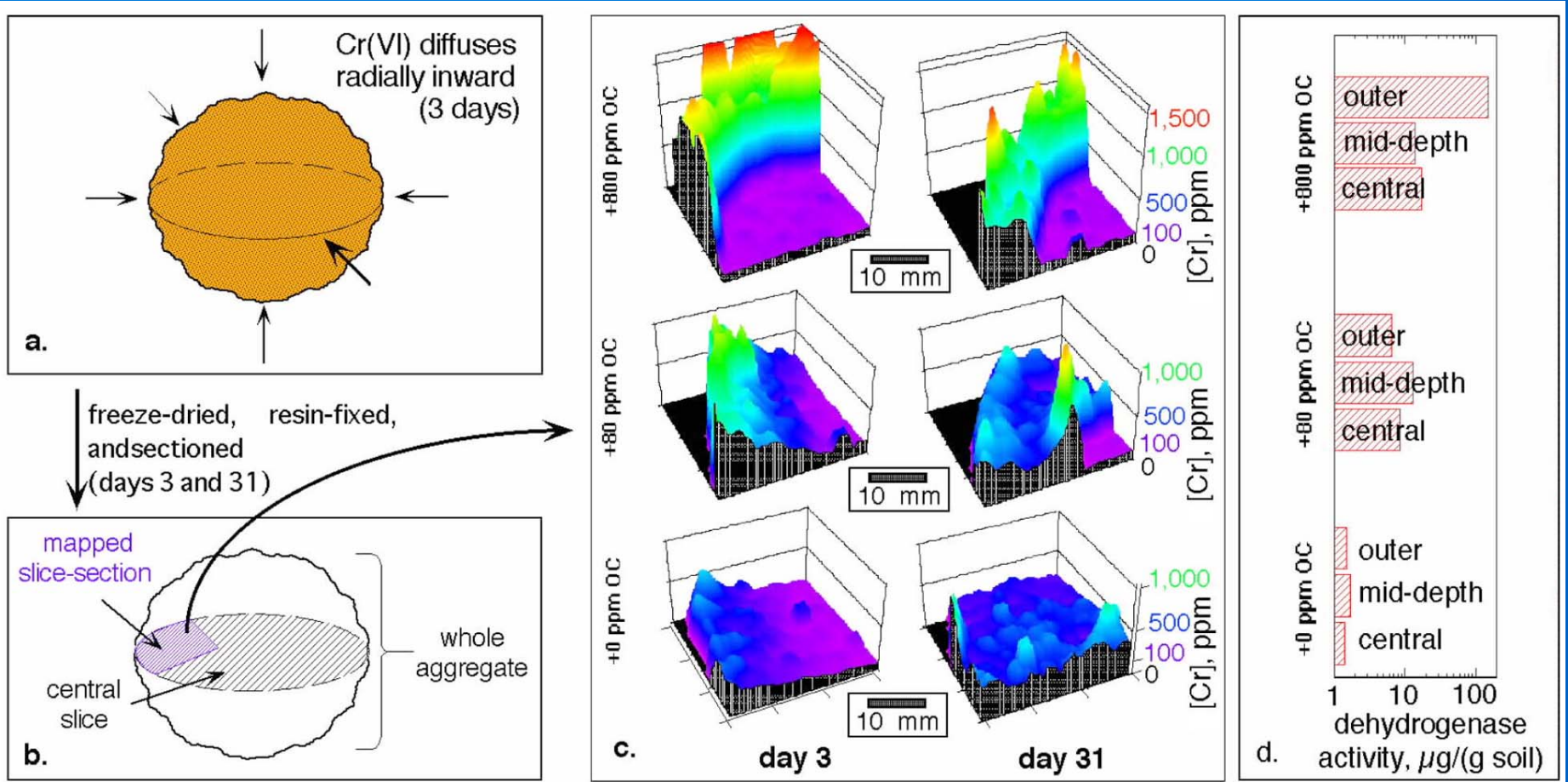
sMMO distribution post test



TCE Mineralization Rates Post Test

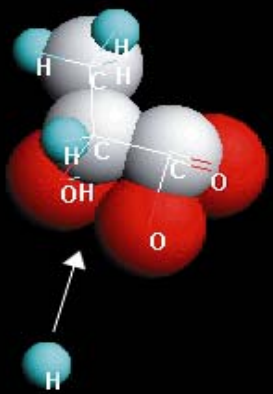


Soil Aggregates Cr Contamination



LBNL collaboration with microXANES light sources at BNL and ANL

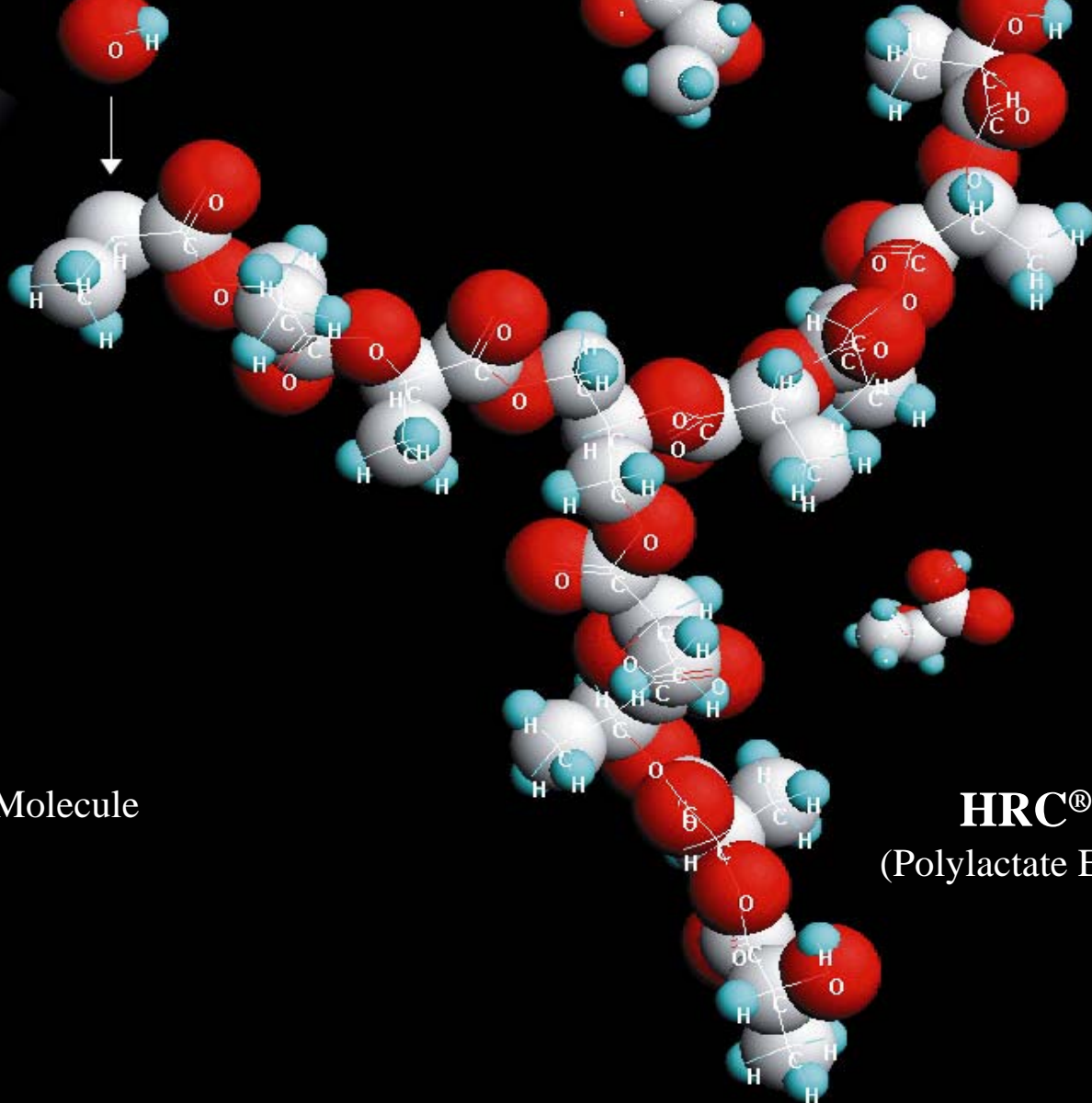
(ES&T, 2001; J. Environ. Qual., 2002, 2003)



H⁺ from water



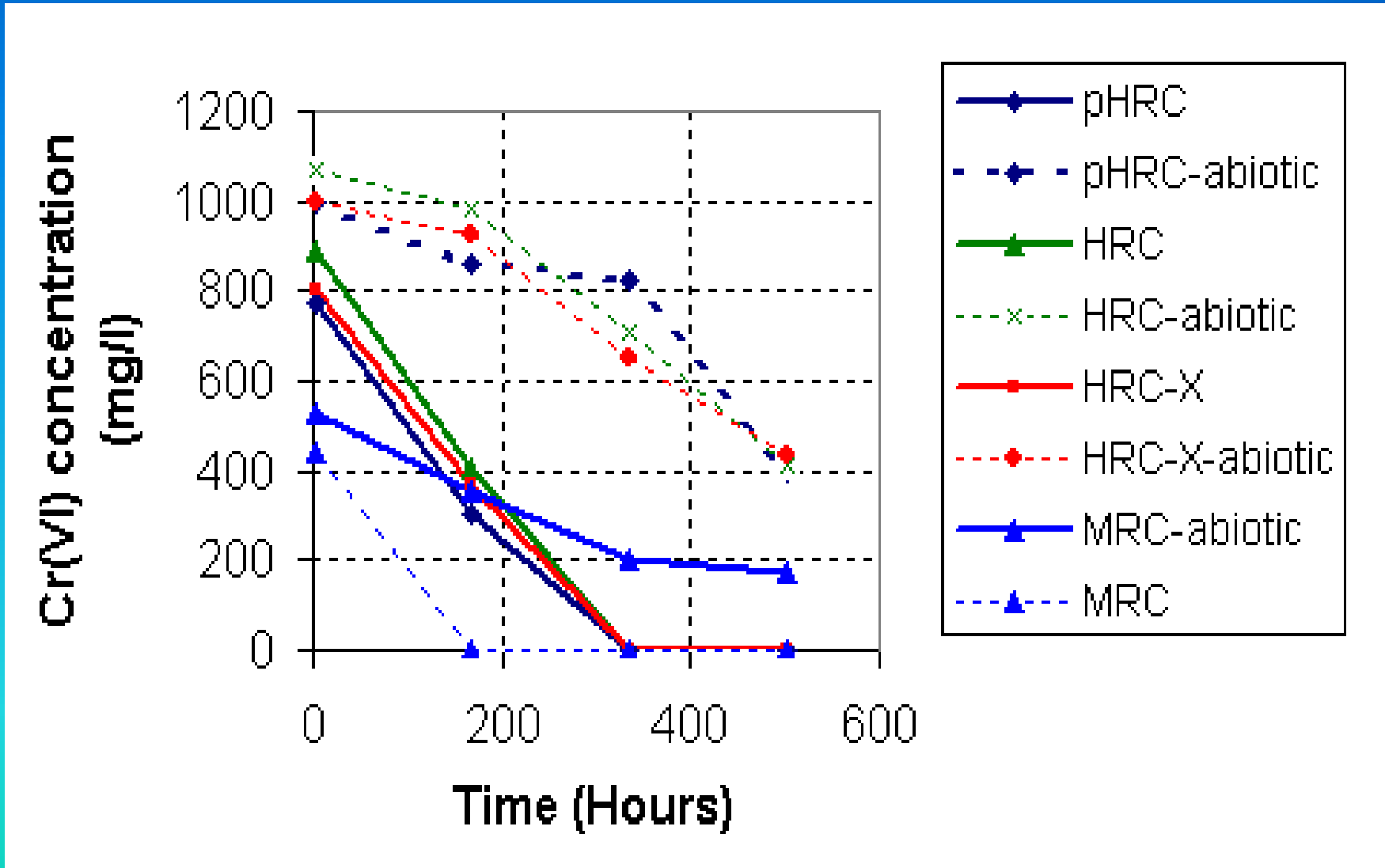
OH⁻ from water



Lactic Acid Molecule

HRC[®]
(Polylactate Ester)

Lactate-Induced Bioreduction of Cr(IV)



August 3, 2004:

- ^{13}C -labeled HRC injection followed by Br-tracer injection into Hanford sediments in Well 699-96-45 over depths of 44-50 ft
- Pumping from Well 699-96-44 started

August 18:

- HRC breakthrough in the monitoring Well 699-96-44

Geophysics

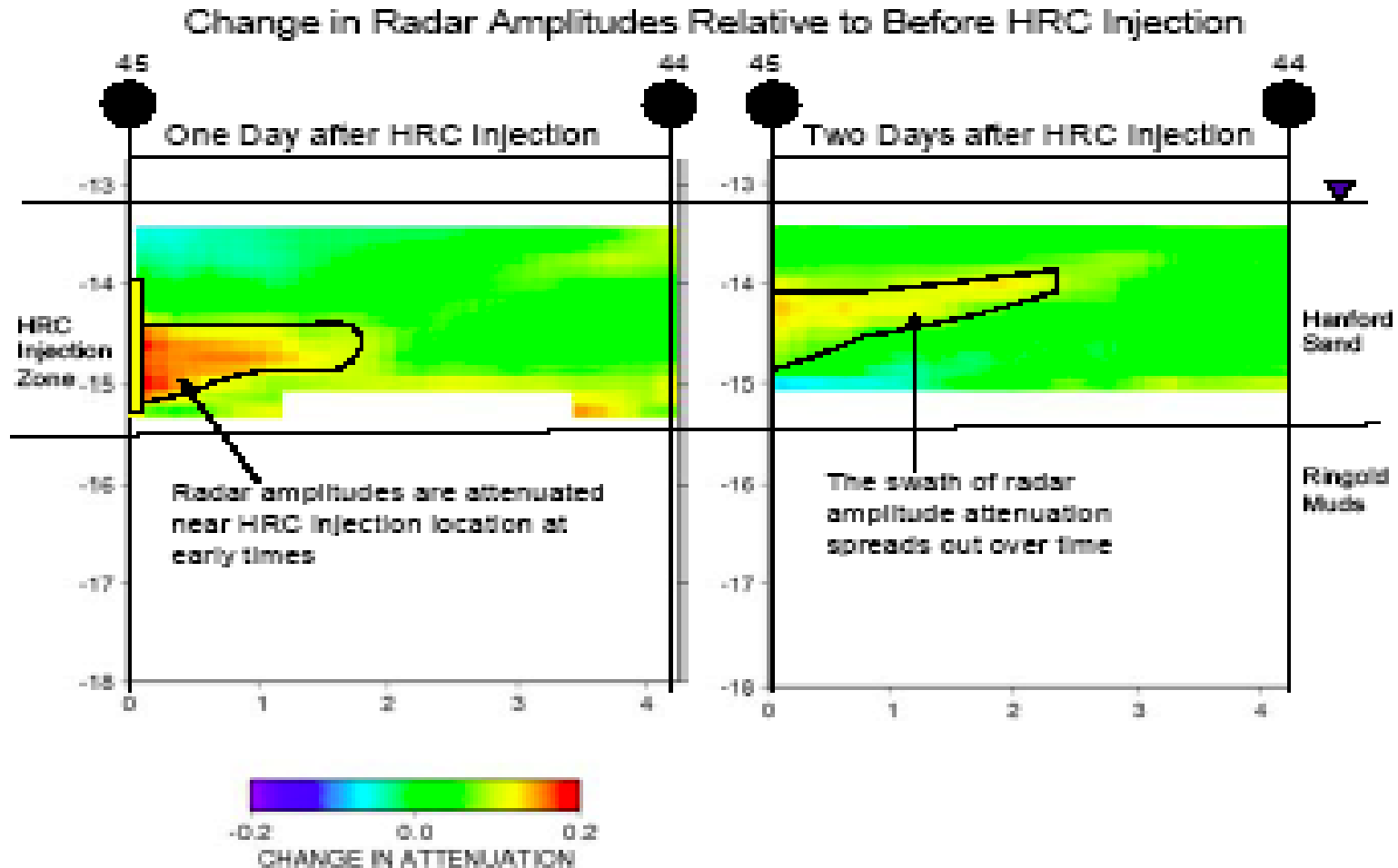
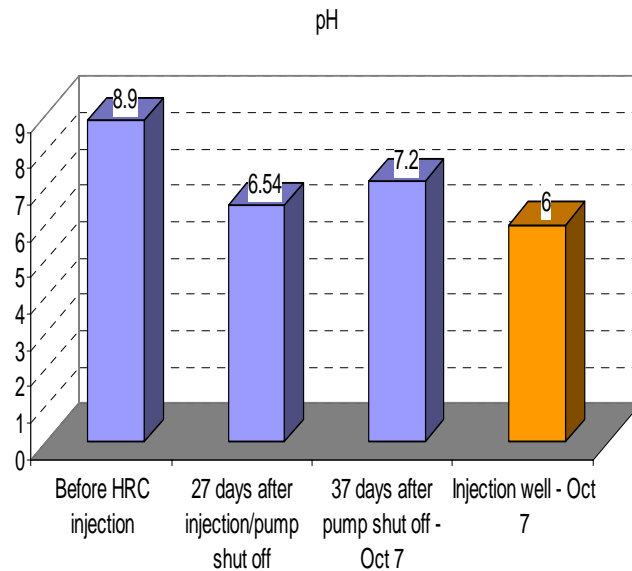
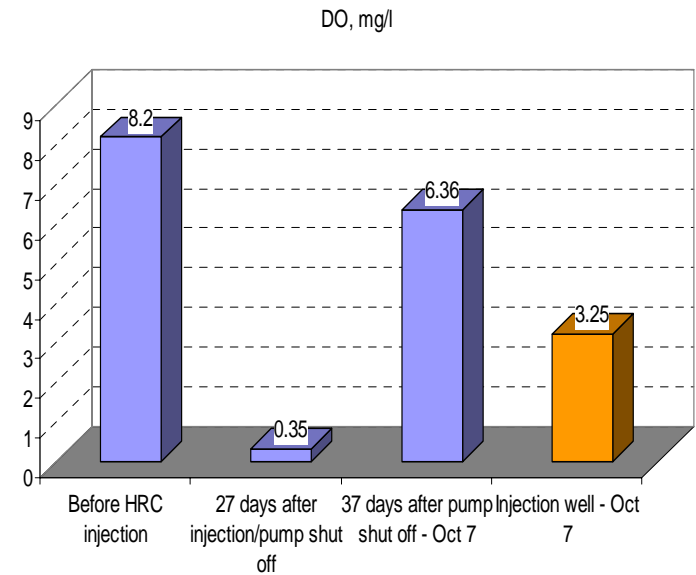
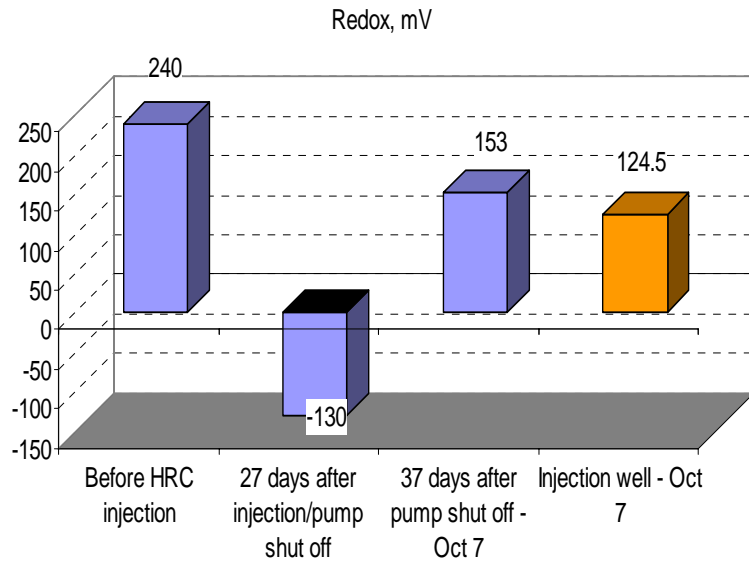


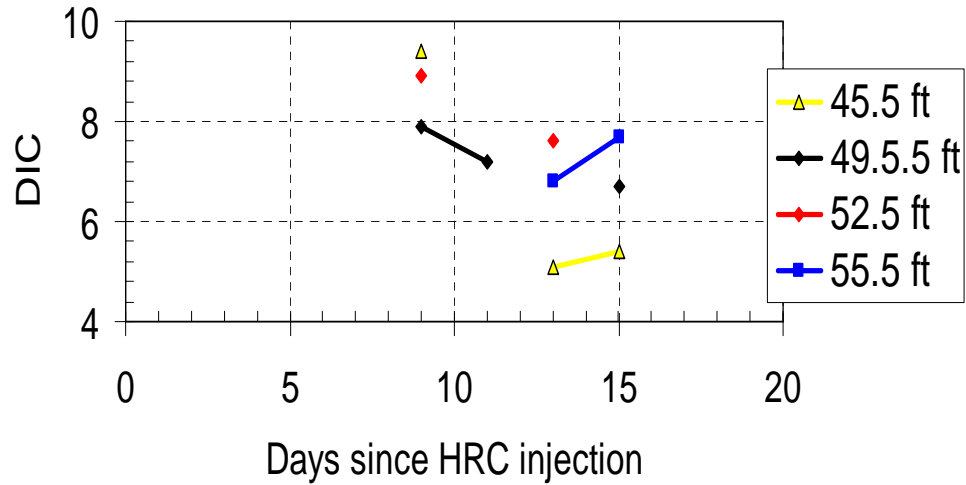
Figure 4 Change in radar attenuation at one day (left) and two days (right) after HRC injection, showing how the interpreted HRC plume spreads over time.

Redox Potential, DO, and pH in Groundwater

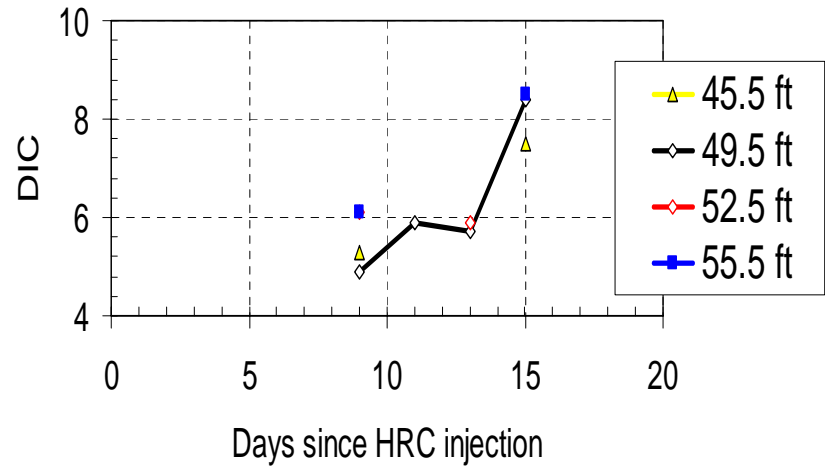


$\delta^{13}\text{C}$ of Dissolved Inorganic Carbon Reflecting Input from ^{13}C -Labeled HRC

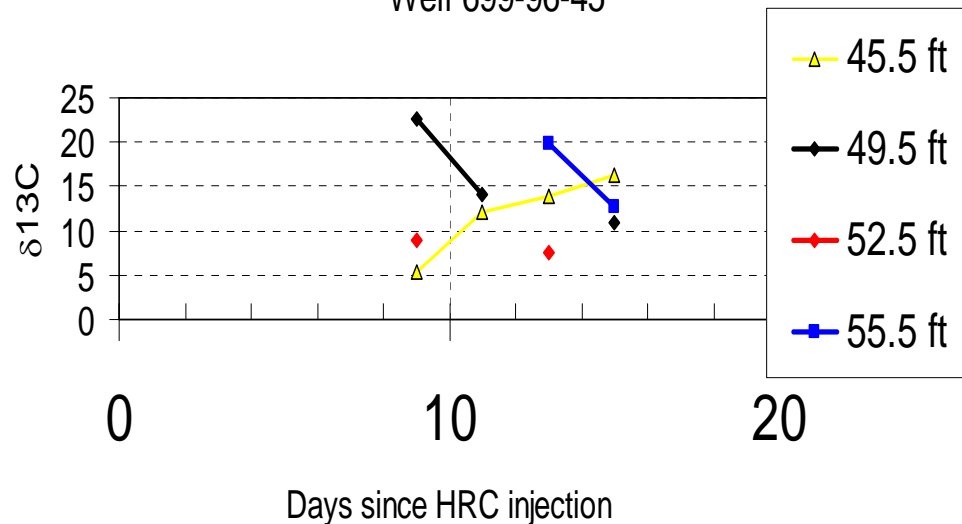
Well 699-96-45



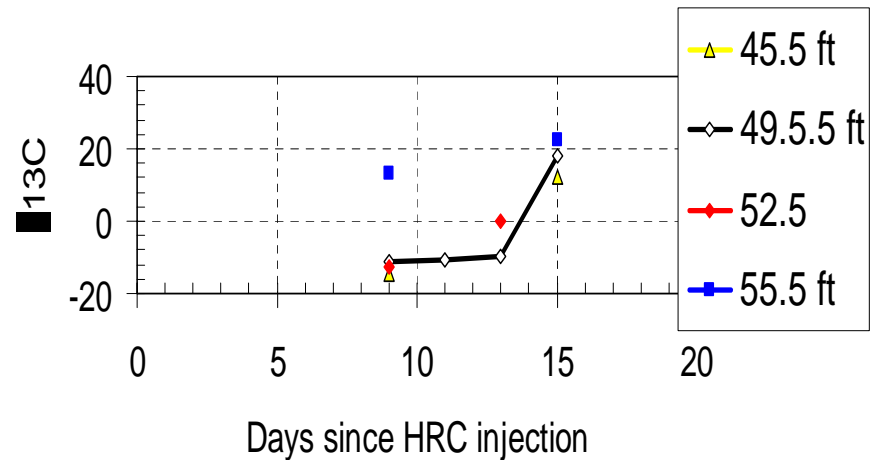
Well 699-96-44



Well 699-96-45



Well 699-96-44





Polish Refinery

Before

**3857 m³ of sludge
contaminated soil
(PAHs, metals)**

After

**18 Months (passive
and active
aeration,
surfactants)**

**120 metric tons
destroyed (81%)**

Green Zone



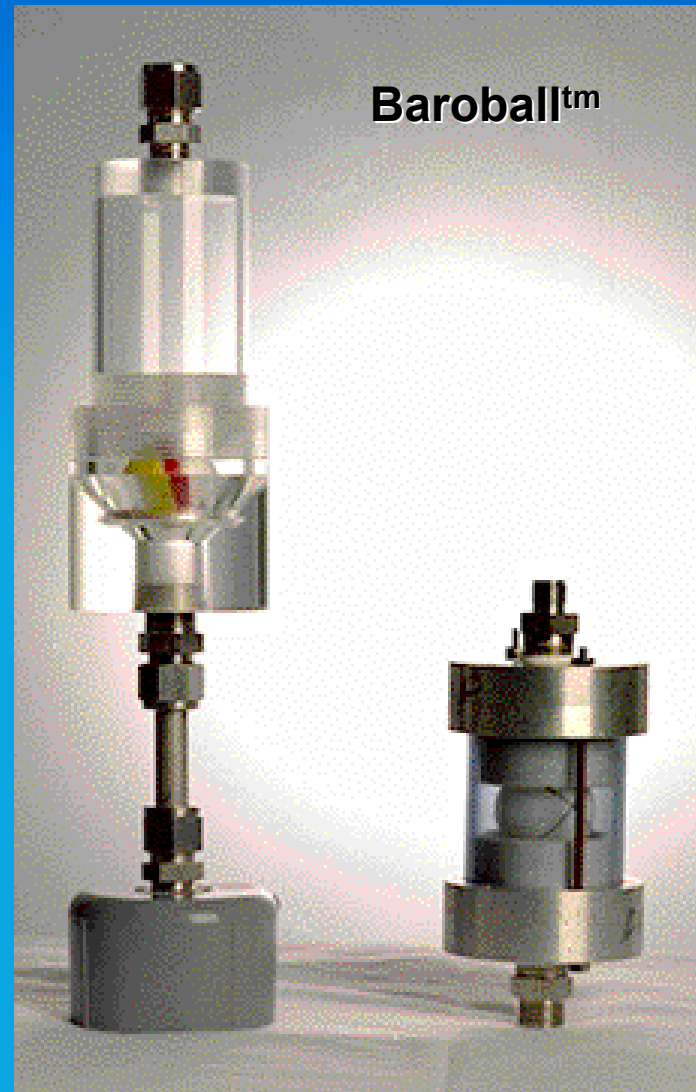
Passive Bioremediation



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



Model vs. Biopile Actual

Model Assumptions

NRI (fraction A) content $\approx 40\%$ of total TP inventory in soil
 Readily available fraction content $\approx 45\%$ of TP inventory in soil
 Sorbed fraction content $\approx 15\%$ of TP inventory in soil
 Soil porosity $\equiv \approx 0.3$

Characteristics of NRI fraction (Fraction A):

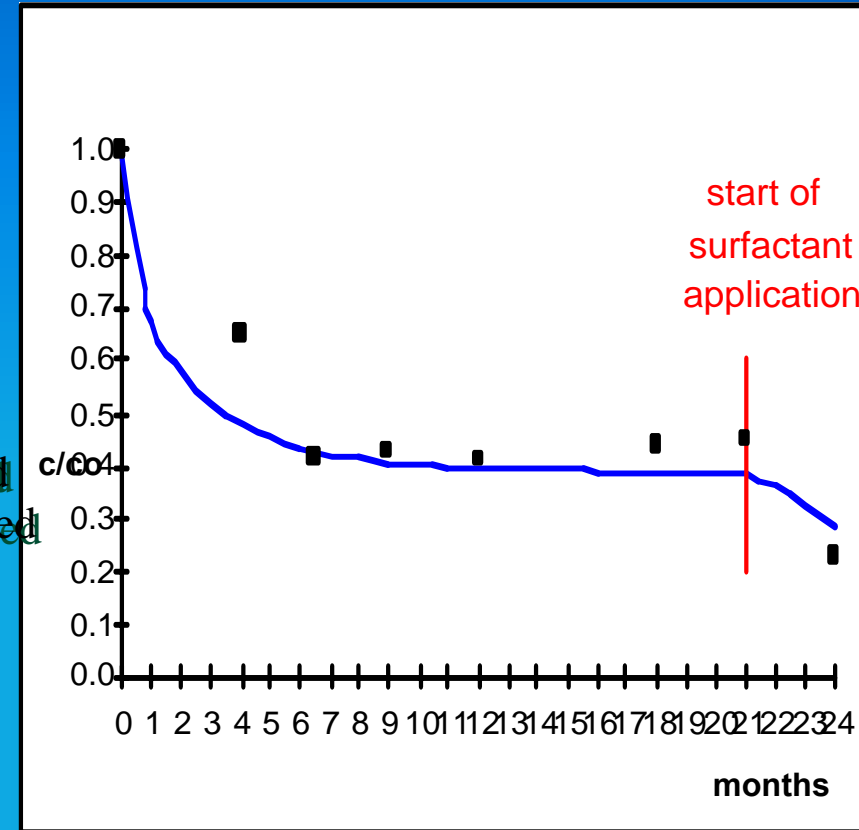
Average radius of aggregates (droplets) $R \equiv 1.0 \mu\text{m}$
 Solubility in water $c \equiv 10 \text{ mg/l}$ before surfactant was added
 $c \equiv 100 \text{ mg/l}$ after surfactant was added

Characteristics of readily available fraction (Fraction B):

Average radius of aggregates $r_0 \equiv 10 \mu\text{m}$
 Desorption coefficient $K_d \equiv 100$
 Pore diffusivity of contaminant $D_{eff} \equiv 5 \times 10^{-10} \text{ m}^2/\text{s}$
 Liquid mass transfer coefficient $k_f \equiv 1 \times 10^{-5} \text{ m/s}$

Characteristics of sorbed fraction (Fraction C):

Average radius of aggregates $r_0 \equiv 30 \mu\text{m}$
 Desorption coefficient $K_d \equiv 1 \times 10^5$
 Pore diffusivity of contaminant $D_{eff} \equiv 5 \times 10^{-10} \text{ m}^2/\text{s}$
 Liquid mass transfer coefficient $k_f \equiv 1 \times 10^{-5} \text{ m/s}$



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

Ecogenomics & Transcriptomics

Ecogenomics - studies of genomes in an environmental context

- **16s rDNA microarrays for community analyses**
- **T-RFLP - terminal restriction fragment length polymorphisms**
- **Metagenome sequencing**
- **Annotation of sequences for environmental context**
- **Microbial Source Tracking for Pathogens**

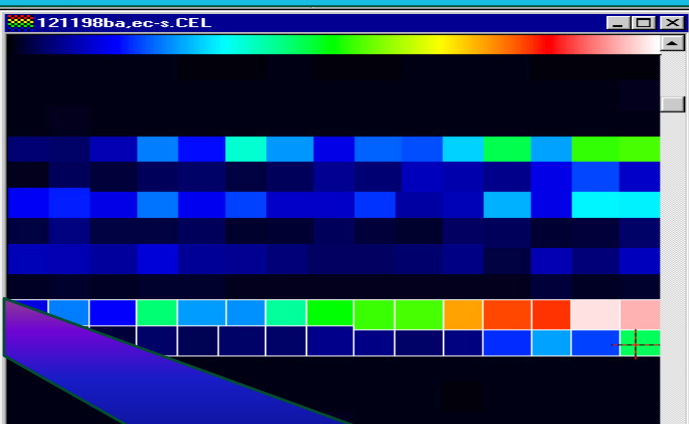
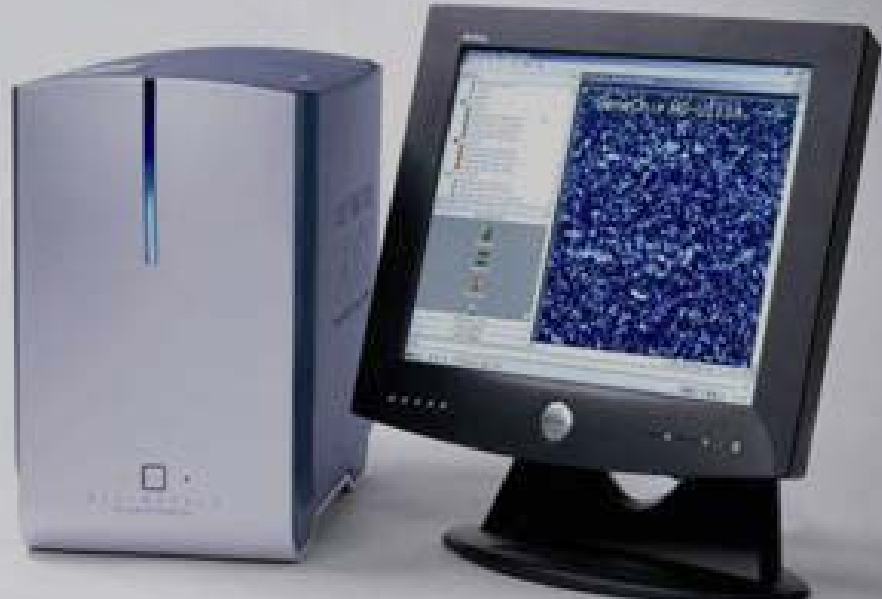
Transcriptomics - gene expression

- **mRNA expression arrays of one organism or functional group**
- **Real-time PCR analyses**

DOE 16s rDNA microarray

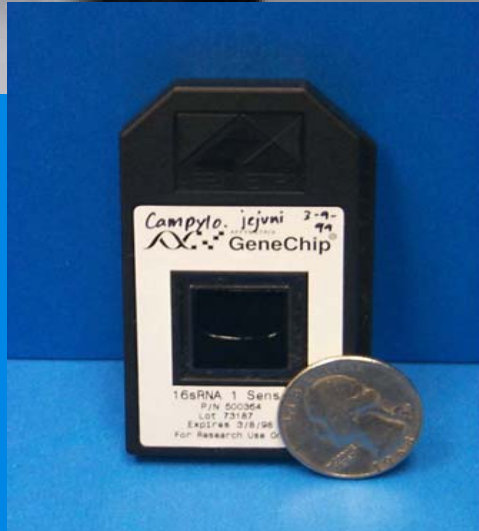
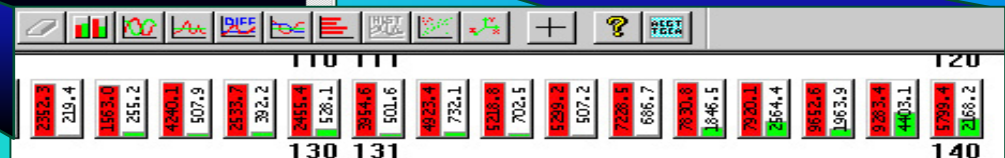


- Rapidly detect the composition and diversity of microbes in an environmental sample
- Massive parallelism - 550,000 probes in a 1.28 cm² array
- all 9,900 species in 16S rDNA database
- Single nucleotide mismatch resolution



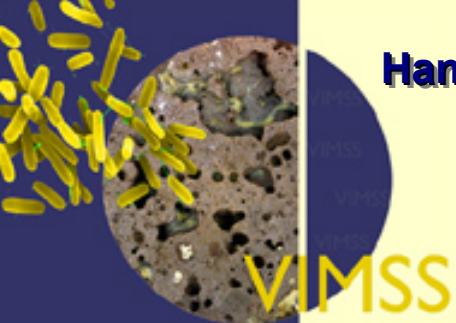
cctagcatgCattctgcata
 cctagcatgGattctgcata

MATCH
MISMATCH



Hanford 100H Chromium-contaminated site

- 16S rDNA genes were only successfully amplified from sediments that had been stimulated with lactate, HRC, or MRC. Further PCR analyses using group specific primers indicated the presence of *Geobacter* sp. and *Desulfovibrio* sp. These amplicons were also assayed with a 16S microarray (Affymetrix GeneChip). The microarray indicated that all five subgroups within the proteobacteria were present, including 2 species of *Desulfovibrio*.
- The biostimulated sediments reduced Cr(VI) from 1000 ppm to non-detect in 1 week.



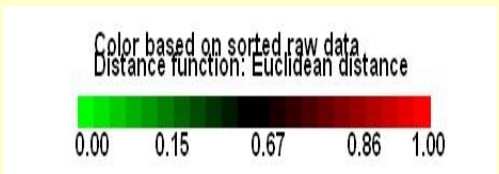
Exp1
Exp5

- 1-Enterococcus_group human infant D8E
- 2-Vr.pantothenicus_subgroup Salibacillus sp. YIM-kkny16
- 3-Brevibacillus agri
- 4-Fillifactor villosus
- 5-Alkalibacterium olivoapovliticus
- 6-B.badius_subgroup Bacillus sp. SAFN-028
- 7-C.litusebürense Clostridium sp. 48
- 8-Planococcus southpolaris
- 9-Bacillus sphaericus
- 10-Bacillus galactosidasius
- 11-Bacillus firmus
- 12-Eub.nodatum_subgroup oral clone P2PB_46 P3
- 13-C.aminobutyricum_subgroup clone IA-19
- 14-Environmental_clone_wchb1-31_group clone SJA-108.
- 15-Alvinella_pompejana_symbionts_subgroup epsilon clone 33-FL76B98
- 16-Mrb.hydrocarbonoclasticus_group Marinobacter sp. MR-1
- 17-Achromatium_assemblage Crater Lake CL500-74
- 18-Sphingobacterium_group CAGY10 strain CAGY10
- 19-Av.avenae_subgroup Acidovorax sp. isolate EfNe1
- 20-Pol.cellulosum_subgroup clone BCTP37
- 21-Mrb.hydrocarbonoclasticus_group clone:pBSB4.12
- 22-Mag.gryphiswaldense_subgroup clone LPB60
- 23-Ectothiorhodospira_group yard-trimming-compost clone S-20
- 24-Ral.eutropha_group soil clone Tc119-F01
- 25-Sphingobacterium_group Wuba47
- 26-Hlm.subglaciescola_subgroup 'A1-UMH 8% pond'
- 27-Rcy.tenuis_subgroup beta clone 8-5
- 28-Lew.nigricans_subgroup clone s22
- 29-Flx.sancti_subgroup clone CC14
- 30-Environmental_clone_wcha2-26_subgroup SR1 clone SRB52
- 31-Petrobacter succinimandens
- 32-Dsv.halophilus_subgroup sp. clone group A7hq
- 33-Saprospira grandis
- 34-Dsv.africanus_subgroup clone SHA-41

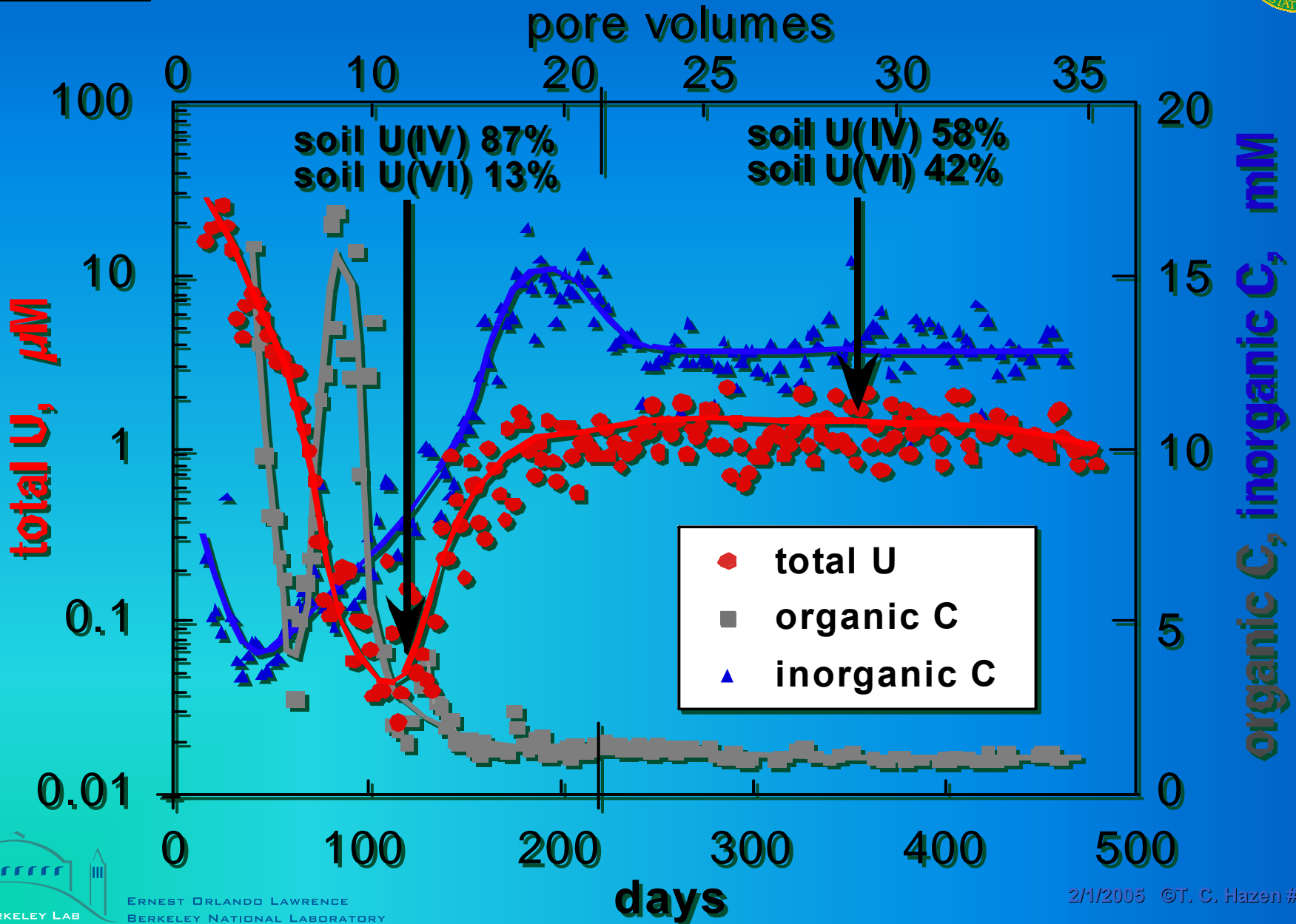
- Enterococcus_Grp, Gram.(+)
- B. megaterium_Grp, Gram.(+)
- Brevibac. Grp, Gram.(+)
- Eubacteria, Gram.(+)
- Carnobacteria, Gram.(+)
- B. megaterium, Gram.(+)
- Eubacteria, Gram.(+)
- B. sphaericus_Grp, Gram.(+)
- B. sphaericus_Grp, Gram.(+)
- B. megaterium_Grp, Gram.(+)
- B. megaterium_Grp, Gram.(+)
- Eubacteria, Gram.(+)
- Eubacteria, Gram.(+)
- Environmental_clone, Gram.(+)
- ε-proteobacteria, Gram.(+)
- γ-proteobacteria, Gram.(+)
- γ-proteobacteria, Gram.(+)
- Sphingobacteria, Gram.(+)
- β-proteobacteria, Gram.(+)
- δ-proteobacteria, Gram.(+)
- γ-proteobacteria, Gram.(+)
- α-proteobacteria, Gram.(+)
- γ-proteobacteria, Gram.(+)
- β-proteobacteria, Gram.(+)
- Sphingobacteria, Gram.(+)
- γ-proteobacteria, Gram.(+)
- β-proteobacteria, Gram.(+)
- Lewinella, Gram.(+)
- Lewinella, Gram.(+)
- Environmental_clone, Gram.(+)
- β-proteobacteria, Gram.(+)
- δ-proteobacteria, Gram.(+)
- Lewinella, Gram.(+)
- δ-proteobacteria, Gram.(+)

Exp1 = Control

Exp5 = lactate stimulated



Reoxidation of Bioreduced Uranium!!!



Reoxidation of Bioreduced Uranium is Microbial!!!



Bacterial diversity estimates based on 16S T-RFLP analysis.

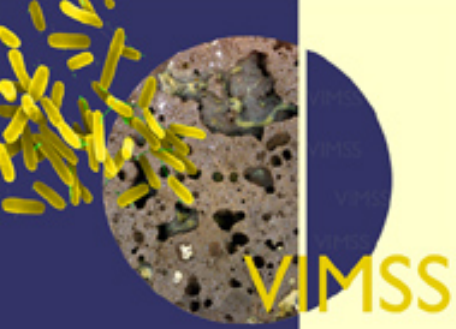
Sample	Richness	Evenness	Diversity‡
Area 2 sediment	108 ± 7a	0.77 ± 0.01a	3.59 ± 0.07a
Net U reduction	112 ± 7a	0.80 ± 0.01b	3.75 ± 0.03a
Net U oxidation	111 ± 9a	0.80 ± 0.00b	3.74 ± 0.06a

‡ Shannon diversity index. Same letter denotes no significant difference ($p > 0.05$) $n=3$.

Representative organism	Group	Area 2	Reduction	Oxidation
<i>Geothrix fermentans</i>	Nitrospina	1.65a (67)	3.37b (100)	3.36b (100)
<i>Pseudomonas spinosa</i>	β -proteobacteria	1.93a (17)	2.92b (91)	2.82b (83)
<i>Geobacter metallireducens</i>	δ -proteobacteria	2.4a (69)	3.35b (100)	3.34b (100)
<i>Geobacter arculus</i>	δ -proteobacteria	1.58a (9)	3.15b (65)	3.23b (96)
<i>Environmental Clone SHA-18</i>	Fibrobacter-Acidobac.	2.25a (17)	2.32a (17)	3.3b (100)
<i>Desulfovibrio africanus</i>	δ -proteobacteria	2.07a (18)	2.22a (14)	3.11b (86)

Phenomics, Proteomics & Lipidomics

- **Phenomics - phenotype expression & physiology**
 - **Phenotypic microarrays**
 - **Real-time analyses using FTIR, etc**
- **Proteomics - protein expression**
 - **ICAT - Isotope-Coded Affinity Tags**
 - **DIGE - Differential In-Gel Electrophoresis**
- **Lipidomics - lipid/fatty acid expression especially as it relates to membranes and cell walls**
 - **FAME - Fatty Acid Methyl Ester**
 - **PLFA - Phospholipid Fatty Acid**

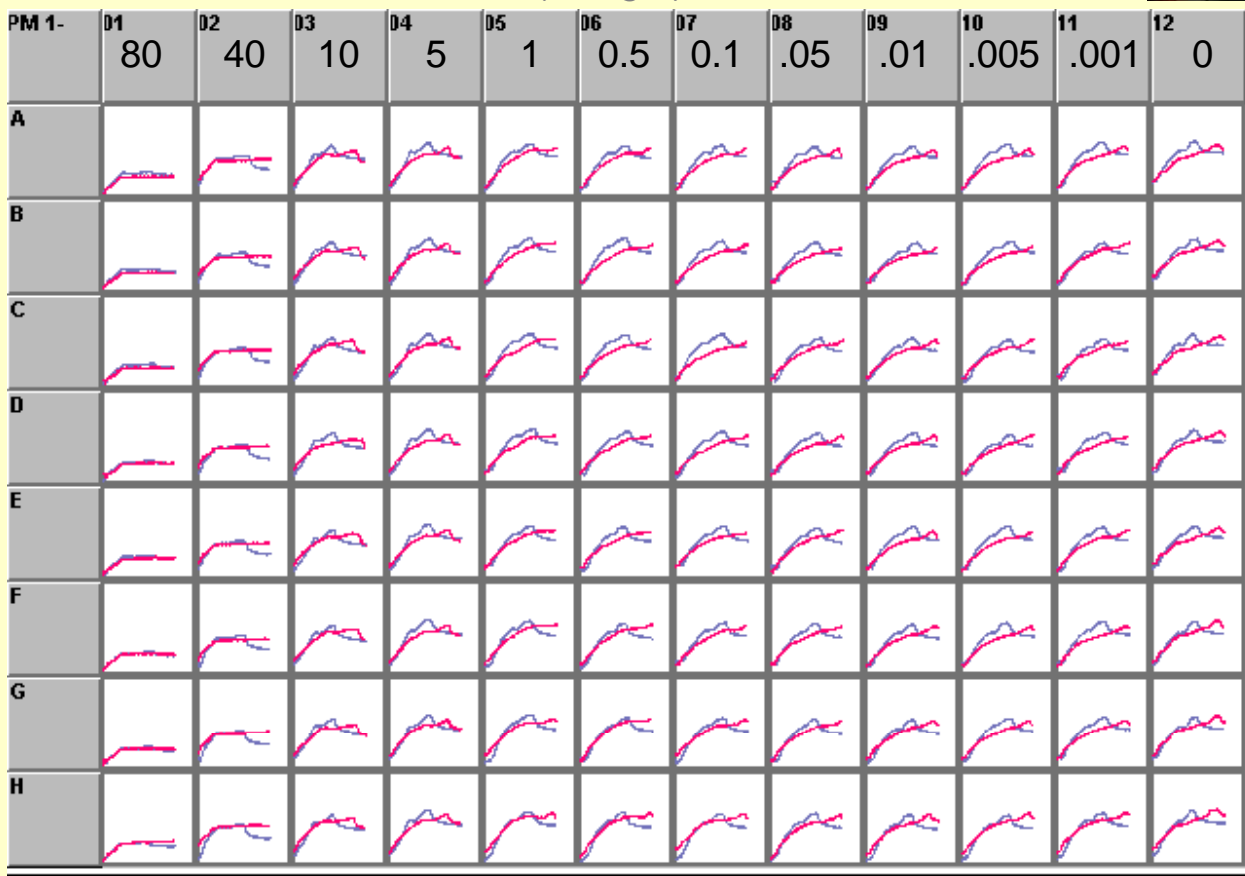


Phenotypic Microarray

Omnilog System - 2000 assays,
50 96-well plates at one time



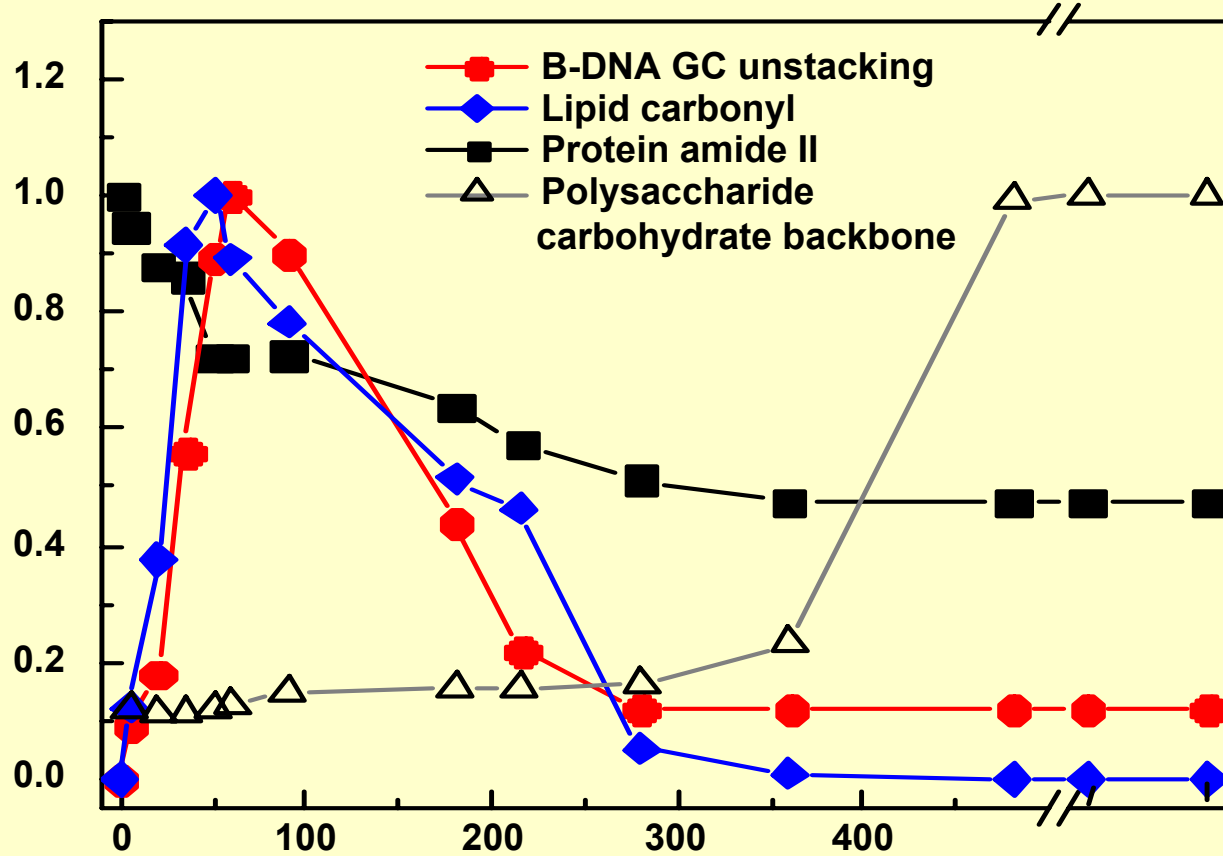
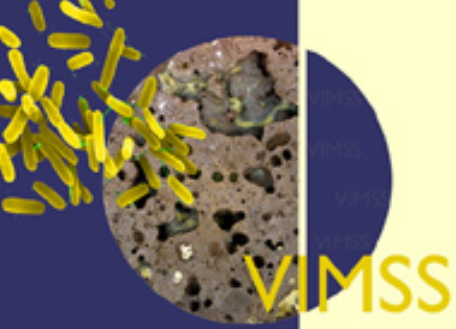
Zn Concentration in LS4D (in mg/L).



Desulfovibrio vulgaris, Hildenborough (blue trace)
DP9 strain from Lake DePue sediments (pink trace)



FTIR Profiling

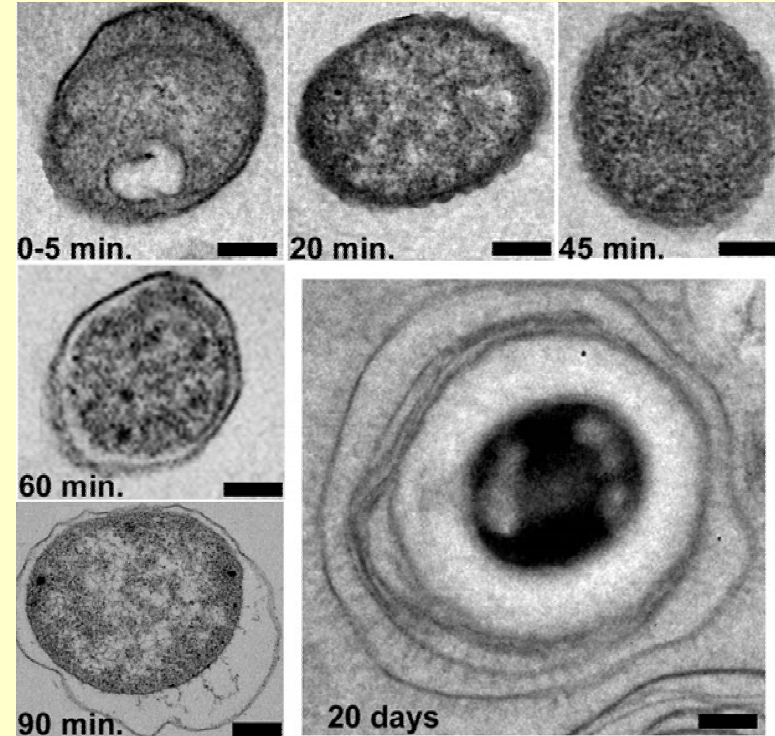
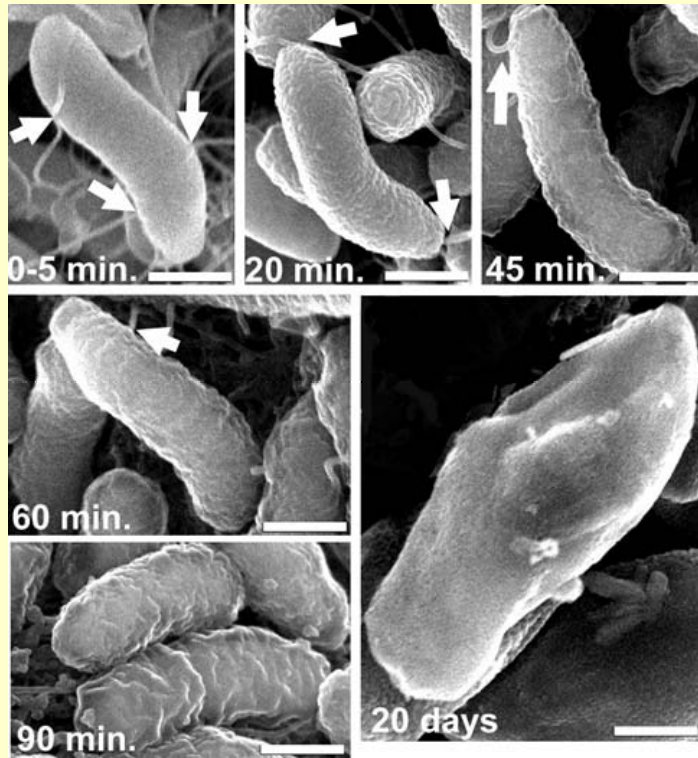


- **Synchrotron FTIR time course of infrared absorption intensity, indicative of oxidative stress levels in different biologically important molecules in *Desulfovibrio vulgaris* after exposure to atmospheric oxygen.**
- **Also found signatures for Cytochrome B hemes**



Electron Microscopy

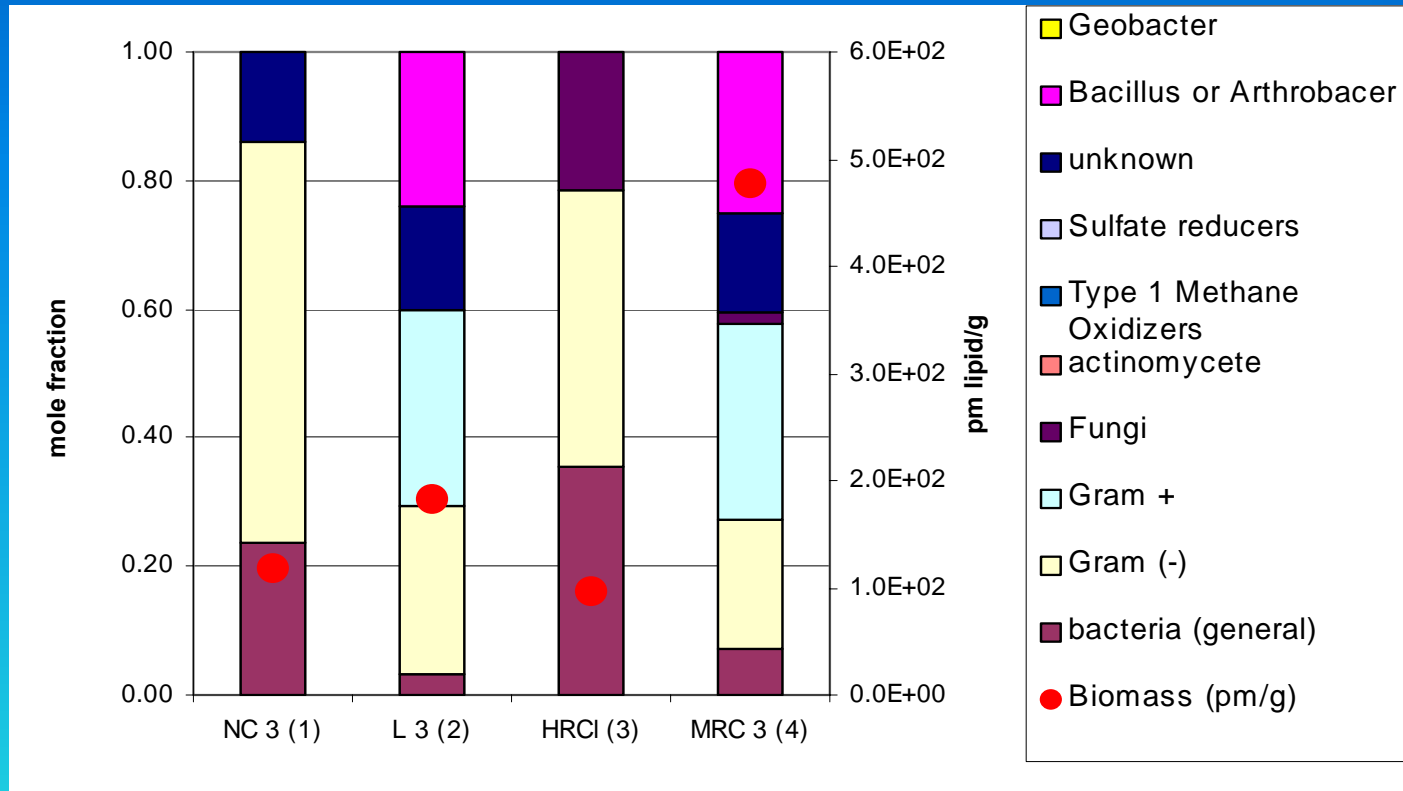
VIMSS



Electron microscopic images of *D. v.* under oxygen exposure



PLFA Analysis of Remediation-Based Enrichment of Hanford Sediments



•NC = no carbon; L = lactate; HRC = hydrogen release compound; MRC = metal remediation compound.

•All enrichments were exposed to 1000 ppb Cr(VI).

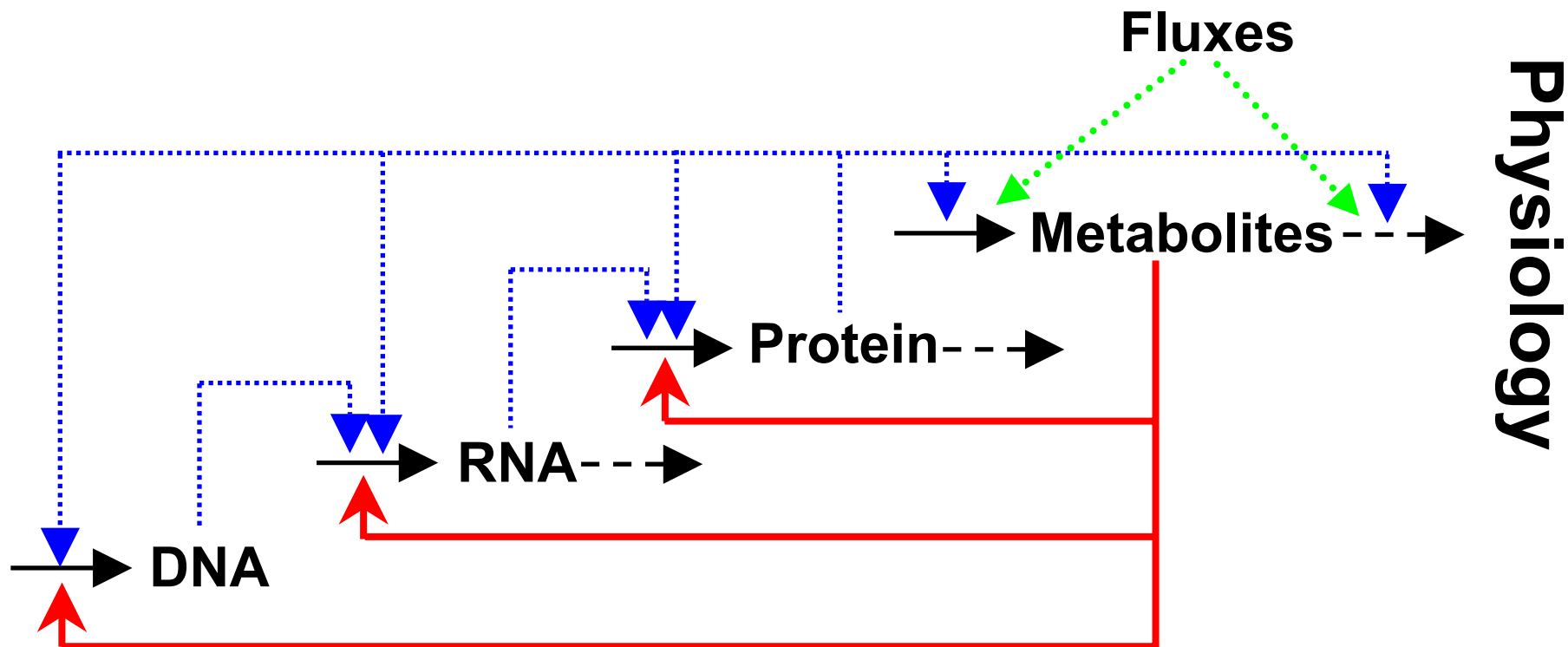
•Left vertical axis is fractions of constituent microorganisms, and right vertical axis is viable biomass, picomole/g

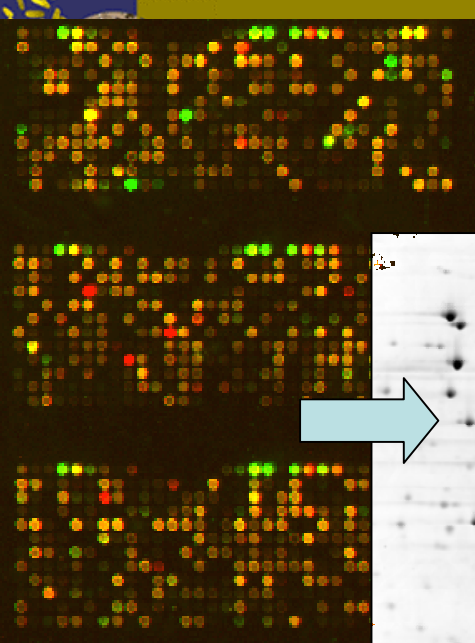
Metabolomics & Fluxomics

- **Metabolomics- metabolite expression**
 - hydrophilic interaction chromatography technique coupled to MS/MS detection and CE-MS methods for amino acids, nucleosides, nucleotides, organic acid CoAs, redox cofactors and the metabolic intermediates of glycolysis, TCA, and pentose phosphate pathway, etc.
- **Fluxomics - studies of rate changes in metabolites**
 - Same techniques as above
- **These two areas are the newest and least developed, lots of development needs, but lots of breakthrough potential.**

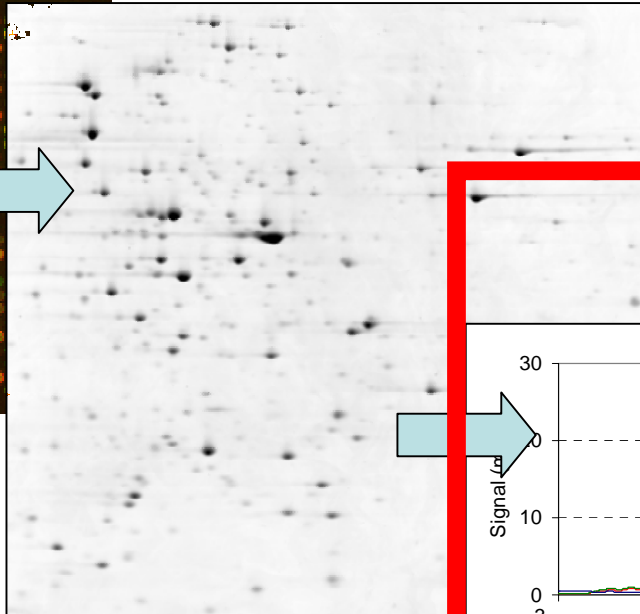


The importance of metabolites and fluxes

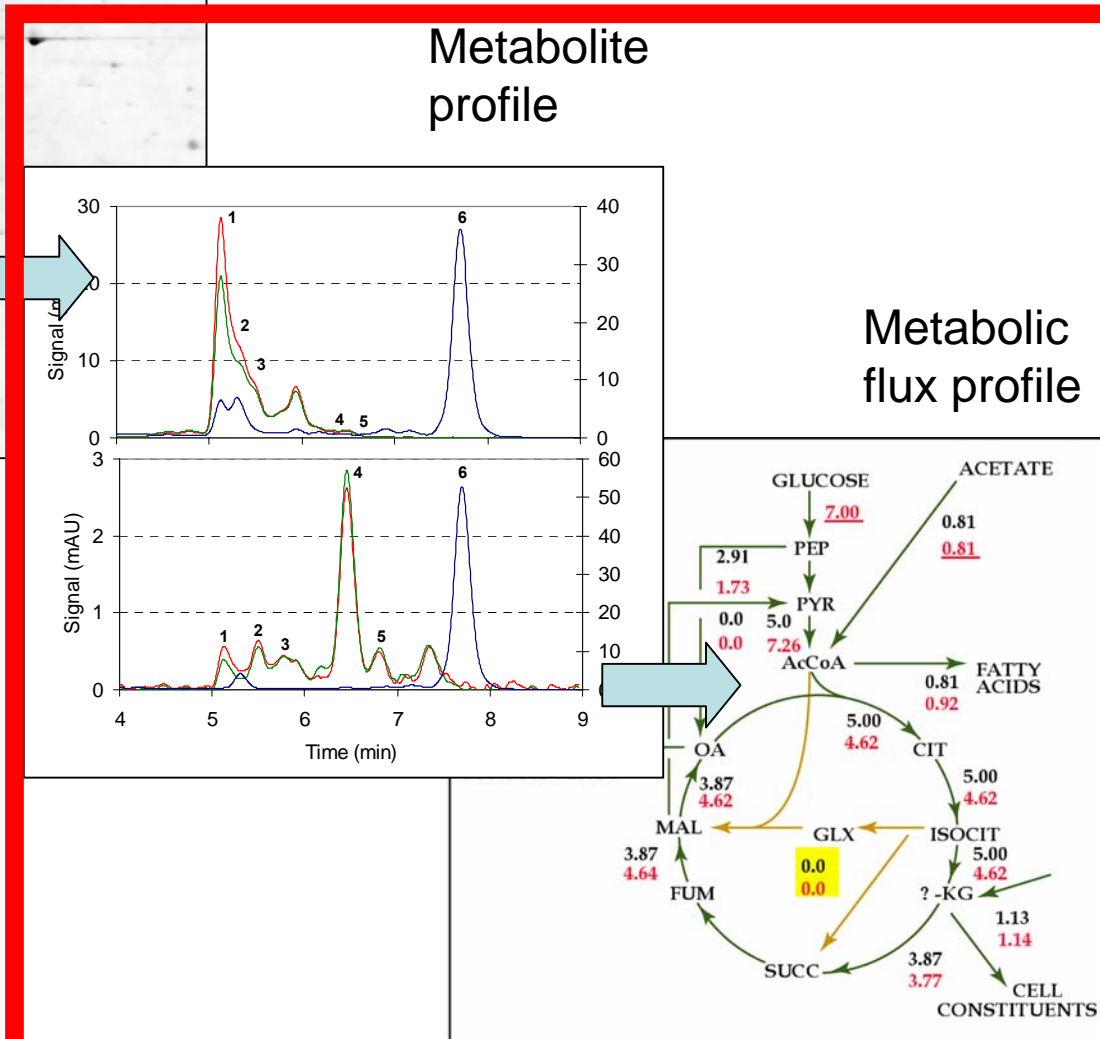




Transcript profile



Protein profile





Applications of Metabolomics

- Assess gene function and relationships to phenotypes
- Understand metabolism and predict novel pathways
- Assess effects of genetic and metabolic engineering
- Assess the effect of environment stress changes that lead to changes in gene expression and metabolite levels

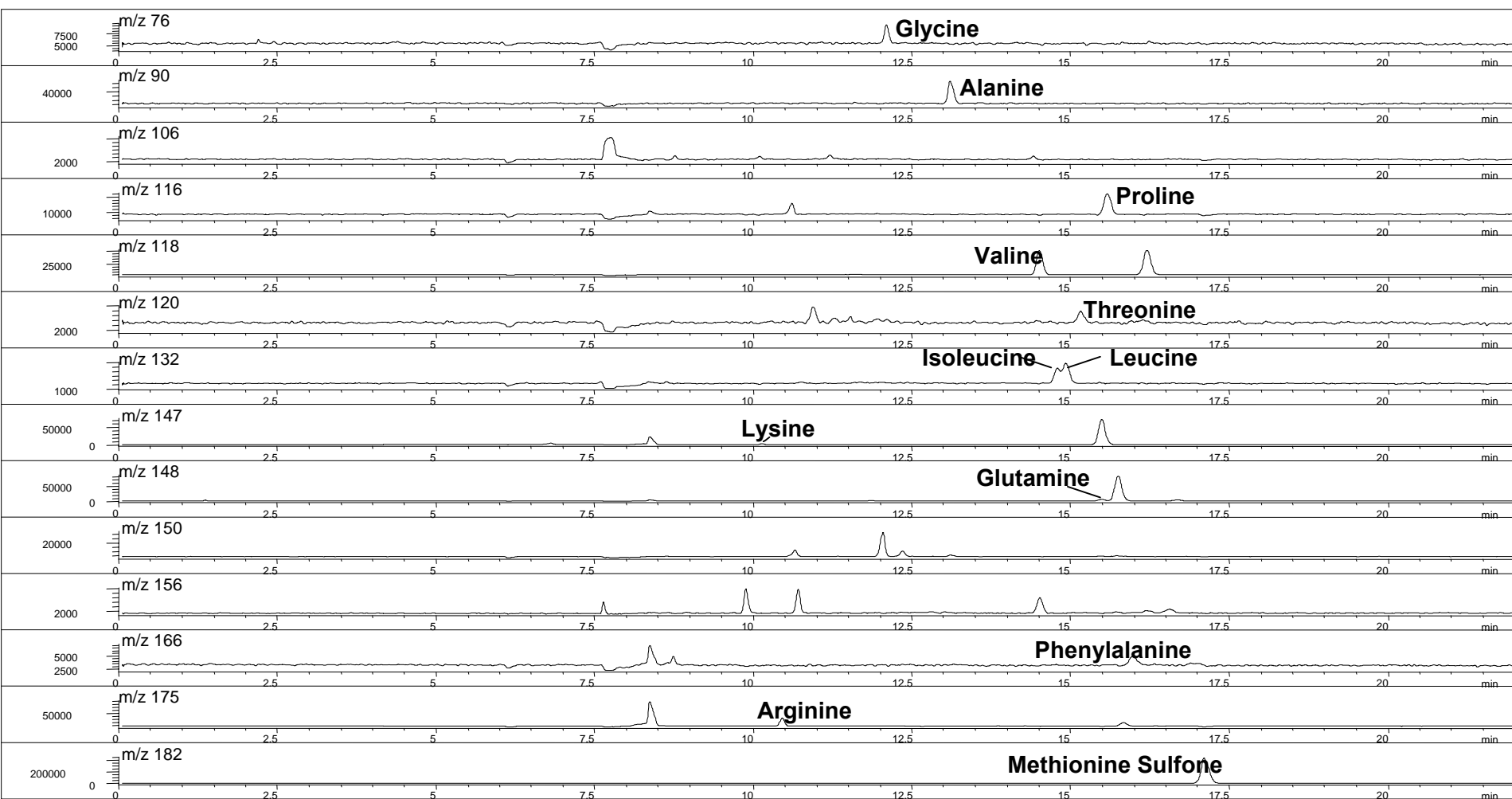


Detection and characterization

-
- The diagram consists of a central list of five detection and characterization methods. To the left of the list is a vertical blue arrow pointing upwards, labeled 'Increasing sensitivity'. To the right is a vertical green arrow pointing downwards, labeled 'Increasing specificity'. The methods are listed from top to bottom: Radiography, FID (flame ionization detection), FT-IR (Fourier transform infrared spectroscopy), Mass Spectrometry (several different types), and NMR (nuclear magnetic resonance). The 'Mass Spectrometry' item is highlighted in red text.
- Radiography
 - FID (flame ionization detection)
 - FT-IR (Fourier transform infrared spectroscopy)
 - Mass Spectrometry (several different types)
 - NMR (nuclear magnetic resonance)



D. vulgaris amino acid profile





Metabolic flux analysis

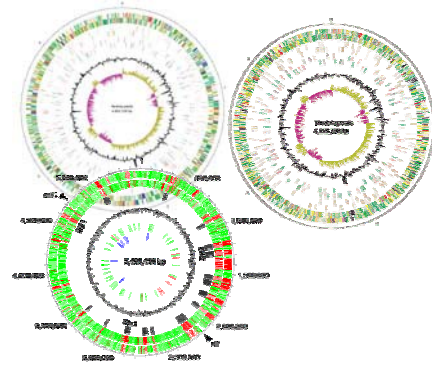
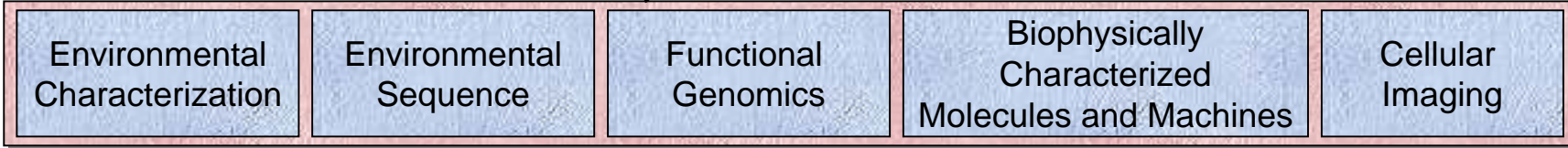
- Rates of production and consumption of metabolites
- Useful for confirming the presence/absence of metabolic pathways
- Useful for assessing potential bottlenecks in metabolic pathways
 - optimization of primary/secondary metabolite production
 - optimization of engineered organism for environmental cleanup

Bioinformatics

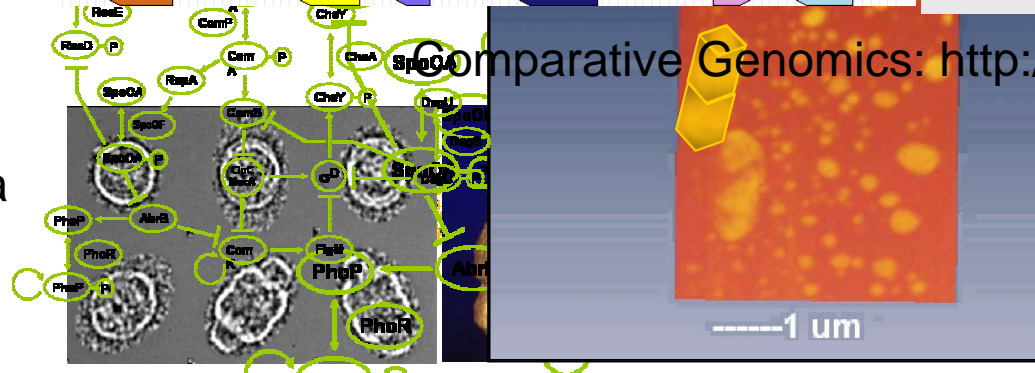
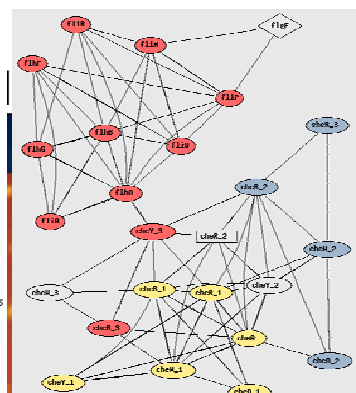
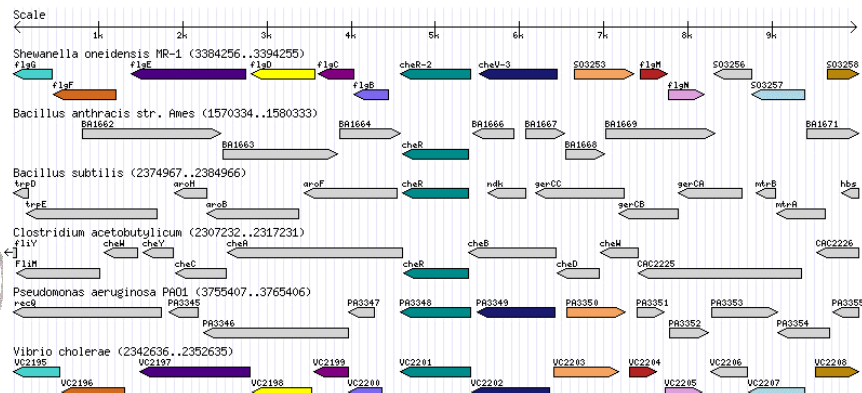
- **Annotation of sequences**
- **Comparative genomics**
- **Integration from Biomolecules to Ecosystems**
- **Models for environmental biotechnology verification and prediction**

***Models, Statistics, and Database Analyses
Galore needed for these new areas***

Centralized, Cross-Referenced Databases



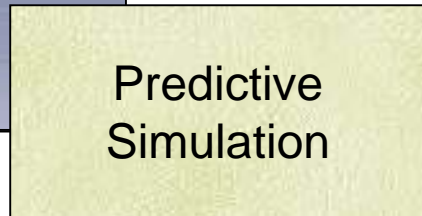
Sequence Data



Microscopy of Molecular Machines
 Deduction of pathways, modules and dynamics
 Cell Imaging



Comparative Genomics: <http://...>



Predictive Simulation



Select Genome(s)

- Favorites
- Escherichia coli K12
- Bacillus subtilis
- Shewanella oneidensis MR-1
- Desulfuromonas spp.
- Desulfovibrio vulgaris
- Desulfovibrio desulfuricans G20
- Geobacter metallireducens
- Geobacter sulfurreducens PCA
- Bdellovibrio bacteriovorus HD100
- Desulfotalea psychrophila
- All
- Archaea
- Bacteria
-
- Bacteria
- Actinobacteria
- Tropheryma whippelii str. Twist
- Tropheryma whippelii TN08/27
- Corynebacterium diphtheriae

[\[help\]](#)

Desulfovibrio vulgaris

Lineage

Bacteria; Proteobacteria; delta/epsilon subdivisions; Deltaproteobacteria; Desulfovibrionales; Desulfovibrionaceae; Desulfovibrio; Desulfovibrio vulgaris

Genomic:

16S (small subunit) ribosomal RNA: 5
 23S (large subunit) ribosomal RNA: 5
 5S ribosomal RNA: 6
 Other non-coding RNA: 2
 Protein-coding gene: 3396
 Pseudogene derived from a protein-coding gene: 3
 Pseudogene derived from an RNA gene: 1
 Transfer RNA: 67

Plasmid:

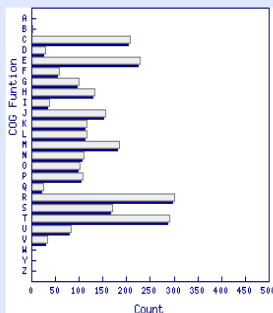
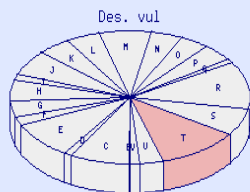
Protein-coding gene: 152

COG

2340 unique proteins assigned

2590 total count of COG Functions

A: 0 **Red:** the fraction of genes in the COG function is ranked in the top 5% among all genomes.
 B: 2
 C: 208
 D: 29 **Blue:** the fraction of genes in the COG function is ranked in the bottom 5% among all genomes.
 E: 229
 F: 58
 G: 100
 H: 132
 I: 38
 J: 156
 K: 117
 L: 117
 M: 185
 N: 109
 O: 101
 P: 107
 Q: 25
 R: 300
 S: 170
 T: 290
 U: 83
 V: 34
 W: 0
 Y: 0
 Z: 0



A: RNA processing and modification
 B: Chromatin structure and dynamics
 C: Energy production and conversion
 D: Cell division and chromosome partitioning
 E: Amino acid transport and metabolism
 F: Nucleotide transport and metabolism
 G: Carbohydrate transport and metabolism
 H: Coenzyme metabolism
 I: Lipid metabolism
 J: Translation, ribosomal structure and biogenesis
 K: Transcription
 L: DNA replication, recombination, and repair
 M: Cell envelope biogenesis, outer membrane
 N: Cell motility and secretion
 O: Posttranslational modification, protein turnover, chaperones
 P: Inorganic ion transport and metabolism
 Q: Secondary metabolites biosynthesis, transport, and catabolism
 R: General function prediction only
 S: Function unknown
 T: Signal transduction mechanisms
 U: Intracellular trafficking and secretion
 V: Defense mechanisms
 W: Extracellular structures
 Y: Nuclear structure
 Z: Cytoskeleton

- >130 full sequenced genomes

- Summary of functional capabilities

- Easy access to sequence and annotations

- Automated annotation of new genomes

- Critica/Glimmer pipeline

- New tools for

- Go assignment

- Operon/Regulon

Prediction

- Community annotation tools

- Analysis workbench



Collection of organismal Info.

Bacterial Species	Metabolism	Habitat	Stress Responses					Notes
			S	T	C	D	M	
Actinobifida spp.	O	soil	+					
Agmenellum quadruplicatum	PO				+			
Alcaligenes spp.	C				+			
Alicyclobacillus spp.	O	soil	+					
Anacystis nidulans	PO			+	+	+	+	
Anaerobacter polyendosporus	O	herbivore guts	+					
Arthromitus spp.	M	guts of wood-eating insects	+					
Azotobacter vinelandii	O	soil, water	+	+	+	+	+	
Bacillus halodurans	O	salt lakes	+	+				
Bacillus subtilis	O	soil, water	+	+	+	+	+	
Camnicella sporogenes	M	deep-sea	+					
Campylobacter jejuni	O	mammalian intestine	+			+		
Chlorobium limicola	PO	mud, stagnant water		+				
Clostridium acetobutylicum	M	soil	+				+	
Clostridium perfringens	M	soil, marine sediments	+				+	
Deinococcus radiodurans	O			+				
Desulfotomaculum spp.	M		+				+	
Gelria glutamica spp.	Me	methanogenic granular sludge	+					
Geobacillus stearothermophilus	O	ocean sediment, heating compost	+					
Haemophilus influenzae	O	mammalian respiratory tract		+	+			pathogenic

- Beginning to relate genotype to microbial lifestyle and phenotypes.

Similar Responses Different Environments

Bacterial Species	Metabolism	Habitat	Stress Responses					Notes
			S	T	C	D	M	
Actinobifida spp.	O	soil	+					
Agmenellum quadruplicatum	PO				+			
Alcaligenes spp.	C				+			
Alicyclobacillus spp.	O	soil	+					
Anacystis nidulans	PO			+	+	+	+	
Anaerobacter polyendosporus	O	herbivore guts	+					
Arthromitus spp.	M	guts of wood-eating insects	+					
Azotobacter vinelandii	O	soil, water	+	+	+	+	+	
Bacillus halodurans	O	salt lakes	+	+				
Bacillus subtilis	O	soil, water	+	+	+	+	+	
Camnicella sporogenes	M	deep-sea	+					
Campylobacter jejuni	O	mammalian intestine	+			+		
Chlorobium limicola	PO	mud, stagnant water		+				
Clostridium acetobutylicum	M	soil	+				+	
Clostridium perfringens	M	soil, marine sediments	+				+	
Deinococcus radiodurans	O			+				
Desulfotomaculum spp.	M		+				+	
Gelria glutamica spp.	Me	methanogenic granular sludge	+					
Geobacillus stearothermophilus	O	ocean sediment, heating compost	+					
Haemophilus influenzae	O	mammalian respiratory tract		+	+			pathogenic

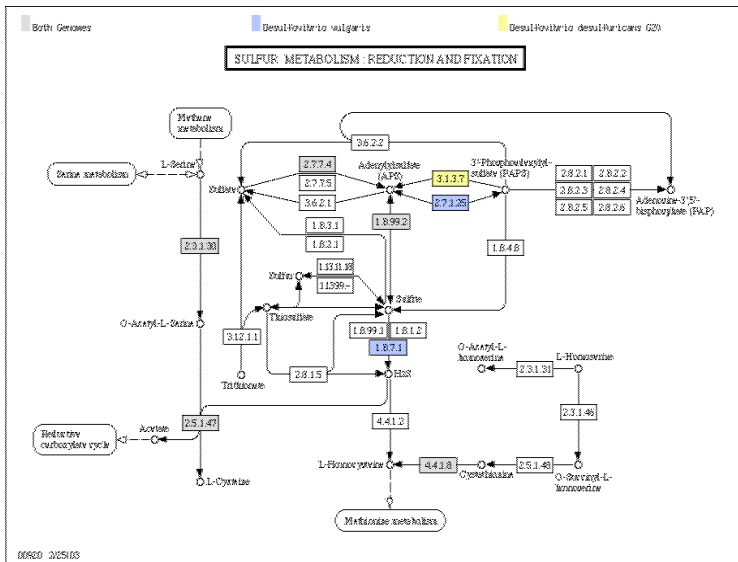
Table 1: Bacterial species vs. stress responses: **S**, sporulation; **T**, natural transformability; **C**, competence for DNA uptake; **D**, degradative enzyme synthesis; **M**, motility/chemotaxis.

Metabolism: O, organotroph; C, chemolithotroph; M, mixotroph; Me, methanotroph; PO, photoorganotroph.

Sources: Bergey 1994; Hurst and Gould 1983; Stewart 1992; Priest 1993; Lorenz and Wackernagel 1994; Bergey 1994; Dubnau 1999; Atlas and Bartha 1998; Madigan et al. 2001; *International Journal of Systematic and Evolutionary Microbiology* (Various issues)



Metabolic Pathway Information



EC: 2.7.7.4 Sulfate adenylyltransferase.

Purine metabolism
Selenoamino acid metabolism
Sulfur metabolism

Desulfovibrio vulgaris

Desulfovibrio desulfuricans G20

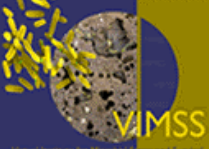
Genes

- | | | | |
|---|--|--|------|
| 1 | G O D H S B VIMSS394579 : . | <i>Desulfovibrio desulfuricans</i> G20 | cart |
| | COG2046, ATP sulfurylase (sulfate adenylyltransferase)
IPR002650, ATP-sulfurylase | | |
| 2 | G O D H S B VIMSS206736 : sat DVU1295,ORF01081 | <i>Desulfovibrio vulgaris</i> cart | |
| | TIGR:sulfate adenylyltransferase
COG2046, ATP sulfurylase (sulfate adenylyltransferase)
IPR002650, ATP-sulfurylase | | |
| 3 | G O D H S B VIMSS207016 : DVU1566,ORF01525 | <i>Desulfovibrio vulgaris</i> cart | |
| | TIGR:phosphoadenosine phosphosulfate reductase, putative
COG175, 3'-phosphoadenosine 5'-phosphosulfate sulfotransferase (PAPS reductase)/FAD synthetase and related enzymes
IPR002500, Phosphoadenosine phosphosulfate reductase | | |
| 4 | G O D H S B VIMSS208715 : engA DVU3194,ORF04289 | <i>Desulfovibrio vulgaris</i> cart | |
| | TIGR:GTP-binding protein EngA
COG1160, Predicted GTPases
IPR005289, GTP-binding domain
IPR005225, Small GTP-binding protein domain
IPR002917, GTP-binding protein, HSR1-related | | |

Rapid assessment of comparative metabolism

Now being linked to molecular profiling data

Now being linked to Flux-Balance Analysis.



Primary Data Management

- All the omics we've talked about to day...
- All the Phenomics...
- All the imaging...

- Are slowly being linked into this infrastructure.
 - Requires development of specialized informatics for each data type to score significant responses.

- First open "Library of Microbial Ecology and Physiology".

The Virtual Institute of Microbial Stress and Survival

<http://vimss.lbl.gov>

Application Goals:

To understand bacterial stress-response to the unique stressors in metal/radionuclide contamination sites

Turn this understanding into a quantitative, data-driven model for exploring policies for natural and biostimulatory bioremediation

To implement proposed policies in the field and compare results to model predictions

Close the experimental/computation cycle by using discrepancies between models and predictions to drive new measurements and construction of new models

Science Goals:

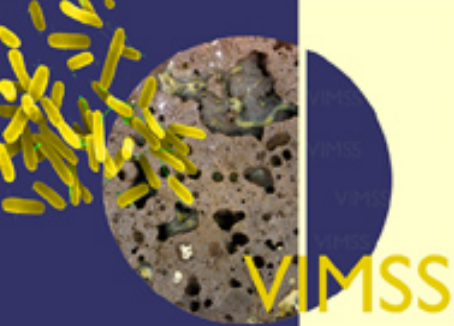
Compare physiological and molecular response of three target microorganisms to environmental perturbation

Deduce the underlying regulatory pathways that control these responses through analysis of phenotype, functional genomic, and molecular interaction data

Use differences in the cellular responses among the target organisms to understand niche specific adaptations of the stress and metal reduction pathways

From this analysis derive an understanding of the mechanisms of pathway evolution in the environment

Ultimately, derive dynamical models for the control of these pathways to predict how natural stimulation can optimize growth and metal reduction efficiency at field sites



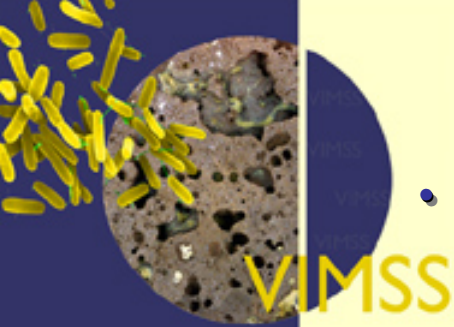
U Washington

OAK RIDGE NATIONAL LABORATORY

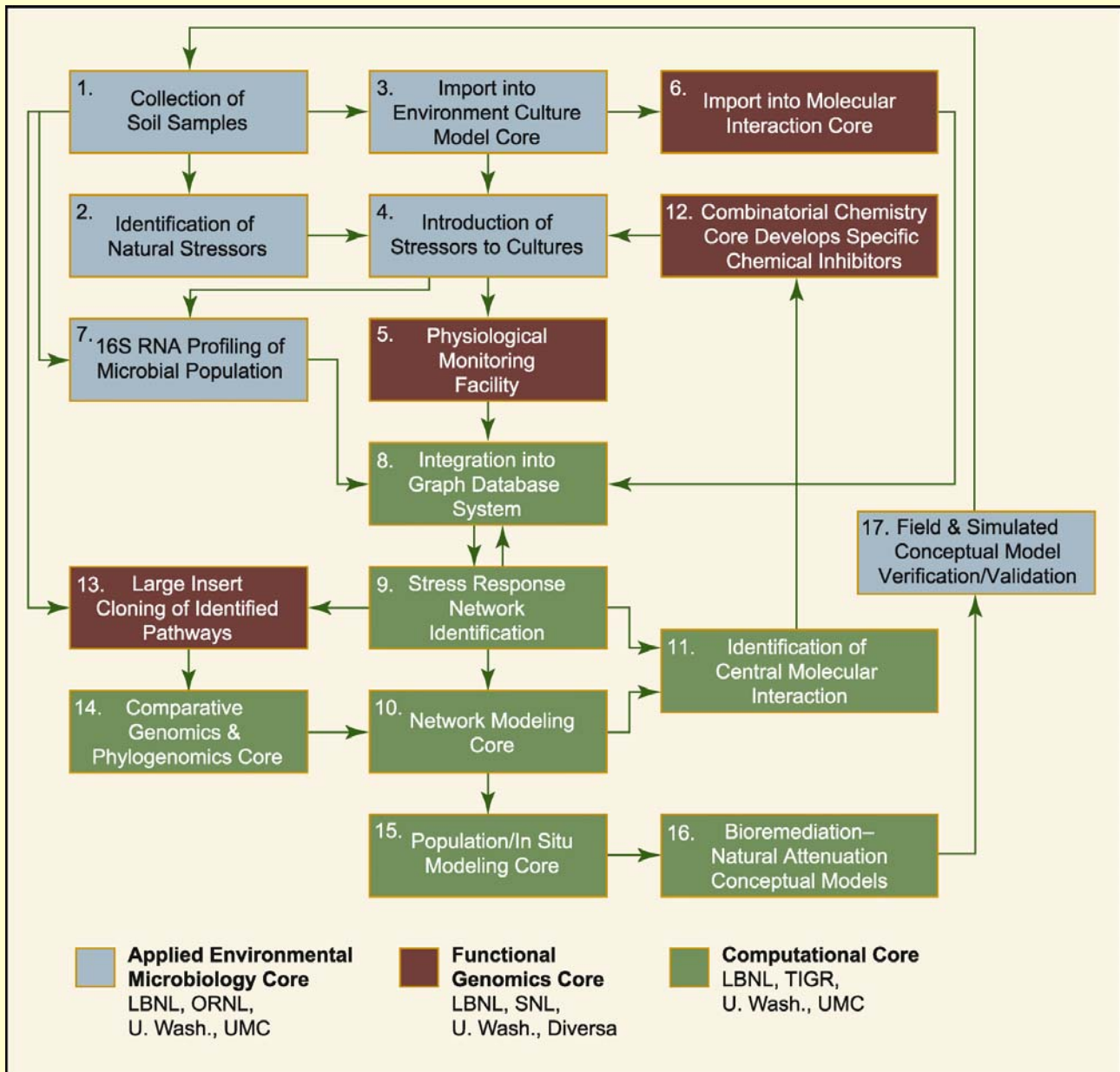
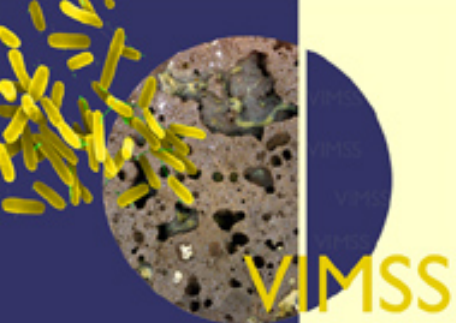


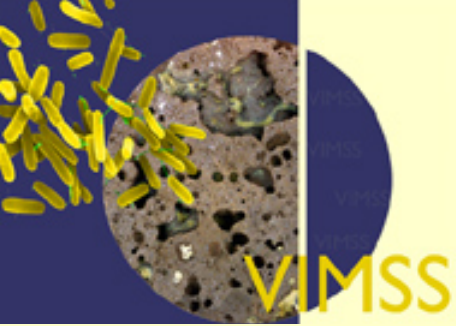
Organisms

- Primary organism:
 - ***Desulfovibrio vulgaris***
 - δ -proteobacteria,
 - “Anaerobic”
 - SRB, uses sulfate and sulfite as terminal electron acceptors for growth.
 - Oxygen, iron, nitrite, chromate, and U(VI) can be reduce but growth is not observed.
 - Does not reduce nitrate
 - Has a megaplasmid containing nitrogen fixation genes
 - Has a number of interesting pathogenicity factors: type III-secretion, adhesins, hemagglutinin
 - common in eutrophic environments, much less known about this organism
- Comparison organisms:
 - ***Shewanella oneidensis* MR-1**
 - γ -proteobacteria
 - “facultative anaerobe”
 - Reduces nitrate
 - Does not have nitrogenase
 - more common in oligotrophic environments
 - ***Geobacter metallireducens***
 - δ -proteobacteria,
 - “Anaerobic”
 - More common in oligotrophic environments
- Stressors: O₂, metals, TEAs, PO₄, nitrate, nitrite, pH, salt, heat



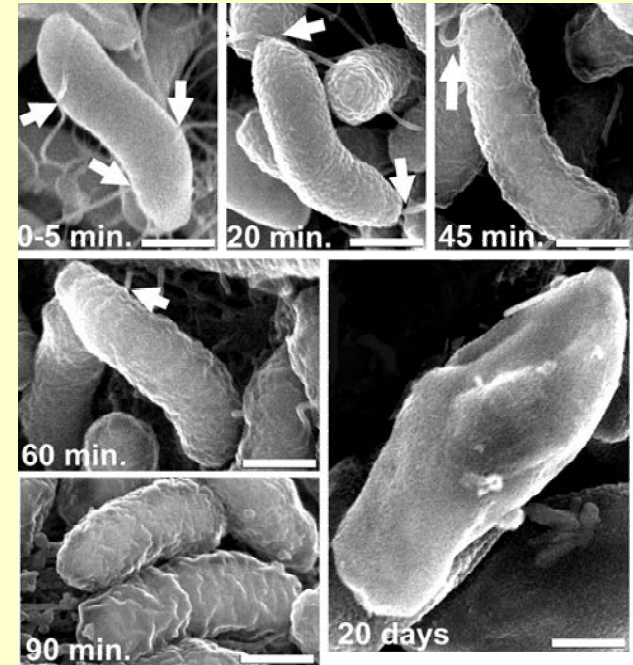
Design of Project





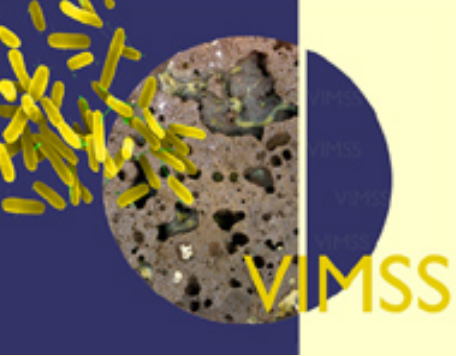
O₂ Stress in *Desulfovibrio vulgaris*

nSig	nUarray	p	GOName
26	142	0.0002	transcription termination
4	6	0.0008	4-diphosphocytidyl-2C-methyl-D-erythritol synth
4	6	0.0008	O-acetyltransferase activity
5	11	0.0017	primary active transporter activity
5	11	0.0017	cell wall
11	51	0.0043	proline-tRNA ligase activity
2	2	0.0082	purine base catabolism
2	2	0.0082	adenine catabolism
2	2	0.0082	phenylalanyl-tRNA aminoacylation
2	2	0.0082	prolyl-tRNA aminoacylation
2	2	0.0082	nucleoside triphosphate metabolism
14	77	0.0109	N-acetyltransferase activity
14	77	0.0109	phosphoenolpyruvate-dependent sugar phosph
2	3	0.0233	acyl-CoA or acyl binding
2	3	0.0233	cobalamin [5'-phosphate] synthase activity
2	3	0.0233	chloramphenicol O-acetyltransferase activity
2	3	0.0233	transferase activity, transferring glycosyl groups
2	3	0.0233	transferase activity, transferring hexosyl groups

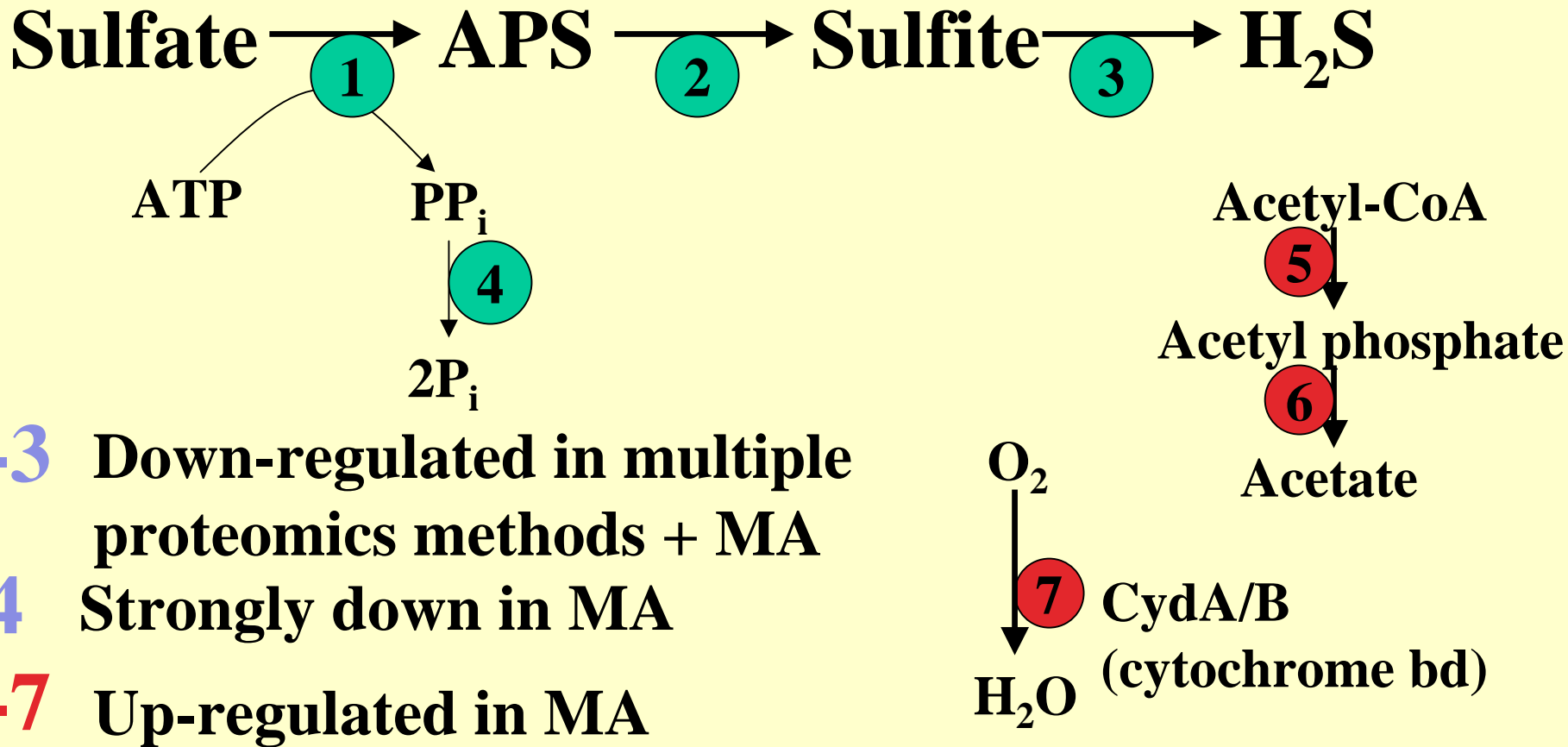


Fischer exact test of GO terms for DE genes as measured by microarrays at 2h revealed numerous up-regulated genes in cell wall and polysaccharide metabolism. Candidates for EPS activity.

Also– why all the sugar activity given D.v. doesn't use hexoses for cell growth?



Down-regulation of Sulfate Reduction Pathway



O₂ Stress: Summary of Results

VIMSS

- Cell wall and various sugar metabolism categories are upregulated in response to O₂ stress.
- This is consistent with the EPS activity observed in the electron micrographs, giving us an initial seed group for elucidating and further characterizing those pathways.
- Apparent down-regulation of the sulfate-reduction pathway observed in MA, and confirmed by several proteomics methods.
- Additional evidence suggests this may be an actual O₂ related change (rather than growth effect) is that pyrophosphatase is significantly down-regulated (pyrophosphate is a byproduct of the second step in sulfate reduction), and several genes involved in substrate-level phosphorylation of ADP are up-regulated (phosphate acetyltransferase and acetate kinase).
- The attractive speculation resulting from all of this is that Dv may be down-regulating sulfate reduction to increase the amount of reducing power available for O₂ reduction.
- One mechanism for such reduction would be the *cydAB* operon (cytochrome bd) recently shown to be essential for oxygen consumption in the strict anaerobe *Bacteroides fragilis*. We note that both *cydA* and *cydB* are significantly up-regulated at 2 hours after air sparging compared to t=0.



Baughn AD, Malamy MH. Nature. (2004) The strict anaerobe *Bacteroides fragilis* grows in and benefits from nanomolar concentrations of oxygen. 427(6973):441-4.

Summary

- **Environmental Biotechnology promises: significant cleanup, safer, lower risk, natural, faster, and cheaper for even the most recalcitrant contaminants**
- **Understanding of subsurface biogeochemistry is critical for successful application and understanding risk**
- **Exciting new science discoveries (gene probes, microarrays, phenotypic microarrays, FTIR, stable isotopes, GFP, Lux reporter, carbon sequestration, adhesion-less, surfactant production, LIF-CPT)**
- **Manipulations of environments may be our only possibility for remediation of some sites (especially low concentrations e.g. endocrine disrupters)**
- **Integration of the latest areas in molecular environmental microbiology promises high-throughput of significant new breakthroughs in science and new technologies for biosustainability**

Dr. Terry C. Hazen tchazen@lbl.gov

Center for Environmental Biotechnology

www-esd.lbl.gov/CEB

Virtual Institute for Microbial Stress and Survival

vimss.lbl.gov

Environmental Remediation Technology Program

www-esd.lbl.gov/ERT

Ecology Department

www-esd.lbl.gov/ECO

Natural and Accelerated Bioremediation Research Program

www.lbl.gov/NABIR

Genomics:GTL Program

doegenomestolife.org