



eobacter Project
www.geobacter.org

Genomics-GTL Addressing DOE Environmental Science Needs in:

- *In situ* characterization of contaminated environments
- Understanding contaminant fate and transport
- Development of strategies for *in situ* control or remediation of contaminated sites

Tools From Genomes-GTL Applicable to DOE Environmental Restoration Needs

- ***In situ* characterization of contaminated environments**

Molecular (mRNA) analysis of the *in situ* metabolic state of the microbial community via whole-genome analysis to reveal:

- environmental stresses
- nutrient requirements

- **Understanding contaminant fate and transport**

Molecular (mRNA) analysis of *in situ* rates of metal reduction from levels of expression of key respiratory gene

- **Development of strategies for *in situ* control or remediation of contaminated sites**

Prediction of fate of contaminants under natural attenuation or engineered bioremediation options by coupling *in silico* microbial models with the appropriate hydrological and geochemical models



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Principle Investigators

Derek Lovley, UMASS, ecology, physiology, and biochemistry of *Geobacteraceae*

Maddalena Coppi, UMASS, genetics of *Geobacteraceae*

Stacy Ciufu, UMASS, bioinformatics, environmental genomics

Barbara Methe, TIGR, bioinformatics, DNA microarray gene expression

Pablo Pomposiello, UMASS, analysis of gene expression in response to stress

Steve Sandler, UMASS, microbial genetics

Cinthia Nunez, UMASS, microbial genetics, regulation of gene expression

Daniel Bond, UMASS, anaerobic microbial physiology, electron transfer to electrodes

Susan Childers, UMASS, microbial physiology, metabolic responses in *Geobacter*

Carol Giometti, Argonne National Laboratory, proteomics

Julia Krushkal, University of Tennessee, bioinformatics

Christophe Shilling and Bernard Palsson, Genomatica, *in silico* modeling

Analysis of the Genetic Potential and Gene Expression of Microbial Communities Involved in the In Situ Bioremediation of Uranium and Harvesting Electrical Energy from Organic Matter

The primary goal of this research is to develop conceptual and computational models that can describe the functioning of complex microbial communities involved in microbial processes of interest to the Department of Energy.

Microbial Communities to be Investigated

1. Microbial community associated with the *in situ* bioremediation of uranium-contaminated groundwater.
2. Microbial community that is capable of harvesting energy from waste organic matter in the form of electricity.

DOE Needs Addressed

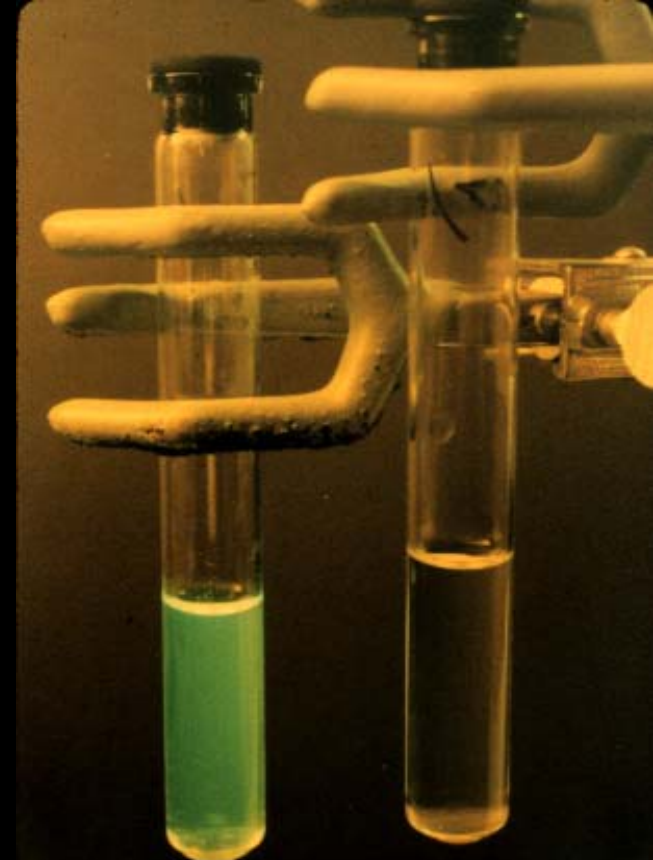
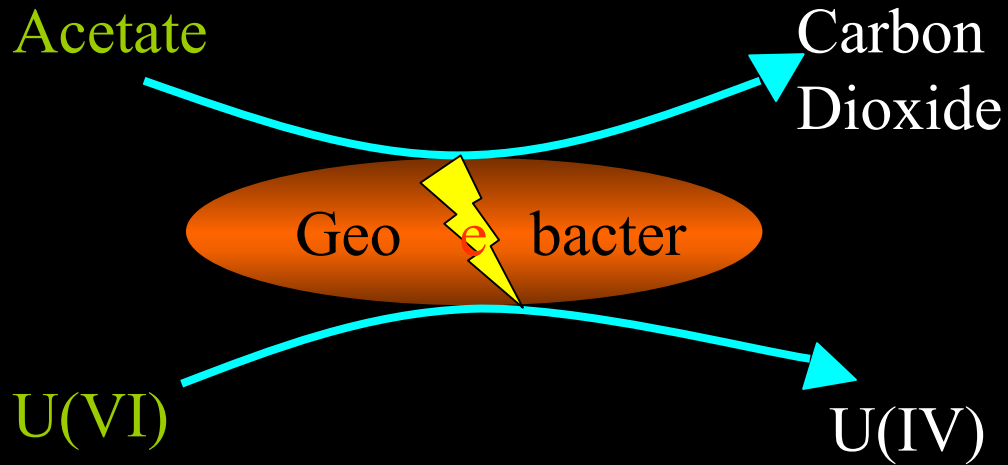
1. Remediation of metals and radionuclides at DOE sites
2. Development of cleaner forms of energy
3. Biomass conversion to energy

Analysis of Microbial Communities Will Focus Exclusively on the *Geobacter* Component in the First Three Years

Rationale:

1. *Geobacters* account for ca. 50-90% of the total microbial community in the environments of interest.
2. *Geobacters* are the primary organisms carrying out the processes of interest in these environments.
3. Environmental genomics studies enhanced by parallel pure culture studies.
4. A meaningful evaluation of the other highly diverse components of the microbial communities in the environments of interest is not currently feasible.

Microbial Bioremediation of Uranium



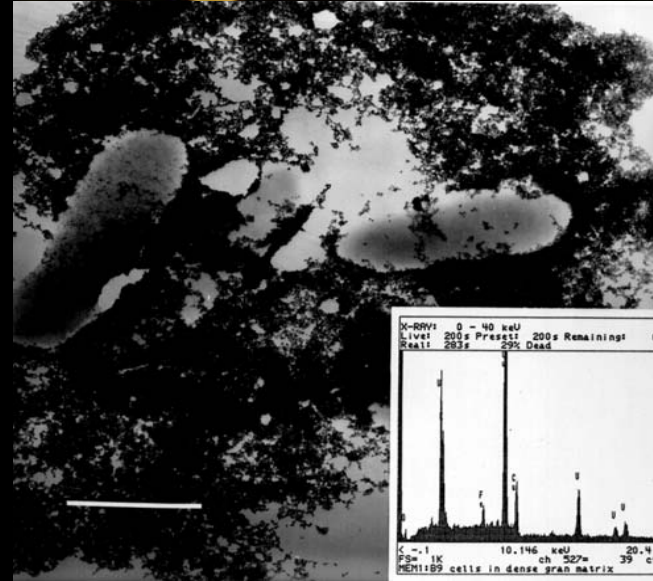
Uranium Contamination Removal

Documented:

Groundwaters from DOE Hanford Site

Surface water from DOI site

Washings from DOD contaminated soil

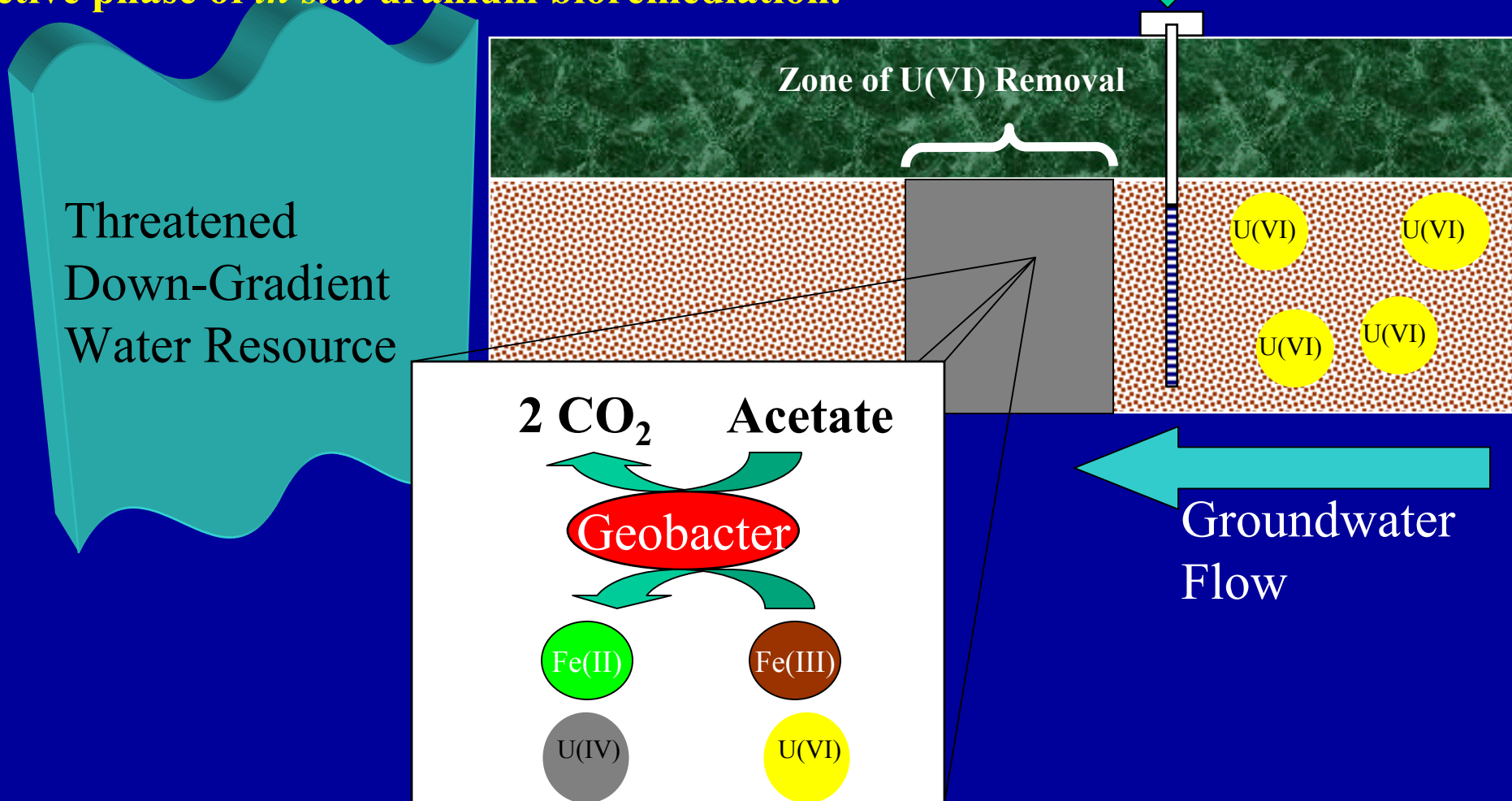


Lovley, D.R., E. J. P. Phillips, Y. A. Gorby, and E. R. Landa.

1991. Microbial reduction of uranium. *Nature* 350:413-416

In situ Uranium Bioremediation Strategy

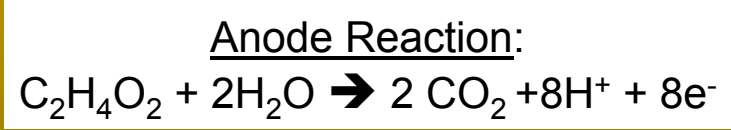
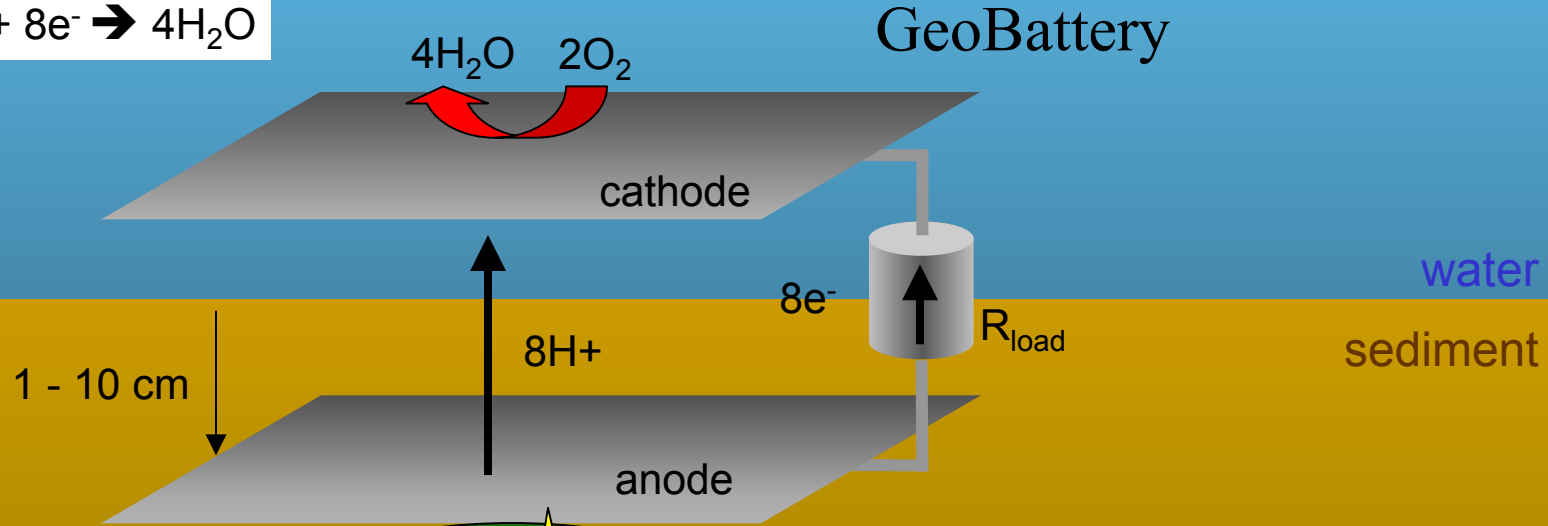
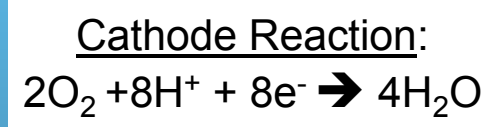
Geobacter species comprise as much as 85% of the microbial community in the subsurface during the most active phase of *in situ* uranium bioremediation.



Anderson et al. 2003. Stimulating the *in situ* activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer Appl. Environ. Microbiol. 69:5584-5891.

Geobacter Can Use Electrodes as an Electron Acceptor

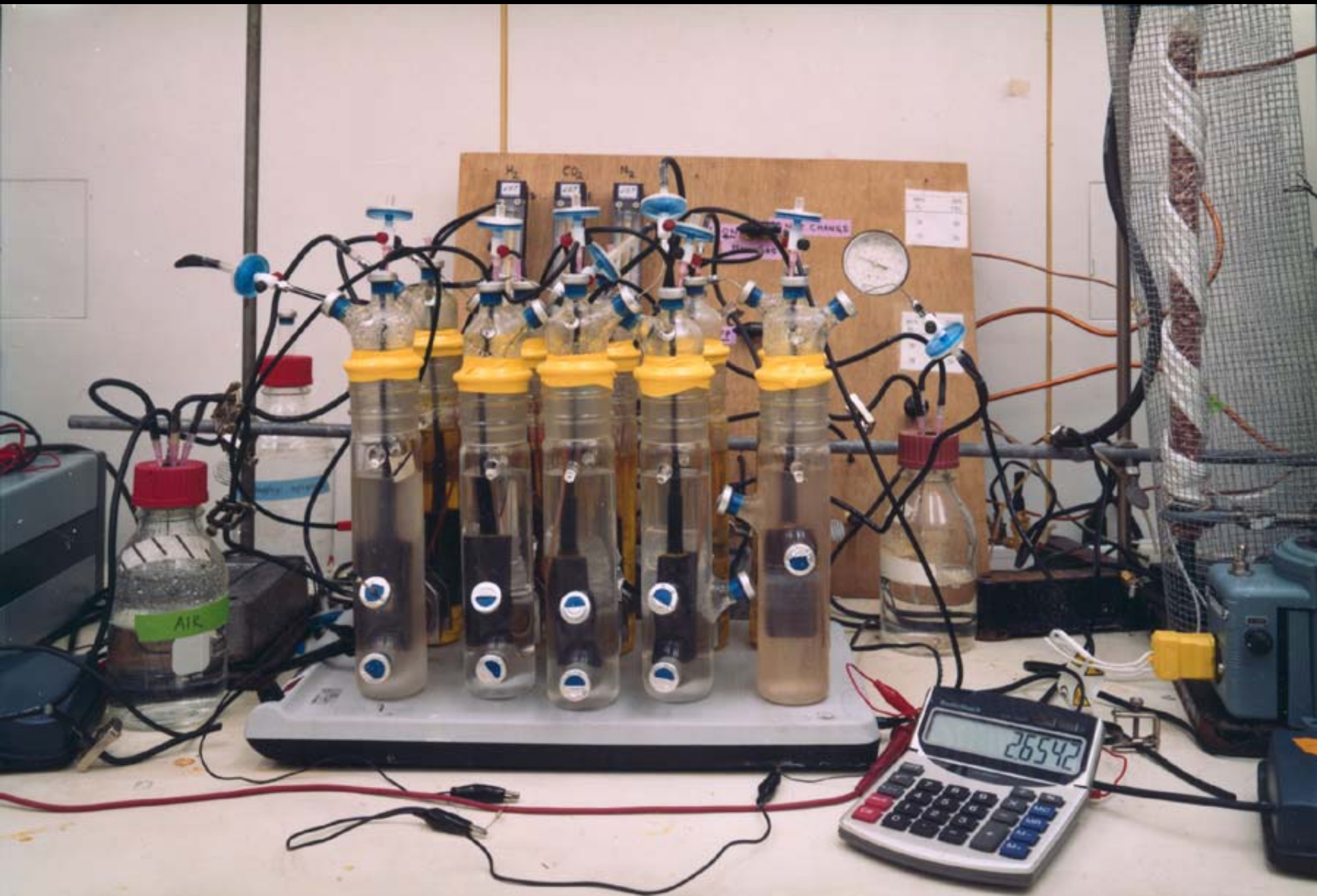
Harvesting Power From Aquatic Sediments and Other Sources of Waste Organics



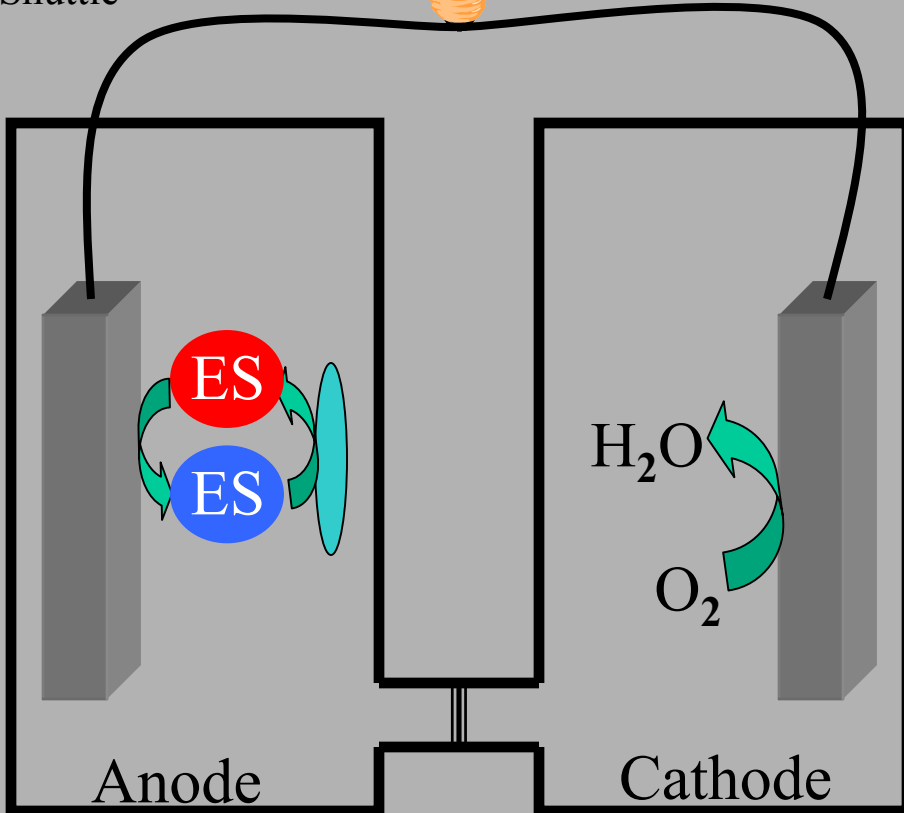
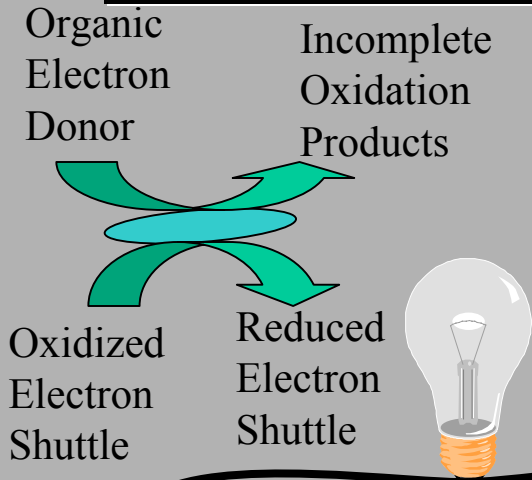
Geobacter species
Comprise ca. 50% of the
Microbial Community
On the Anode

2 CO₂ Acetate
Fermentation Sediment Organic Matter

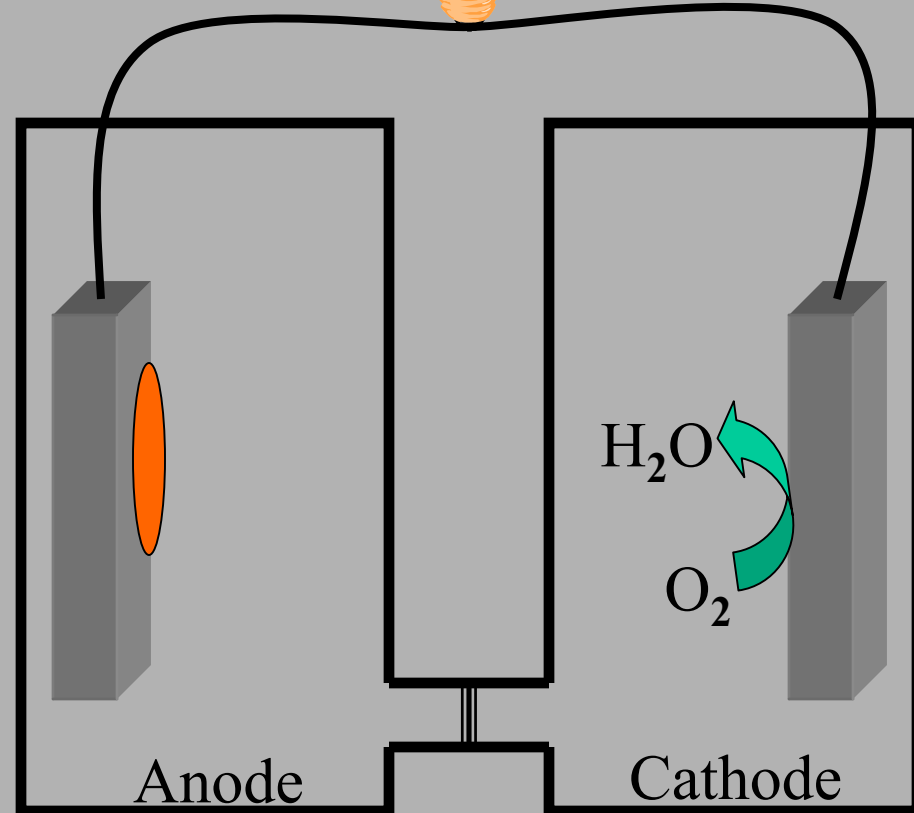
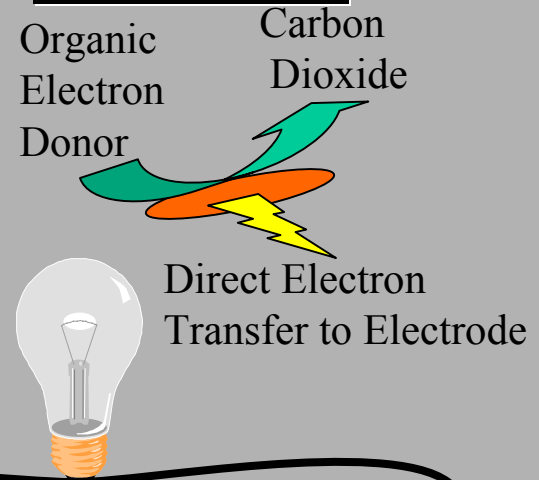
Pure Culture Geobatteries



Traditional Microbial Fuel Cell



GeoBattery



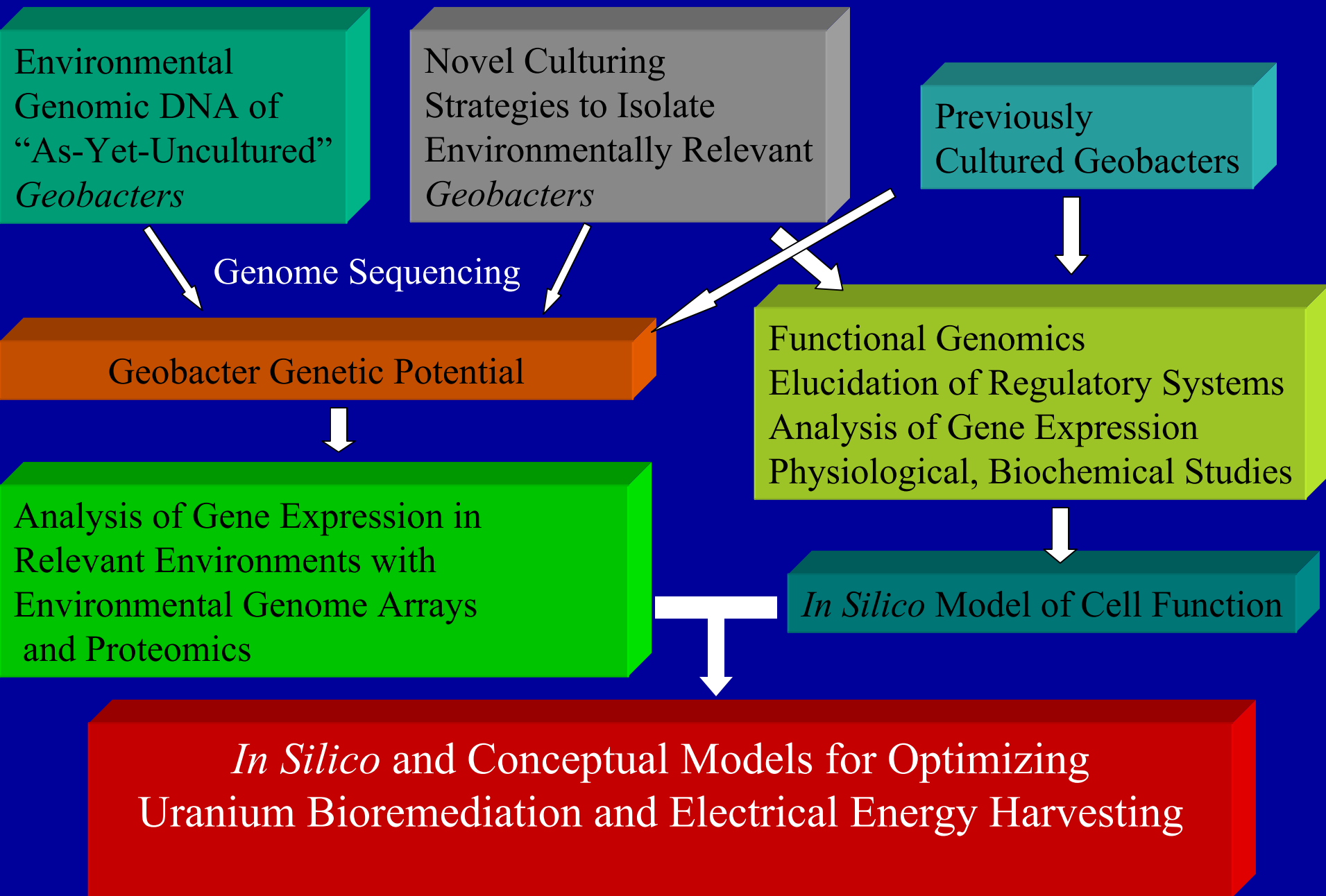
Comparison of Geo Batteries with Previous Microbial Fuel Cells

	Previous Microbial Fuel Cells	GeoBattery
Oxidation of Organic Fuel	Incomplete	Complete to Carbon Dioxide
Requirement for Toxic Electron Shuttles to Function	Yes	No
Recovery of Electrons As Electricity	1-50%	80-95%
Long-Term Stability	Poor	Excellent
Ability to Function in “Open” Environments	No	Yes

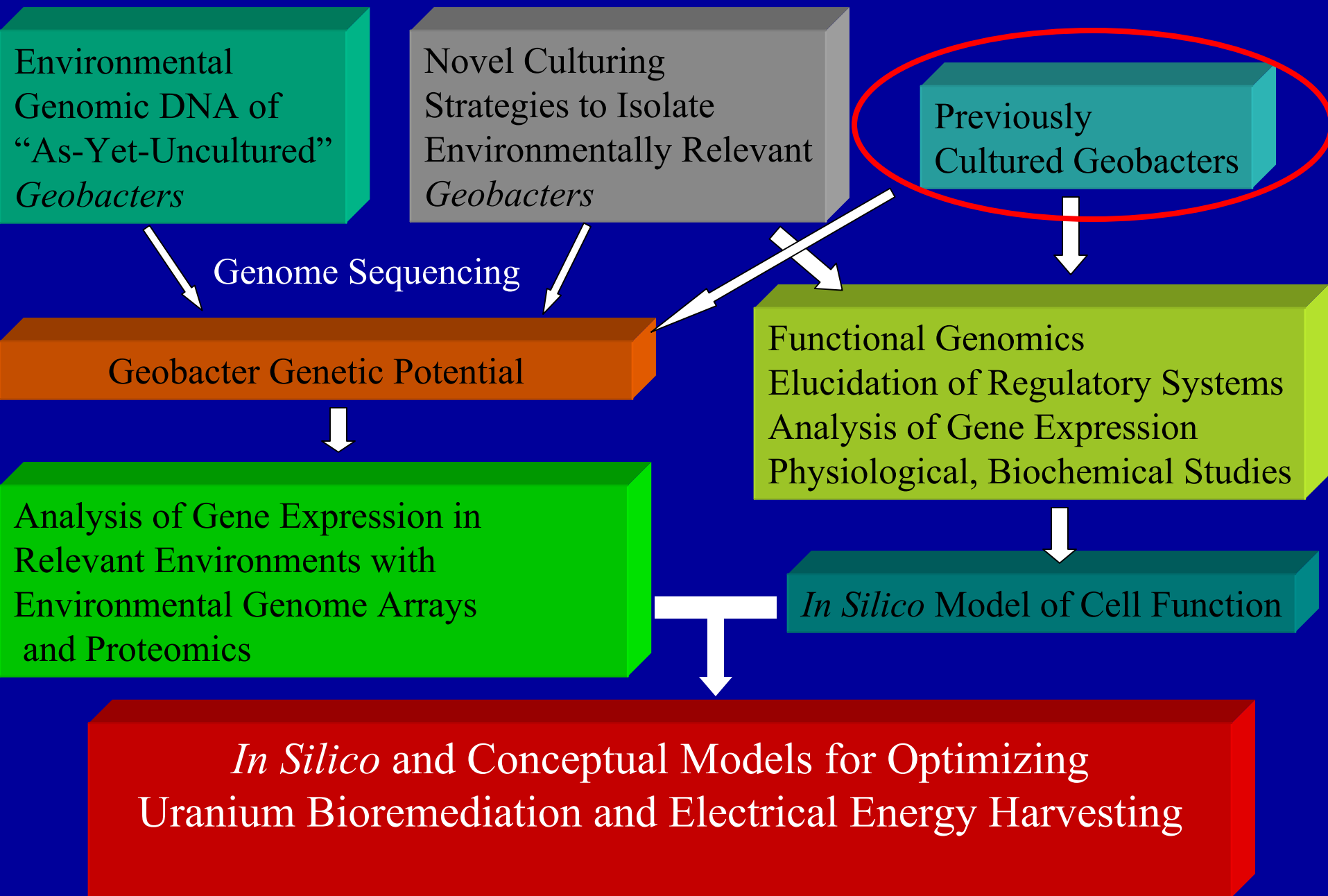
Potential Applications of GeoBatteries

- Powering Monitoring Devices in Remote Locations
- Powering Electronic Devices from Renewable Energy Sources
- “Gastrobots”-robots fueled from food or organic waste
- Decentralized domestic power source
- Novel sensing devices
- Conversion of waste organic matter to electricity instead of methane
- Conversion of renewable biomass to electricity instead of ethanol
- Bioremediation of contaminated environments
- Powering automobiles

Application of Environmental Genomics and Systems Biology to Uranium Bioremediation and Harvesting Electricity from Waste Organic Matter



Application of Environmental Genomics and Systems Biology to Uranium Bioremediation and Harvesting Electricity from Waste Organic Matter



Status of *Geobacteraceae* Sequencing Projects

Organism	Sequencing Group	Status
<i>Geobacter sulfurreducens</i>	TIGR	Complete
<i>Geobacter metallireducens</i>	JGI/UMASS	Nearly Complete
<i>Desulfuromonas acetoxidans</i>	JGI/UMASS	10 gaps
<i>Pelobacter carbinolicus</i>	JGI/UMASS	Draft
<i>Pelobacter propionicus</i>	JGI/UMASS	Draft
<i>Geobacter uranibioremediacens</i>	JGI/UMASS	Underway

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Discoveries from GTL with Direct and Immediate Application to NABIR

- Demonstration that *Geobacter* species can grow with oxygen as the terminal electron acceptor
- Elucidation of novel mechanism for *Geobacter* species to find and access Fe(III) oxides
- Elucidation of genes encoding for key respiratory proteins
- Elucidation of systems regulating expression key respiratory genes
- Elucidation of systems regulating:
 - growth under slow, environmentally relevant conditions
 - response to environmental stress
 - response to nutrient limitation
- Elucidation of novel central metabolism genes

Discoveries from GTL with Direct and Immediate Application to NABIR

(continued)

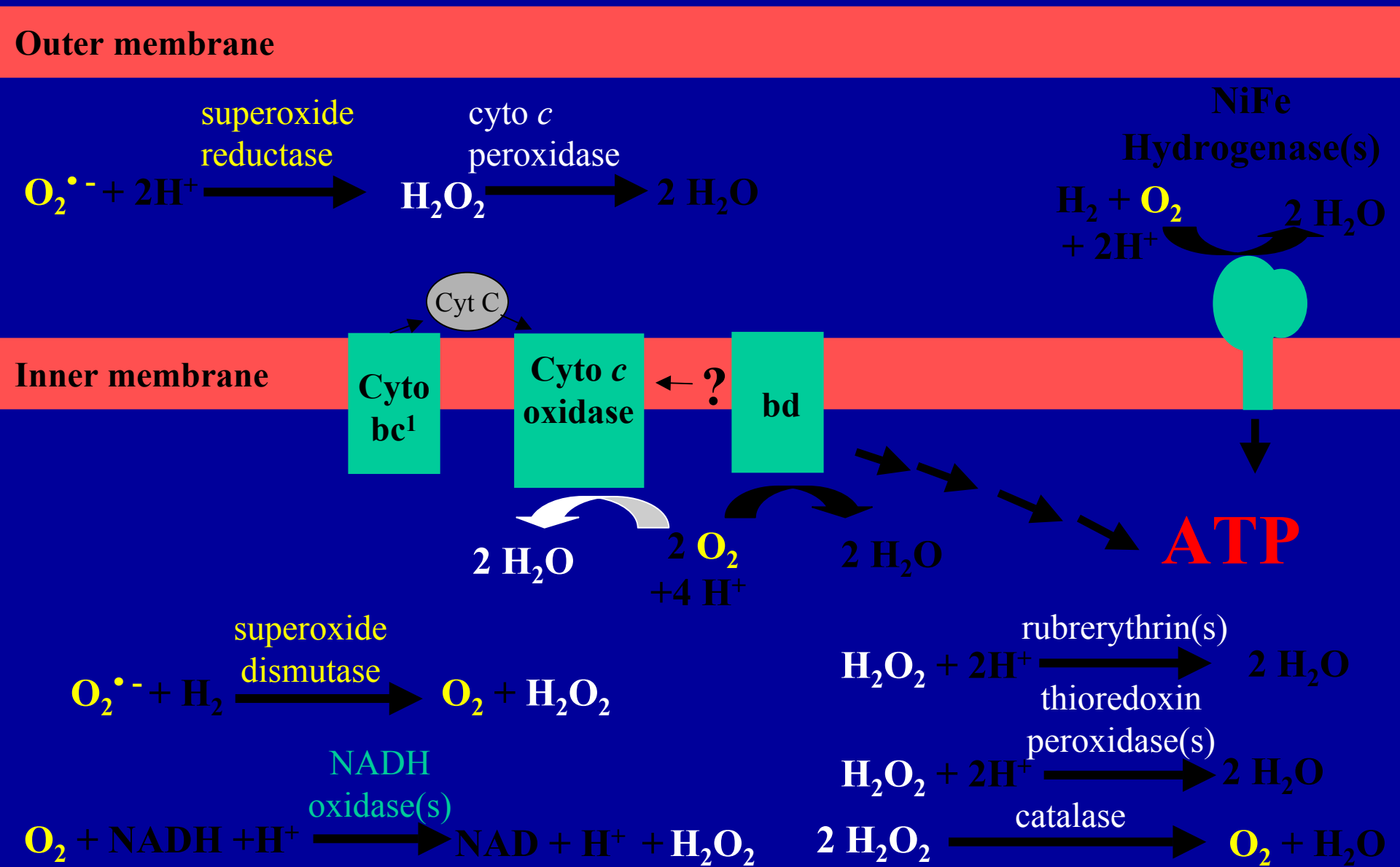
- Development of an *in silico* model that can:
 - Predict the response of *Geobacter* to different environmental conditions including strategies for manipulating the environment to promote bioremediation
 - Aid in elucidating the likely outcome of genetically engineering novel metabolic capabilities in *Geobacter*
- Discovery of significant similarities in genomes of as-yet-uncultured *Geobacter* species and pure cultures of *Geobacter* species

Discoveries from GTL with Direct and Immediate Application to NABIR

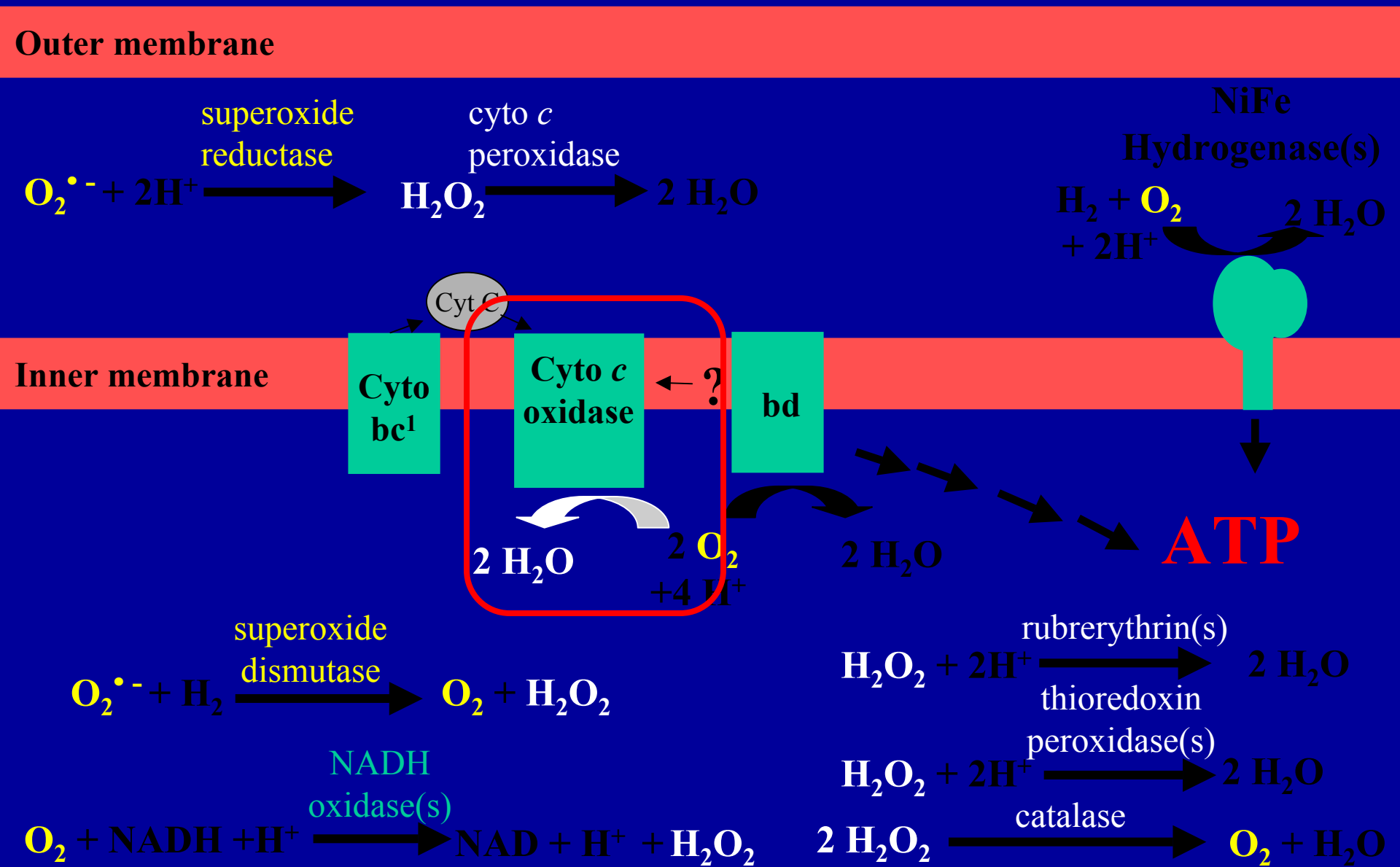
- Demonstration that *Geobacter* species can grow with oxygen as the terminal electron acceptor

Provides explanation for the reservoir of *Geobacter* species in aerobic aquifers that can so rapidly respond to introduction of acetate and immediately start removing uranium from contaminated groundwater.

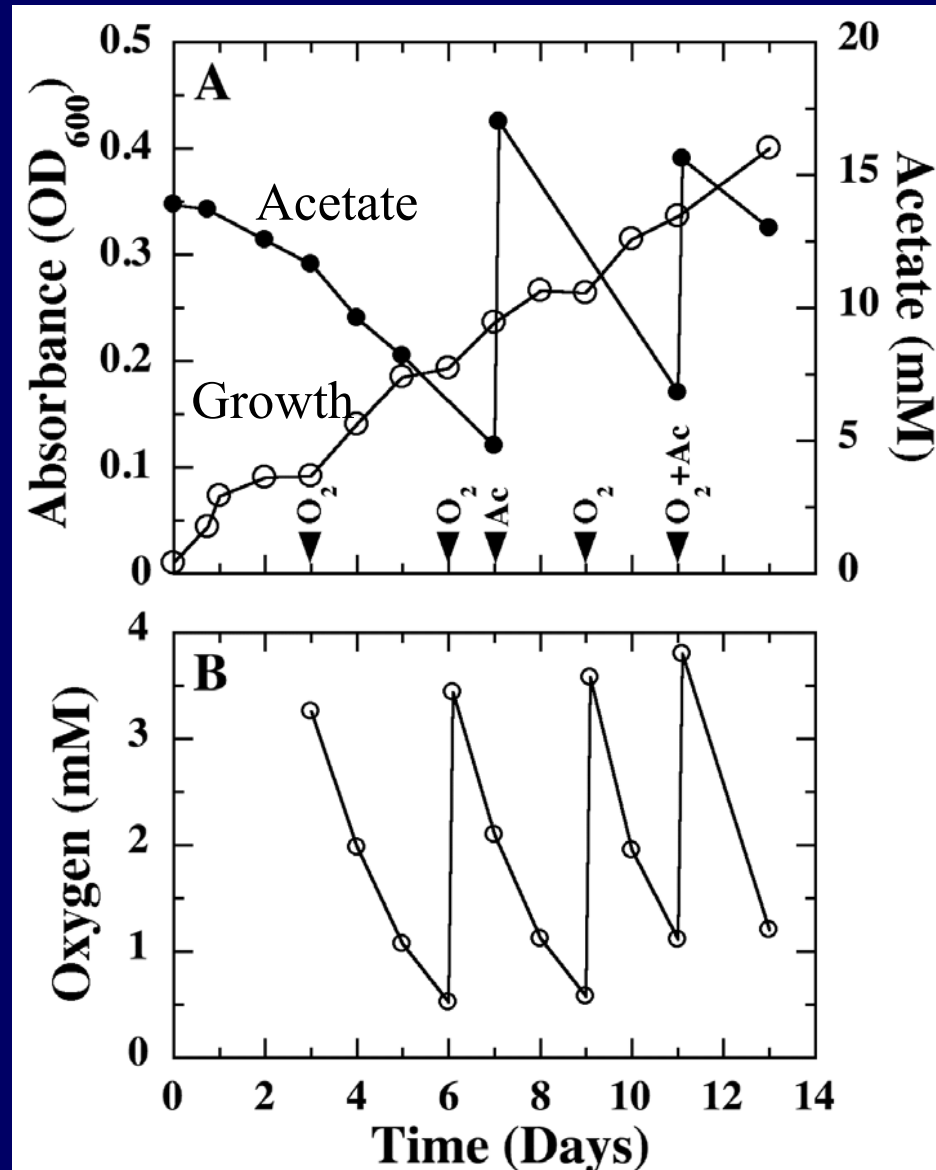
Genome-based Model for the Reduction of Oxygen and the Detoxification of Reactive Oxygen Species by *G. sulfurreducens*



Genome-based Model for the Reduction of Oxygen and the Detoxification of Reactive Oxygen Species by *G. sulfurreducens*



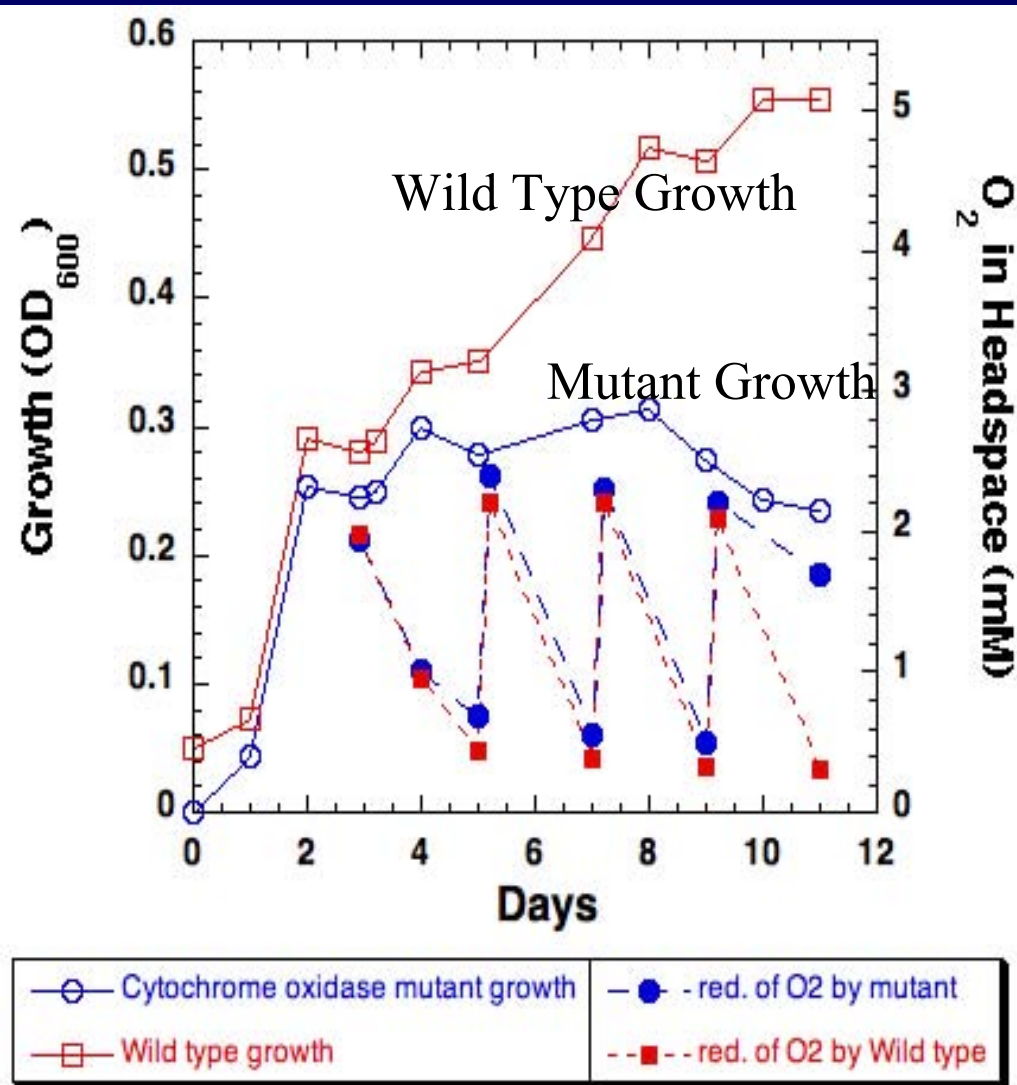
Growth of *G. sulfurreducens* can grow with oxygen as the sole terminal electron acceptor



Growth of *Geobacter* on oxygen provides an explanation for how *Geobacter* survives in low numbers in aerobic subsurface environments and then rapidly responds to the development of anaerobic conditions when uranium bioremediation is initiated.

Lin, Coppi, and Lovley. 2004. *Geobacter sulfurreducens* can grow with oxygen as a terminal electron acceptor. *Appl. Environ. Microbiol.* 70: (in press).

Knocking out the cytochrome oxidase genes inhibits growth of *G. sulfurreducens* on oxygen

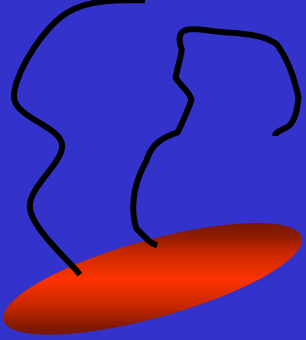


- Cytochrome oxidase is comprised of four genes, ORFs 374, 376, 378, and 380.
- Mutant is a deletion of 374, 376 and replaced with antibiotic resistance cassette.
- Mutant does not grow with O₂ but still can consume O₂.
- Implications:
 - Terminal oxidase is responsible for growth with low % O₂.
 - Inactivation of terminal oxidase does not affect the activity of oxidative stress enzymes.

Discoveries from GTL with Direct and Immediate Application to NABIR

- Elucidation of novel mechanism for *Geobacter* species to find and access Fe(III) oxides

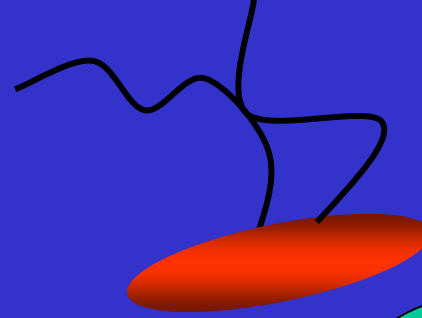
Solves the mystery of how *Geobacter* species, which were thought to be non-motile, can efficiently access Fe(III) oxides via chemotaxis and thus compete for Fe(III) oxides even though *Geobacter* species require direct contact with Fe(III) oxides in order to reduce them.



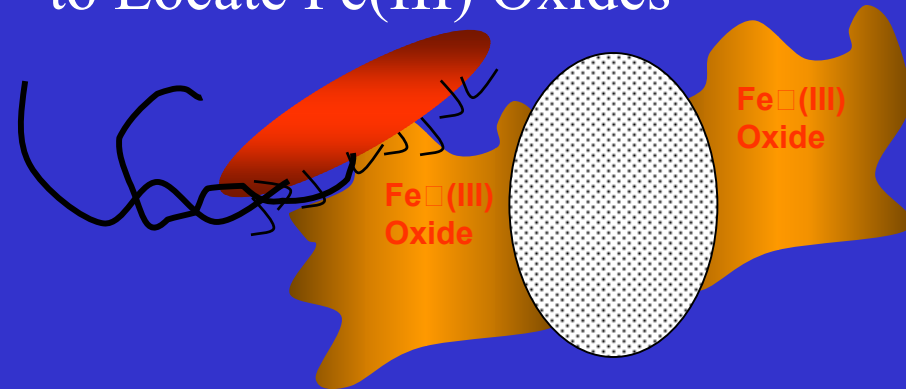
Geobacter Specifically Expresses Flagella when only Fe(III) Oxide is Available as an Electron Acceptor



Geobacter May use Flagella to Make Initial Contact with Fe(III)



Geobacter follows Fe(II) Gradient to Locate Fe(III) Oxides



Geobacter uses Pili to “Twitch” Along Sediment Surface and Contact Fe(III)

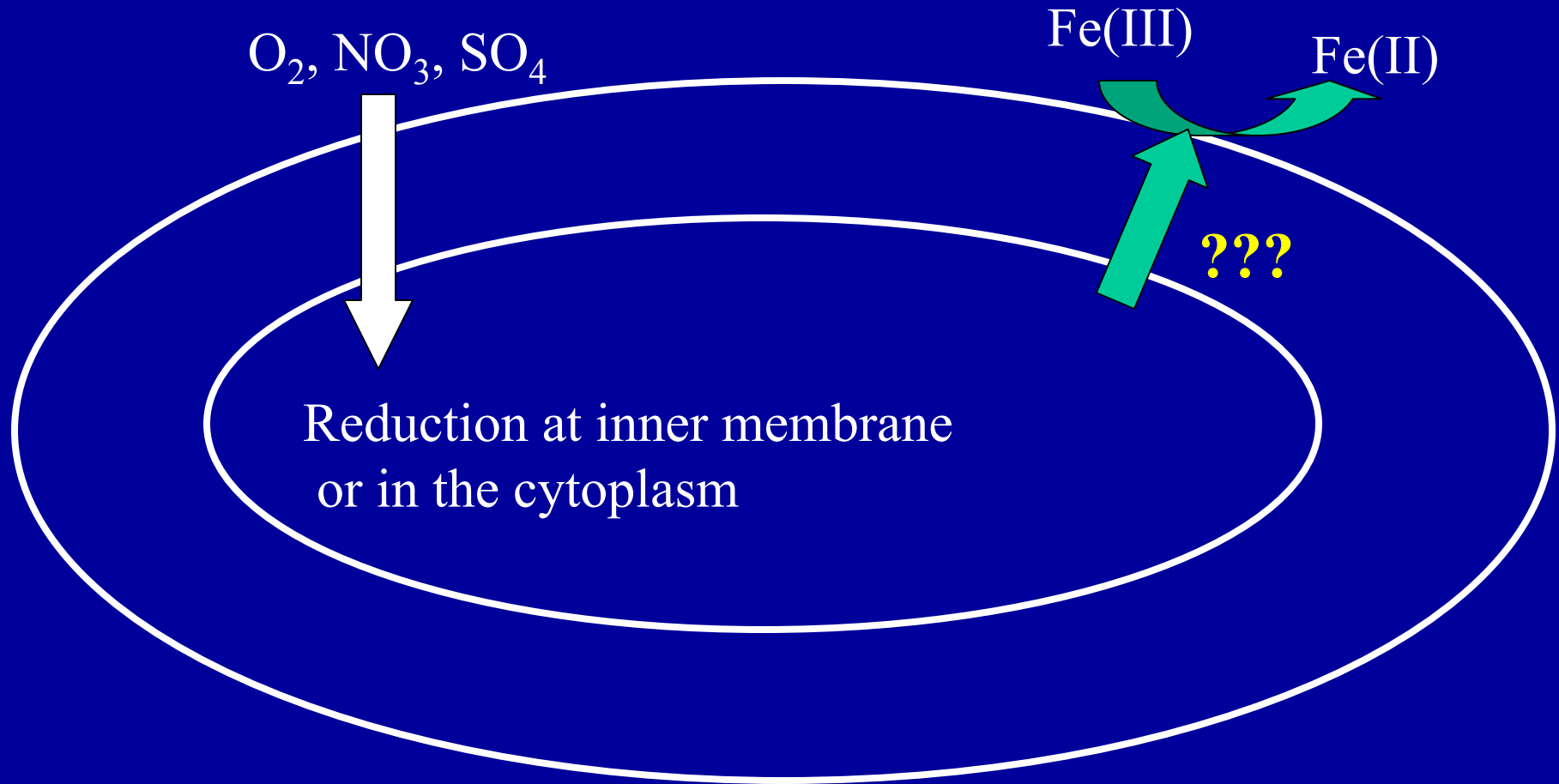
Childers S.E., S. Ciuffo, and D. R. Lovley. 2002. *Geobacter metallireducens* access Fe(III) oxide by chemotaxis. *Nature* 416:767-769

Discoveries from GTL with Direct and Immediate Application to NABIR

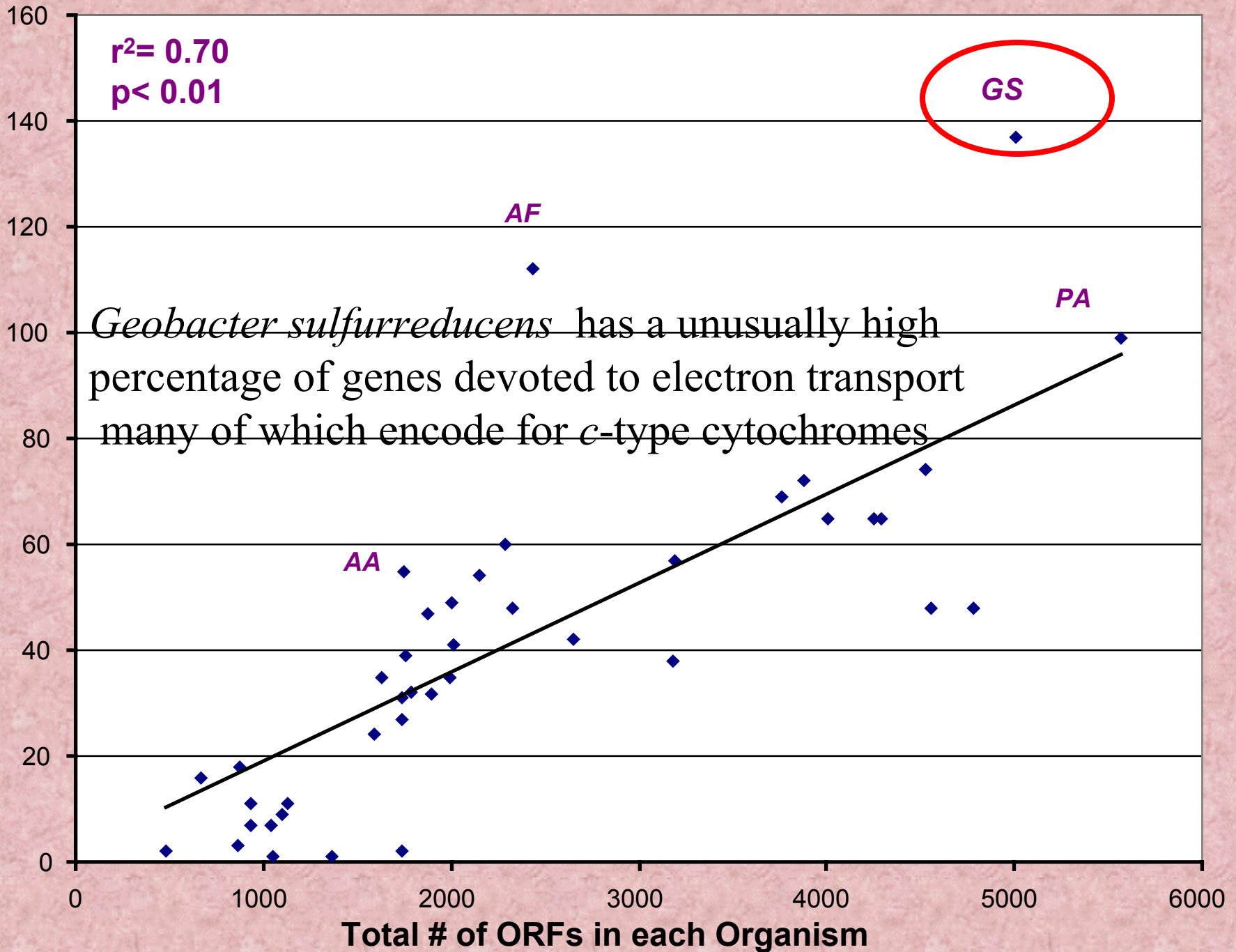
- Elucidation of genes encoding for key respiratory proteins

Provides molecular targets for estimating rates of metal reduction in the subsurface.

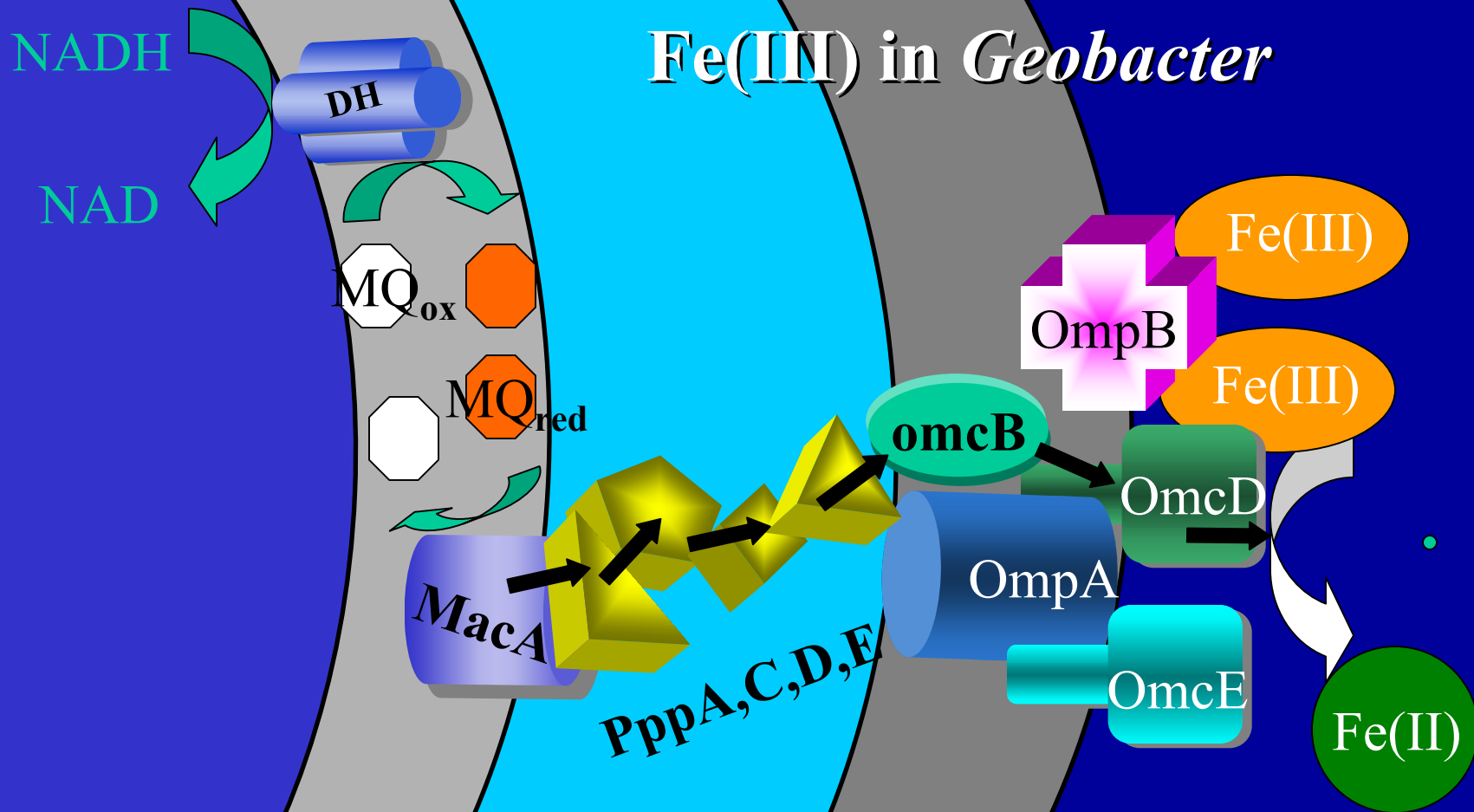
Electron Transfer to Extracellular Electron Acceptors Such as Metals and Electrodes is Fundamentally Different than the Reduction of Commonly Considered Soluble Electron Acceptors



of ORFs- Electron Transport



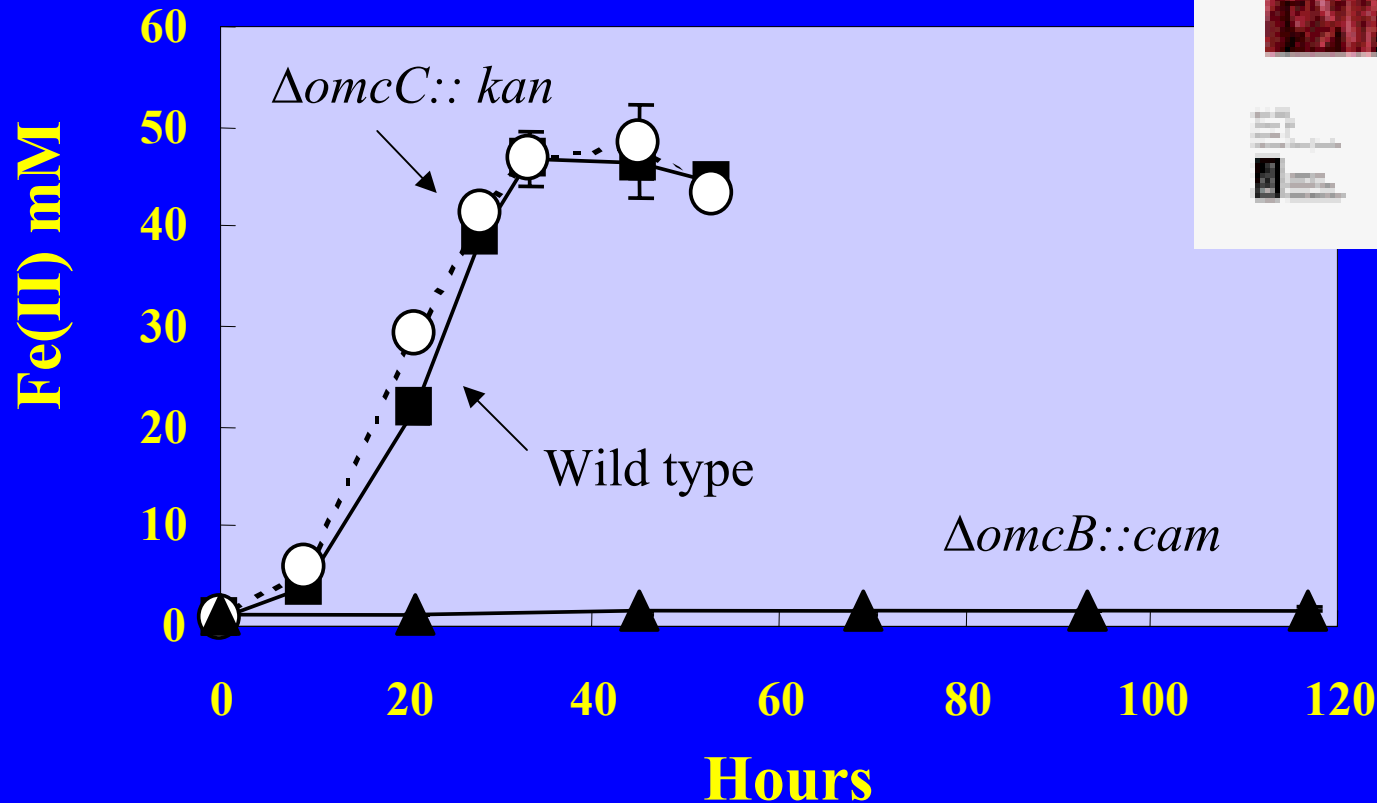
Model for Electron Transfer to Fe(III) in *Geobacter*



OmcB but not OmcC is Required for Fe(III) Reduction

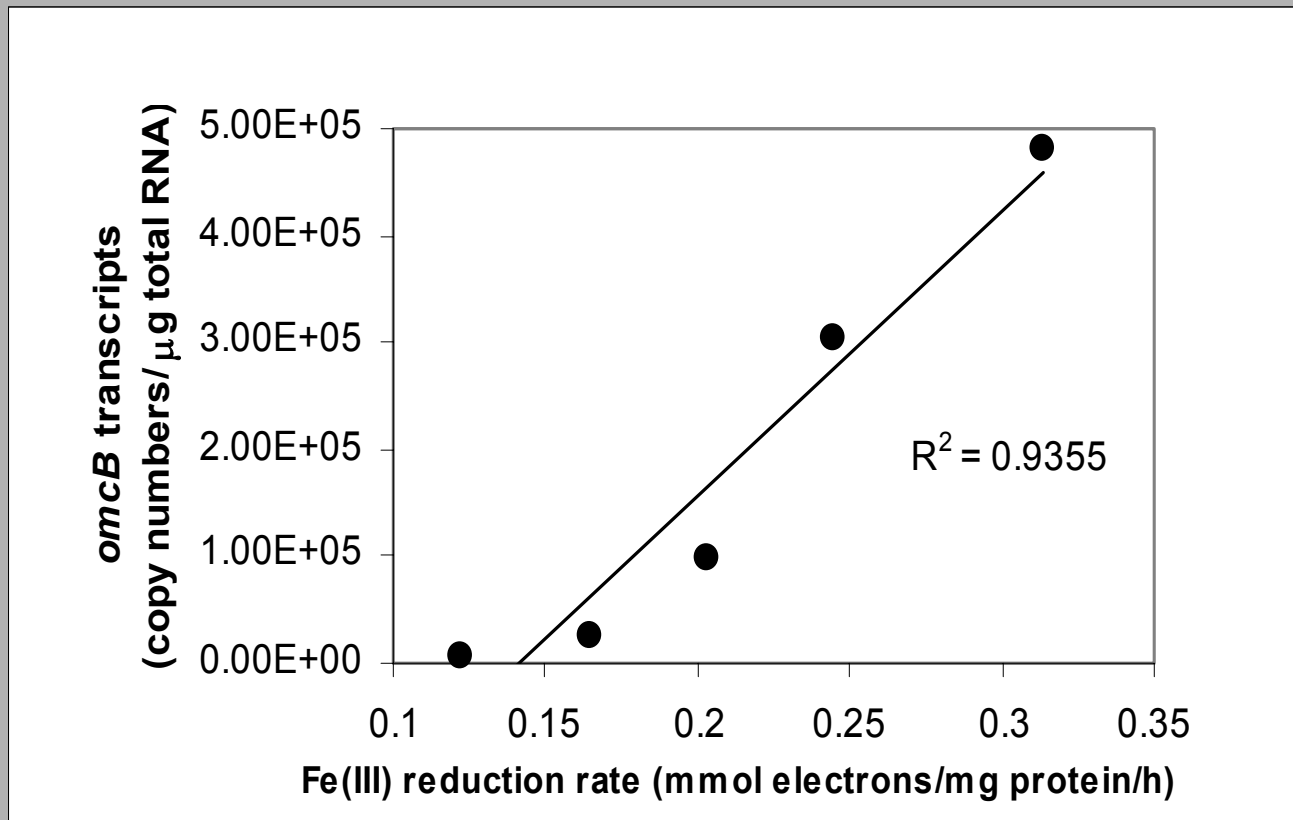


JB
Journal of Bacteriology



Leang, C., M. V. Coppi, and D. R. Lovley. 2003. OmcB, a c-Type polyheme cytochrome, involved in Fe(III) reduction in *Geobacter sulfurreducens*. J. Bacteriol. 185:2096-2113.

Direct Correlation Between levels of *omcB* mRNA and Rates of Fe(III) Reduction in Acetate-Limited Chemostats of *Geobacter sulfurreducens*



Discoveries from GTL with Direct and Immediate Application to NABIR

- Elucidation of systems regulating expression key respiratory genes

This makes it possible to predict under which environmental conditions respiratory genes necessary for metals bioremediation will be expressed.

**ENVIRONMENTAL
STIMULI**

RESPONSE

Signals

Differential
gene expression

REGULATORY CASCADES

TO CONTROL TRANSCRIPTION

SIGMA FACTORS

**TWO COMPONENT
SYSTEMS**

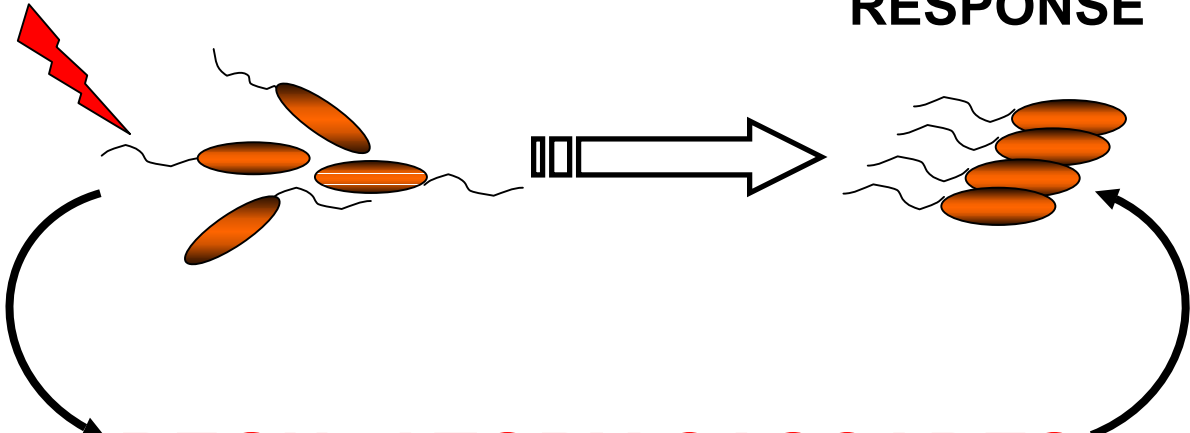
**OTHER GLOBAL
REGULATORS**

RpoS RpoE

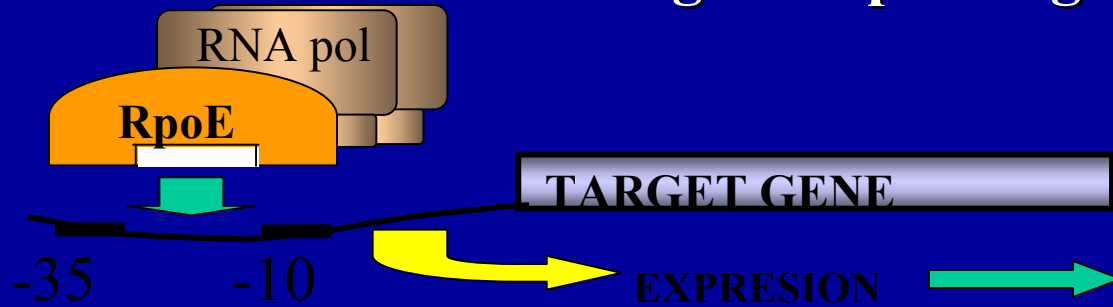
Histidine kinases
Response regulators

RelA Fur

ELECTRON TRANSFER



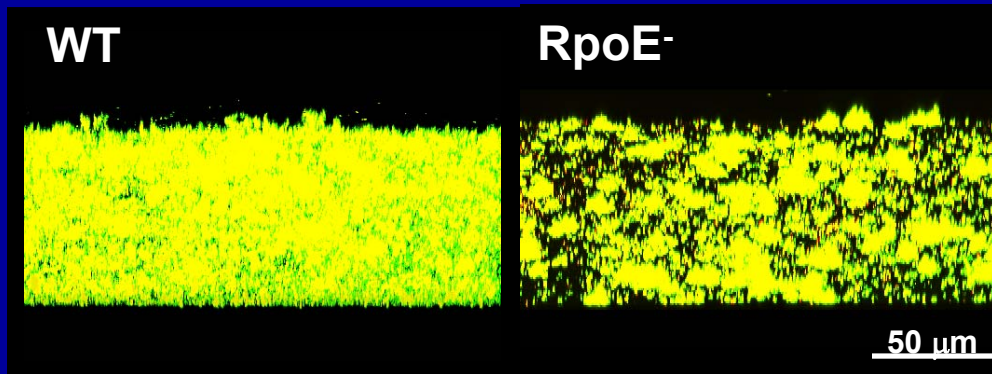
Defining the RpoE regulon in *G. sulfurreducens*



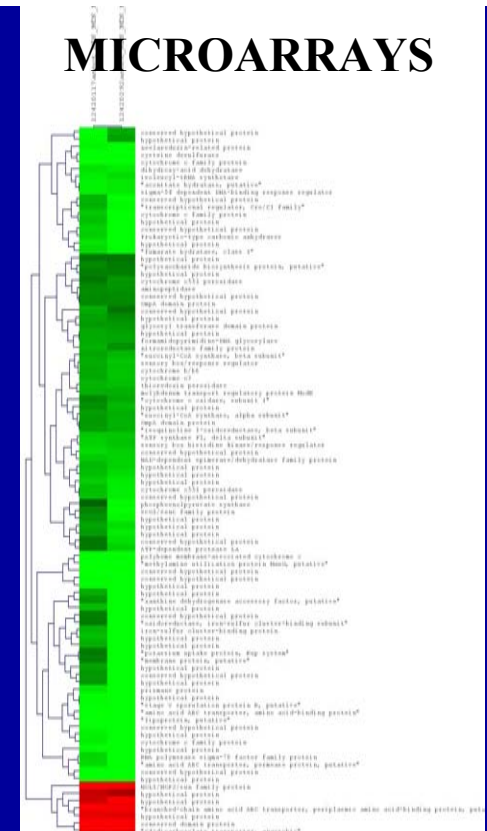
An *rpoE* mutation affects the expression of at least **200** other genes

• RpoE REGULON:

1. **Cytochrome genes** (7) and cytochrome biogenesis genes involved in Fe(III) reduction
2. **Oxidative stress** regulon different from RpoS
3. **Biofilm metabolism and development**



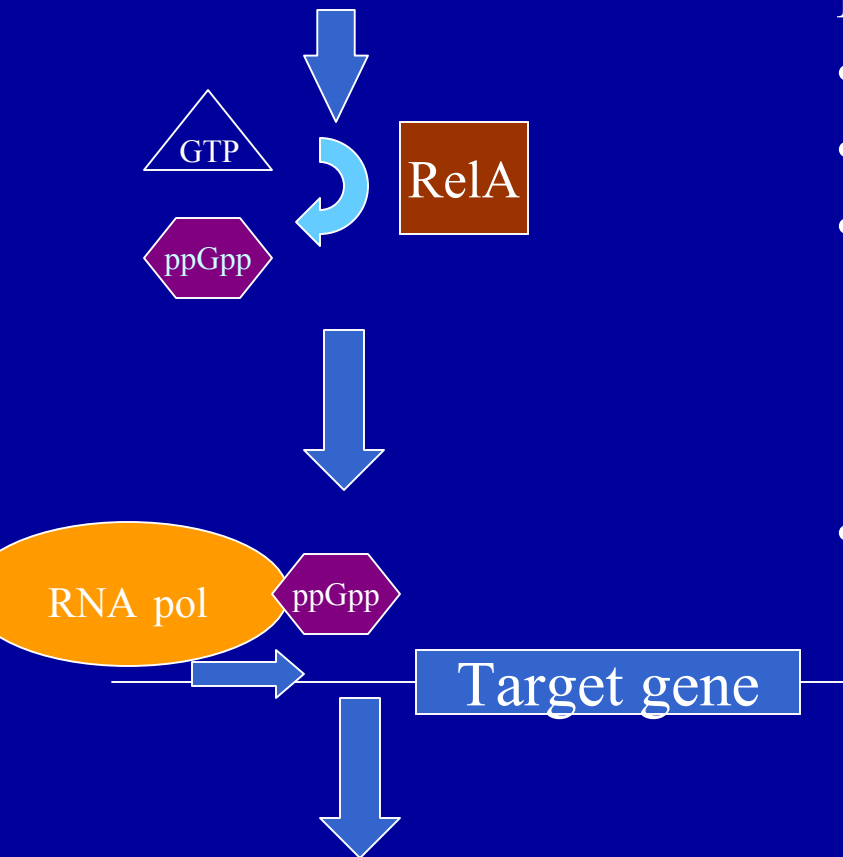
MICROARRAYS



4. **Biofilm electron transfer via H₂**

Rel A Plays an Important Role in Regulating Growth and Metabolism in *Geobacter sulfurreducens* under Environmentally Relevant Conditions

Starvation



Phenotype of *relA* Mutant

- Increase growth under nutrient limitation
- Decrease growth in presence of oxygen
- Upregulation of genes involved in:
 - protein biosynthesis
 - cell division
 - transport
- Downregulation of genes for:
 - stress response
 - signal transduction
 - insoluble Fe(III) reduction**

Slower Growth (protein synthesis, nutrient transport)

Increased resistance to Oxidative Stress Resistance

Increased production of cytochromes for Fe(III) Reduction

Microarray Results Comparing Wild Type to the *fur* Mutant

Regulators

Up regulation of 9 regulatory genes was found, including *dtx*, another iron regulated repressor.

Metabolism

Up regulation of 15 genes involved in metabolism including *HydB*, the hydrogenase responsible for hydrogen dependant growth.

Metal Uptake

Up regulation of 16 possible metal uptake genes was found, including *FeoB*, a ferrous iron cytoplasmic membrane transporter.

Cytochromes

Up regulation of 7 cytochrome genes was found, including *OmcB* and *OmcD*.

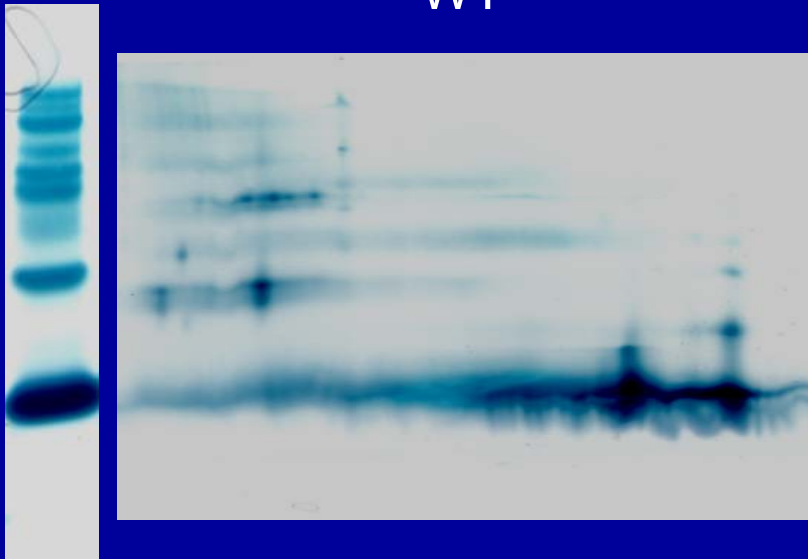
Unknown Proteins

Up regulation of 32 genes with an unknown function.

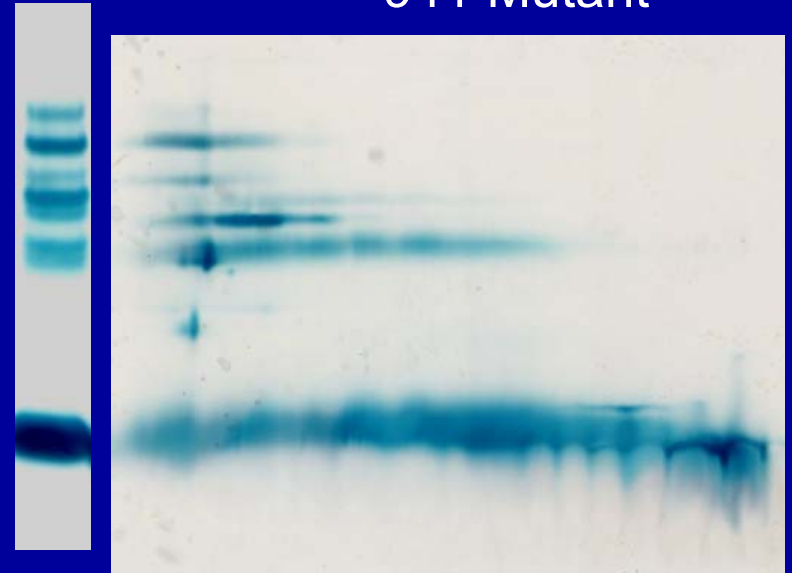
Knocking out a Histidine Kinase Sensor Inhibits Cytochrome Production



WT

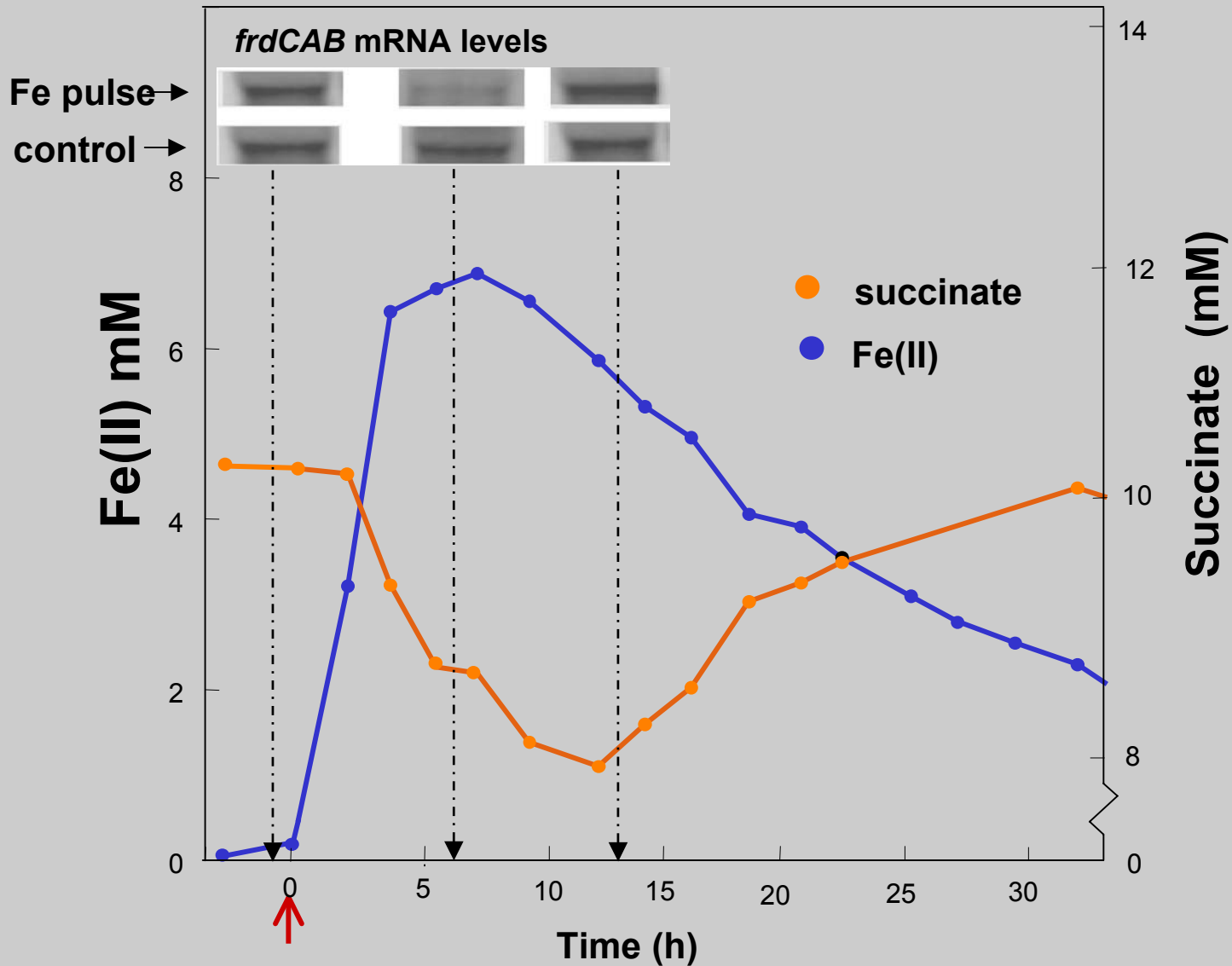


941 Mutant



1-D and 2-D SDS-PAGE stained for c-type cytochromes (heme)

Fe(III)-Specific Regulation of Fumarate Respiration



Fe(III) pulse

Abraham Esteve-Núñez, Cinthia Núñez and Derek R. Lovley. 2004.
J. Bacteriol. (in press).

Discoveries from GTL with Direct and Immediate Application to NABIR

- Elucidation of systems regulating:
 - growth under slow, environmentally relevant conditions
 - response to environmental stress
 - response to nutrient limitation

Provides information necessary to interpret the *in situ* metabolic state of *Geobacter* species in the subsurface.

Discoveries from GTL with Direct and Immediate Application to NABIR

- Elucidation of novel central metabolism genes

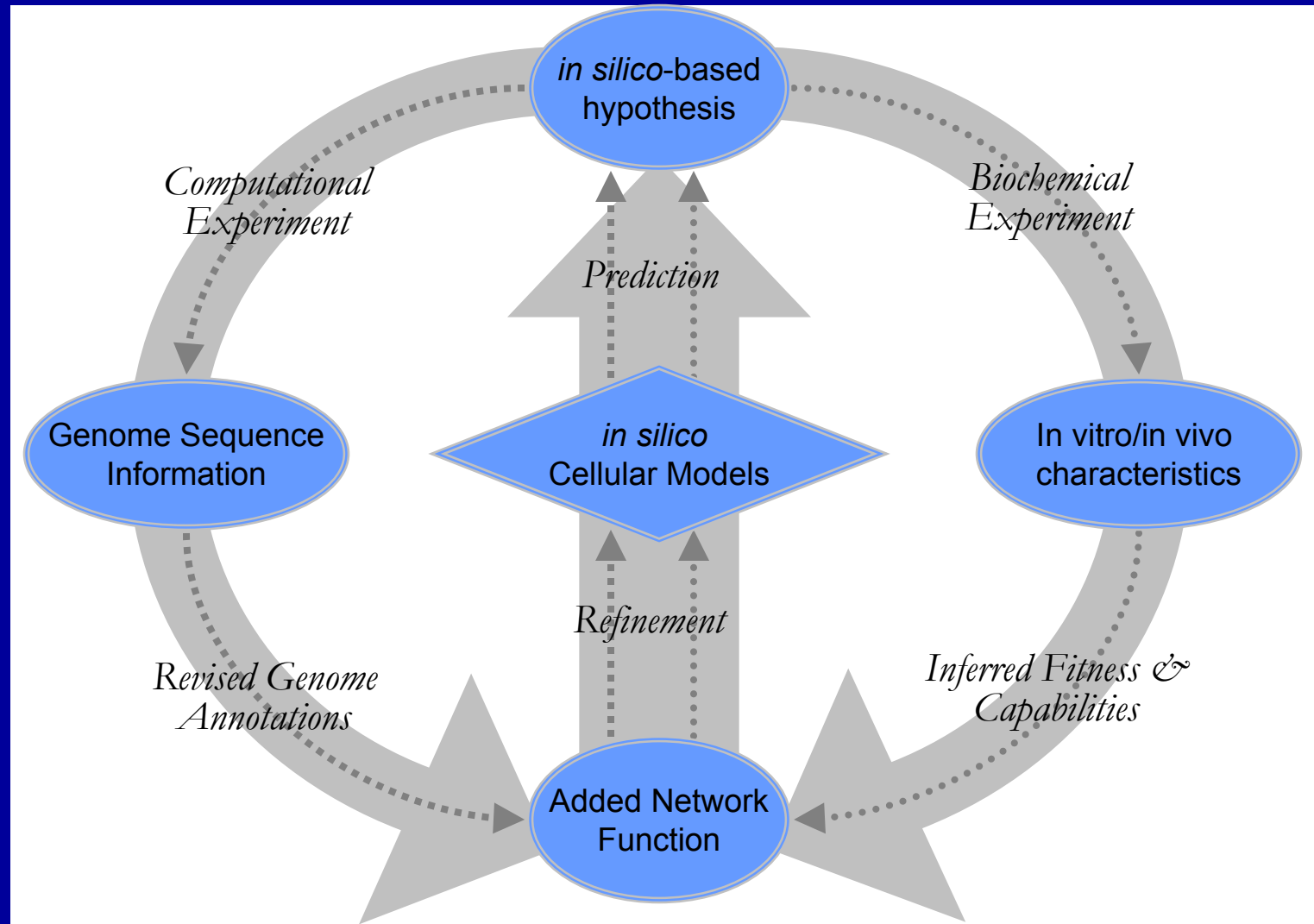
For example, the novel, eucaryotic-like citrate synthase provides a unique molecular marker for tracking *Geobacter* species and their activity in the subsurface.

Discoveries from GTL with Direct and Immediate Application to NABIR

(continued)

- Development of an *in silico* model that can:
 - Predict the response of *Geobacter* to different environmental conditions including strategies for manipulating the environment to promote bioremediation
 - Aid in elucidating the likely outcome of genetically engineering novel metabolic capabilities in *Geobacter*

Contributions of Iterative *In Silico* Model Building to Understanding of the Environmental Responses of *Geobacter*



Genome-scale model of *G. sulfurreducens*

Total Number of Genes: 3532

Included Genes: 583 (17 %)

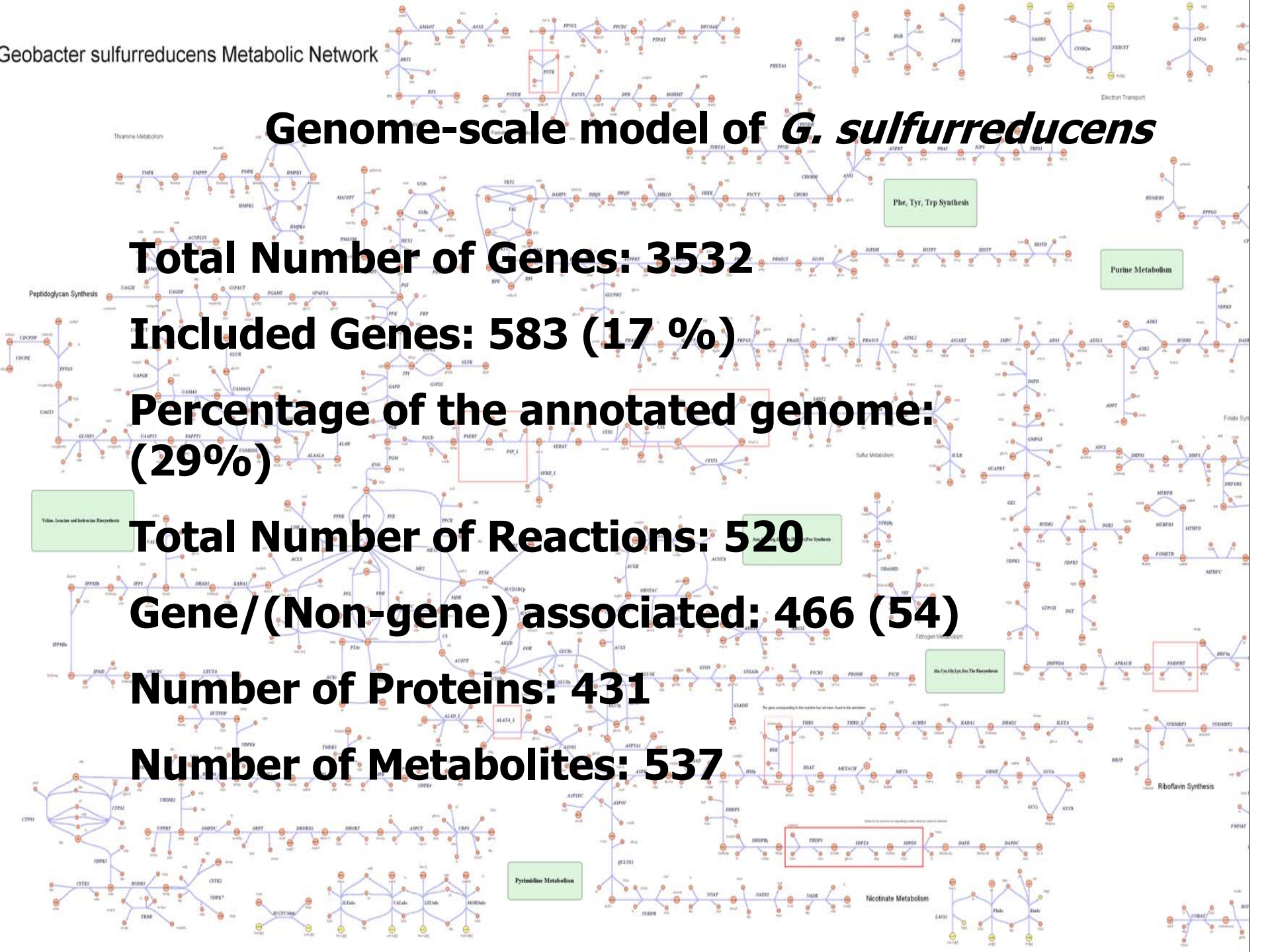
**Percentage of the annotated genome:
(29%)**

Total Number of Reactions: 520

Gene/(Non-gene) associated: 466 (54)

Number of Proteins: 431

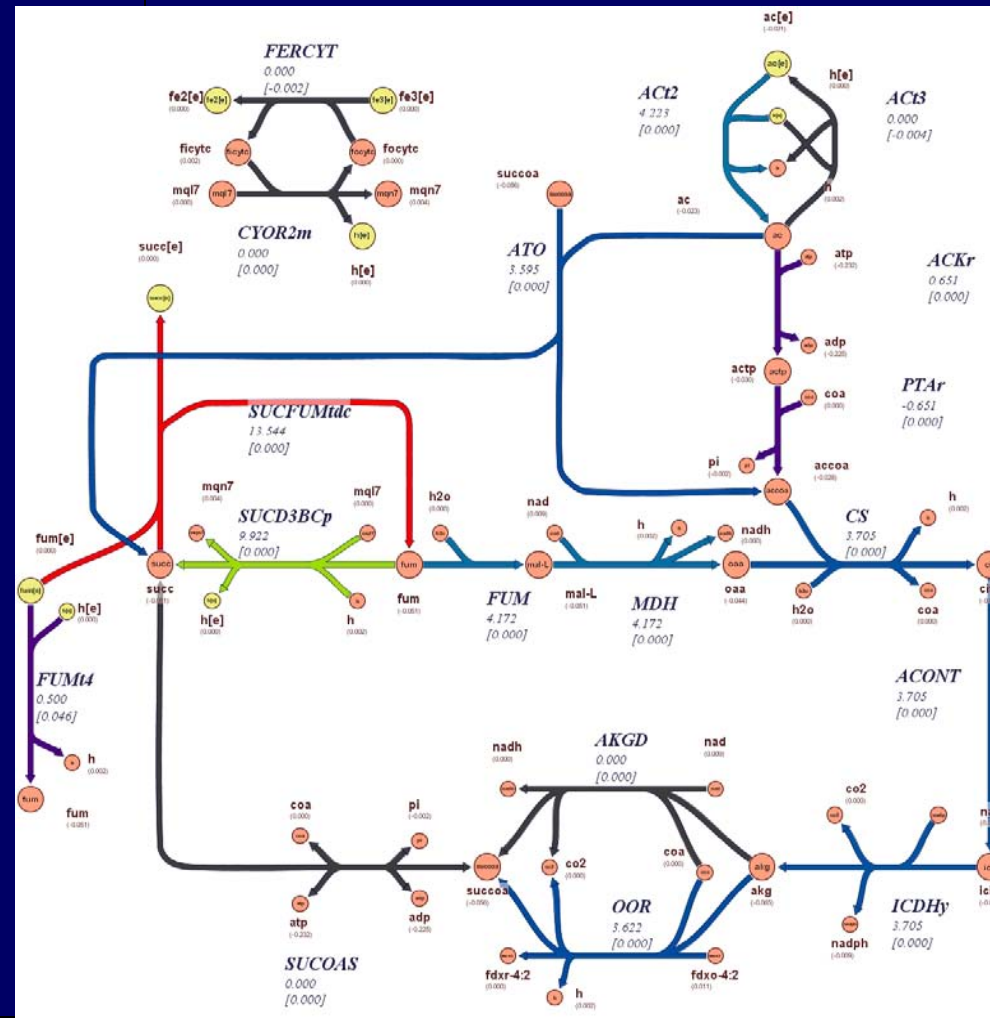
Number of Metabolites: 537



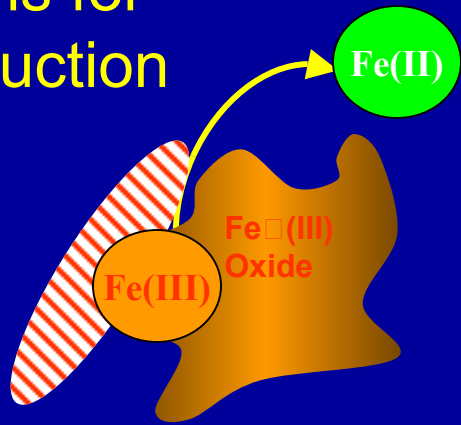
Given a limited number of constraints, growth rate, yield, and flux through metabolic network can be quantitatively predicted

Predicted growth rate vs. observed

Flux (example)



Phylogenetically Distinct Fe(III) Reducers Have Different Mechanisms for Fe(III) Reduction



Geobacter has to directly contact Fe(III) oxide in order to reduce it

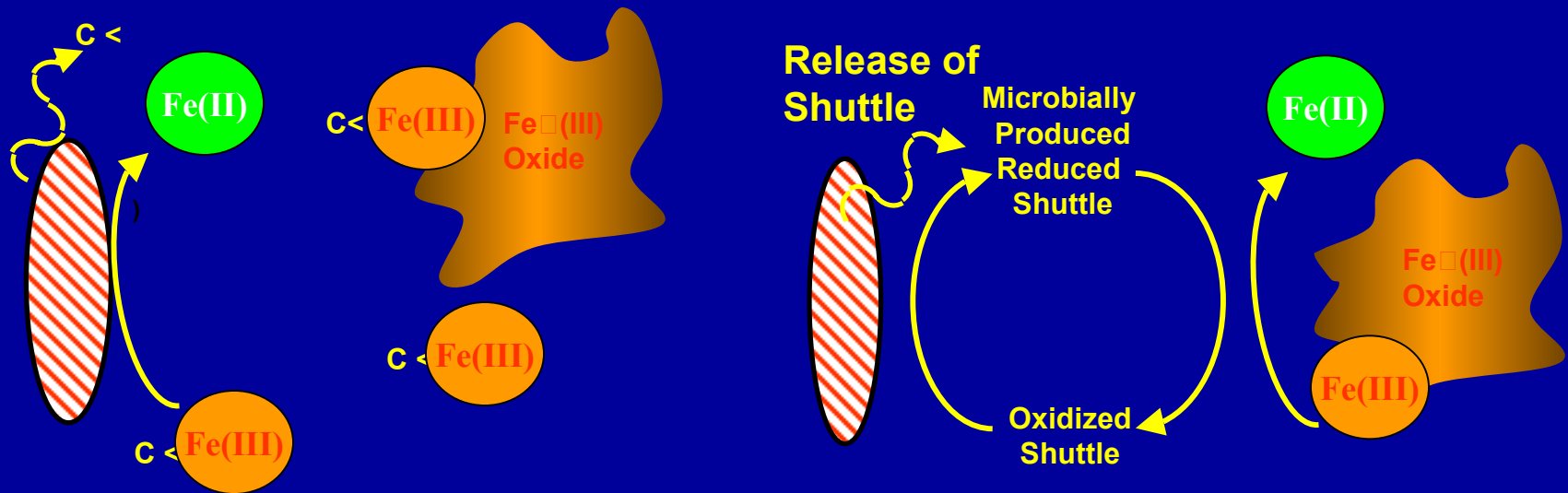
Nevin, K. P., and D. R. Lovley. 2000.

Appl. Environ. Microbiol. 66:2248-2251

Childers S.E., S. Ciuffo, and D. R. Lovley. 2002.

Nature 416:767-769

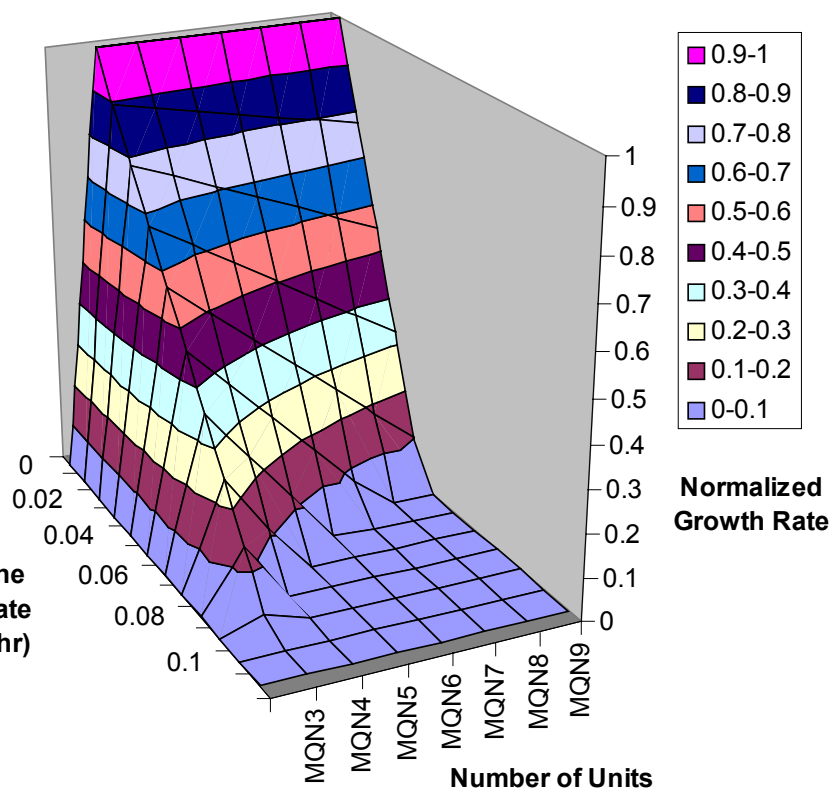
Geothrix releases electron shuttles and chelators which alleviate the need for direct microbe-Fe(III) oxide contact



Nevin, K. P., and D. R. Lovley. 2002. Appl. Environ. Microbiol. 68:2294-2299

Analysis of Metabolic Cost to Release a Quinone-Based Electron Shuttle

Energetics of Menaquinone Secretion
(10 mmol/gdw hr Acetate Uptake)



Simulations carried out for varying

- Quinone secretion rates
- Different Sized Molecules

Significant growth rate reduction due to both ATP requirements and carbon requirements for shuttle synthesis

Provides likely explanation for the predominance *Geobacter* over *Geothrix* in subsurface environments

Discoveries from GTL with Direct and Immediate Application to NABIR (continued)

- Discovery of significant similarities in genomes of as-yet-uncultured *Geobacter* species and pure cultures of *Geobacter* species

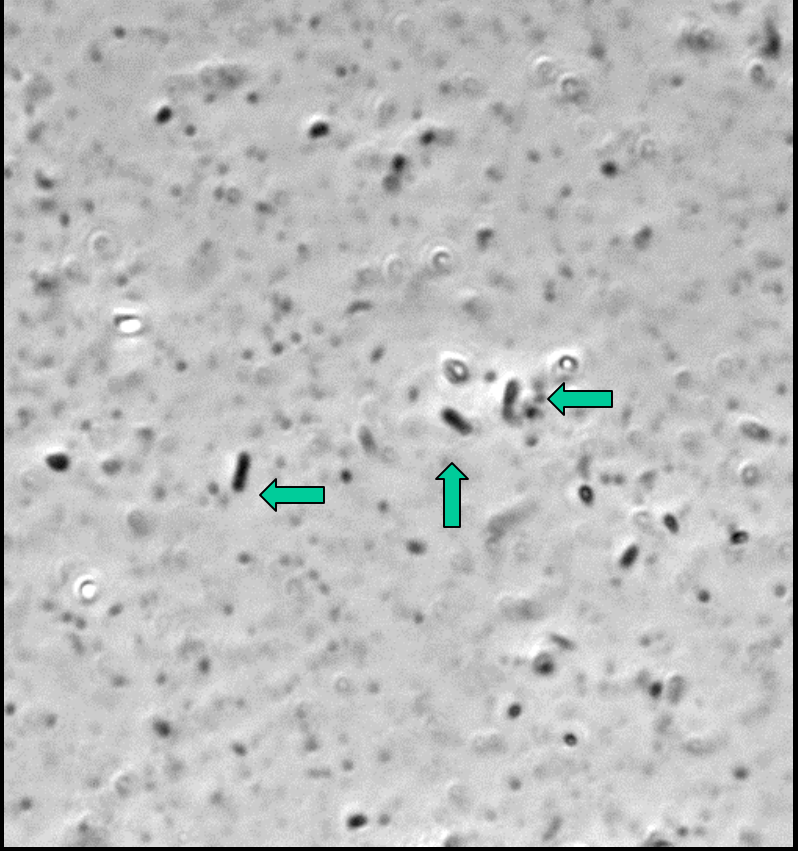
Suggests that models based on intensively studied pure cultures may have applicability to predicting the activity of as-yet-uncultured *Geobacter* species that predominate in uranium-contaminated subsurface environments.

Geobacter uranibioremediacens: Isolate from Rifle, Colorado Field Site

16S rDNA sequence identical with predominant *Geobacter* sequence in groundwater during uranium bioremediation

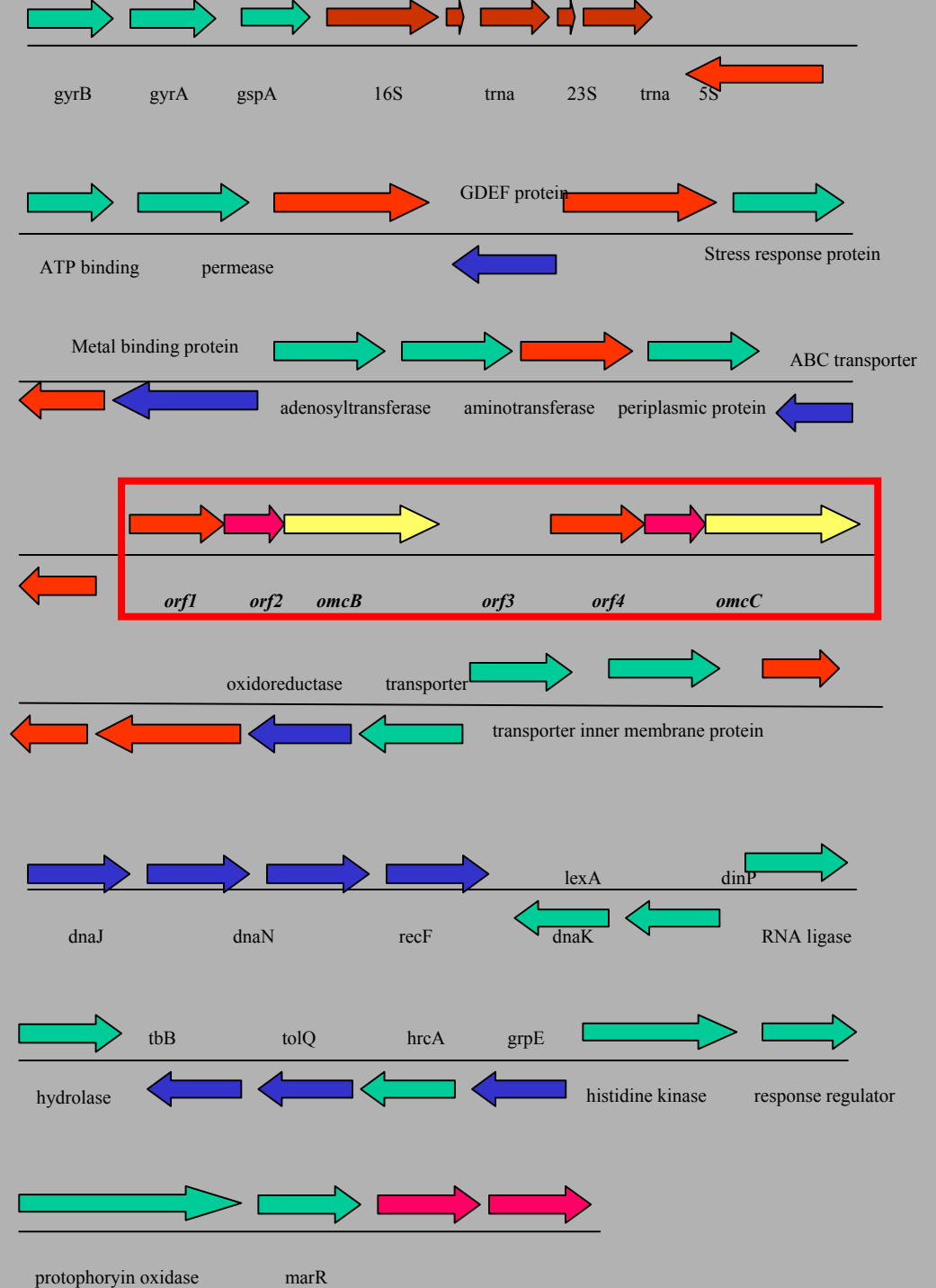


Direct Isolation on Solidified Medium with Aquifer Clay Fraction Serving as Fe(III) Source

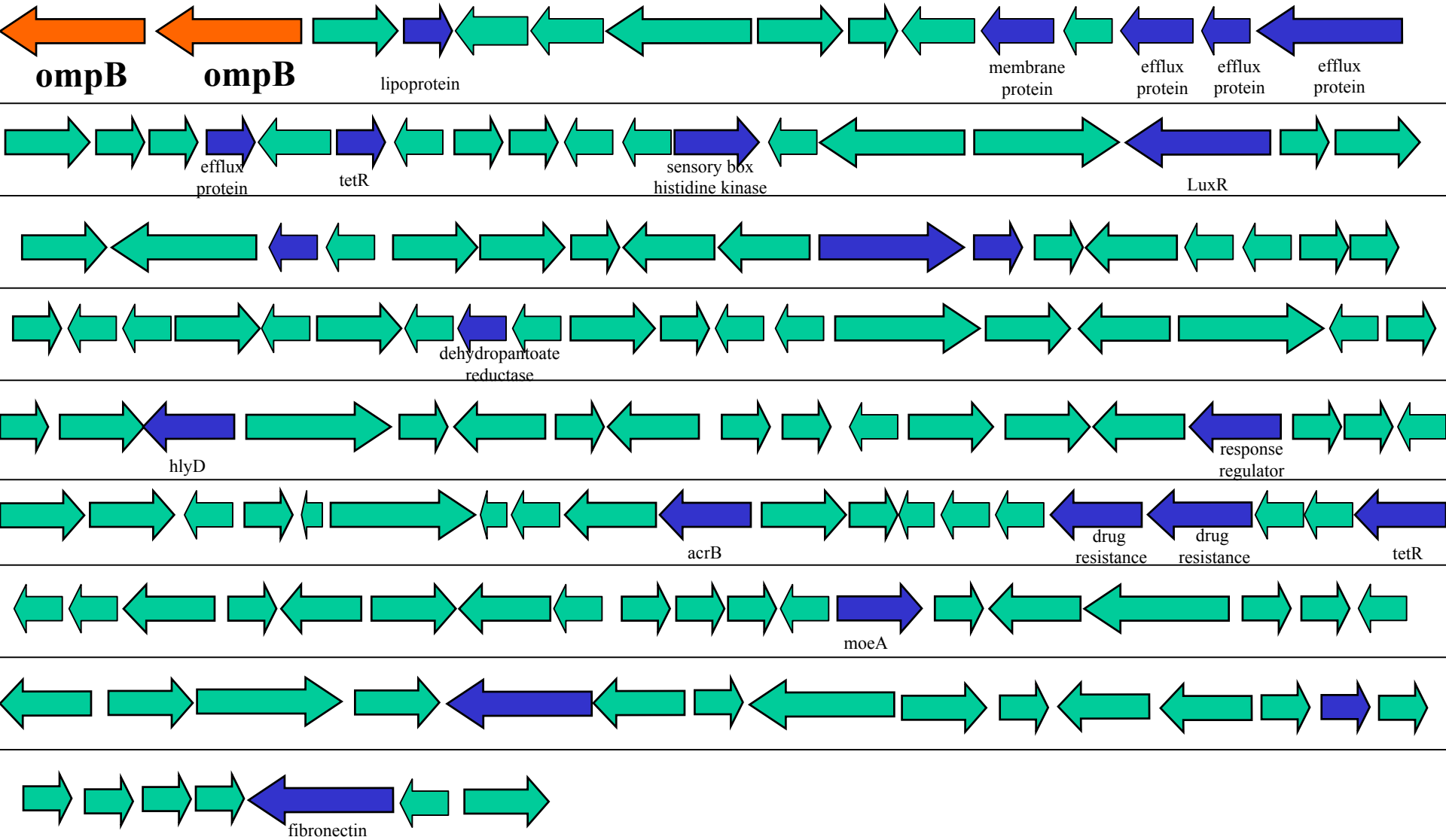


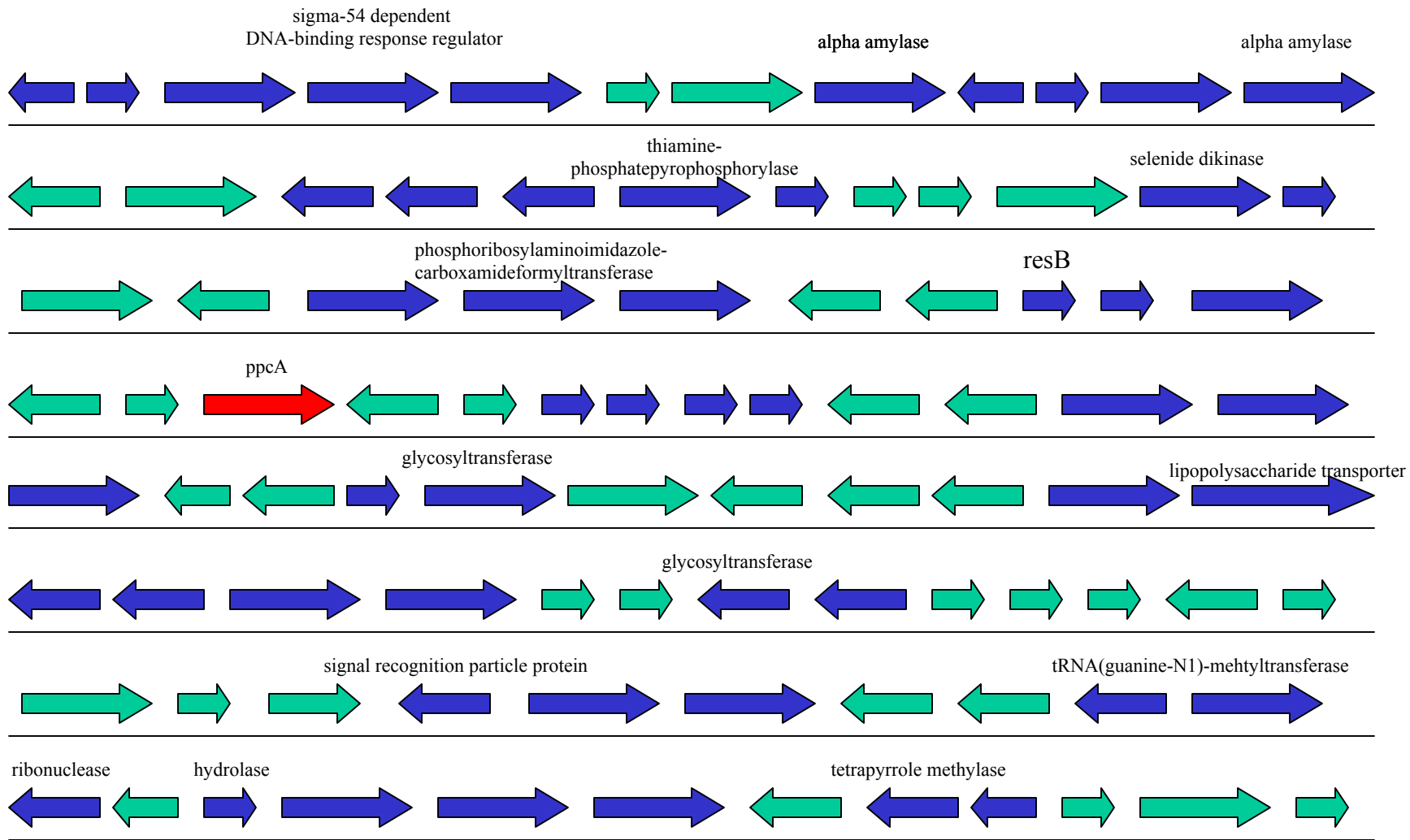
Phase Contrast Micrograph of Cells Amongst Sediment Clay Fraction In Ground-Water Amended Medium

BAC clone from as-yet-uncultured *Geobacter* from subsurface sediments contains *omcB*, a gene for an outer-membrane cytochrome required for Fe(III) reduction in *G. sulfurreducens* in the same gene organization as seen in *G. sulfurreducens*



Map of BAC 109

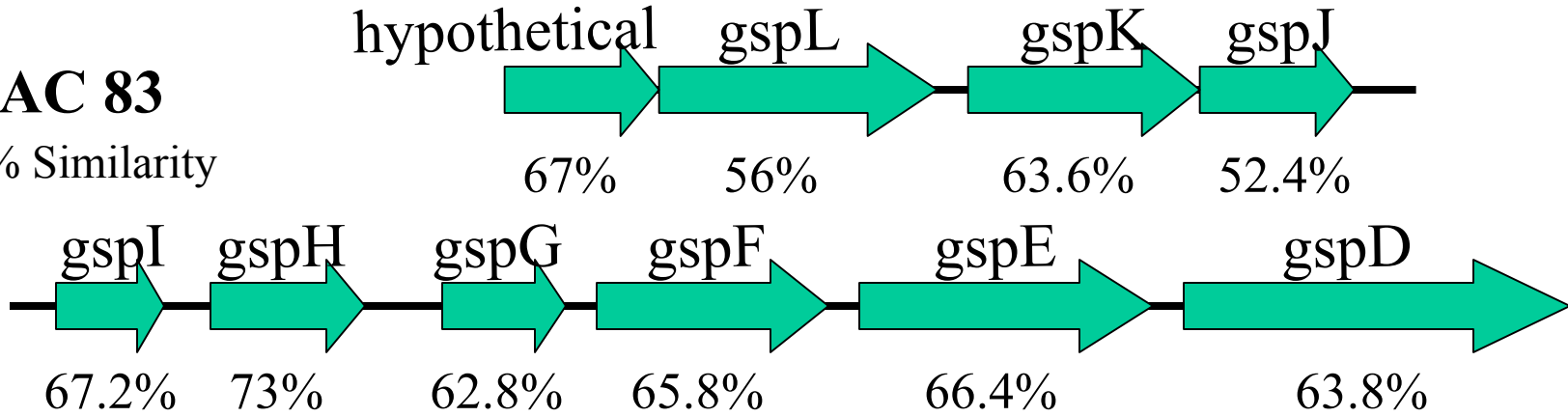




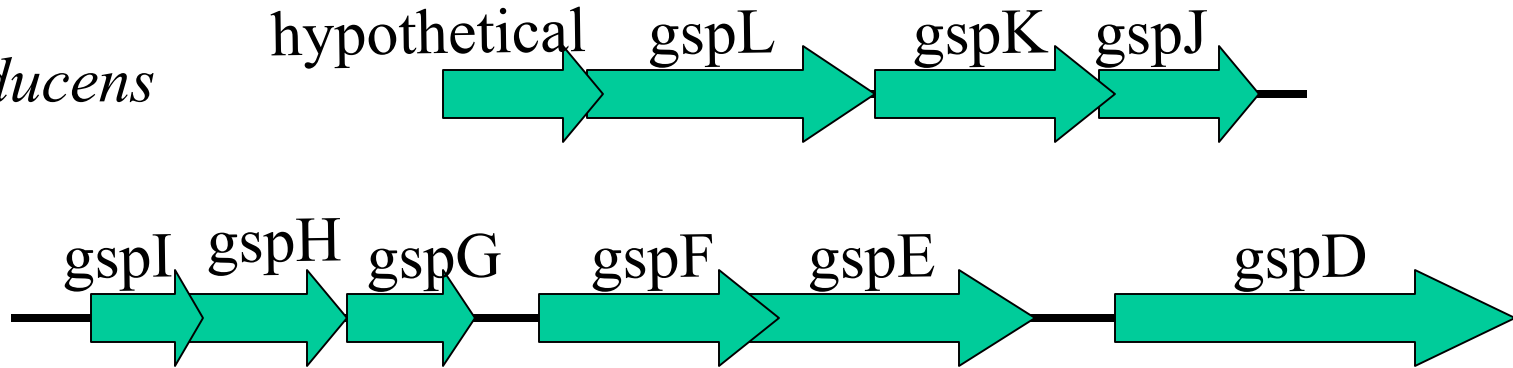
General Secretion Pathway

BAC 83

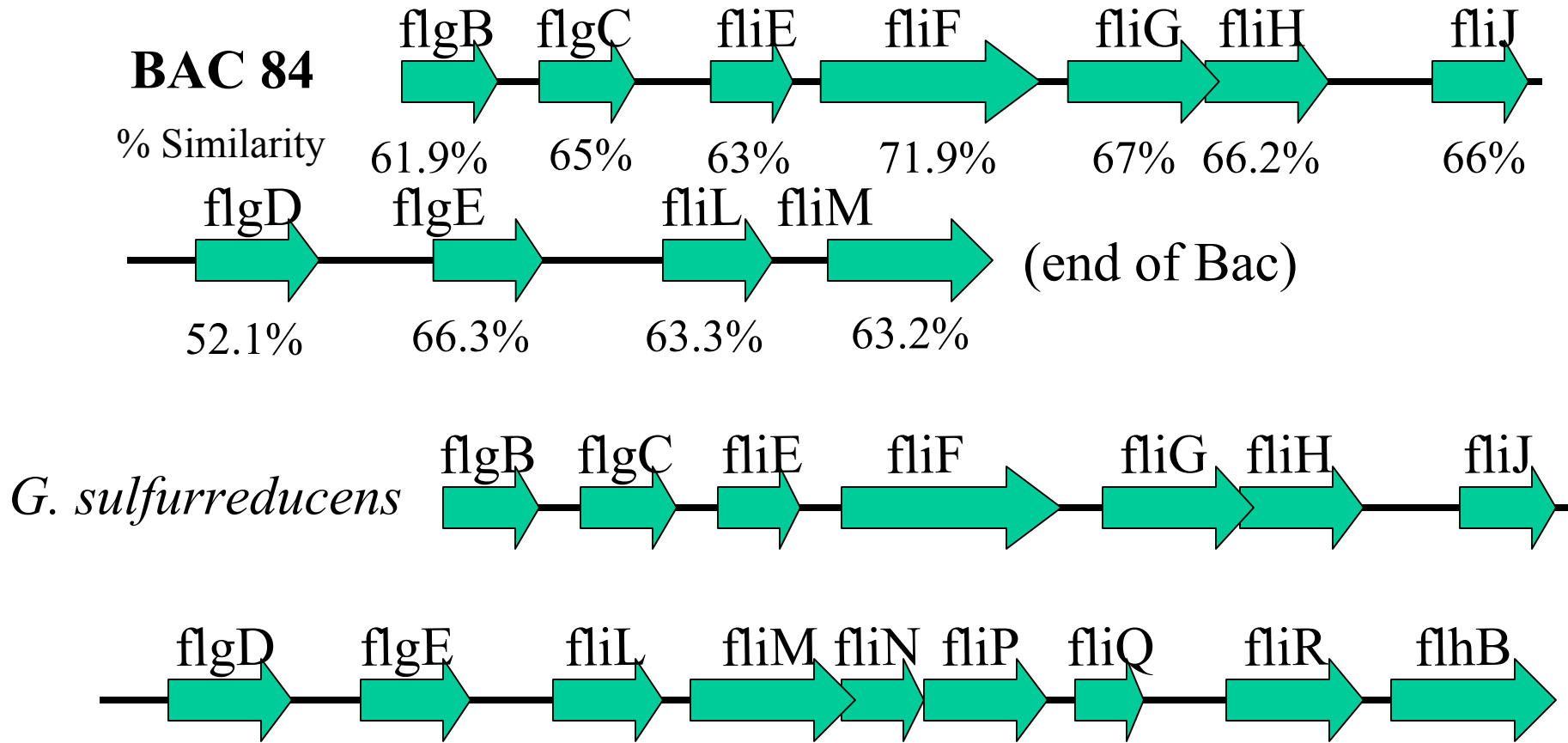
% Similarity



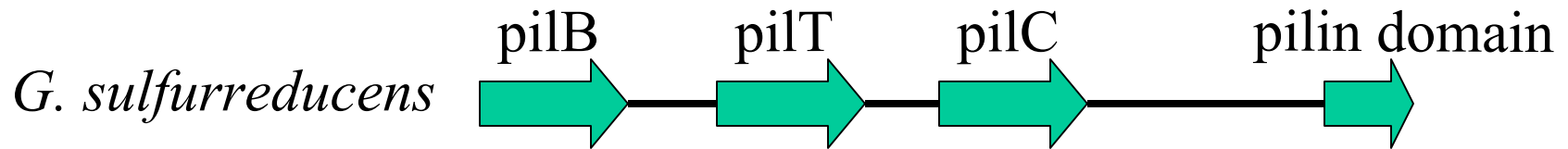
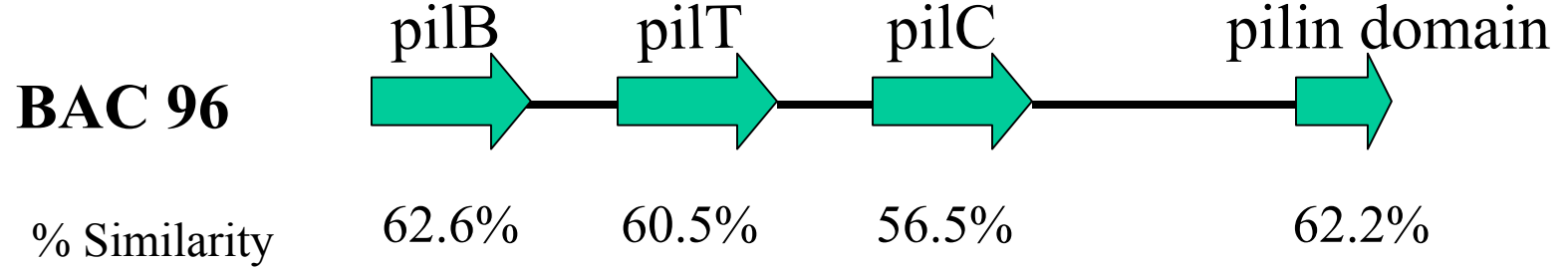
G. sulfurreducens



Flagella



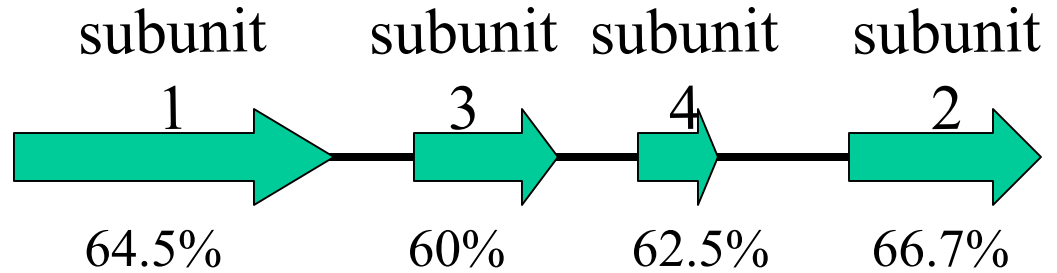
Pilin



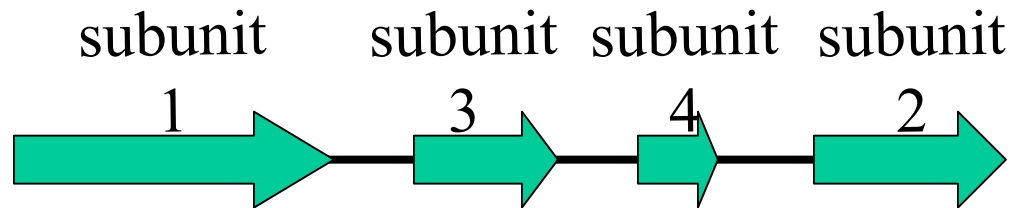
Cytochrome Oxidase

BAC 82

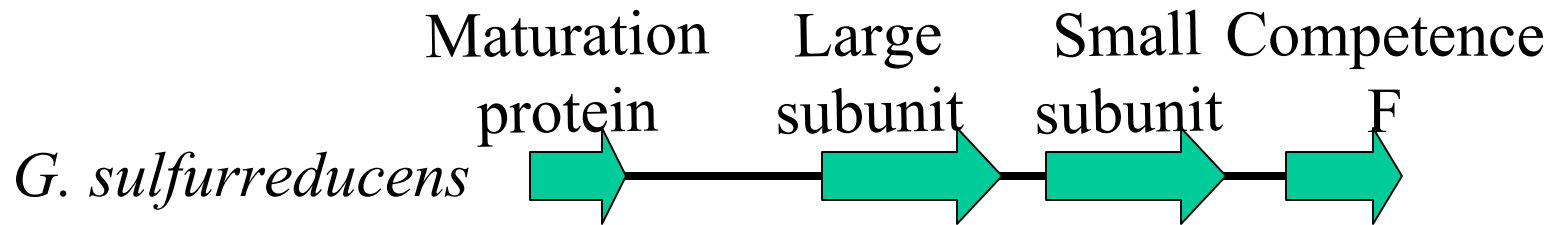
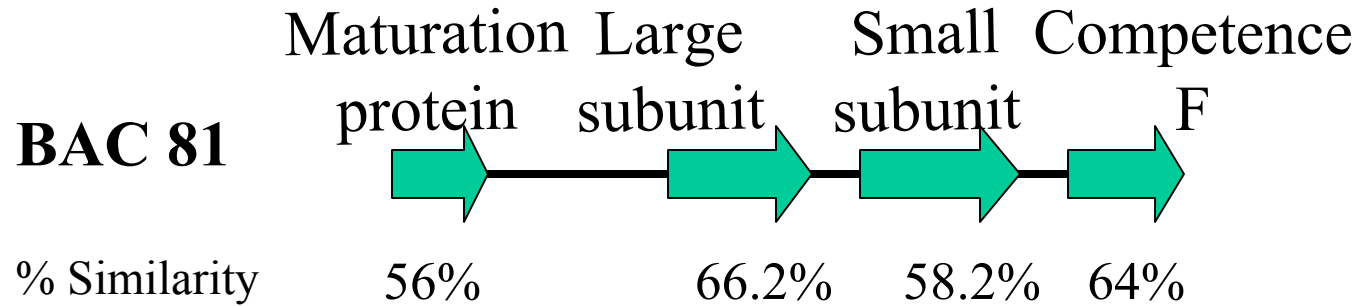
% Similarity



G. sulfurreducens



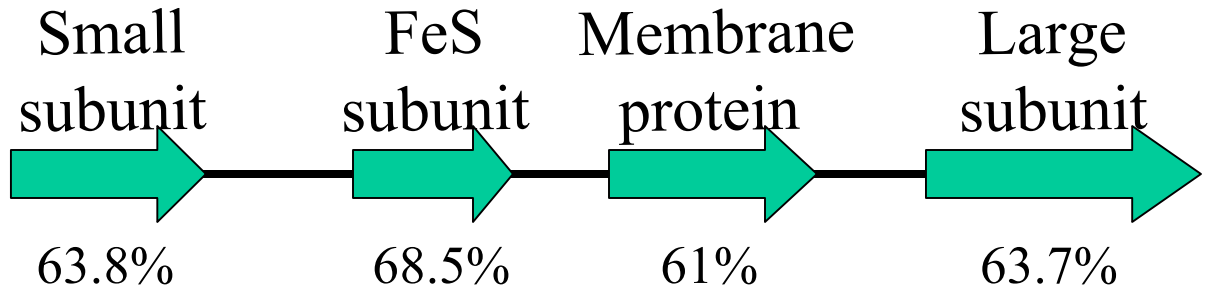
Hydrogenase A



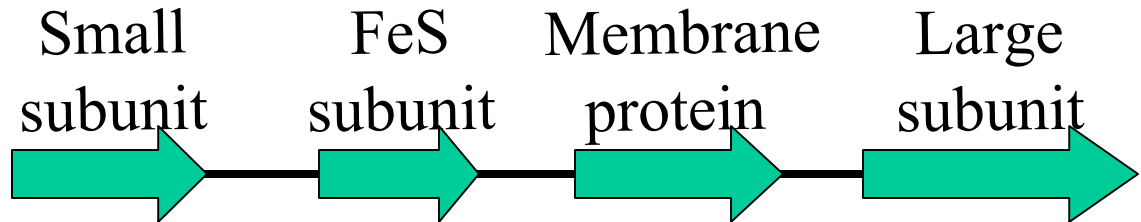
Hydrogenase B

BAC 88

% Similarity

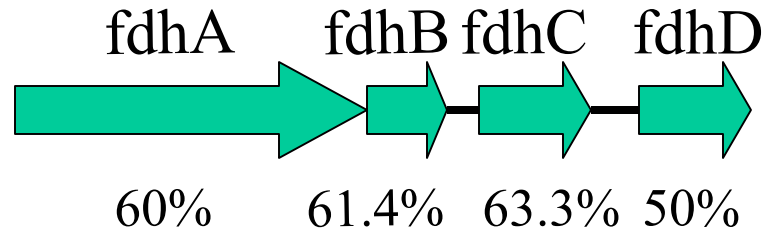


G. sulfurreducens

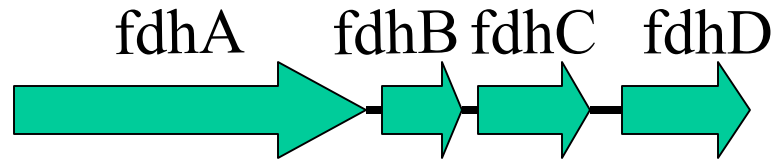


Formate Dehydrogenase

BAC 147



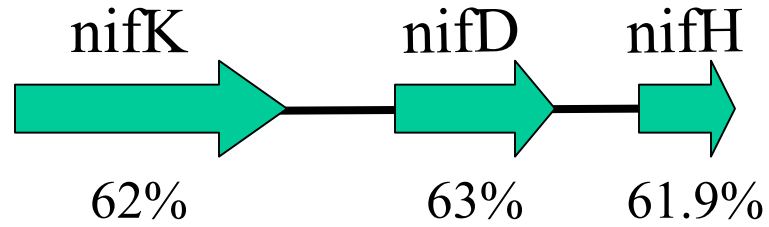
G. sulfurreducens



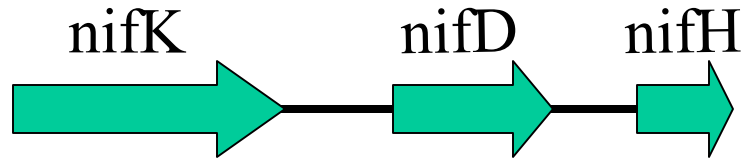
Nitrogen Fixation Genes

BAC 74

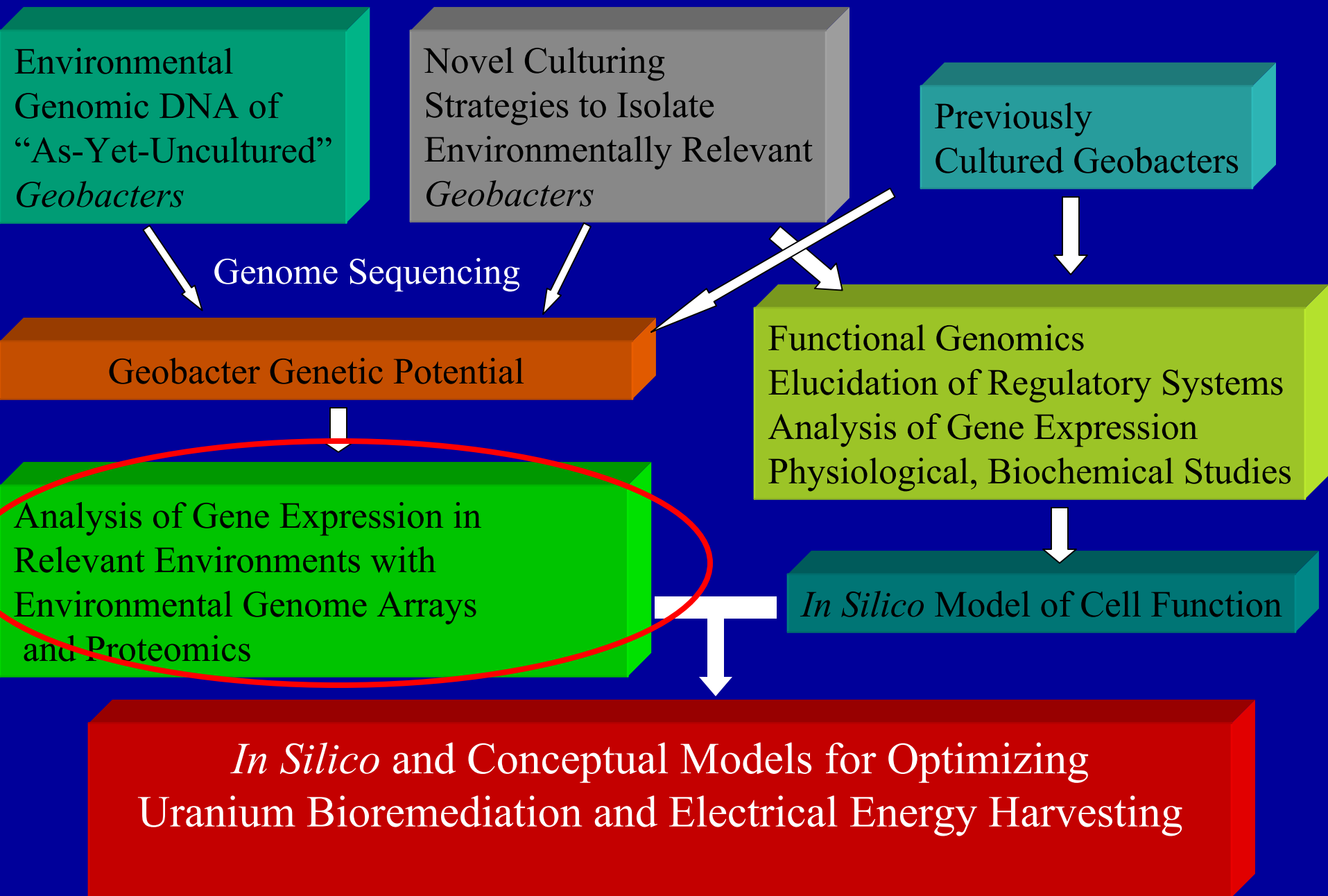
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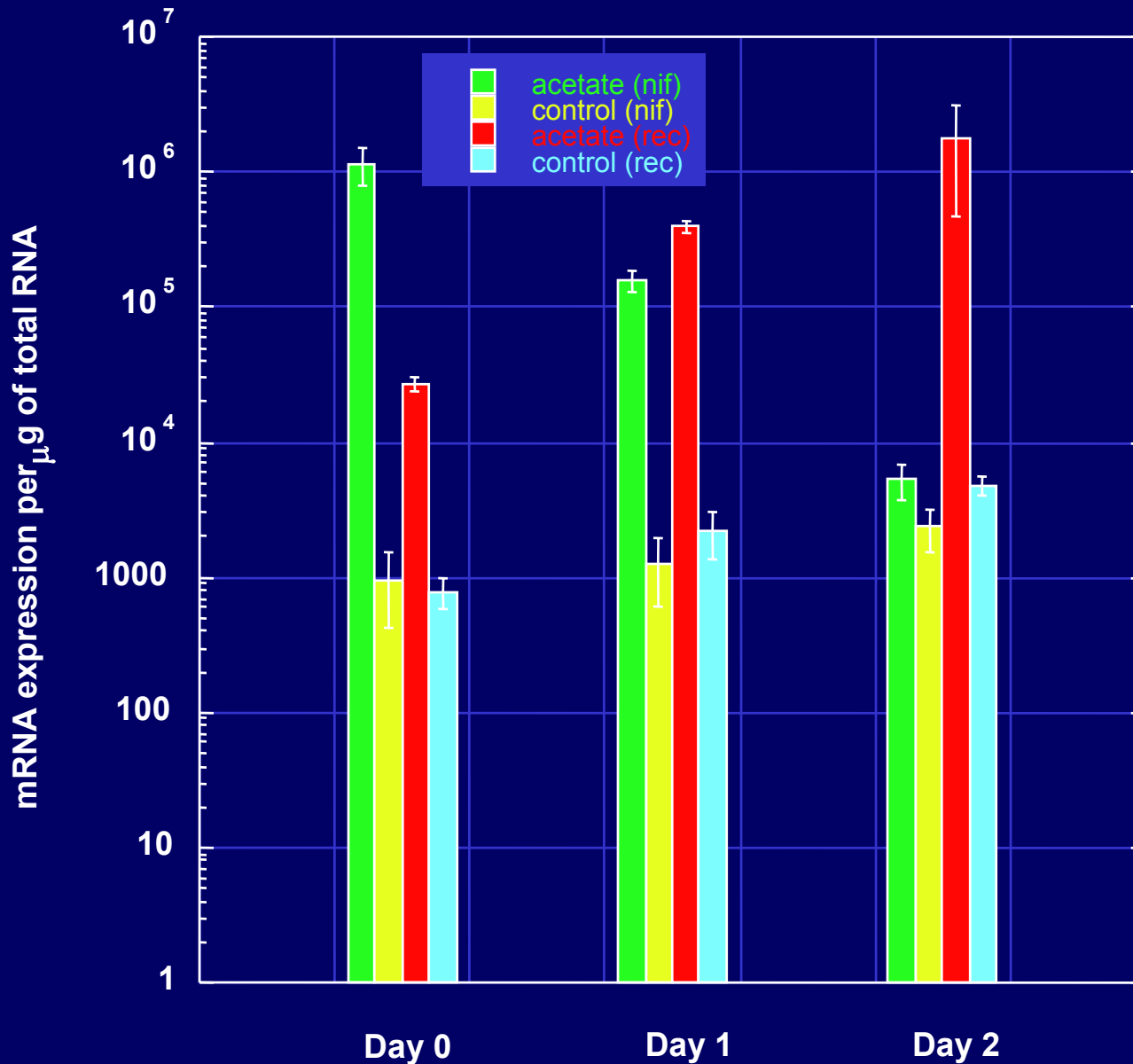
G. sulfurreducens



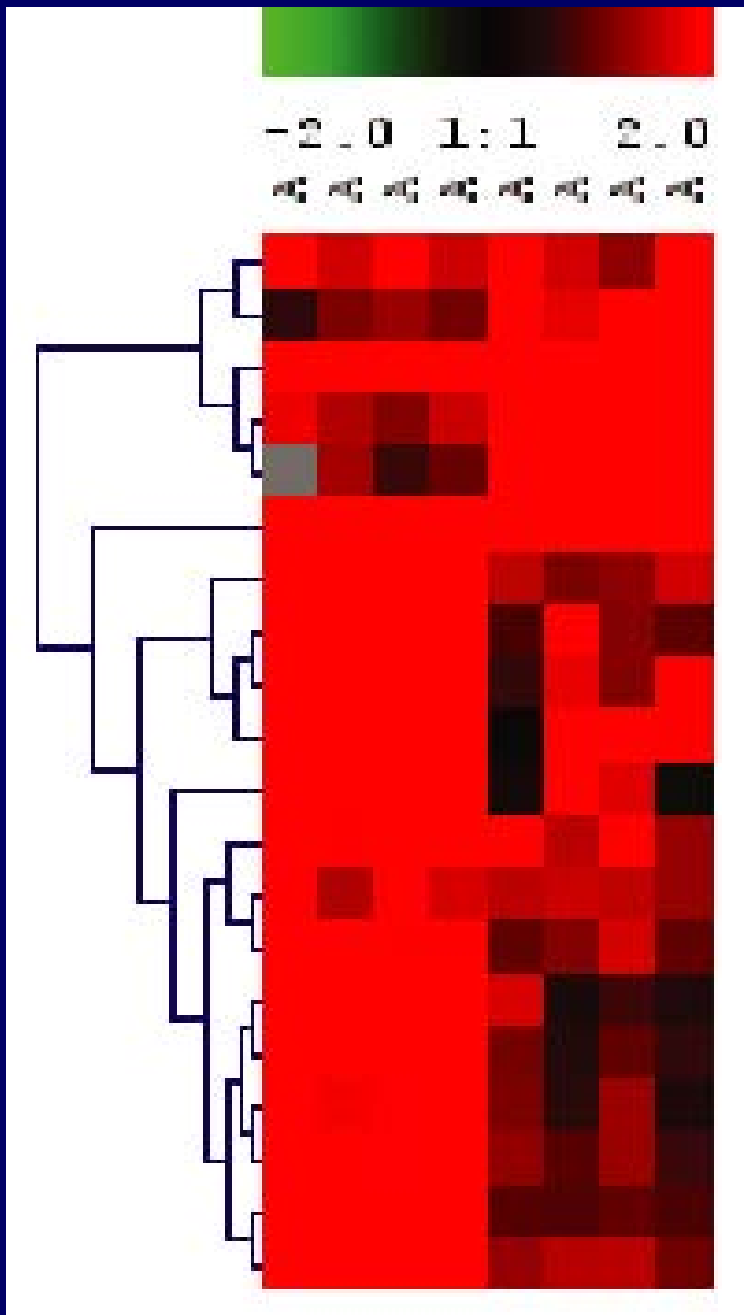
Application of Environmental Genomics and Systems Biology to Uranium Bioremediation and Harvesting Electricity from Waste Organic Matter



nifD and *recA* expression in Acetate-amended and Control Subsurface Sediments before and after adding 100 μM NH_4Cl

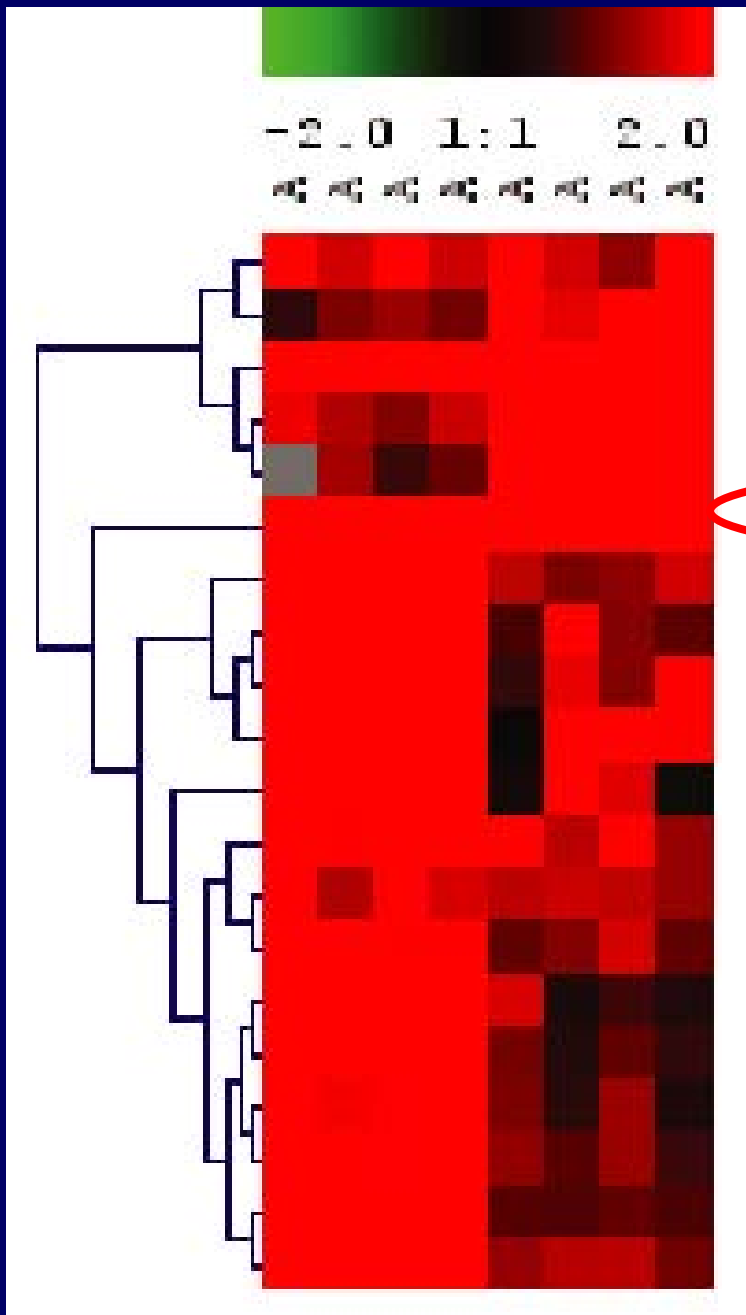


Geobacter Genes Upregulated During Growth on Electrodes



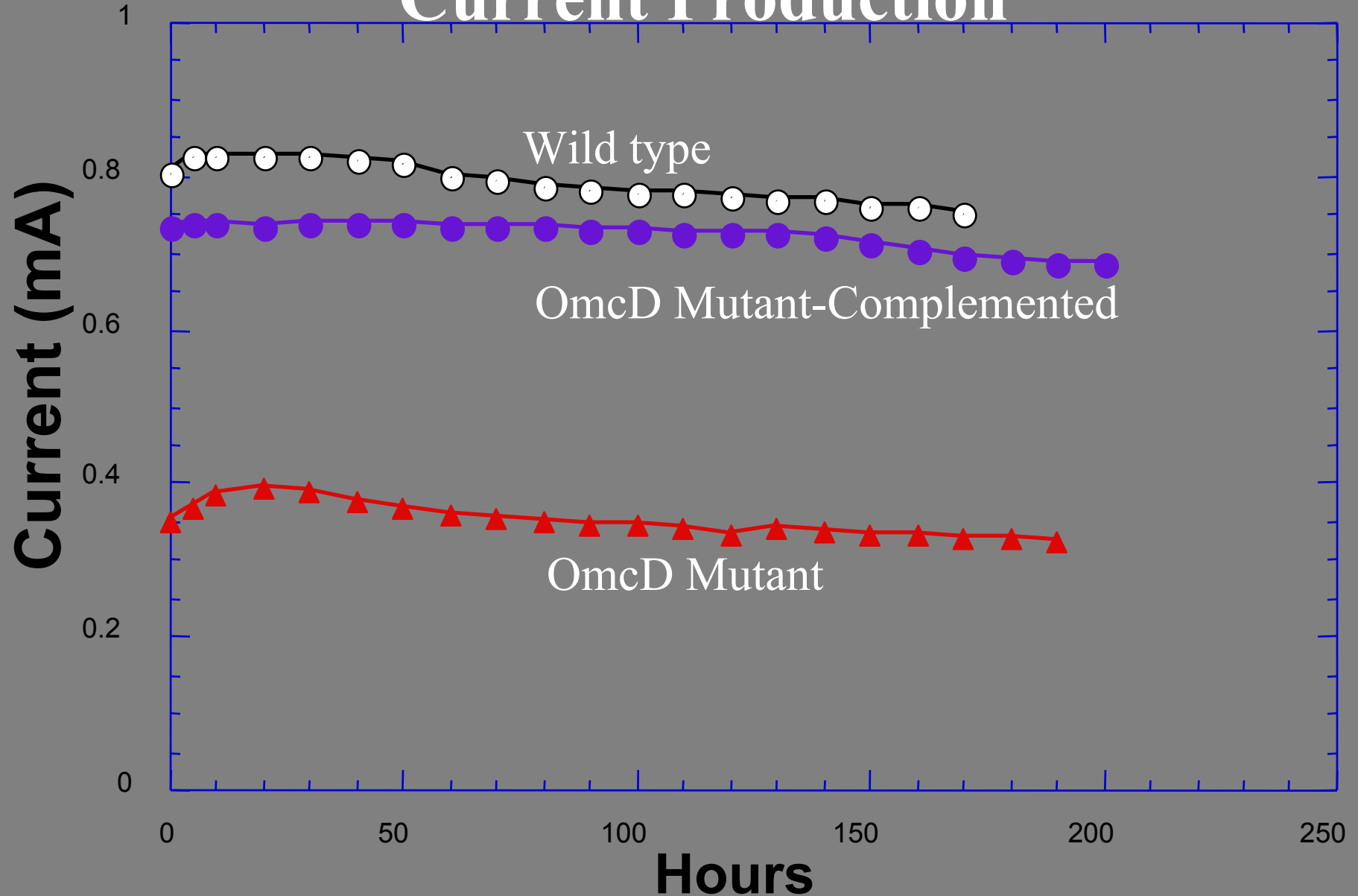
heat shock protein, Hsp20 family
dnaJ domain protein
heat shock protein, Hsp20 family
heat shock protein, Hsp20 family
heat shock protein, Hsp20 family
cytochrome c family protein
hypothetical protein
NOL1/NOP2/sun family protein
conserved domain protein
C4-dicarboxylate transporter, anaerobic
cytochrome c family protein, putative
cytochrome c family protein
ClpB protein
NHL repeat domain protein
metal ion efflux outer memb. prot. family, put.
hypothetical protein
hypothetical protein
ABC transporter, permease protein
transcriptional regulator, MerR family
hypothetical protein

Geobacter Genes Upregulated During Growth on Electrodes



- heat shock protein, Hsp20 family
- dnaJ domain protein
- heat shock protein, Hsp20 family
- heat shock protein, Hsp20 family
- heat shock protein, Hsp20 family
- cytochrome c family protein OmcD**
- hypothetical protein
- NOL1/NOP2/sun family protein
- conserved domain protein
- C4-dicarboxylate transporter, anaerobic
- cytochrome c family protein, putative**
- cytochrome c family protein**
- ClpB protein
- NHL repeat domain protein
- metal ion efflux outer memb. prot. family, put.
- hypothetical protein
- hypothetical protein
- ABC transporter, permease protein
- transcriptional regulator, MerR family
- hypothetical protein

Effect of Deletion Mutation in *omcD* on Current Production



Support of NABIR by GTL in the Future

- Use of molecular techniques to assess *in situ* rates of metal reduction.
- Whole-genome analysis of *in situ* gene expression to determine the *in situ* metabolic state of microorganisms during uranium bioremediation which will help direct implementation of bioremediation strategies.
- Coupling *in silico* microbial models with geochemical and hydrological models to accurately predict the rate and extent of bioremediation in diverse environments under various bioremediation strategies.