# Update on Microbiology Research at the Waste Isolation Pilot Plant (WIPP)

Los Alamos National Laboratory—Carlsbad Operations
Actinide Chemistry & Repository Science Program Team

April 16, 2013

LA-UR-13-22647

# BACKGROUND

# CONCEPTUAL MODEL of MICROBIAL EFFECTS on NUCLEAR WASTE REPOSITORIES

- Microbial activity may affect actinide oxidation state
- Organic waste components, including high molecular weight organics (cellulose, plastic, and rubber), will be degraded
- The degradation of organics leads to production of
  - gas
  - organic ligands
- Microorganisms can adsorb actinides and serve as vectors for transport away from repository
- Microbial activity may affect backfill materials

**ASSUMPTION VS. REALITY**

# ASSUMPTIONS VERSUS REALITY in SALT-BASED WASTE REPOSITORIES

| ASSUMPTION | REALITY |
|---|---|
| Near-field homogeneity means microorganisms have access to all waste components | Near-field will be heterogeneous with microenvironments |
| Basic requirements for optimal growth (water, nutrients, substrates, electron donors and acceptors) are present and accessible | Basic requirements are not always met and rarely ideal for halophiles; heterogeneity and solubility limit accessibility |
| Cellulose will be completely degraded | Partial degradation may occur if cellulolytic organisms are present, active, and have access to cellulosics |

# ASSUMPTIONS VERSUS REALITY in SALT-BASED WASTE REPOSITORIES

| ASSUMPTION | REALITY |
|---|---|
| Gas generation from consumption of organics | Generation rates are likely optimistic; $CO_2$ accounted for by MgO at WIPP |
| Hydrocarbon degradation will lead to gas generation | Known haloarchaeal hydrocarbon degraders exist, but all are obligate aerobes; unlikely survival of halophilic bacterial degraders in repository brine, but more research needed |
| All organisms adsorb actinides; all organisms are mobile | Differential sorption behavior; presence of EDTA diminishes sorption; some cells motile, some sessile; cells may lyse between near- and far-fields |
| Is far-field taken into consideration? | Far-field microbial activity far greater than near-field |

# MICROBIAL ECOLOGY of the WIPP

# SURVIVAL of MICROORGANISMS at HIGH IONIC STRENGTH

- Must be able to maintain osmotic balance with external environment
  - Haloarchaea and some Bacteria (order *Halanaerobiales*) maintain high concentrations of $K^+$ or $Cl^-$ internally
  - All other Bacteria and all eukaryotes use mostly organic solutes

- Maintaining osmotic balance is energetically costly

- All metabolic processes are limited by thermodynamics; weigh cost of maintaining osmotic balance with benefit of energy obtained through given metabolic reaction

# METABOLIC PROCESSES of HALOPHILIC MICROORGANISMS

| MODE OF METABOLISM | HALOPHILIC BACTERIA | HALOARCHAEA | CONSTRAINTS OTHER THAN [SALT] |
|---|---|---|---|
| Aerobic | + | + | Oxygen availability |
| Nitrate Reduction | + | + | Inventory |
| Metal Reduction | + | − | Solubility |
| Sulfate Reduction | + | − | Few limits |
| Methanogenesis | − | − | Adequate substrate |
| Fermentation | + | + | Primary lytic species; substrate |

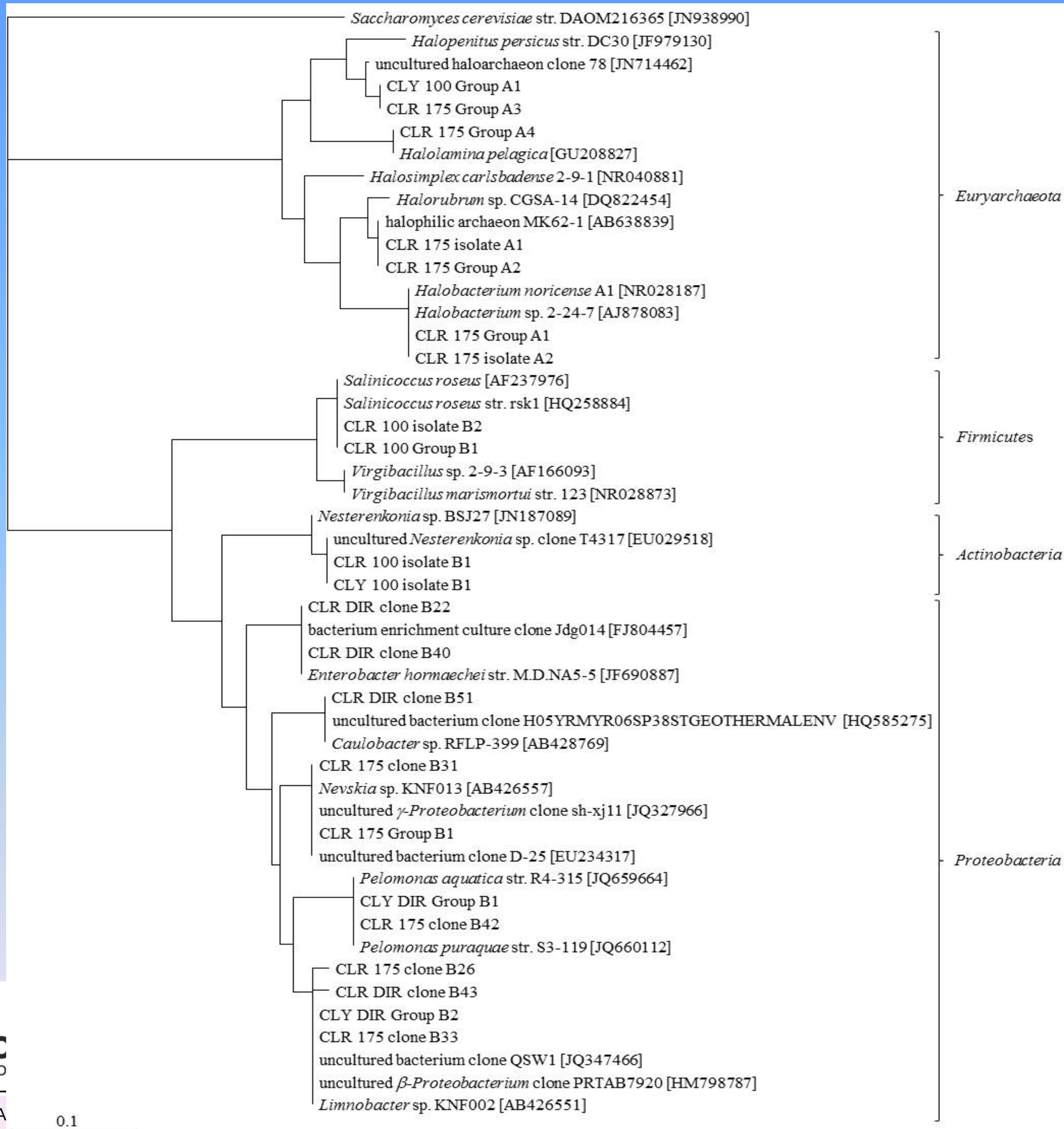
# WIPP HALITE—"Near-field Environment"

- Extremely halophilic *Archaea* (all members of class *Halobacteria*):
  - *Halobacterium* (sorption experiments)
  - *Halorubrum*-like (unclassified)
  - *Halolamina*
  - *Natronomonas*

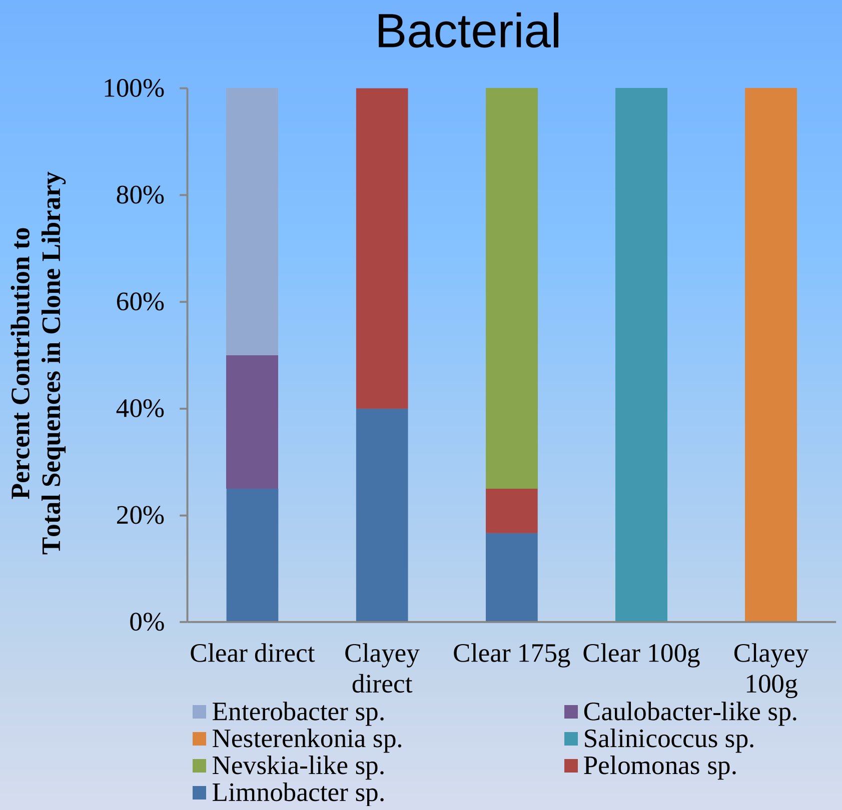
- Halotolerant to moderately halophilic *Bacteria*:
  - *Proteobacteria*
  - *Actinobacteria*
  - *Firmicutes* (Vreeland et al., 2000)

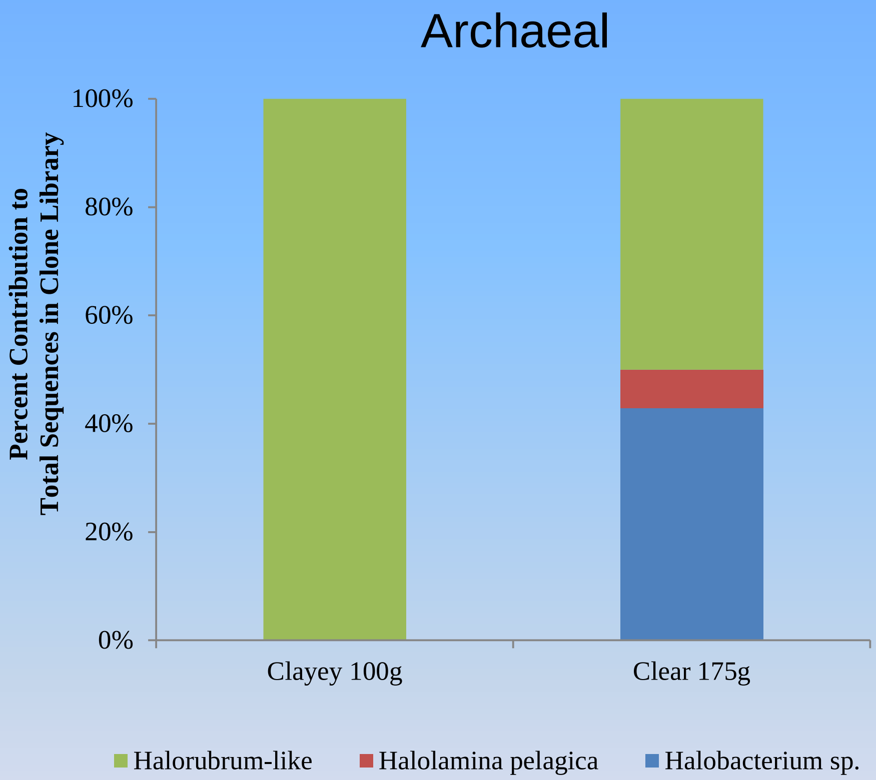
- Halotolerant to moderately halophilic *Fungi* (all members of phylum *Ascomycota*):
  - *Cladosporium*
  - *Engyodontium*
  - *Phoma*

*ALL FROM AEROBIC INCUBATIONS*
*NO GROWTH IN ANAEROBIC INCUBATIONS*


Saccharomyces cerevisiae str. DAOM216365 [JN938990]
Halopenitus persicus str. DC30 [JF979130]
uncultured haloarchaeon clone 78 [JN714462]
CLY 100 Group A1
CLR 175 Group A3
CLR 175 Group A4
Halolamina pelagica [GU208827]
Halosimplex carlsbadense 2-9-1 [NR040881]
Halorubrum sp. CGSA-14 [DQ822454]
halophilic archaeon MK62-1 [AB638839]
CLR 175 isolate A1
CLR 175 Group A2
Halobacterium noricense A1 [NR028187]
Halobacterium sp. 2-24-7 [AJ878083]
CLR 175 Group A1
CLR 175 isolate A2
Euryarchaeota
Salinicoccus roseus [AF237976]
Salinicoccus roseus str. rsk1 [HQ258884]
CLR 100 isolate B2
CLR 100 Group B1
Virgibacillus sp. 2-9-3 [AF166093]
Virgibacillus marismortui str. 123 [NR028873]
Firmicutes
Nesterenkonia sp. BSJ27 [JN187089]
uncultured Nesterenkonia sp. clone T4317 [EU029518]
CLR 100 isolate B1
CLY 100 isolate B1
Actinobacteria
CLR DIR clone B22
bacterium enrichment culture clone Jdg014 [FJ804457]
CLR DIR clone B40
Enterobacter hormaechei str. M.D.NA5-5 [JF690887]
CLR DIR clone B51
uncultured bacterium clone H05YRMYR06SP38STGEOTHERMALENV [HQ585275]
Caulobacter sp. RFLP-399 [AB428769]
CLR 175 clone B31
Nevskia sp. KNF013 [AB426557]
uncultured γ-Proteobacterium clone sh-xj11 [JQ327966]
CLR 175 Group B1
uncultured bacterium clone D-25 [EU234317]
Pelomonas aquatica str. R4-315 [JQ659664]
CLY DIR Group B1
CLR 175 clone B42
Pelomonas puraquae str. S3-119 [JQ660112]
CLR 175 clone B26
CLR DIR clone B43
CLY DIR Group B2
CLR 175 clone B33
uncultured bacterium clone QSW1 [JQ347466]
uncultured β-Proteobacterium clone PRTAB7920 [HM798787]
Limnobacter sp. KNF002 [AB426551]
Proteobacteria
0.1

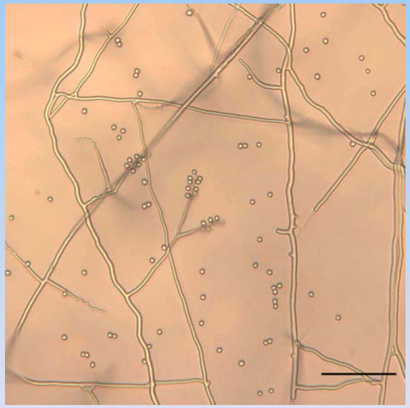
# HALITE PROKARYOTIC DIVERSITY

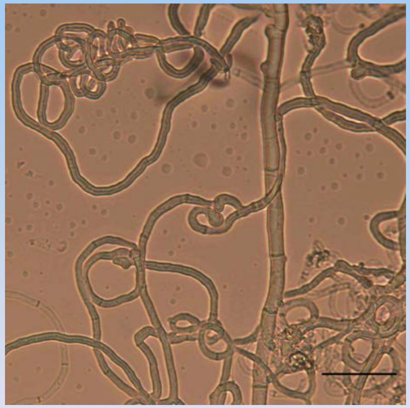


# HALITE EUKARYOTIC DIVERSITY

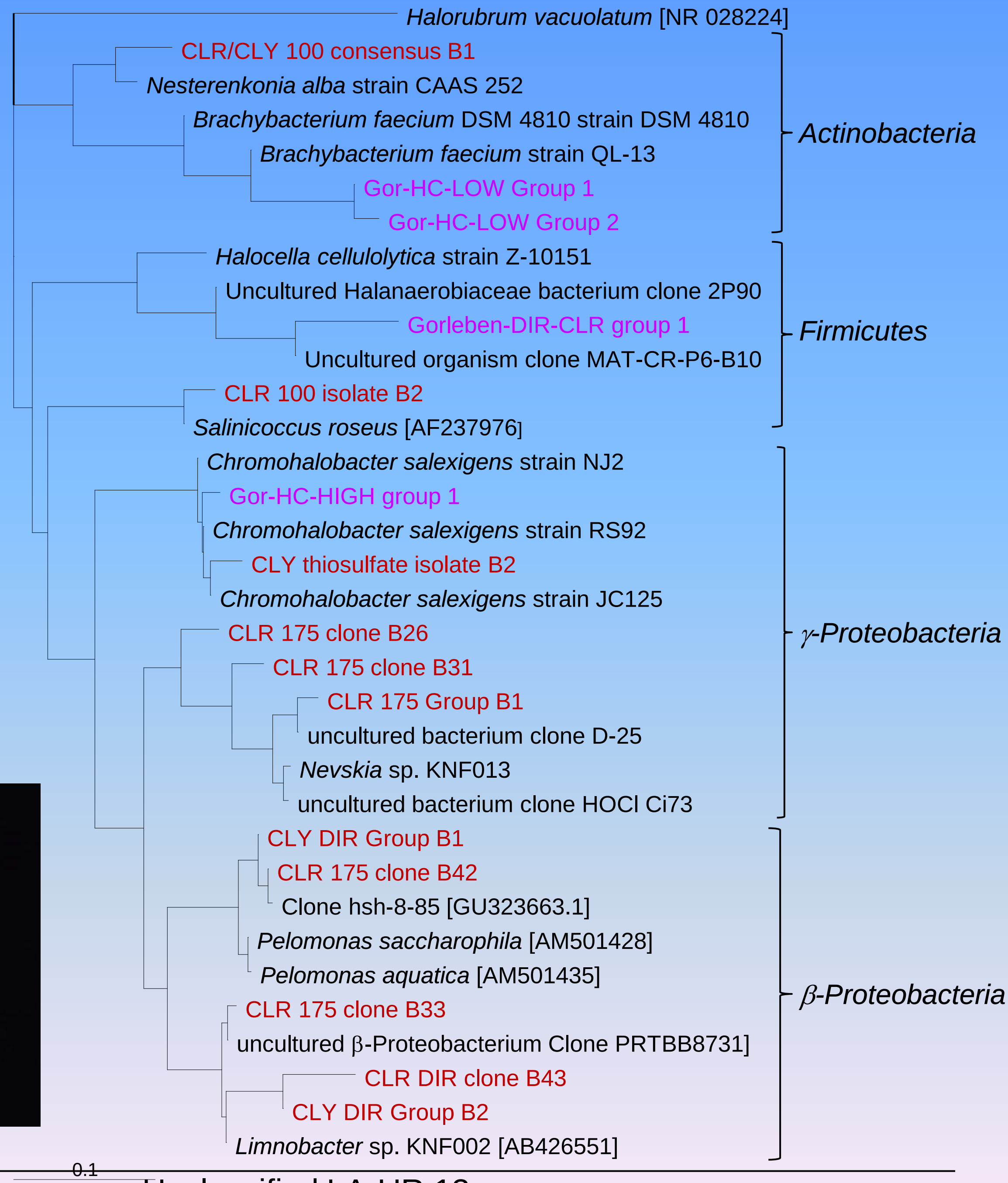
- Three fungal isolates:
  - *Cladosporium*
  - *Engyodontium*
  - *Phoma*
- All obligate aerobes



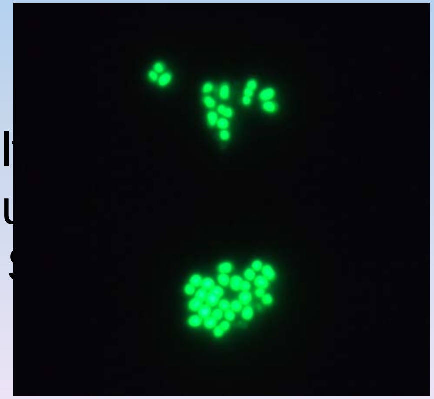


# COMPARISON of SALADO and GORLEBEN HALITES—*BACTERIA*

Shared organism:
*Chromohalobacter*
Not found in Salado:
*Brachybacterium, Halocella*-like




Archaeal resul
negative for Eu
direct extract, 
in culture



# WIPP GROUNDWATERS

- Far-field environment
- Range of ionic strengths (~1.5-5 M)
- Low in DOC, nitrate; high in sulfate (25-74 mM), $Mg^{2+}$, $K^{+}$, $Ca^{2+}$

- *Bacteria* with diverse metabolic capability:
  - Nitrate reducers
  - Metal reducers
  - Sulfate reducers
  - Fermenters (including extremely halophilic *Bacteria*)

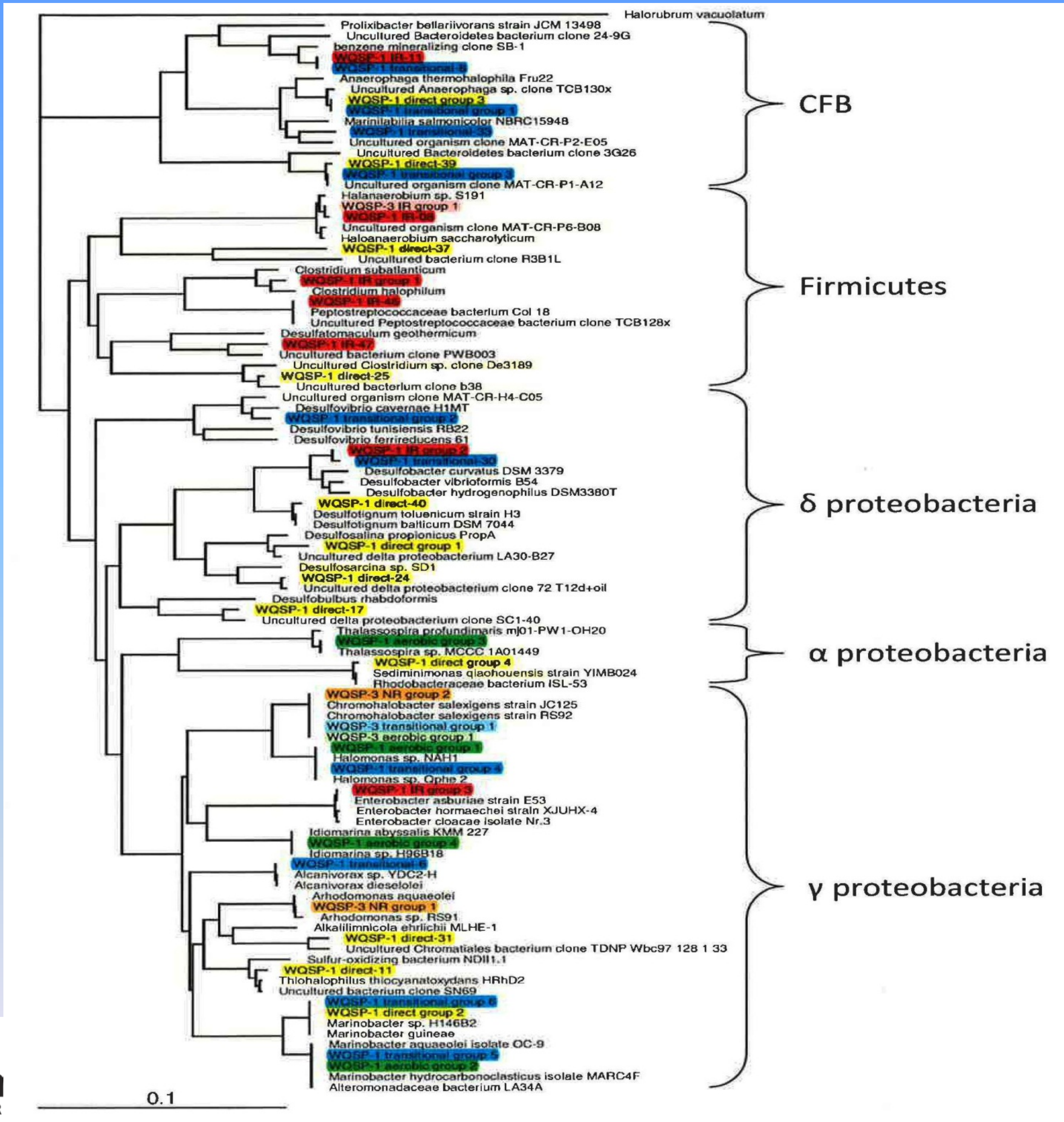
- When metals are present, incubations result in precipitation of metal-sulfide; both metal-reducers and sulfate-reducers can be detected

# WIPP GROUNDWATERS—AEROBIC

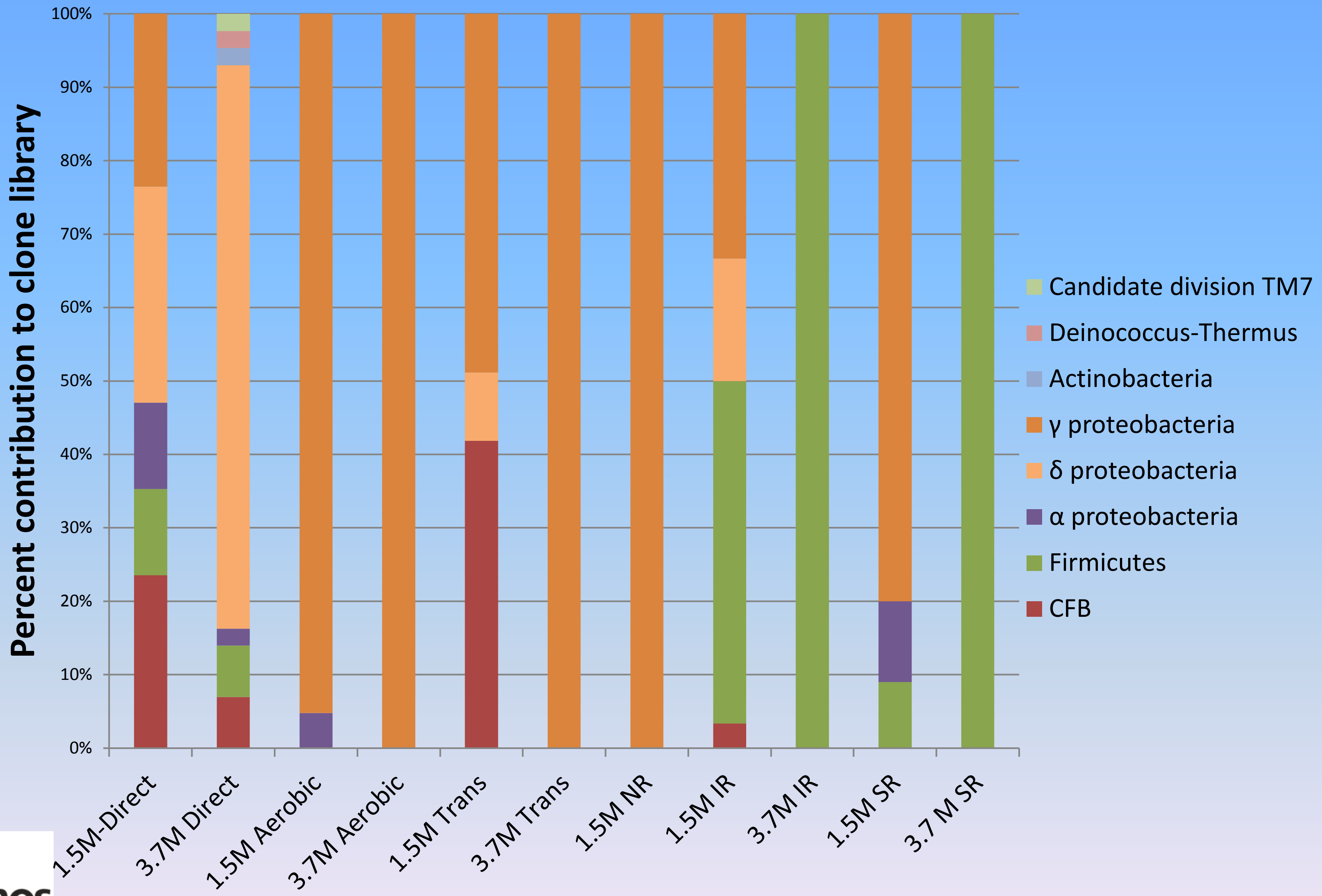
- Moderate to extremely halophilic *Archaea* (all order *Halobacteriales*):
  - *Halococcus*
  - *Natrinema*
- Halotolerant to extremely halophilic *Bacteria:*
  - *Proteobacteria* (*Chromohalobacter* spp.)
  - *Firmicutes* (*Virgibacillus*, *Pontibacillus, Bacillus*)
- Halotolerant *Fungi* (only in 5 M groundwater; not yet characterized)

# WIPP GROUNDWATERS—ANAEROBIC

- Moderate to extremely halophilic *Archaea* (all *Halobacteriales*), viable but not growing anaerobically:
  - *Natrinema*
  - *Haloferax*
  - *Haloarcula*
- Halotolerant to moderately halophilic *Bacteria:*
  - *Proteobacteria* (including denitrifiers, iron- and sulfate-reducers)
  - *Bacteroidetes*
  - *Firmicutes* (including iron-reducers and fermenters)


Halorubrum vacuolatum
Prolixibacter bellariivorans strain JCM 13498
Uncultured Bacteroidetes bacterium clone 24-9G
benzene mineralizing clone SB-1
WQSP-1 IR-11
WQSP-1 transitional-6
Anaerophaga thermohalophila Fru22
Uncultured Anaerophaga sp. clone TCB130x
WQSP-1 direct group 3
WQSP-1 transitional group 1
Marinilabilia salmonicolor NBRC15948
WQSP-1 transitional-33
Uncultured organism clone MAT-CR-P2-E05
Uncultured Bacteroidetes bacterium clone 3G26
WQSP-1 direct-39
WQSP-1 transitional group 3
Uncultured organism clone MAT-CR-P1-A12
Halanaerobium sp. S191
WQSP-3 IR group 1
WQSP-1 IR-08
Uncultured organism clone MAT-CR-P6-B08
Haloanaerobium saccharolyticum
WQSP-1 direct-37
Uncultured bacterium clone R3B1L
Clostridium subatlanticum
WQSP-1 IR group 1
Clostridium halophilum
WQSP-1 IR-48
Peptostreptococcaceae bacterium Col 18
Uncultured Peptostreptococcaceae bacterium clone TCB128x
Desulfatomaculum geothermicum
WQSP-1 IR-47
Uncultured bacterium clone PWB003
Uncultured Clostridium sp. clone De3189
WQSP-1 direct-25
Uncultured bacterium clone b38
Uncultured organism clone MAT-CR-H4-C05
Desulfovibrio cavernae H1MT
WQSP-1 transitional group 2
Desulfovibrio tunisiensis RB22
Desulfovibrio ferrireducens 61
WQSP-1 IR group 2
WQSP-1 transitional-30
Desulfobacter curvatus DSM 3379
Desulfobacter vibrioformis B54
Desulfobacter hydrogenophilus DSM3380T
WQSP-1 direct-40
Desulfotignum toluenicum strain H3
Desulfotignum balticum DSM 7044
Desulfosalina propionicus PropA
WQSP-1 direct group 1
Uncultured delta proteobacterium LA30-B27
Desulfosarcina sp. SD1
WQSP-1 direct-24
Uncultured delta proteobacterium clone 72 T12d+oil
Desulfobulbus rhabdoformis
WQSP-1 direct-17
Uncultured delta proteobacterium clone SC1-40
Thalassospira profundimaris mj01-PW1-OH20
WQSP-1 aerobic group 3
Thalassospira sp. MCCC 1A01449
WQSP-1 direct group 4
Sediminimonas qiaohouensis strain YIMB024
Rhodobacteraceae bacterium ISL-53
WQSP-3 NR group 2
Chromohalobacter salexigens strain JC125
Chromohalobacter salexigens strain RS92
WQSP-3 transitional group 1
WQSP-3 aerobic group 1
WQSP-1 aerobic group 1
Halomonas sp. NAH1
WQSP-1 transitional group 4
Halomonas sp. Qphe 2
WQSP-1 IR group 3
Enterobacter asburiae strain E53
Enterobacter hormaechei strain XJUHX-4
Enterobacter cloacae isolate Nr.3
Idiomarina abyssalis KMM 227
WQSP-1 aerobic group 4
Idiomarina sp. H96B18
WQSP-1 transitional-8
Alcanivorax sp. YDC2-H
Alcanivorax dieselolei
Arhodomonas aquaeolei
WQSP-3 NR group 1
Arhodomonas sp. RS91
Alkalilimnicola ehrlichii MLHE-1
WQSP-1 direct-31
Uncultured Chromatiales bacterium clone TDNP Wbc97 128 1 33
Sulfur-oxidizing bacterium NDII1.1
WQSP-1 direct-11
Thiohalophilus thiocyanatoxydans HRhD2
Uncultured bacterium clone SN69
WQSP-1 transitional group 6
WQSP-1 direct group 2
Marinobacter sp. H146B2
Marinobacter guineae
Marinobacter aquaeolei isolate OC-9
WQSP-1 transitional group 5
WQSP-1 aerobic group 2
Marinobacter hydrocarbonoclasticus isolate MARC4F
Alteromonadaceae bacterium LA34A
0.1
CFB
Firmicutes
δ proteobacteria
α proteobacteria
γ proteobacteria

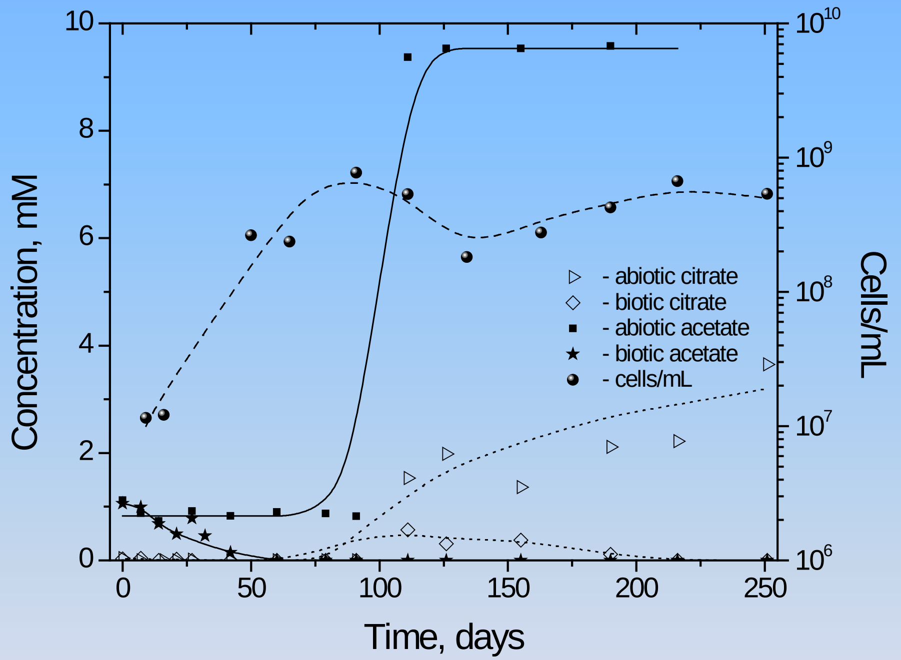
# GROUNDWATER COMPARISON-BACTERIA



# BIODEGRADATION of ORGANIC WIPP WASTE

# DEGRADATION of LOW MOLECULAR WEIGHT ORGANICS—COMPLEXING AGENTS

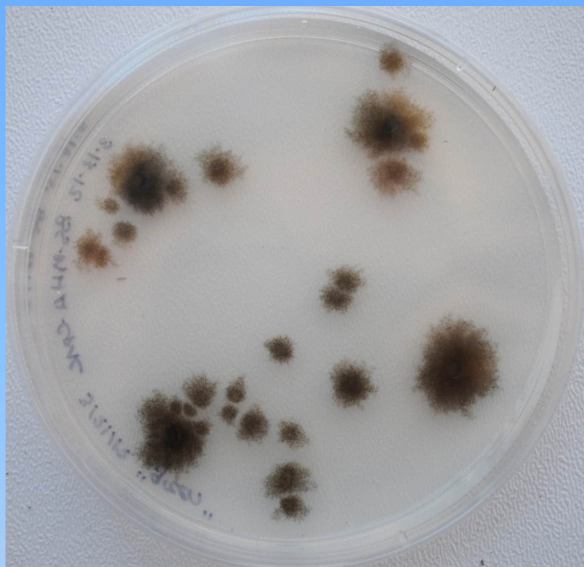
- Aerobic degradation of acetate, oxalate, and citrate
- Degradation of citrate under denitrifying conditions not shown (shown by Francis et al., 2000, using organism enriched from area brine lake); co-oxidation of citrate with acetate being tested



# DEGRADATION of HIGH MOLECULAR WEIGHT ORGANICS—CELLULOSICS

- Degradation of cellulose and exhibition of cellulase activity under aerobic conditions
  - two isolates (fungal and bacterial) currently growing on solid agar with either carboxymethylcellulose or Kimwipes as sole carbon source
  - cellulase-positive bacterial isolates (*Salinicoccus*, *Nesterenkonia*)
- Potential for anaerobic fermentation in far-field
  - glucose fermenter isolated (*Halanaerobium* sp.)
  - sequences related to *Halocella* detected

# AEROBIC CELLULOSE UTILIZATION



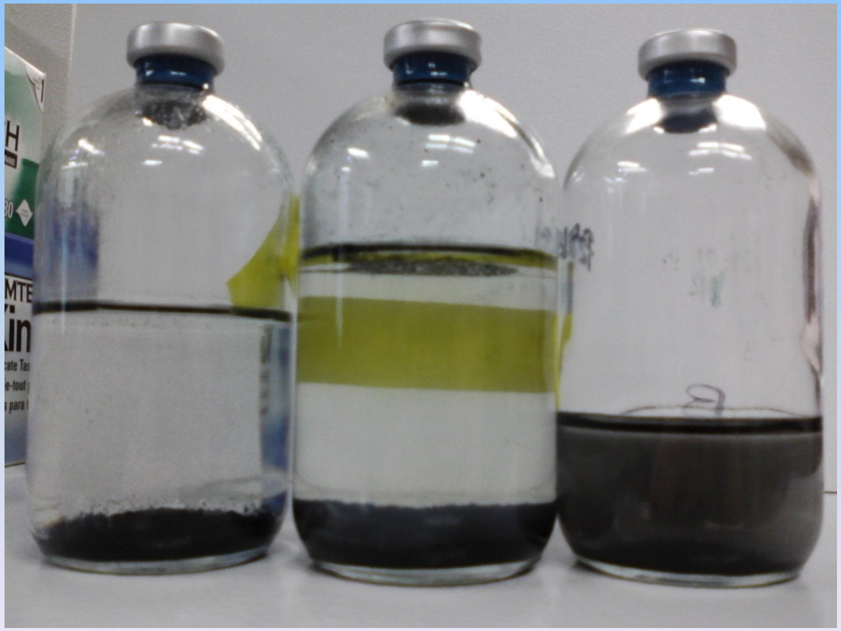
carboxymethylcellulose

No Kimwipe Kimwipe

*Cladosporium* sp.
pH range: 5-10, optimum 6
NaCl range: 0-20%, optimum 10%

Nutrient-rich medium

# METAL REDUCTION and PRECIPITATION
# in
# HIGH IONIC STRENGTH SYSTEMS



# IMPLICATIONS for SALT-BASED WASTE REPOSITORIES

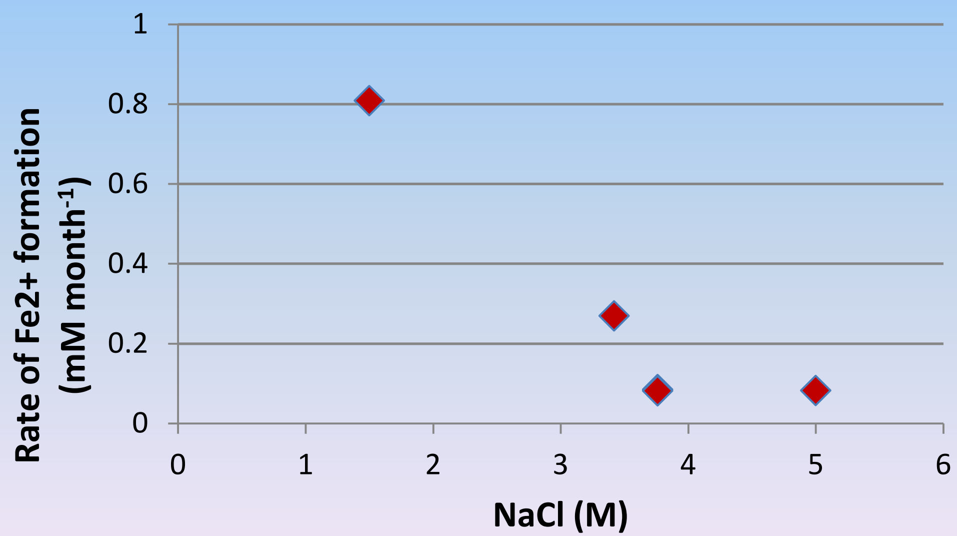
- Ability to reduce iron may translate to ability to reduce actinides
- Reduced iron precipitates with sulfide
- Reduced sulfate precipitates both oxidized and reduced iron species
- Metal-sulfide precipitate removes toxic sulfide
- Microorganisms induce metal precipitation as carbonate species, regardless of oxidation state

# METAL REDUCTION in GROUNDWATER INCUBATIONS

- Fe(IIII) solubility in saline systems is low; usually associated with POM and sediment in marine systems

- Iron reduction observed in *abiotic* incubations as function of ionic strength; slower rate than *microbially-induced* reduction

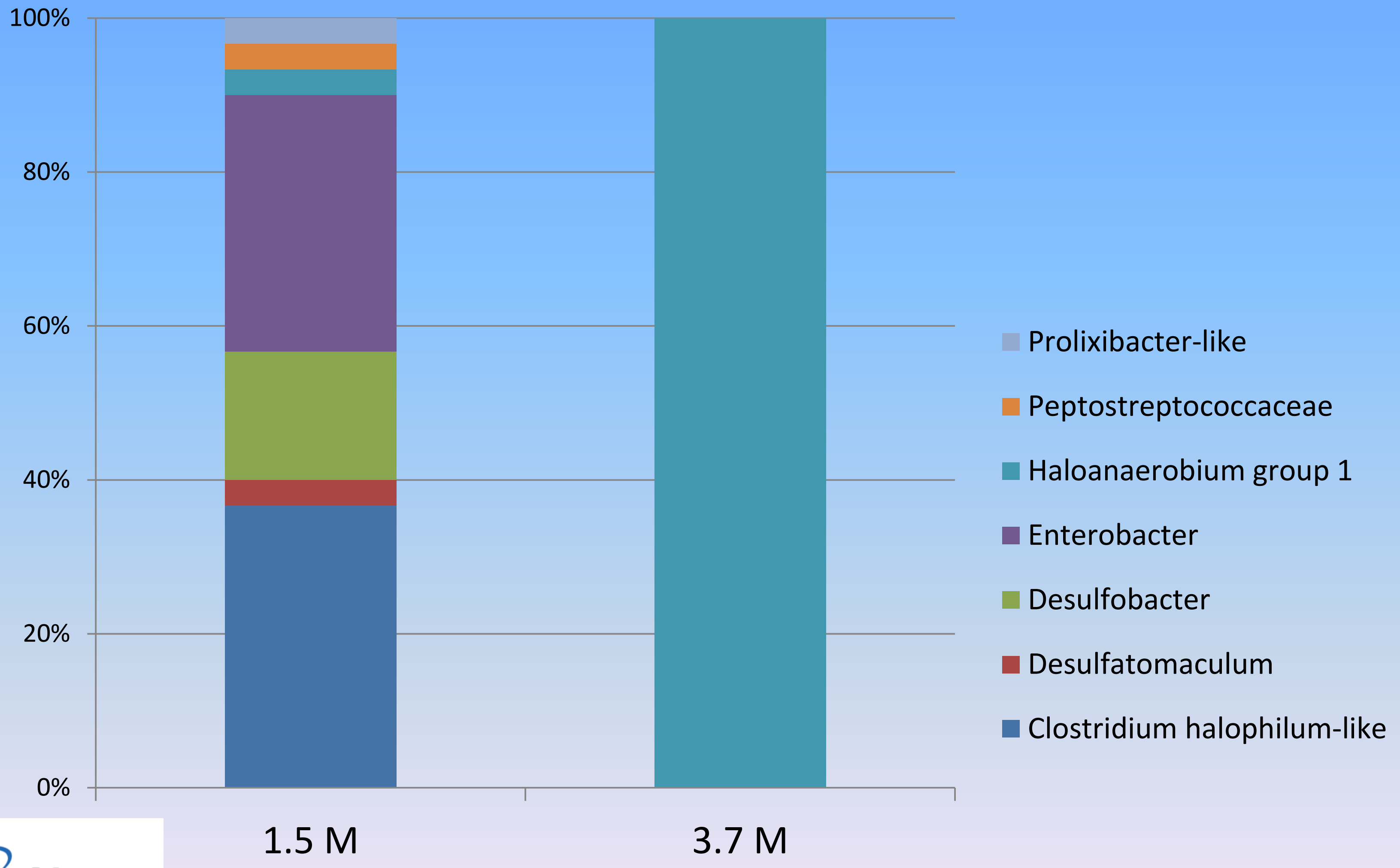
- Rates of reduction decreases with increasing [NaCl]



# ORGANISMS FOUND in IRON-REDUCING INCUBATIONS

- 1.5 M: consortium of fermenters, iron-reducers, and sulfate-reducers (*Bacteroidetes*, *Halanaerobium*, *Clostridium*, *Peptostreptococcus*, *Desulfobacter*)
- 3.7 M: mixed culture of halophilic *Bacilli* (*Virgibacillus*, *Pontibacillus*); likely obligate aerobes
- 5 M: mixed culture of *Archaea* (*Haloferax*, *Haloarcula*) and *Bacteria* (see 3.7 M)

# MICROBIAL DIVERSITY ASSOCIATED WITH IRON REDUCTION



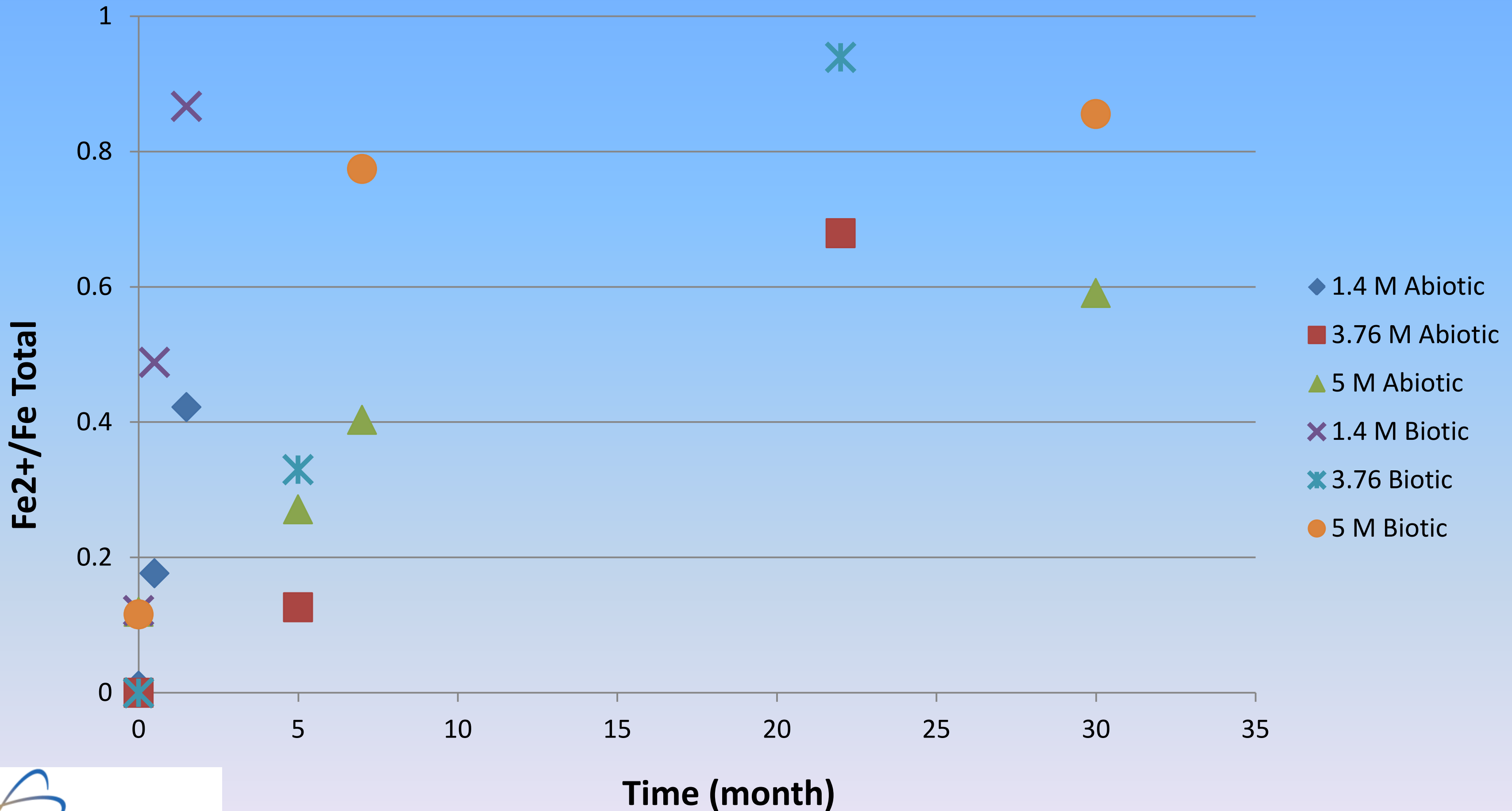
# POSSIBLE MODES of IRON REDUCTION at HIGH IONIC STRENGTH

- Direct reduction: appears limited to lower ionic strengths, both iron and sulfate reducers (1.5 M culture)

- Indirect reduction via:
  - Fermentation
  - Sulfate reduction
  - Release of reducing agents during sporulation or halocyst formation?

- Microbially-induced precipitation as iron carbonate, metal sulfide

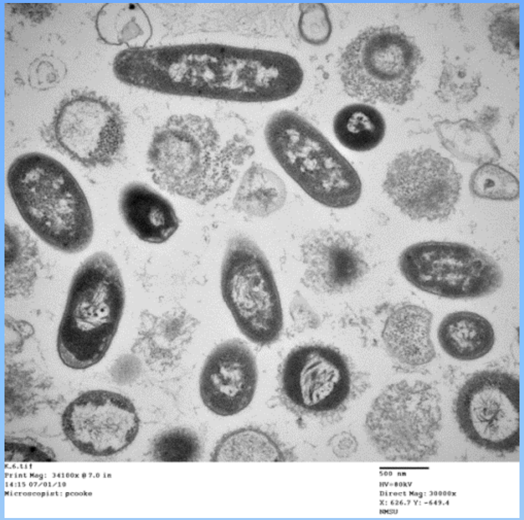
# IRON REDUCTION in BIOTIC and ABIOTIC INCUBATIONS at DIFFERENT IONIC STRENGTH

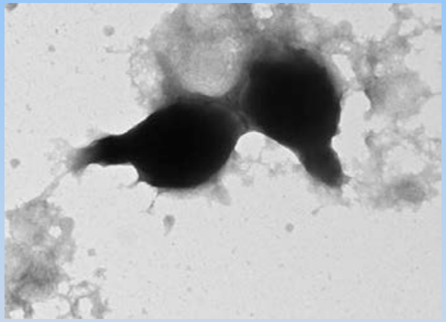


# BIOSORPTION

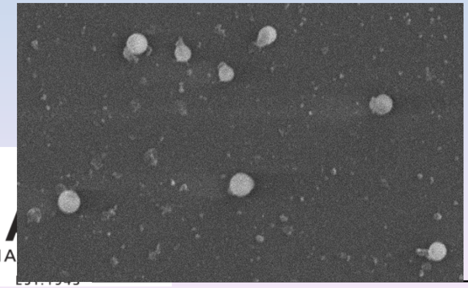
# BIOSORPTION– TEST ORGANISMS

*Chromohalobacter* sp.—isolated from area groundwater, borehole seep, WIPP and Gorleben halites; strain used tolerates pH 5-9, 0.9-4.3 M NaCl; 0.3-0.5 x 1.5-2 µm size



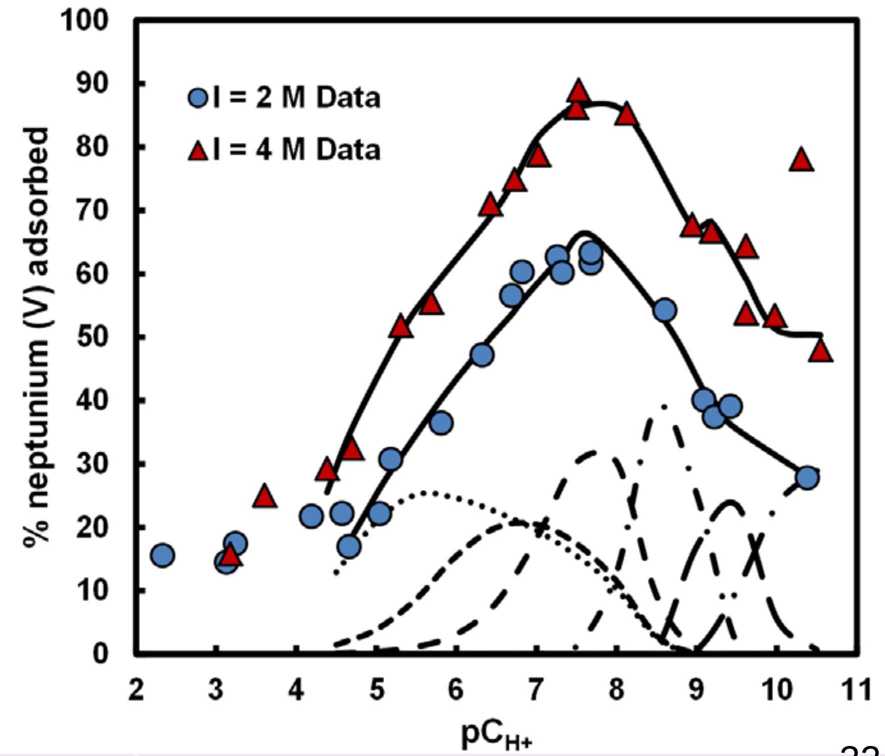


*Halobacterium noricense*—isolated from incubations of halite in generic media and in WIPP brines; detected in other subterranean salts worldwide (including Germany); requires 2.5-5 M NaCl, tolerates pH 6-10; 0.3-1.5 x 0.3-1.5 µm size

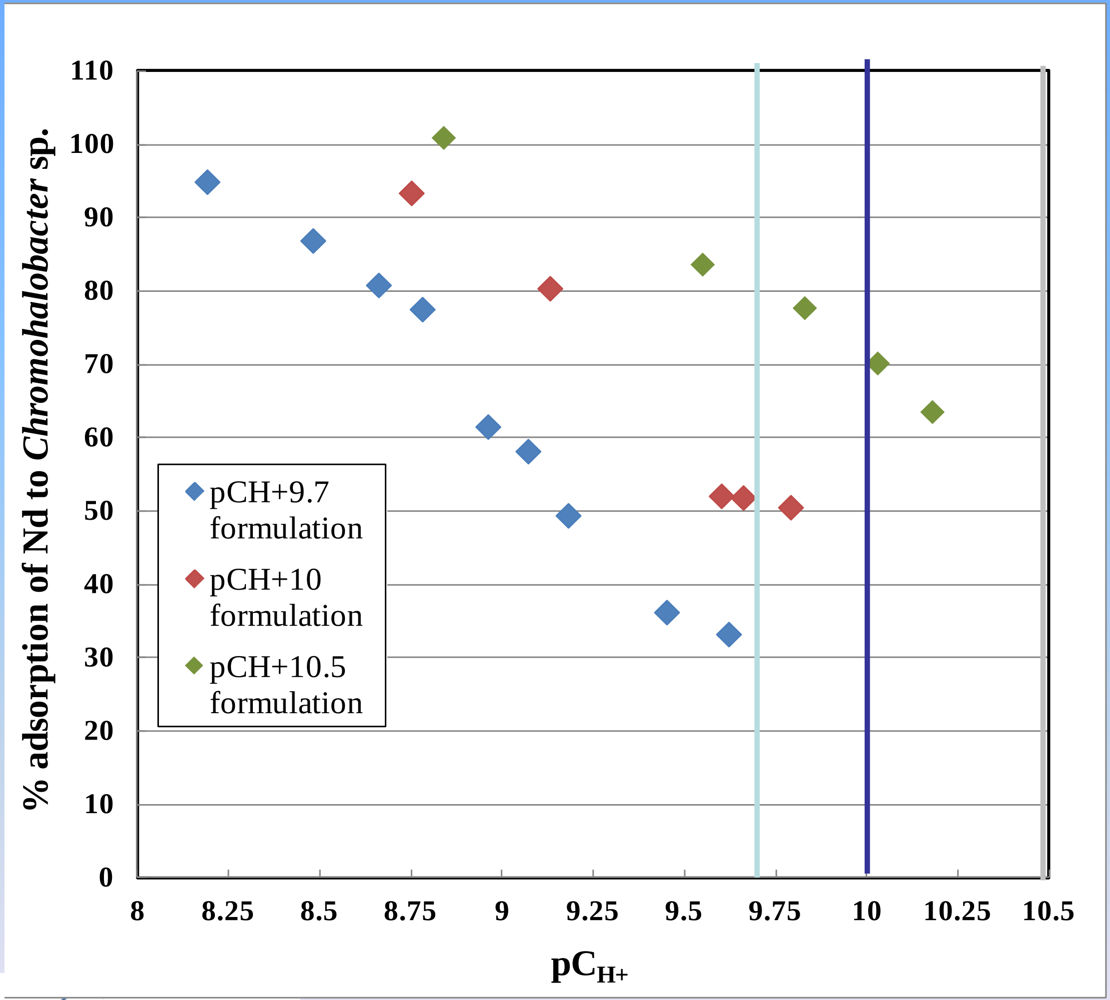


# BIOSORPTION—Np onto *Chromohalobacter*

- Titration data at 2 and 4 M similar to each other
- Titration data similar to other organisms at low ionic strength
- Best-fit model invokes 4 surface complexation sites
  - Differences observed insorption at 2 and 4M over $pC_{H+}$ range
  - Model invoking carbonate complexation reactions fits best at higher $pC_{H+}$
  -

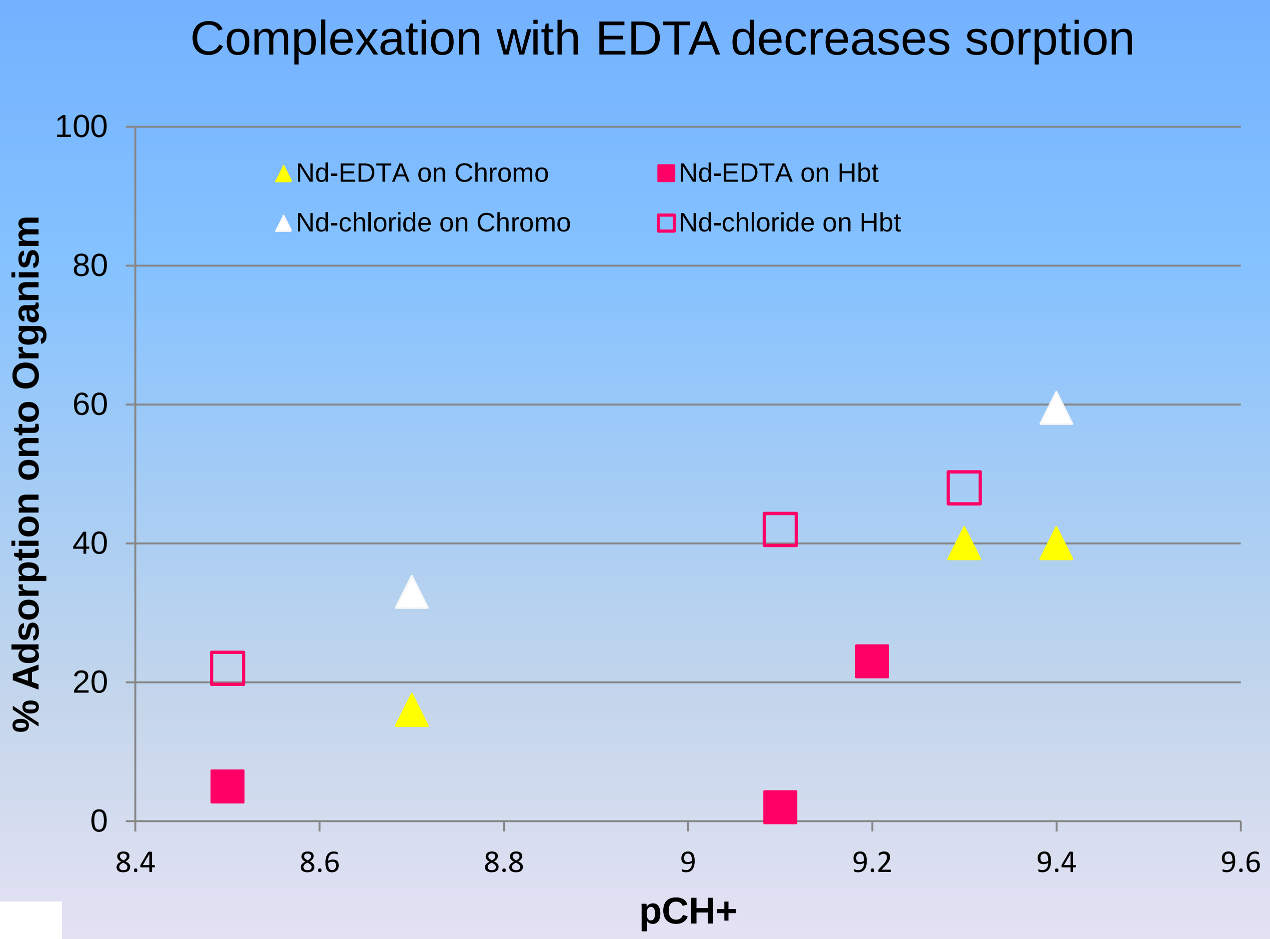


# BIOSORPTION—Nd onto *Chromohalobacter*

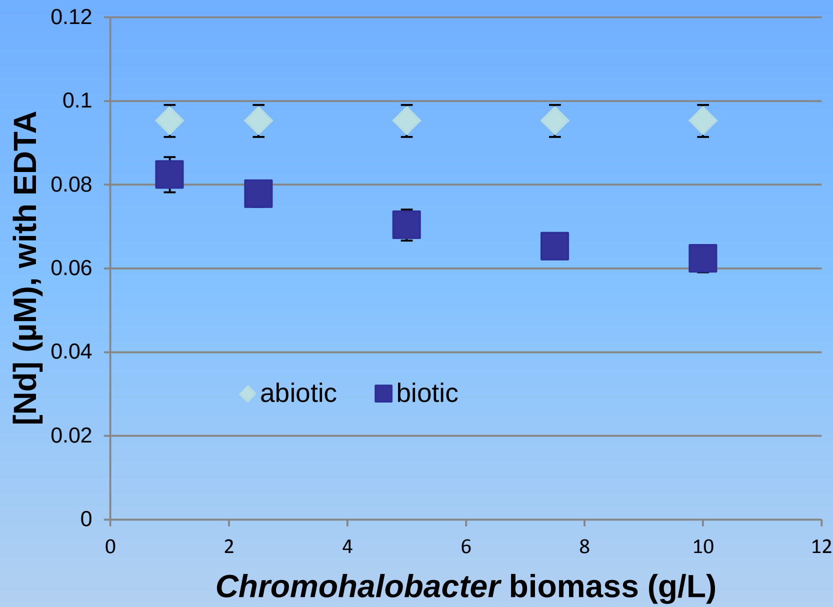



- Nd sorption to *Chromohalobacter* in 3 reacted ERDA formulations; carbonate in system
- Significant differences in sorption behavior as function of $pC_{H+}$ and formulation (Mg effect)
- Note: this is at limit of *Chromohalobacter* pH tolerance

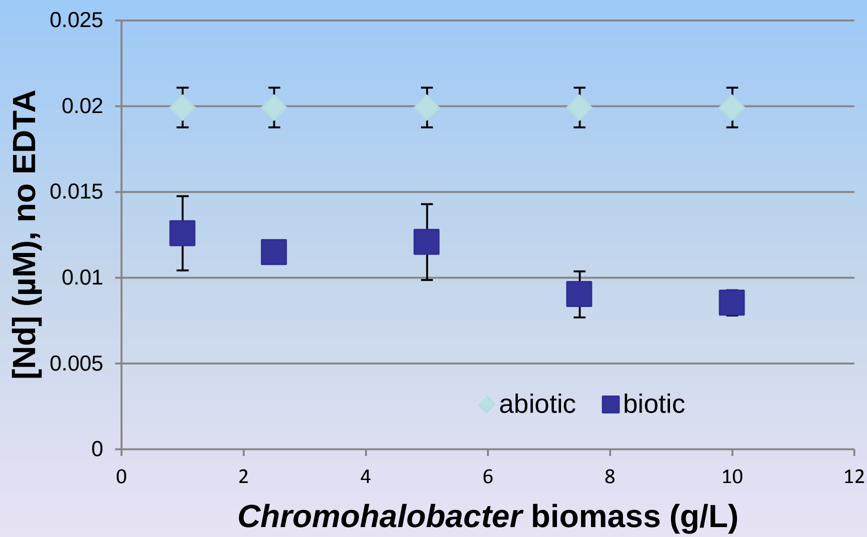
# ASSOCIATION of Nd with CELLS as a FUNCTION of $pC_{H+}$



# ASSOCIATION of Nd with CELLS as a FUNCTION of BIOMASS




Sorption increases with increasing biomass



# SUMMARY of BIOSORPTION DATA at EXPECTED WIPP $pC_{H+}$

- **Nd(III)**
  - *Archaea*: 25-50% sorption
  - *Bacteria*: 40-65% sorption
  - EDTA reduced sorption in both cases by ~ 40%
- **Th(IV)**
  - *Archaea*: 40-65% sorption
  - *Bacteria*: 81% (no EDTA) and 57% (EDTA) sorption
  - At $pC_{H+} > 9$, cannot differentiate between sorption and precipitation, due to colloids and slow hydrolysis

# CONCLUSIONS

- Structural and functional diversity of WIPP microbial communities differs between near- and far-field environments
- *Aerobic* degradation of organic complexing agents and cellulose by WIPP-indigenous organisms occurs; *Anaerobic* degradation of organic complexing agents has not been shown with halite-derived organisms; bacterium similar to only known halophilic, anaerobic, cellulolytic microorganism detected in WIPP groundwater and associated with Gorleben halite
- Both abiotic and microbially-mediated metal reduction occur in hypersaline systems; rates dependent upon [Na]
- Actinide sorption to microorganisms shows ionic strength, $pC_{H+}$, organism, and actinide dependence

# ACKNOWLEDGMENTS

This work is sponsored by the US Department of Energy—Carlsbad Field Office

We especially thank:

- Russ Patterson, DOE program manager and support
- WIPP and Washington TRU Solutions for sampling assistance