THE PROJECTED IMPACT OF MICROBIAL ACTIVITY ON THE PERFORMANCE OF A SALT-BASED NUCLEAR WASTE REPOSITORY: THE WASTE ISOLATION PILOT PLANT

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ABC-Salt V March, 2017

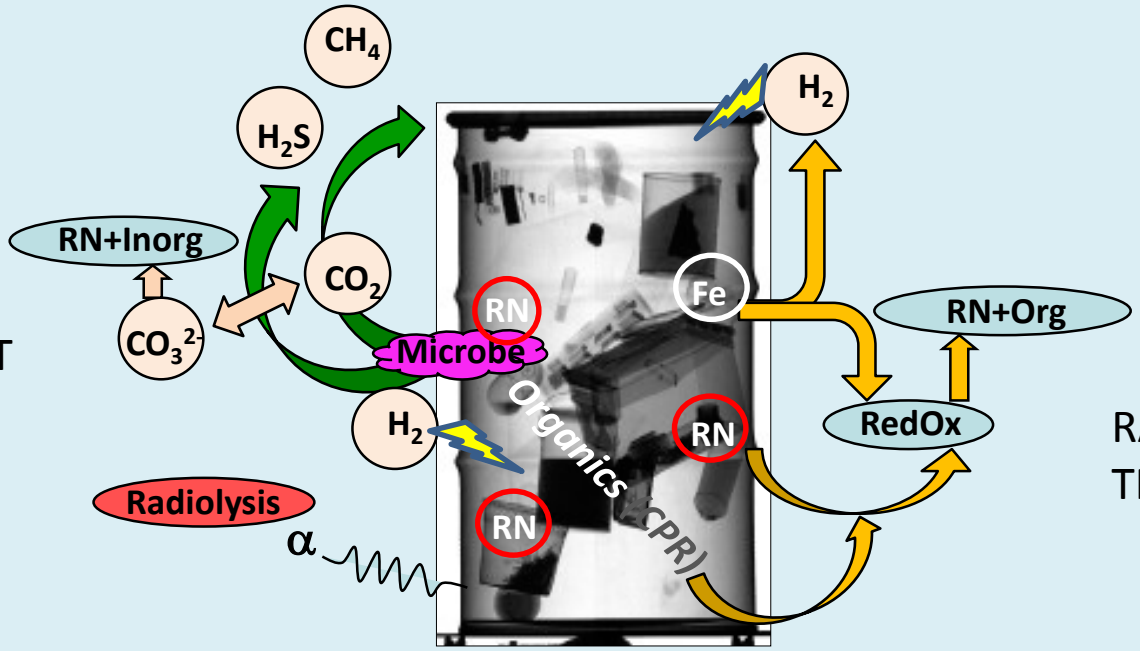


MICROBIAL ACTIVITIES CONSIDERED TO BE "SIGNIFICANT PROCESSES"

ANY PROCESS THAT INFLUENCES RADIONUCLIDE SOLUBILITY OR TRANSPORT

GAS GENERATION FROM ORGANIC DEGRADATION

BIOCOLLOID TRANSPORT VIA SORPTION OR OTHER UPTAKE



COMPLEXANT GENERATION

CANISTER CORROSION

RADIONUCLIDE TRANSFORMATION

WHERE WE ARE IN UNDERSTANDING MICROBIAL EFFECTS

RANKING OF IMPORTANCE FOR A SALT-BASED REPOSITORY

NEAR-FIELD

- Biocolloid transport
- Biologically-induced effects on solubility
- Ligand generation

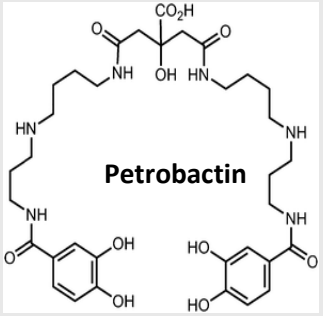
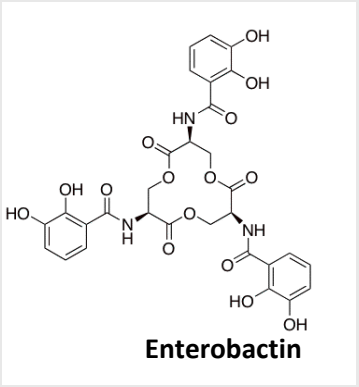
FAR-FIELD

- Redox reactions and transformation
- Ligand generation
- Sorption
- Mineralization

WHERE WE ARE IN UNDERSTANDING MICROBIAL EFFECTS

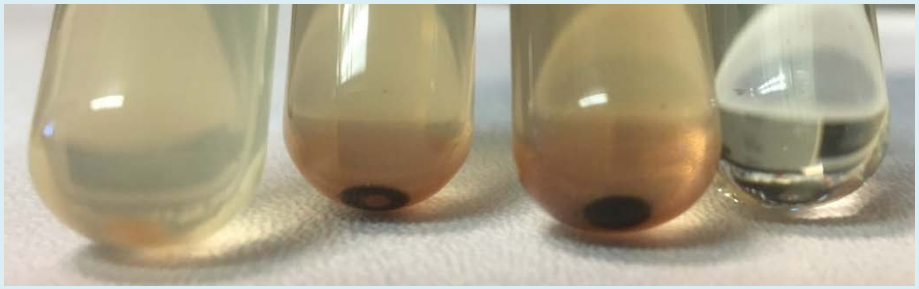
NEAR-FIELD	WHAT IS UNDERSTOOD
Biocolloid transport	• Association behavior differs between archaea and bacteria • Association behavior differs with radionuclide • Biomass dependence • Concentration dependence
Biologically induced effects on solubility	• Influence on brine chemistry • Precipitation/mineralization • Competition for available water

WHERE WE ARE IN UNDERSTANDING MICROBIAL EFFECTS

NEAR-FIELD	WHAT IS UNDERSTOOD
Ligand generation (apart from gas generation)  Petrobactin  Enterobactin	• Siderophore production capability within drums • Xenosiderophore use by haloarchaea • Limited siderophore production by haloarchaea • Sulfidogenic extreme halophiles
Mineralization	• Phosphate, carbonate (see Bader)

WHERE WE ARE IN UNDERSTANDING MICROBIAL EFFECTS

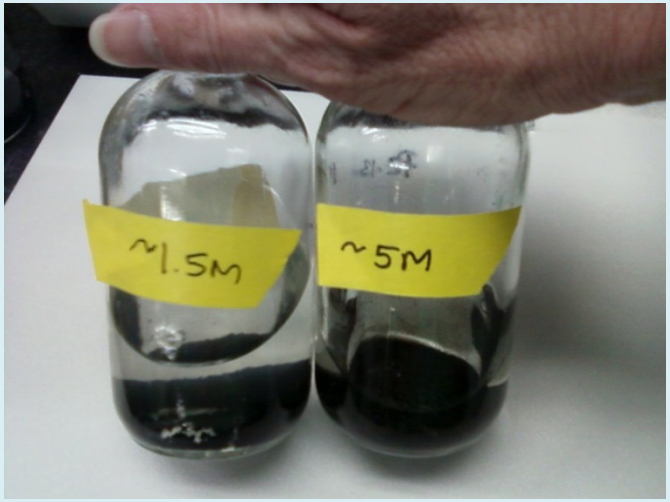
FAR-FIELD	WHAT IS UNDERSTOOD
Redox reactions and transformations	• Indirect effects via iron(II), sulfide, and hydrogen generation • Enzymatic respiratory (U, Tc only; Se?) • Detoxification or inadvertent
Sorption	• See Dittrich
Mineralization	• Phosphate, carbonate



REDOX REACTIONS/REDOX POTENTIAL in the WIPP FAR-FIELD

- H_2-generating fermenters, iron-reducers, and sulfate-reducers
  - *Bacteroidetes*
  - *Halanaerobium*
  - *Clostridium*
  - *Peptostreptococcus*
  - *Desulfobacter*

- Organisms found at WIPP match those detected in hypersaline sediments enriched under iron-reducing conditions; fermentation hypothesized to be cause of iron reduction



Mixed iron(II/III)-sulfides in solids

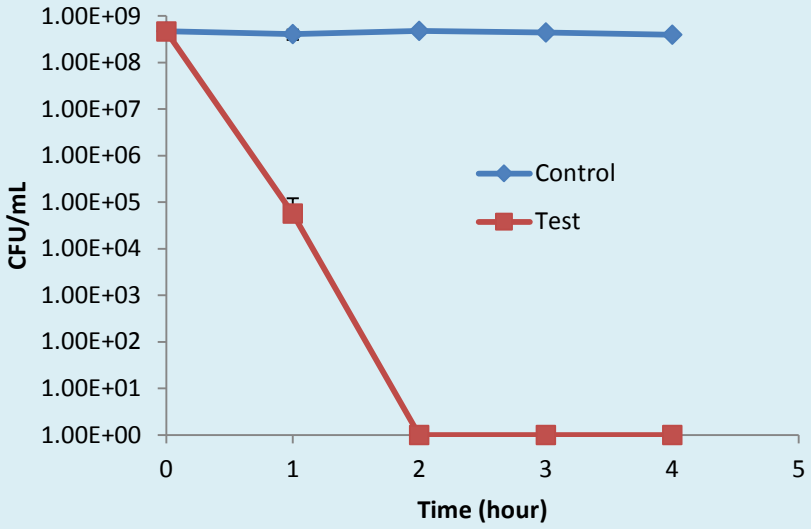
WHERE WE ARE IN UNDERSTANDING MICROBIAL EFFECTS

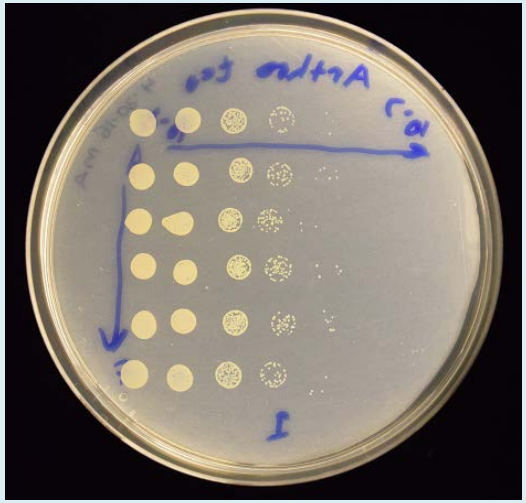
FAR-FIELD	WHAT IS UNDERSTOOD
Ligand generation (not derived from waste)	• Phosphate detoxification; sulfidogenesis • Siderophore production
Sorption	• Competition between organism and geological matrix; See Dittrich
Mineralization	• Phosphate, carbonate

WHAT IS STILL LACKING

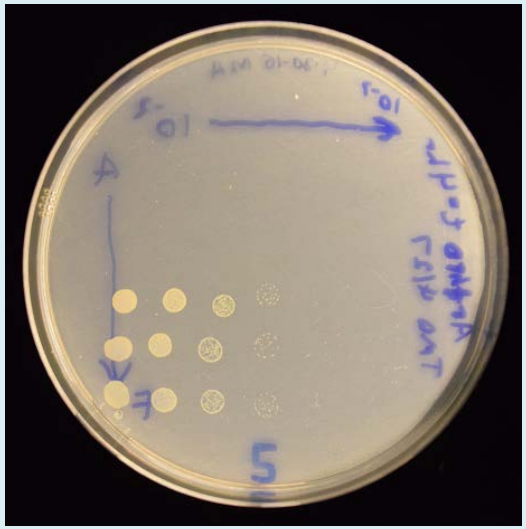
NEAR-FIELD	UNANSWERED QUESTIONS
Biocolloid transport	• Are cells motile/mobile? • Will cells lyse with dramatic change in ionic strength? • What happens to sorbed radionuclide if cell lyses?
Presence of sulfate reducers and other sulfidogens	• None have been detected in the near-field. Are they present? • Are other sulfidogens present?
Presence of methylated amines	• Are they present? • Can they support growth of halophilic methanogens?
L/ILW vs HLW; heat-generating waste vs non-heat generating; radiative effects	• Will certain repository configurations permit survival and growth of organisms? • At what distance from canister is activity ok?

WHAT IS STILL LACKING:
Example-Radiative Effects





t = 0



t = 4

Arthrobacter sp. isolated from WIPP-bound waste

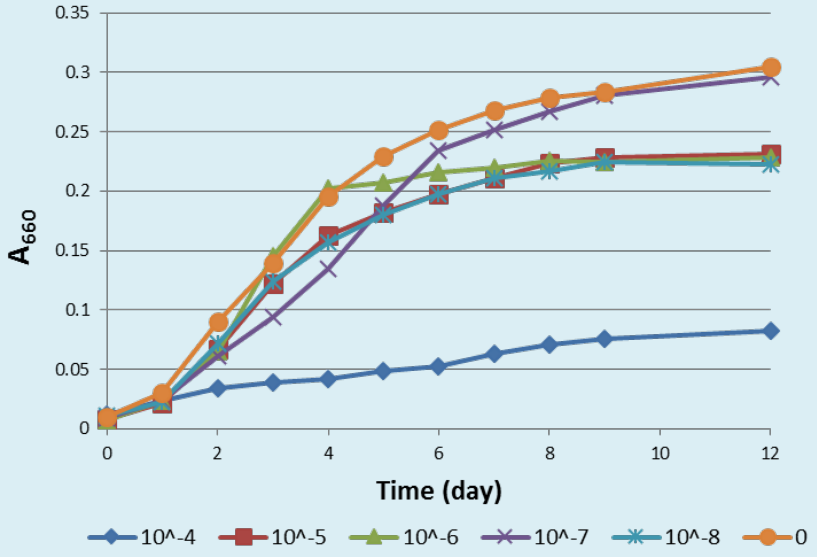
4-log reduction within 1 hour at 146 krad dose

Organism known to be radiation tolerant; repeated subcultures outside of drum lead to loss of resistance functions

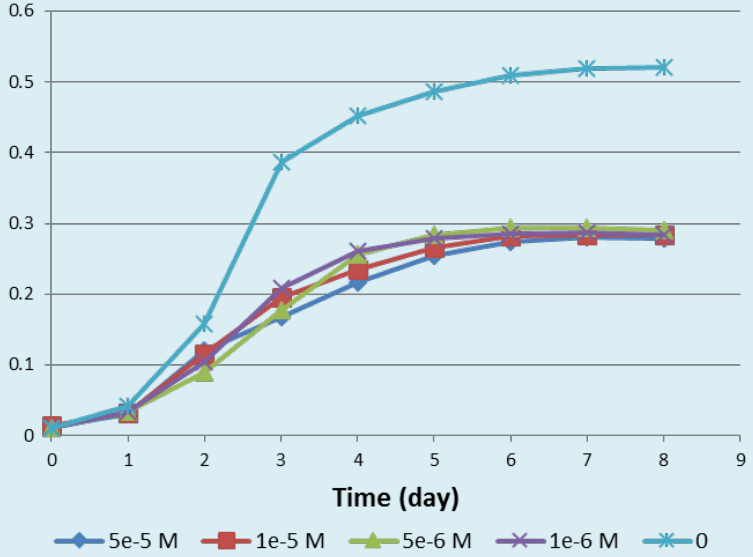
WHAT IS STILL LACKING

NEAR-FIELD	UNANSWERED QUESTIONS
Interactions with barrier components, including canisters	• Are organisms present and active in clay/salt mixes?
Radionuclide toxicity	• What are effects of different oxidation states and ligands? • Are persistor cells present?

Pu-242



Np-237



Halobacterium noricense growth in presence of Pu, Np

WHAT IS STILL LACKING: Example-Toxicity Effects

Pu-242

Solubility in brine in absence of ligands ~10^{-6} M

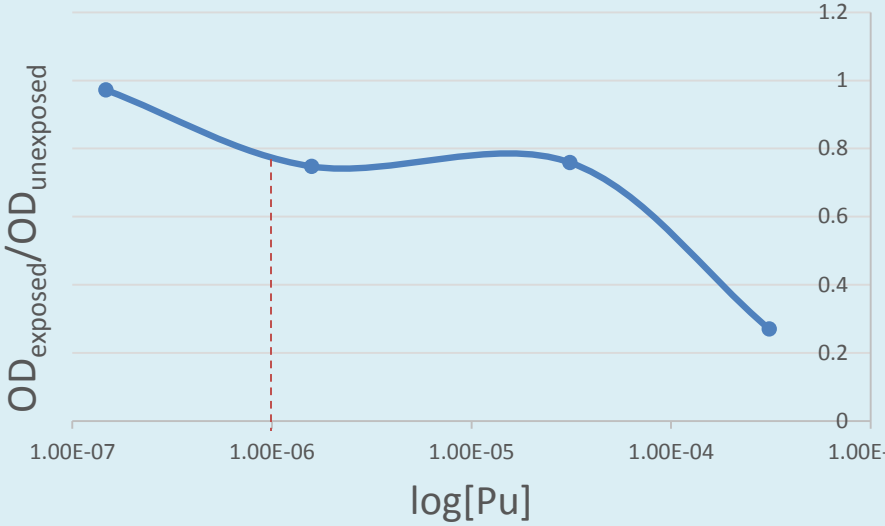
At this concentration, ~80% inhibition of growth

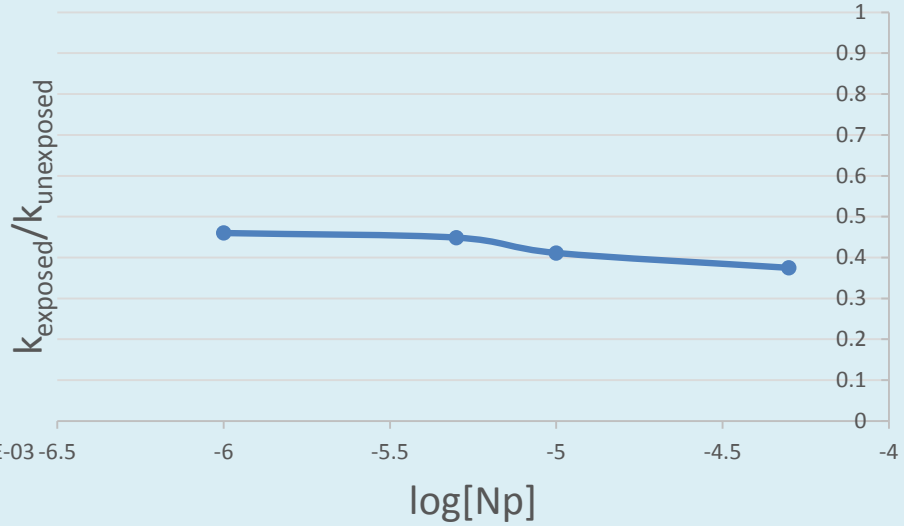
Np-237

No dose-response in tested range

Solubility in brine in absence of ligands ~10^{-7} M

Projected ~50% inhibition?

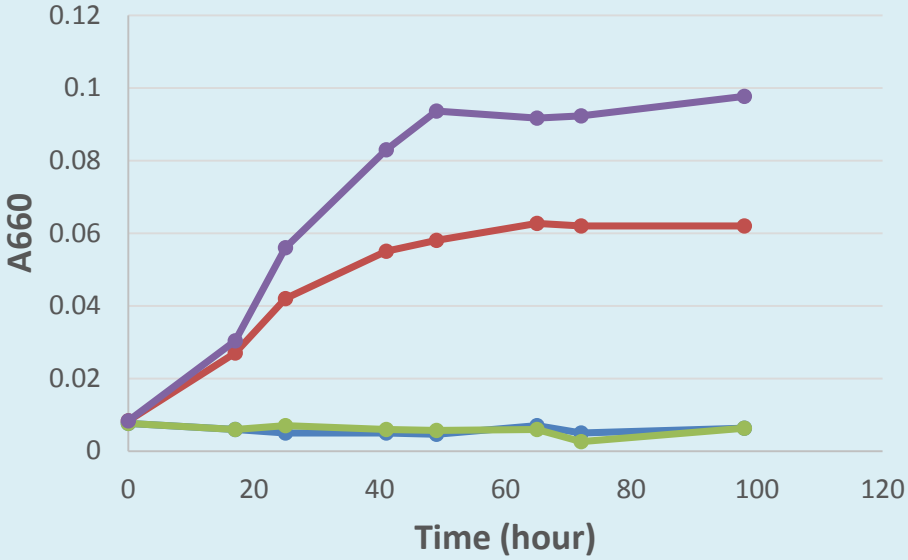


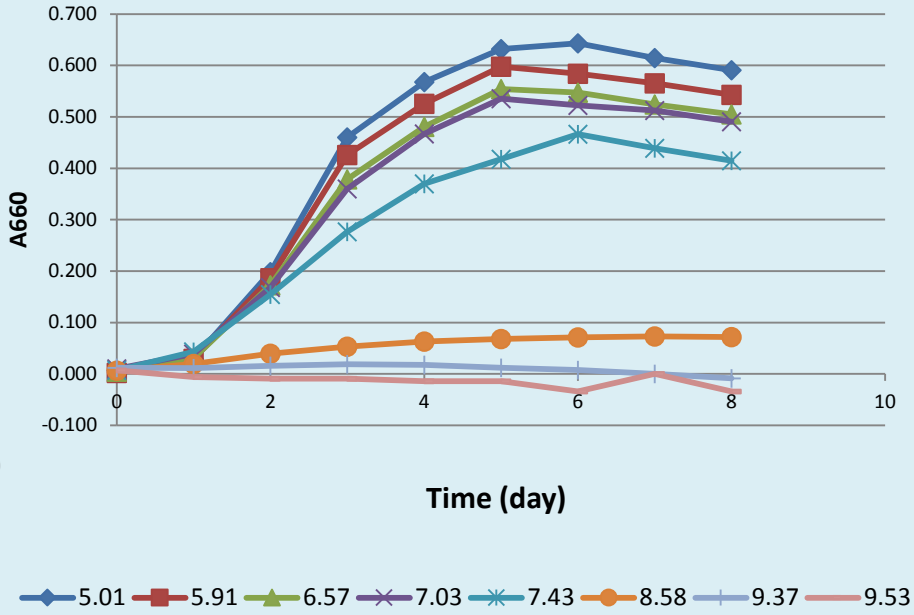


Halobacterium noricense

WHAT IS STILL LACKING:
Effects on Barrier Components

- Enhanced growth of *Halobacterium noricense* in presence of gluconate
- No growth above pH 9





SUMMARY OF GAS GENERATION RESULTS

- 1970's (Barnhart et al.; Molecke et al.):
  - Inocula: bacilli isolated from simulated waste, soil (possibly from waste burial site)
  - Waste used: simulated organic waste, sawdust, asphalt
  - Aerobic, anaerobic, humid, brine-inundated, nutrients added, varied temperature

- $+CO_2$; $-CH_4$
- Some temperature effect
- No brine effect
- Cellulose accounted for all gas generated

- No microbe-specific data generated
- Possible composting effect

UNEXPLAINED RESULTS:

How is there no brine effect when the inocula contain no halophilic organisms?

Why were rates in sterile controls often greater than rates in tests?

SUMMARY OF GAS GENERATION RESULTS

- 1980's (Caldwell et al.):
  - Inoculum: soil from TA-54 burial site
  - Waste used: simulated organic waste, sawdust, asphalt
  - Aerobic, anaerobic, humid, inundated, nutrients added, varied temperature
  - Pu added

- $+CO_2$; $-CH_4$
- Inhibitory effect of Pu

- No microbe-specific data generated

SUMMARY OF GAS GENERATION RESULTS

- 1990's (Brush et al.; Leonard et al.):
  - Inocula: sewage sludge, laboratory dust, halophile co-culture
  - Waste used: "heterogeneous and homogeneous" sludges?
  - Aerobic, anaerobic, fermentative; inundated with GWB-like brine
- +CO_2 or CO_2 + N_2 when glucose was substrate; no gas with cellulose as substrate
- No growth of non-halophiles; halophile growth only in complex medium
- Small number of viable cells in 5% of containers

SUMMARY OF GAS GENERATION RESULTS

- 2000's (Francis and Gillow):
  - Inocula: halophile co-culture, halite, G-seep brine, brine lake water and sediment
  - Waste used: cellulose
  - Aerobic, anaerobic; humid, inundated; +/- bentonite
- +CO_2 or CO_2 + N_2, CH4 in small quantities
- Positive correlation between gas generation and presence of nutrients/nitrate
- Anaerobic rates > aerobic
- Fermentation by-products detected

WHAT TO CONCLUDE FROM PREVIOUS WORK?

- A rich inoculum is required in order to see significant gas generation
- Radionuclides will have adverse affect on gas generation
- The presence of nutrients will increase gas generation
- Cellulose is not an ideal substrate for halophilic organisms

UPCOMING EXPERIMENTS

- Vary inoculum
  - Compare realistic halite (poor inoculum) versus brine lake sediment (rich inoculum)
- Vary moisture
  - Compare humid versus inundated
  - Look for activity of halotolerant bacteria and fungi under humid conditions

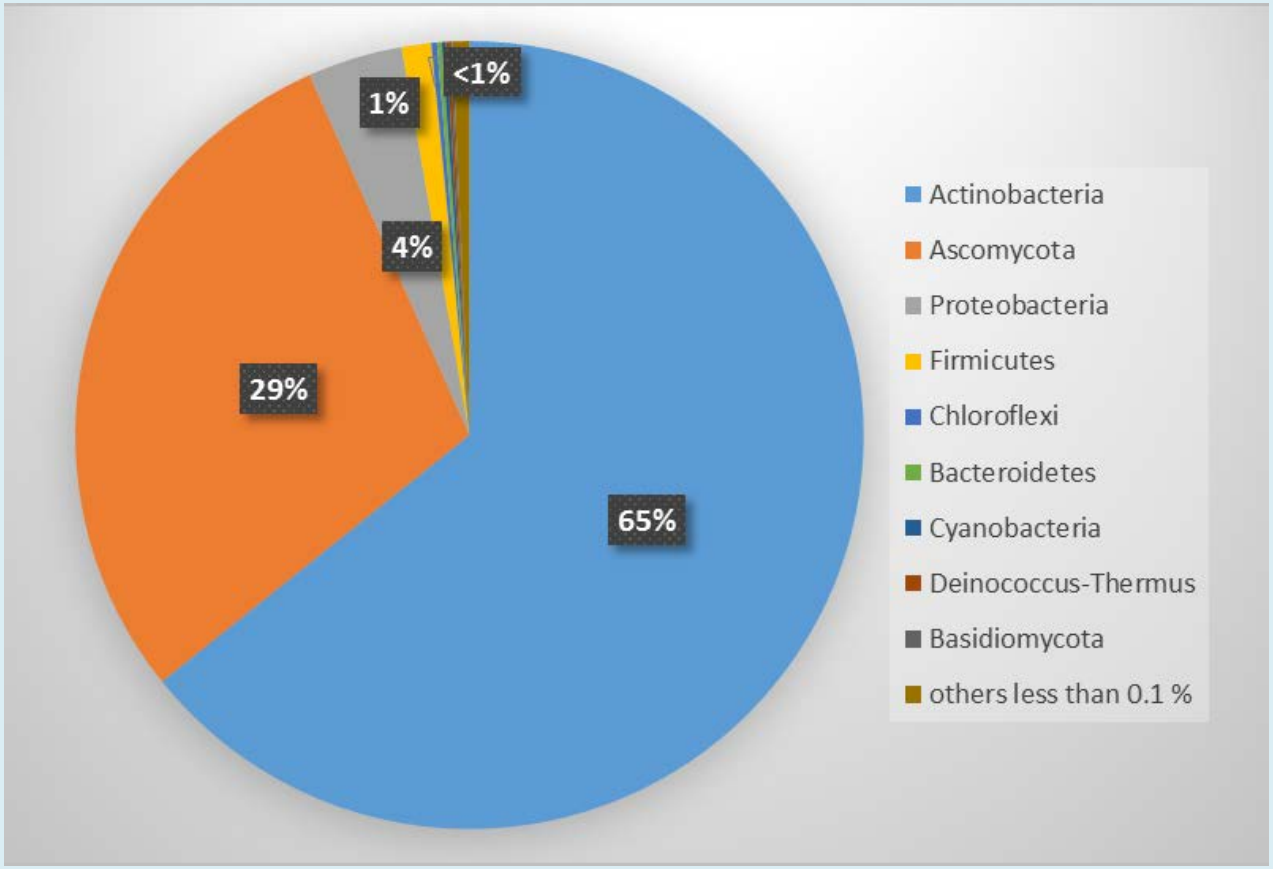
PARALLEL DATA

- Continued use of microbial ecology to infer metabolic capability of indigenous and introduced communities

- Use of genomic/metagenomic data to support above inferences and direct research

  The DNA from an individual *organism* or an entire *community* can be fully sequenced and reviewed for genes encoding enzymes involved in biochemical pathways or other features of interest

EMPLACED POPULATION: WHO IS THERE?

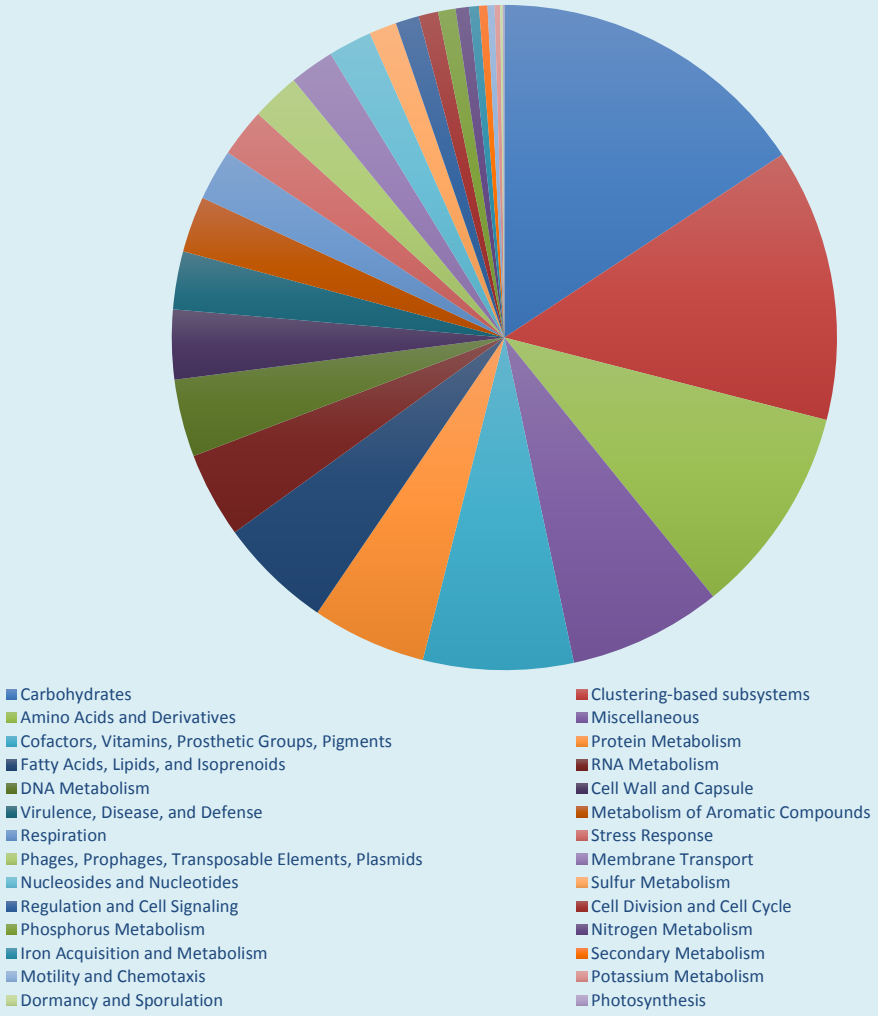






Dominant organisms are those most resistant to desiccation

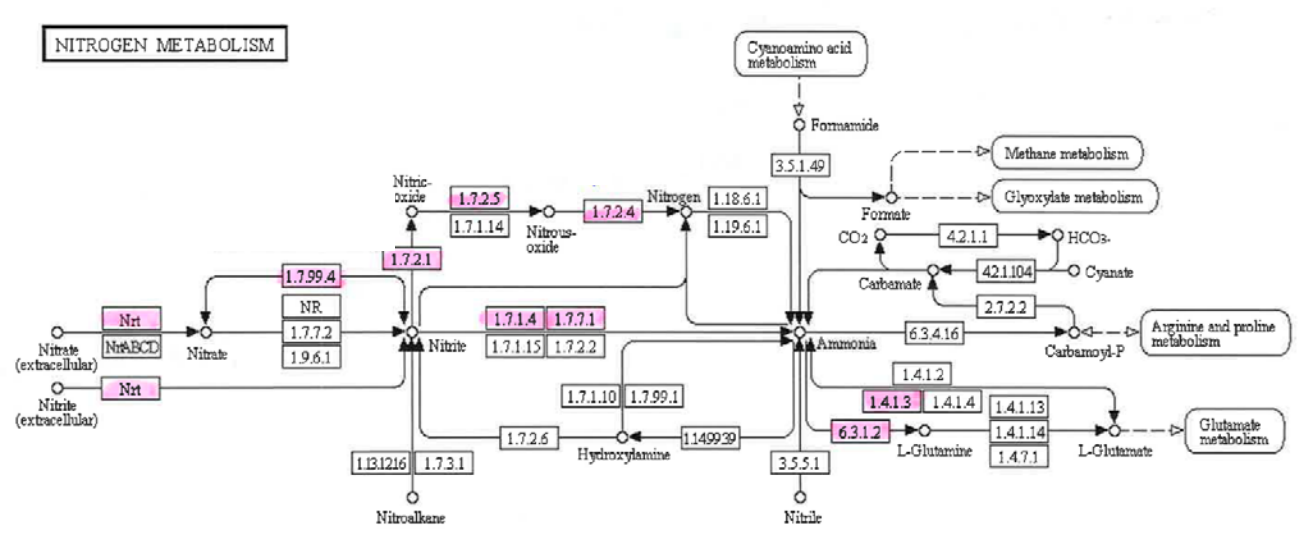
EMPLACED POPULATION: WHAT CAPABILITIES DID/DO THEY HAVE?



- Metagenomic analysis is underway
- Examples:
  - 17% of stress response genes encode for osmotic stress
  - carbohydrate genes suggest ability to metabolize carboxylic acids (e.g., lactate, pyruvate, propionate, succinate), sugars, natural cellulosics (e.g., xylan)
  - Presence of genes encoding aromatic hydrocarbon degradation

INDIGENOUS POPULATION: WHAT CAPABILITIES DO THEY HAVE?

- Presence of gene suggests, but does not guarantee, capability of function; function must be shown experimentally
- Example: genes encoding denitrification detected in *Halobacterium noricense*, but organism is NOT capable of this mode of metabolism
- Is organism capable of arsenate and selenate transformation?



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- THANK YOU FOR YOUR TIME AND ATTENTION

