Reassessment of the Impact of Microbial Activity on the Performance of the Waste Isolation Pilot Plant

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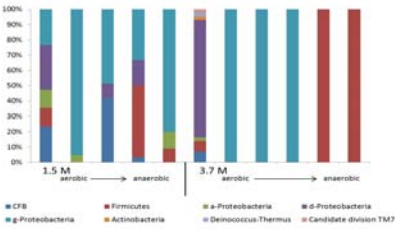
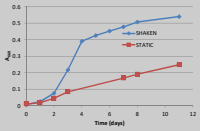
INTRODUCTION. The projected impact of microbial activity on the performance of the Waste Isolation Pilot Plant has changed little from the initial certification period to the most recent recertification application submitted in 2014 [1-3]. The areas of greatest concern were, and still are: 1) carbon dioxide generation from the consumption of organic waste; 2) bioreduction of actinides; and 3) biocolloid migration potential. Over time, the reasoning behind certain model assumptions has changed somewhat (e.g., methanogenesis initially accounted for >90% of all carbon dioxide generated; currently, it is not included in the model), but the overall conservativism remains unchanged. Reviews of microbial survival and activity in high ionic strength matrices and recent work on WIPP-indigenous microorganisms suggest that the gas generation model is more conservative than originally thought, that bioreduction of actinides is most likely to occur in the far-field, and that biocolloid migration continues to be an area of uncertainty.

- WIPP microbial community divided into near-field (halite, see image to left) and far-field (overlying water-bearing horizon, see image to right) compartments
- Chief determinant of community structure is [NaCl]
- Halophilic archaea (order *Halobacteriales*) dominate near-field and high-salt conditions
- Bacteria dominate the far-field and low-salt conditions
- Groundwater Bacteria enriched under nitrate-reducing, iron-reducing, and sulfate-reducing conditions; diversity decreases with increasing [NaCl] and increasing anaerobicity



GAS GENERATION

Chief constraints for <u>all</u> projected modes of metabolism:
- [NaCl]
- Lack of ideal substrates, i.e. cellulose

Projected Mode of Metabolism/ Gas Generation	Constraints to Projected Mode of Metabolism and Gas Generation
Aerobic	Salt concentration will constrain some Fungi and Bacteria Unknown [O_2] at repository closure Low O_2 solubility in brine: 5 M NaCl (ERDA): ~2.6 mg/L Haloarchaea prefer aeration, even in oxygenated setting 
Denitrification	Salt concentration may constrain some Bacteria Only 2 genera of haloarchaea known to denitrify; neither has been isolated from WIPP halite WIPP *Halobacterium* sp. possesses denitrification genes, but function has not been shown Nitrate reduction by haloarchaea can occur without gas production
Sulfate Reduction/ Sulfidogenesis	Discrepancies between *in situ* and *in vitro* rates of sulfidogenesis; rates of sulfate reduction decrease with increasing [NaCl] Levels of borate in WIPP brines may inhibit SRB Halophilic sulfur-reducing bacteria and archaea exist; thiosulfate-reducing archaea exist No known sources of elemental sulfur or thiosulfate

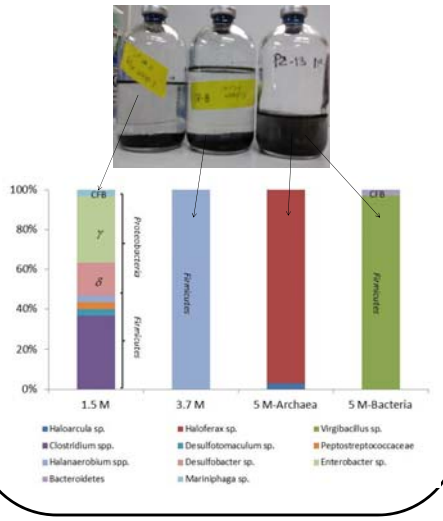
- Most likely modes of metabolism in the WIPP near-field:
  - Aerobic respiration
  - Nitrate reduction
  - Fermentation
- **No organisms have been enriched from WIPP halite under high-salt, anaerobic conditions with ideal substrates**
- Methanogenesis at high ionic strength is only due to methylated amines
- Sources of methylated amines in WIPP are unknown.

(BIO)REDUCTION OF IRON/ACTINIDES

Studies are carried out with complexed Fe(III) to enrich for organisms with potential actinide reduction capability

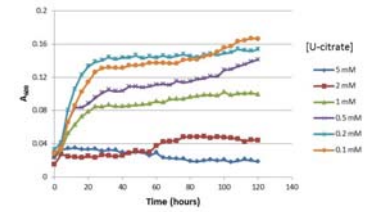
Iron Reduction in the Near-Field (High Ionic Strength)
- No organisms have been enriched from WIPP <u>halite</u> under high-salt (3.42 M), iron-reducing conditions; however,…
- …Iron reduction/precipitation observed over time in both biotic and abiotic incubations but is more notable in biotic

Iron Reduction in the Far-Field (Low to High Ionic Strength (1.7-5 M):
- Organisms isolated from WIPP groundwaters:
  - 1.7 M: *Clostridium* sp.-direct and indirect reduction via sulfate; acetate as e^- donor
  - 3.7 M: *Halanaerobium* spp.-indirect reduction via pyruvate fermentation
  - 5 M: organisms isolated are not known to reduce metals but have also been detected in hypersaline, iron-reducing sediments [4]
- Indirect reduction of actinides may occur as a result of lower redox and sulfidogenesis

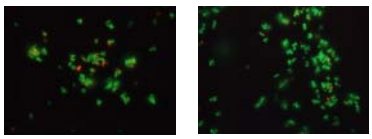


BIOCOLLOID MIGRATION

Via Waste-Indigenous Organisms:
- Organisms isolated from WIPP waste may have high tolerance to radionuclides
  - No upregulation of stress response genes in *Arthrobacter* isolate: EF, phosphatase, hsp, protease, methyl-transferase
- Also resistant to desiccation
- Survive in WIPP brines for extended period of time and do not lyse after death
- In preliminary experiments, *Arthrobacter* cells adsorbed ~90% of soluble Nd

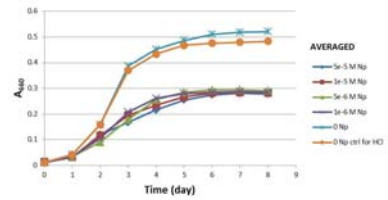


Growth of Arthrobacter isolate in presence of U-citrate



Arthrobacter cells in GWB (high Mg brine, left) and ERDA-6 (high NaCl brine, right) after 6 weeks

Via WIPP-Indigenous Organisms:
- *Halobacterium* sp. tolerance to radionuclides is under investigation
- Organism remains viable and intact for extended periods at ionic strengths found in WIPP groundwaters



Effect of Np on *Halobacterium* growth

CONCLUSIONS. Given a low-diversity community with limited metabolic capabilities, the projected rates of gas production under near-field repository conditions can be deemed optimistic. If actinides migrate into the far-field, they may be reduced directly or indirectly by indigenous organisms there. Biocolloid enhancement of migration by indigenous organisms is likely to be less than projected by PA for some actinides, especially americium. Uncertainty exists as to the role of the emplaced organisms in within-drum waste degradation and biocolloid migration.

REFERENCES

[1] U.S. Department of Energy. 2004. Title 40 CFR Part 191 Compliance Recertification Application for the Waste Isolation Pilot Plant. DOE/WIPP 2004-3231. Carlsbad, NM: Carlsbad Field Office.
[2] U.S. Department of Energy. 2009. Title 40 CFR Part 191 Subparts B and C Compliance Recertification Application 2009, Appendix PA, Attachment SOTERM. DOE Carlsbad Field Office. Carlsbad, NM.
[3] U.S. Department of Energy. 2014. Title 40 CFR Part 191 Subparts B and C Compliance Recertification Application 2014, Appendix SOTERM-2014. DOE Carlsbad Field Office. Carlsbad, NM.
[4] Emmerich M et al. 2012. Applied and Environmental Microbiology 78(12): 4386-4390.

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