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Title: ~~An Overview of Research on Microbial Interactions in the~~
Waste Isolation Pilot Plant (WIPP) *microbiology*

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An Overview of Research on Microbial Interactions in the Waste Isolation Pilot Plant

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INTRODUCTION

Microorganisms in a nuclear waste repository can affect actinide solubility by changing the redox environment, adsorbing actinides, degrading or generating complexants, or by changing actinide oxidation states^{1,2}. While research on the microbiology of subsurface igneous and clay repositories has made many advances, there are fewer data available on the microbial effects in salt-based repositories, such as the Waste Isolation Pilot Plant (WIPP) in southeastern New Mexico. Microorganisms that grow in hypersaline systems may not be as metabolically versatile or active as those found in subsurface soils or groundwaters³, and extrapolations of their interactions with emplaced waste cannot be easily made from data obtained with organisms at other repositories. This research is being conducted to gain a more realistic understanding of the potential effects of WIPP-indigenous organisms on repository performance.

DESCRIPTION OF THE WORK

The goals of this work are manifold: 1) to characterize the microbial ecology of the Salado formation and surrounding groundwaters in order to predict the metabolic capability of indigenous populations, 2) to investigate the potential degradation of emplaced organic waste components, such as complexing agents and cellulose, 3) to measure the sorption of actinides onto WIPP-relevant microorganisms, and 4) to determine if metal/actinide bioreduction occurs at the high ionic strength expected in the repository.

RESULTS

Microbial characterization.

Clone libraries constructed from bacterial and archaeal 16S (and eukaryotic 18S) rRNA-encoding genes reflect a low-diversity community (see Figure 1). At higher salt concentrations (> 2.5 M), haloarchaea, such as *Halobacterium* sp., *Halorubrum*-like sp., *Halopelagica* sp., and *Natronomonas* sp. dominate the community structure. Similar organisms have been retrieved from other subsurface halite formations in the US and Europe, suggesting that findings from this research may be useful to other salt-based repositories. At lower salt

concentrations (2.5 M), *Bacteria* (*Limnobacter* sp., *Pelomonas* sp., and *Nesterenkonia* sp.) were detected along with two archaeal sequences (*Halobacterium noricense* and a *Halorubrum*-like sp.). Fungi (*Coniothyrium* sp., *Cladosporium* sp., *Engyodontium* sp.) also appeared in incubations at 2.5 M NaCl; these were likely introduced into the WIPP from above-ground. Anaerobic enrichment incubations are also underway. Organic complexing agent degradation and solubility. Acetate and citrate were readily degraded aerobically by a mixed culture of haloarchaea cultivated from halite, but only after oxalate had precipitated from solution (see Figure 2)⁴. Oxalate was weakly degraded by an organism previously isolated from WIPP halite, but its disappearance was mainly due to low solubility in brine. Acetate was also degraded by a halophilic bacterium in diluted brines. Degradation experiments under nitrate-reducing conditions are currently underway. The potential for cellulose degradation is being investigated using the fungal and actinomycetal cultures isolated from halite.

Bioreduction of metals/actinides.

Iron solubility in brines is a limiting factor in its reduction by halotolerant/halophilic bacteria, and haloarchaea have not been shown to reduce metals. In incubations of lower ionic strength (~1.5 M) groundwaters from the WIPP site, the precipitation of metal sulfides was noted. XANES and XRD analyses are being performed to determine the metal oxidation states in these precipitates, as it is possible that sulfate reduction alone led to metal precipitation without actual reduction. However, both iron-reducing and sulfate-reducing bacteria were detected in these incubations. In groundwater and halite incubations at higher ionic strength (3.5 M), iron is also reduced abiotically, albeit at a slower rate.

Biosorption.

Adsorption of Np (V) onto a Gram-negative, halophilic bacterium in 4 M NaClO₄ was enhanced relative to adsorption in 2 M NaClO₄ over the majority of the pH range evaluated (see Figure 3). This was apparently due to the effect of increasing aqueous ion activity coefficients at high ionic strength. Sorption studies using a Gram-positive halophilic bacterium and a haloarchaeon isolated from WIPP environs are underway.

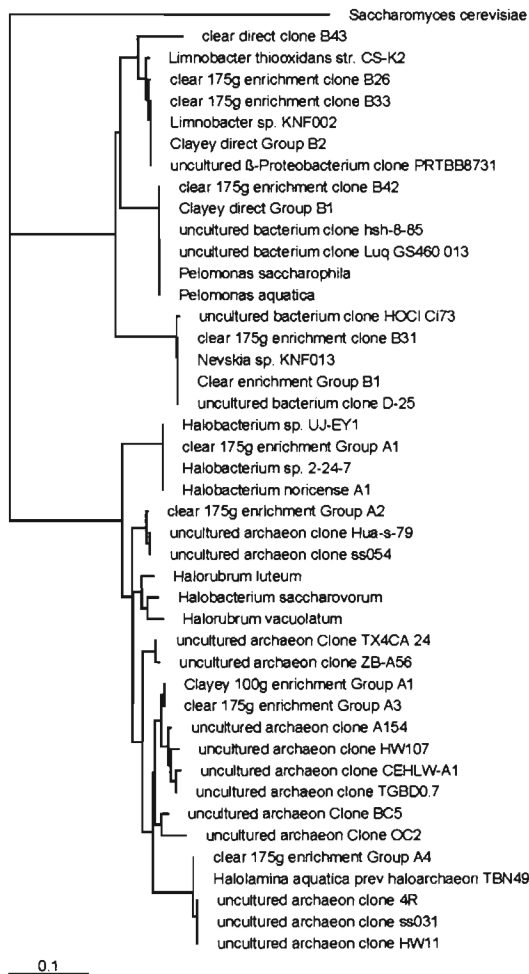


Fig. 1: Phylogenetic tree showing the relatedness of DNA sequences retrieved from samples of clear and clayey halite. Reference sequences included.

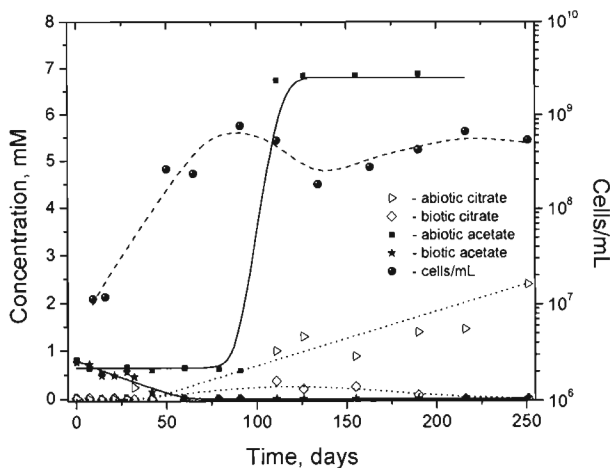


Fig. 2: Degradation of acetate and citrate in high Mg^{2+}

brine by a mixed culture of haloarchaea, as evidenced by the increase in abiotic concentrations, but not in biotic, after oxalate disappearance.

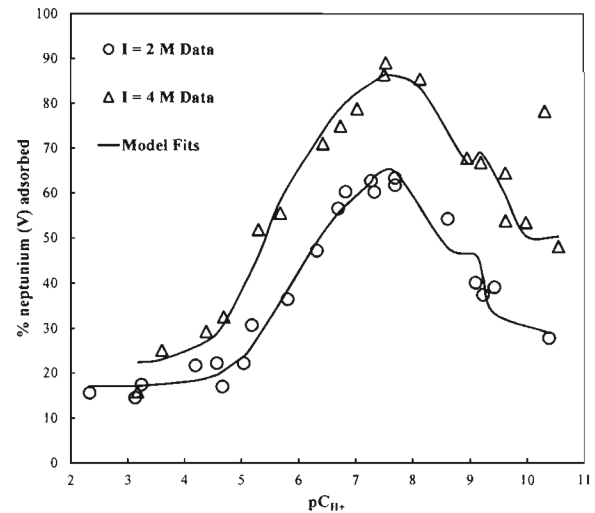


Fig. 3: Sorption of Np (V) onto *Chromohalobacter* sp. at 2 M and 4 M perchlorate.

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Microbiology in the Waste Isolation Pilot Plant (WIPP)



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WIPP CONCEPTUAL MODEL- MICROBIAL EFFECTS

- High molecular weight organics (cellulose, plastic, and rubber) will be biodegraded; low molecular weight organic chelating agents are not considered
- Once oxygen is depleted, biodegradation will proceed sequentially through denitrification and sulfate reduction
- The creation of a reduced environment by microbial processes is expected to lead to actinide reduction
- Colloid formation by actinide adsorption to microbial surfaces is significant

Title 40 CFR Part 191/Subparts B and C/Compliance Recertification Application for the WIPP/ Appendix
MgO-2009

REVIEW OF GOALS

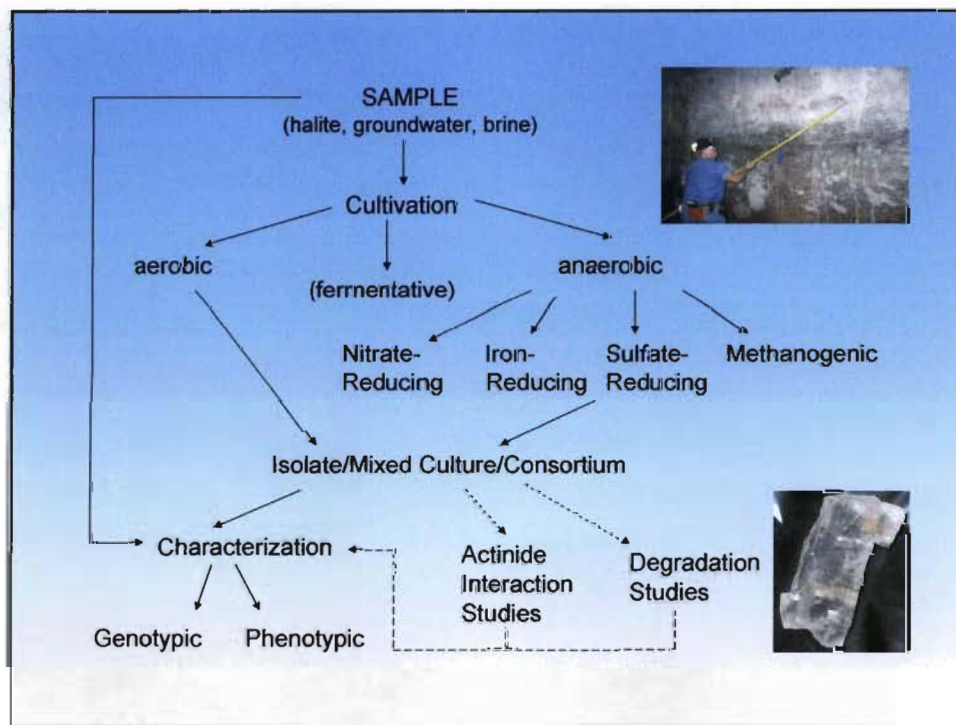
- To characterize the microbial community in the WIPP and surrounding groundwaters in order to better predict its metabolic capability and the effects of those processes on WIPP waste
- To obtain isolates, mixed cultures, and/or consortia of WIPP microorganisms for study on:
 - Interactions with organics in waste
 - Interactions with actinides in waste

Metabolic processes potentially occurring in WIPP

- All processes are limited by thermodynamics: organisms must weigh energy obtained versus energy required to maintain osmotic balance in high ionic strength environment
- Predominant communities in hypersaline environments are Archaea
- Bacterial metabolic capabilities broader than those of Archaea; however, Archaea more likely to be dominant in near-field
- Potentially significant differences in microbial community structure in near-field versus far

Metabolic processes (cont'd)

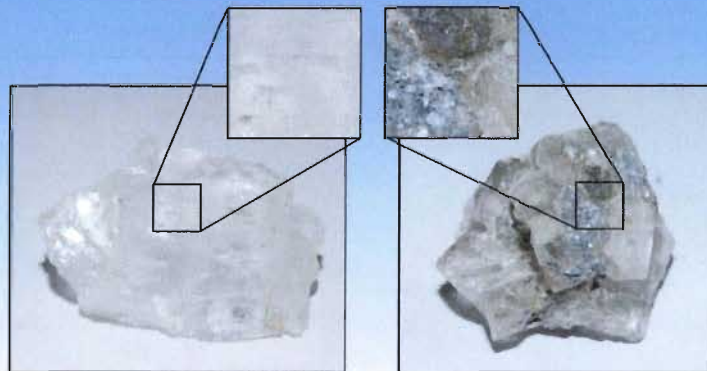
- *Aerobic respiration* (limited time frame)
- *Nitrate reduction* (nitrate not a limiting factor but reducing potential of environment may be); both Bacteria and Archaea
- *Fermentation* (citrate; cellulose may depend upon prior degradation); both extremely halophilic Bacteria and Archaea are capable
- *Metal reduction* (MnO_2 ; iron is solubility-limited); Bacteria only
- *Sulfate reduction* (likely to predominate); Bacteria only
- *Methanogenesis* (unlikely to occur at ionic strengths expected in WIPP, but may occur in far-field); Archaea only

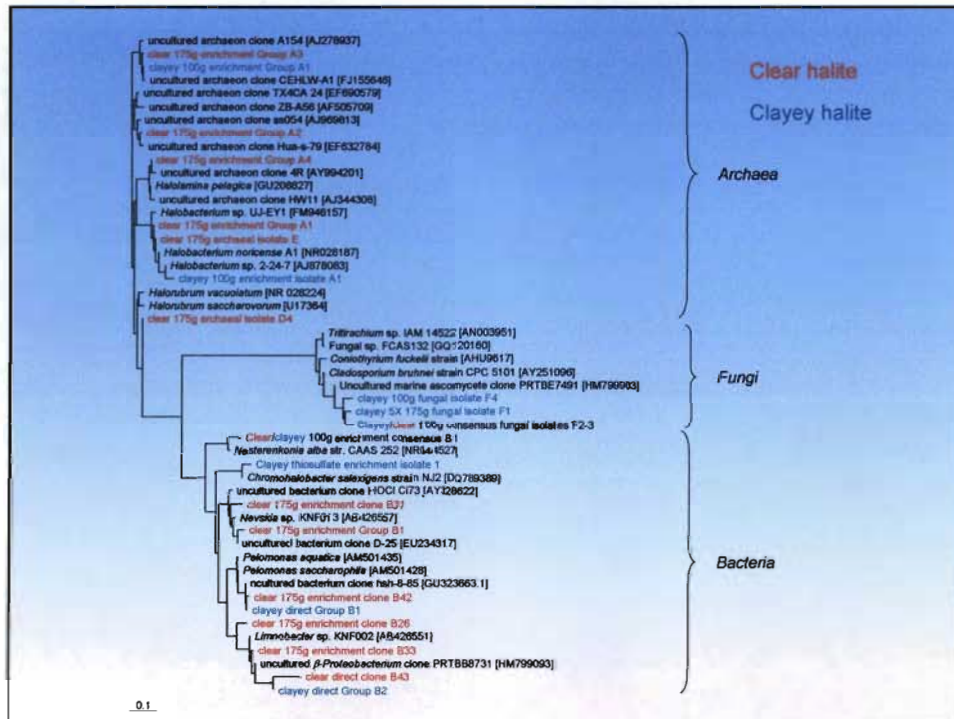


MICROBIAL CHARACTERIZATION

Clear versus clayey halite

- Does the presence of clay influence the composition of the microbial population?
 - osmoprotection
 - associated organics
 - higher B, Br, Mg, K





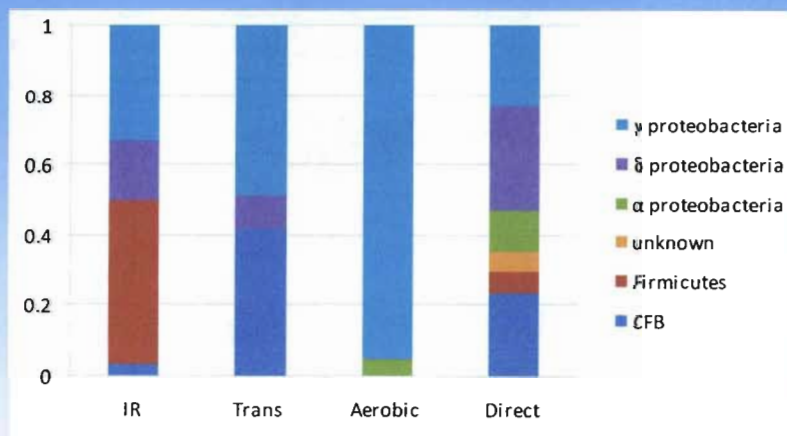
Factors most affecting microbial community in WIPP

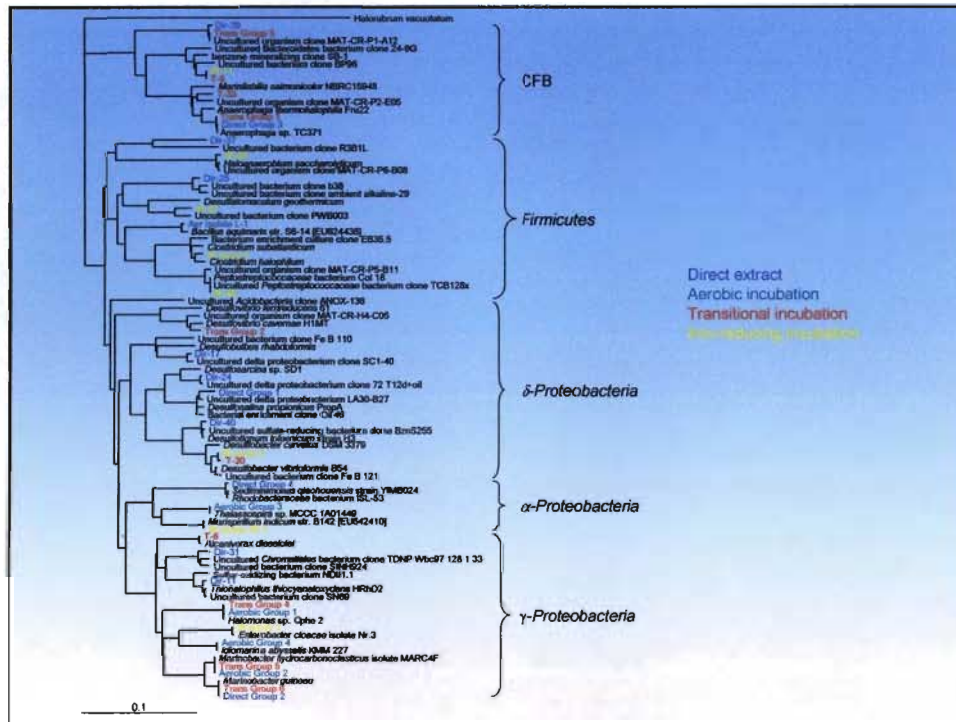
- [Na]
- lack of oxygen: anaerobic enrichment incubations underway (transitional, NR, IR, SR), no growth as yet
- [Mg]
- pH

WIPP groundwaters

- Far-field environment
 - range of ionic strengths (~1.5-5 M)
 - Bacteria predominant and more diverse in lower ionic strength waters
 - Metal reducers
 - Sulfate reducers
 - Fermentors
 - Community proceeds toward sulfate reduction
 - Results in precipitation of metals (iron) in both oxidized and reduced state

WIPP groundwater: $I \approx 1.5$ M

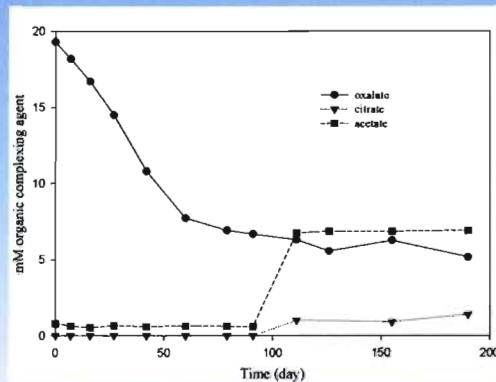




BIODEGRADATION of ORGANIC WIPP WASTE

Effects of solubility on degradation: solubility affects bioavailability

- The solubilities of acetate and oxalate are lower than inventory-predicted concentrations
- Acetate and citrate solubility in GWB (high Mg) brine is affected by oxalate
- Steady-state concentrations of organics are not statistically significantly different between GWB and ERDA-6 brines
- EDTA is soluble in both brines at inventory relevant concentrations



Degradation of Organic Waste Components by Microorganisms Indigenous to the WIPP

- Degradation of organic complexing agents (OCAs) under aerobic and anaerobic conditions
 - aerobic degradation of acetate, oxalate, and citrate
 - anaerobic degradation of citrate under denitrifying conditions in progress
 - anaerobic enrichments ongoing
- Gas generation from the degradation of high-molecular weight organics under aerobic and anaerobic conditions, focus on cellulose
 - testing fungal and actinomycetal cultures for ability to degrade cellulose aerobically; no growth anaerobically

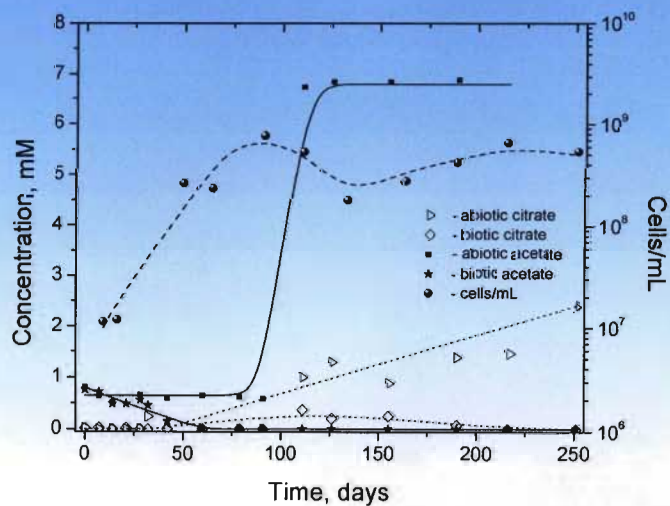
Summary of aerobic OCA degradation

Organic	<i>Halomonas halodenitrificans</i>		<i>Halosimplex carlsbadense</i>		Mixed Halophile Culture	
	GWB	ERDA	GWB	ERDA	GWB	ERDA
Acetate	+	+	-	-	+	+
Oxalate	-	-	++	++	-+	--+
Citrate	+	+	-+	-+	++	++
EDTA	-	-	-	-	-	-

+ degradation during original incubation

+ growth when subcultured on agar plate containing organic as sole carbon source

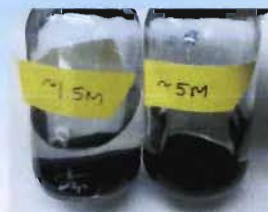
Aerobic degradation of acetate and citrate in high Mg brine by mixed halophile culture



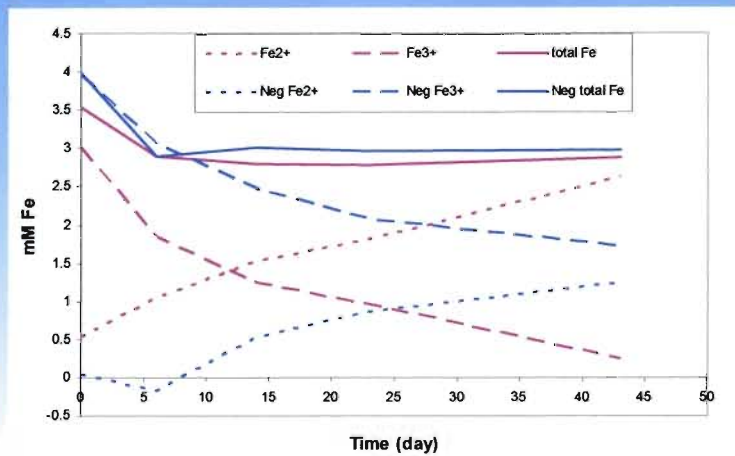
METAL REDUCTION in HIGH IONIC STRENGTH SYSTEMS

Metal reduction by halophilic microorganisms

- if organisms can reduce iron, can they reduce actinides?
- iron reduction observed in both biotic and abiotic incubations but at a slower rate in abiotic
- rates also slower as ionic strength increases
- precipitation of both oxidized and reduced iron as sulfides in groundwater incubations



Iron Reduction at 1.4 M NaCl



Iron reduction at 3.8 M NaCl

