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LCO-ACP-32, Revision 0

Biologically Influenced Transport of the +3 Actinide Oxidation State from the WIPP Environment, Using Neodymium as an Analog

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History of Revisions

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0	12-15-2022	All	Original Release

ACRONYMS AND ABBREVIATIONS

CFU	colony-forming unit
DPA	dipicolinic acid (dipicolinate)
EPS	extracellular polymeric substance
ERDA	Energy Research and Development Administration Well 6
GWB	Generic Weep Brine
ICP-MS	inductively-coupled plasma mass spectrometry
L	liter
M	molar; moles/liter
µM	micromolar; micromoles/liter
Nd	neodymium
OD	optical density
PA	performance assessment
pC_{H+}	negative log of the hydrogen ion concentration
pH	negative log of the hydrogen ion activity
sp.	species (singular)
WIPP	Waste Isolation Pilot Plant

EXECUTIVE SUMMARY

This report presents results of experiments designed to update the microbial contribution to the +3 actinide biocolloid parameters for the Waste Isolation Pilot Plant (WIPP) and provides a basis for biocolloid parameters to be recommended for the 2024 Compliance Recertification Application.

Bioassociation experiments with neodymium were carried out on several WIPP isolates from different phyla and with different characteristics. The extent of biological influence varied initially, but over time, this influence approached 100 percent, regardless of the organism tested (the exception is in the presence of bacillus spores that produce the ligand, dipicolinate; DPA). This suggests that surface complexation is the predominant controlling factor at first, but that other factors control the long-term solubility of neodymium in the presence of microorganisms.

Experiments in WIPP brines suggest that the test matrix chemistry significantly affects biological influence. Little to no influence was noted in experiments carried out in high magnesium Generic Weep Brine (GWB), while NaCl matrices—such as Energy Research and Development Administration well 6 brine (ERDA) or simplified NaCl—led to more apparent influence. This is hypothesized to be due to several factors, including competition between magnesium and neodymium for surface sites and biologically-induced mineral precipitation masking as apparent sorption. Ultimately, more experiments are needed to determine the exact mechanism.

These new data will be used to update biocolloid parameters for the +3 oxidation state.

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1.0 INTRODUCTION

Potential biocolloid transport of actinides is an ongoing area of research for the Waste Isolation Pilot Plant's Compliance Recertification Application (WIPP CRA). This report provides an update on this work and presents a justification for possible new enhancement parameters for WIPP Performance Assessment (PA). The experiments described herein are only for the +3 actinide oxidation state.

Microorganisms can have varied influence on actinides in solution. In terms of performance assessment, "biocolloid" formation assumes mobilization and potential release. The term "bioassociation" has been used to encompass certain actinide-microbial interactions including surface complexation (and potential surface mineralization) and internal uptake. The term "biological influence", while inclusive of association phenomena, can also include induced precipitation, solubilization with microbially-generated complexants, or even redox transformations. This work is not focused on redox issues, but the generation of complexants is included where relevant.

To date, three Archaea and five Bacteria have been isolated from aerobic cultures of WIPP halite and/or brine seep stalactites at NaCl concentrations ranging from 10-27.5% (1.7-4.7 M; Swanson et al. 2012; Swanson and Reed, 2018).

Table 1. Organisms isolated from WIPP halite.

Organism	Domain/Phylum	Optimum [NaCl] for Growth, M (range)
Halobacterium sp. (*noricense*)	Archaea/Euryarchaeota	3.42 (1.71-saturated)
Isolate PB-1a		4.28 (2.14-saturated)
Isolate Trans-1b		2.99 (1.71-saturated)
Chromohalobacter sp. (*salexigens*)	Bacteria/Proteobacteria	2.57 (0.86-4.28)
Salinicoccus sp. (*roseus*)	Bacteria/Firmicutes	1.71 (0.43-2.99)
Nesterenkonia sp.	Bacteria/Actinobacteria	0.43 (0-2.57)
Thalassobacillus sp. (*cyri*)	Bacteria/Firmicutes, spore forming organisms	0.76 (0.43-2.14)
Bacillus sp.		0.33 (0.33-0.86)

One of the archaea, *Halobacterium* sp., has been used as a representative organism for study in ACRSP experiments, since it is also one of the most commonly found haloarchaea in subterranean salts (Jaakkola et al., 2016). It has been tested for its ability to associate with uranium, neodymium, and cadmium (Showalter et al., 2016; Bader et al., 2017; Swanson, 2019; Liu et al., 2020). Neptunium(V) biosorption was tested with the halophilic bacterium, *Chromohalobacter* sp., and some preliminary data also exist for this organism with neodymium and thorium (Ams et al., 2013; Reed, 2013). Finally, Nd association experiments were carried out using spores of a *Bacillus* sp. that was isolated from WIPP-bound waste (Swanson et al., 2021).

Of the above list, only the three archaea and *Chromohalobacter* sp. are able to grow in WIPP brines amended with substrates and nutrients. In comparison, all organisms from Table 1 are

able to survive exposure to GWB and ERDA brines for a period of weeks (ranging from 3-39 weeks) at high biomass concentrations before resuscitation becomes impossible. At lower biomass concentrations, survival is shorter, but these timeframes are inconsequential when compared to the 10,000-year repository lifetime. Those that form spores survive much longer and could remain throughout this timespan.

This report presents data on the behavior of neodymium (Nd) with several WIPP isolates in simple NaCl solutions and for a subset of isolates also in WIPP brines. Neodymium was used as an analog for the +3 actinide oxidation state. Simplified NaCl was used to screen the potential for biological influence at each organism's optimum salt concentration, since published data on biosorption are most often generated in simple matrices. These experiments were extended to anoxic WIPP brines, whose chemistry and pH can significantly affect outcome.

2.0 SUMMARY OF EXPERIMENTS

All organisms were grown in their optimal media at their optimal NaCl concentration. At early stationary phase, cells were harvested and washed five times with solution containing optimal NaCl, and the cell pellet was stored overnight at 4°C. Pellets were resuspended in NaCl solution to achieve a target optical density (OD) equivalent to ~10^9 cells/ml. OD values and cell numbers were correlated prior to the start of each bioassociation experiment.

Concentrated neodymium stock solutions (~ 2 mM) were prepared by adding solid $NdCl_3$ to each salt solution. Working solutions containing twice the target Nd concentration (2X) were prepared from these stocks, also in each respective solution (working solutions of WIPP brines were prepared using the 25% NaCl-Nd stock). The cell suspension was then mixed 1:1 with the 2X Nd-NaCl working solution. Cell suspensions were placed on a rotator at ambient temperature, and samples were withdrawn periodically for analysis by ICP-MS.

Optical density readings were taken at each sampling point, after which samples were passed through an Amicon 100 kD centrifugal filter and then diluted in 2% nitric acid. Measurements of Nd in the filtrate are considered to be "dissolved" Nd. The filtration step captures surface sorption, internal uptake, and induced precipitation and/or mineralization either at the cell's surface or from the bulk solution. Abiotic controls were run parallel to the biotic samples to account for any precipitation not caused by the presence of organisms. Pooled filtrates were used to measure pH. Direct microscopic counts or colony-forming unit (CFU) counts were also obtained periodically.

Experiments in WIPP brines were conducted in a nitrogen-filled glovebox using anoxically prepared, 90% formulations of GWB and ERDA-6 brines. Cell pellets were brought into the glove box and resuspended in each brine prior to mixing with brine-2X Nd solutions. All other steps were the same.

The percentage of biologically-influenced Nd was calculated as:

$$\{[(Nd\ in\ abiotic\ sample) - (Nd\ in\ biotic\ sample)]/(Nd\ in\ abiotic\ sample)\} * 100$$

3.0 RESULTS

To date, five new organisms have been tested for bioassociation with Nd: archaeal isolate PB-1a, *Chromohalobacter* sp., *Salinicoccus* sp., *Nesterenkonia* sp., and spores of *Thalassobacillus* sp. Results for the previously tested *Halobacterium* sp. and waste *Bacillus* sp. will be presented for comparison.

3.1. Bacteria

3.1.1. *Chromohalobacter* sp. (putatively *salexigens*)

This organism is a member of the Gamma Proteobacteria. It is a Gram-negative, borderline extreme halophile, capable of slow growth in WIPP brines but growing optimally at 2.57 M NaCl. Members of this genus have been found to produce extracellular polymeric substances (EPS) that may contribute to metal or actinide surface complexation (Biswas and Paul, 2014). This isolate has been tested previously for its ability to adsorb neptunium in perchlorate solutions (Ams et al., 2013) and neodymium in WIPP brine.

Ninety-seven percent of the neodymium in NaCl solution with *Chromohalobacter* was immediately removed and remained so throughout the time course of the experiment (Figures 1-2). Abiotic levels of Nd in solution also dropped over time by 31%.

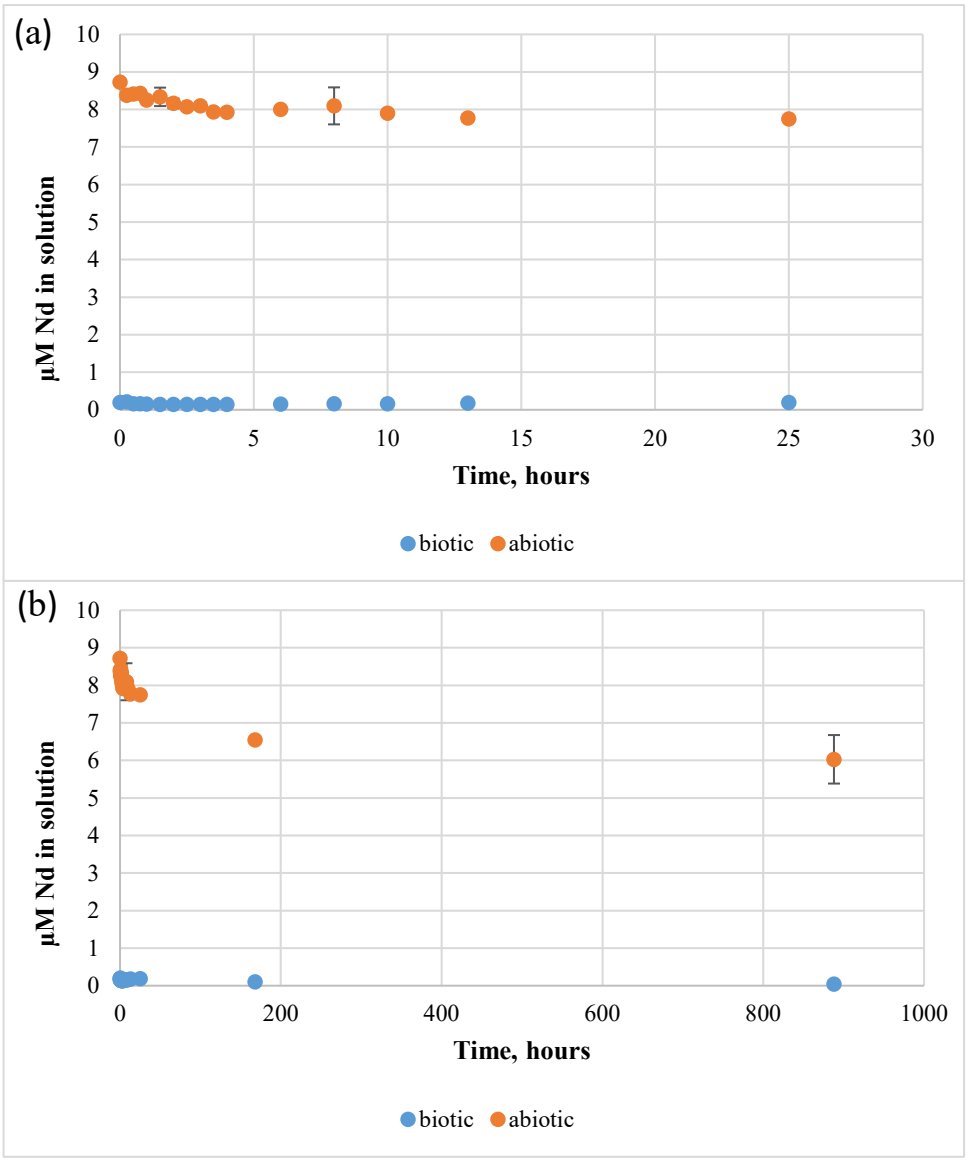


Figure 1. Neodymium concentrations in *Chromohalobacter* sp. biotic samples and abiotic controls during a) the first 24 hours and b) ~ 5 weeks. Error bars represent the standard deviation of three replicate samples.

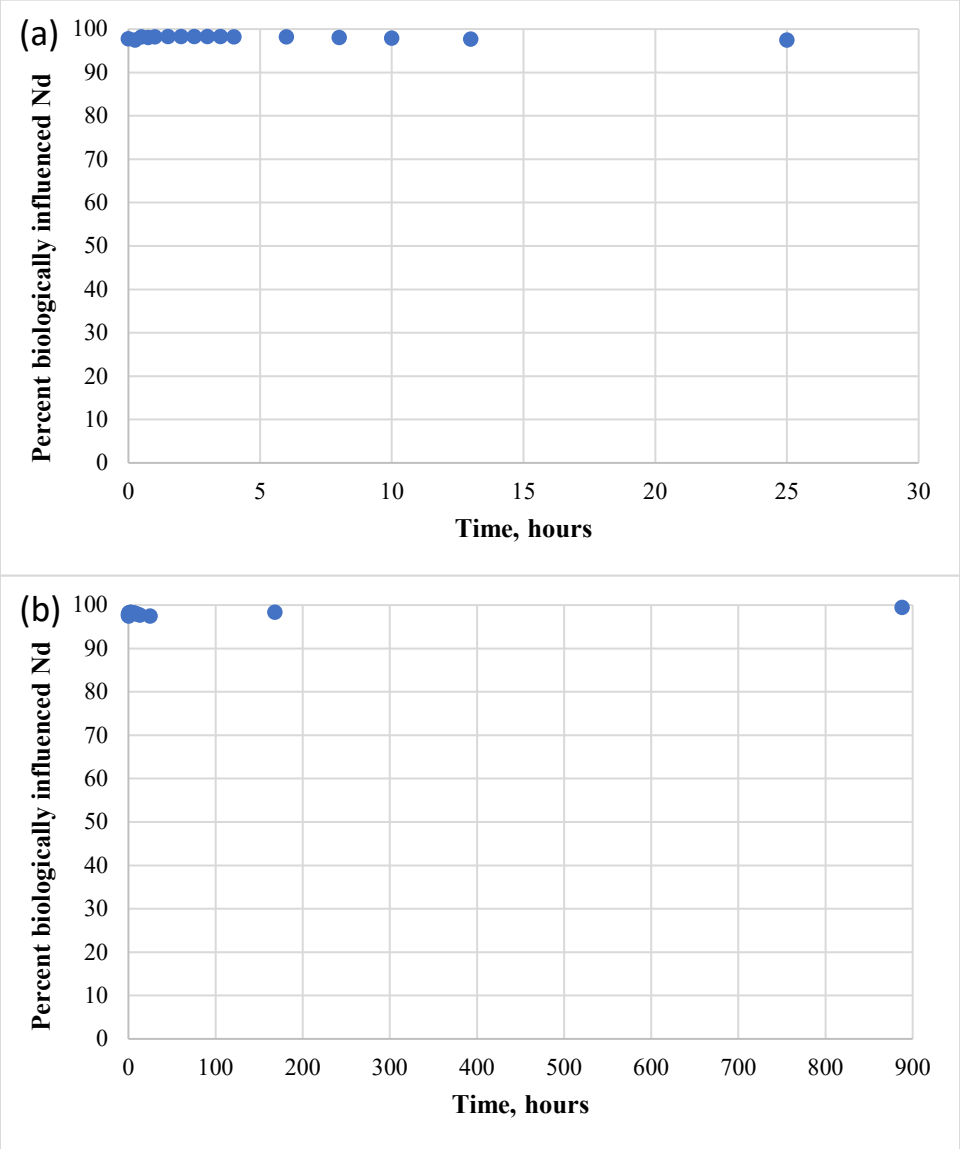


Figure 2. Percent of biologically influenced neodymium in *Chromohalobacter* sp. experiments over a) the first 24 hours and b) ~ 5 weeks.

3.1.2. *Salinicoccus* sp. (putatively *roseus*)

This organism is a member of the Phylum Firmicutes. It is a moderately halotolerant, pink-pigmented, Gram-positive coccus and obligate aerobe. Members of this genus produce EPS and form clumps when grown in broth that are often difficult to measure by optical density or microscopic counts. By microscopy, it often appears in tetrads or larger aggregates of up to 12 cells. It cannot grow in WIPP brines, but it can be resuscitated after exposures of 23-25 weeks in GWB and ERDA, respectively, at high biomass concentrations (~1E+09 cells/ml).

Neodymium biosorption onto *Salinicoccus* was immediate and measured approximately 39%. It increased to 55% over a 24 hour period and leveled off at 60% by one week (Figure 3). However, at one month, apparent bioassociation was 97% (Figure 4). Nd was also lost from the abiotic samples over time.

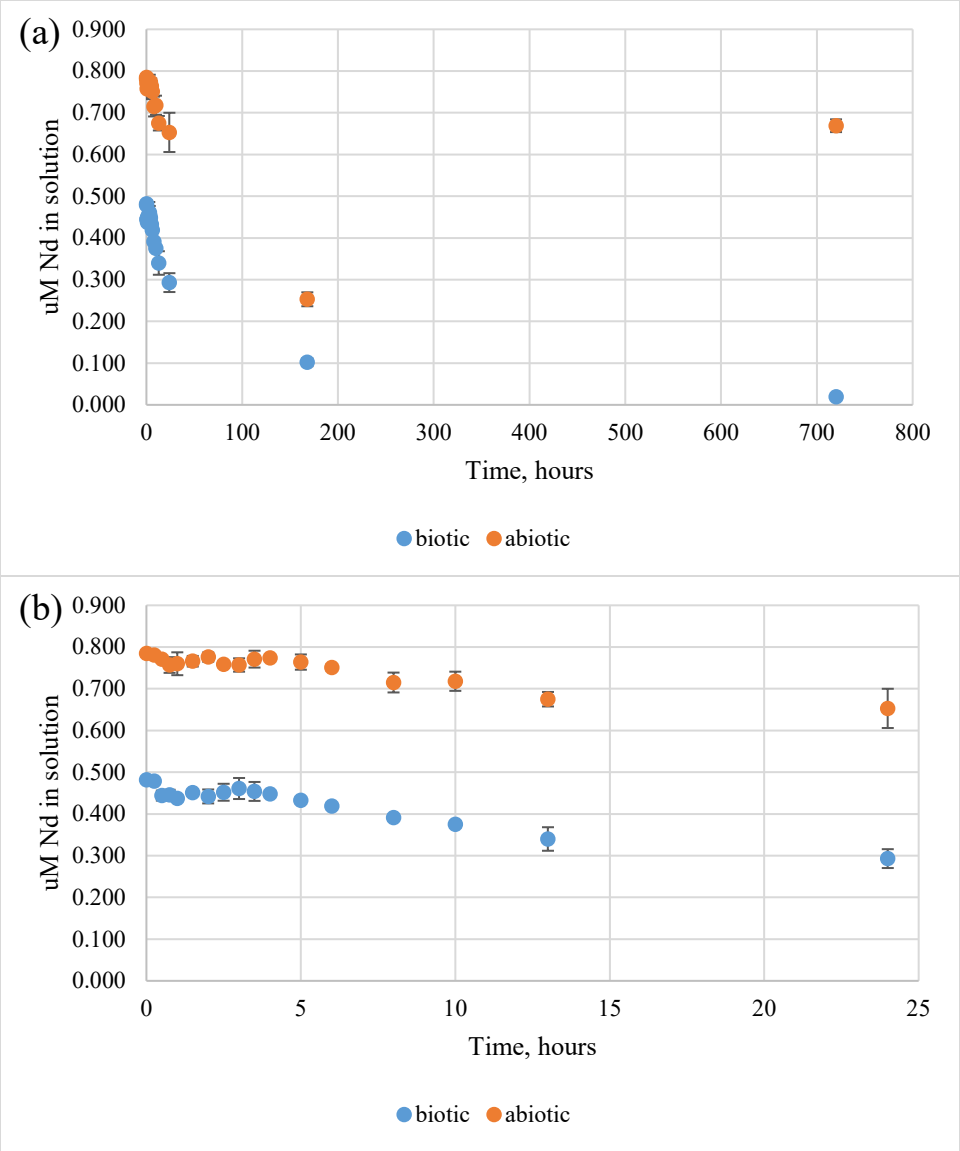


Figure 3. Neodymium concentration in *Salinicoccus* sp. biotic samples and abiotic controls over a) the first 24 hours and b) one month. Error bars represent the standard deviation of triplicate samples.

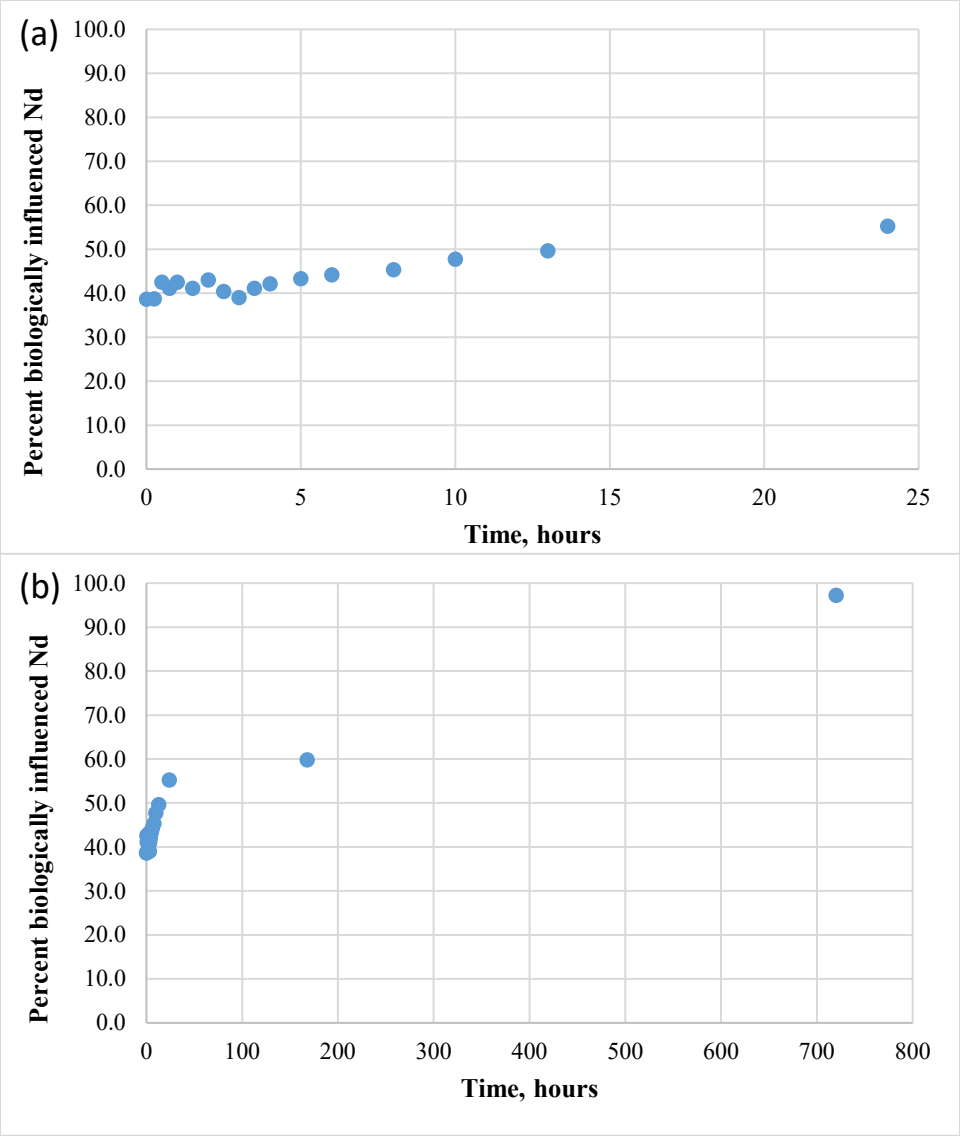


Figure 4. Percent of biologically influenced neodymium in *Salinicoccus* sp. experiments over a) the first 24 hours and b) one month.

3.1.3 *Nesterenkonia* sp.

Nesterenkonia sp. is a member of the Phylum Actinobacteria. It was isolated from two different samples of WIPP halite (near-pure halite and argillaceous halite) both incubated in generic halophile broth containing 10% NaCl. It is Gram-positive and halotolerant with an optimum NaCl concentration for growth of ~2% (~0.03 M), but it is capable of growth between 0-17% NaCl (~2.9 M). Members of the Actinobacteria are notable for their resistance to environmental stressors, such as pH and temperature extremes, heavy metals, solvents, ionizing radiation, and desiccation.

Neodymium removal by *Nesterenkonia* was immediate and near complete, with over 99% loss from solution throughout the course of the experiment (Figures 5-6). *Nesterenkonia* cells, like *Salinicoccus*, often appear as couplets or tetrads. In these experiments, morphology remained coccoid, although these cells can form short rods during their log growth phase, as do many Actinobacteria.

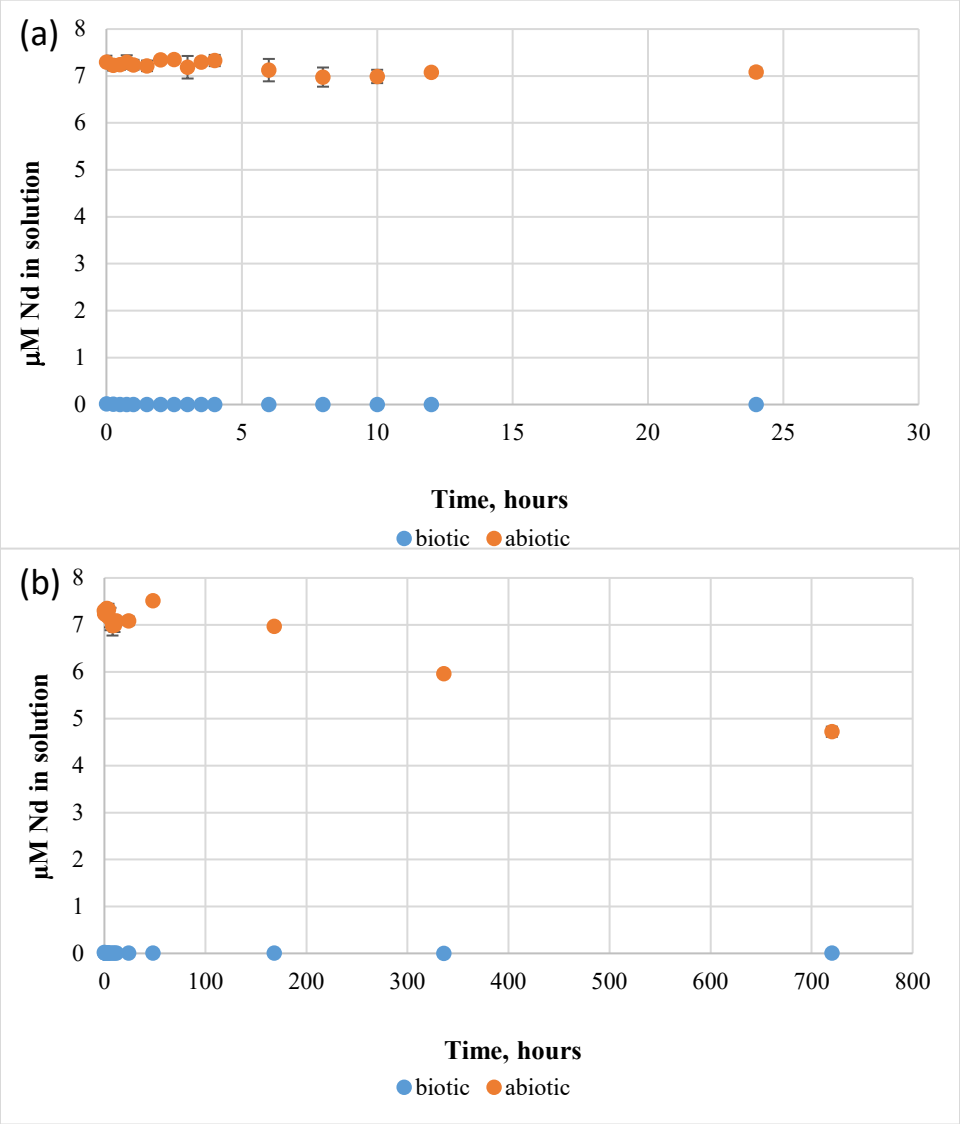


Figure 5. Neodymium concentration in *Nesterenkonia* sp. biotic samples and abiotic controls during a) the first 24 hours and b) one month. Error bars represent the standard deviation of triplicate samples.

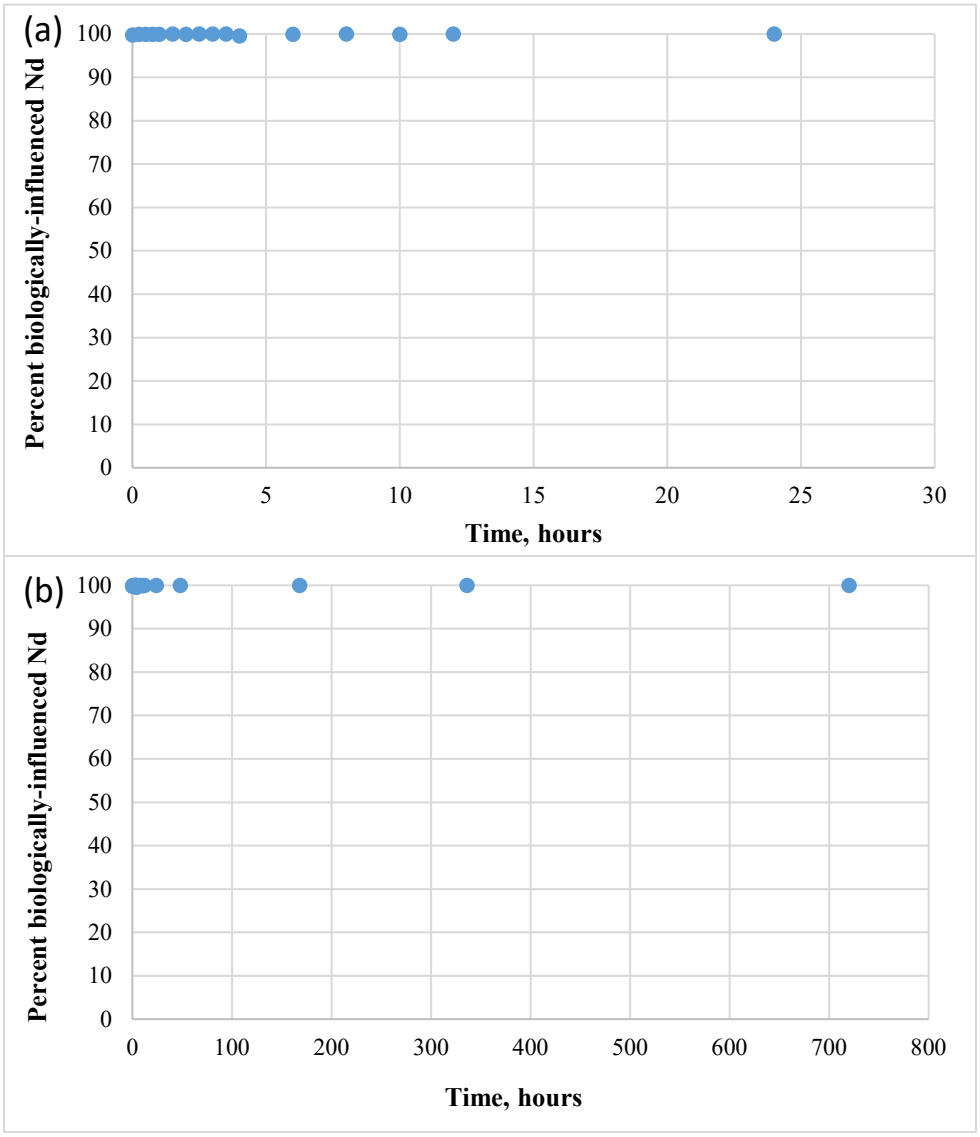


Figure 6. Percent of biologically influenced neodymium in *Nesterenakonia* sp. experiments over a) the first 24 hours and b) one month.

3.1.4 *Thalassobacillus* sp. spores

Thalassobacillus sp. was isolated from a stalactite sample formed from brine seep and incubated in growth medium containing 10% NaCl (1.71 M). It is a Gram-positive, slightly halophilic spore forming bacillus and does not grow in the absence of NaCl. In its spore form, it can likely survive for long periods in WIPP brines (longest tested was 39 weeks). Prior to beginning these experiments, spores were purified with and without lysozyme and all wash solutions were tested for the presence of dipicolinate (DPA), a ligand produced and released by some spores that can enhance actinide solubility. These spores were not as susceptible to lysozyme treatment as a previously tested species (Swanson et al., 2021), but since some DPA was detected (~8 µM), lysozyme was omitted from the procedure.

Thalassobacillus spore influence on neodymium in solution was immediate, with approximately 78% loss from solution at time zero (Figures 7-8). This increased slowly over time until 98% of Nd was lost by one month. This is in contrast to previous experiments with spores from a different organism, where the release of DPA caused all Nd to return to solution (Swanson et al., 2021). Samples taken from the original *Thalassobacillus* spore suspension that had been stored for the duration of these kinetics experiments had approximately 0.17 µM DPA, which might not have been enough to cause solubilization in these samples. Optical density also decreased slightly, as did CFU counts.

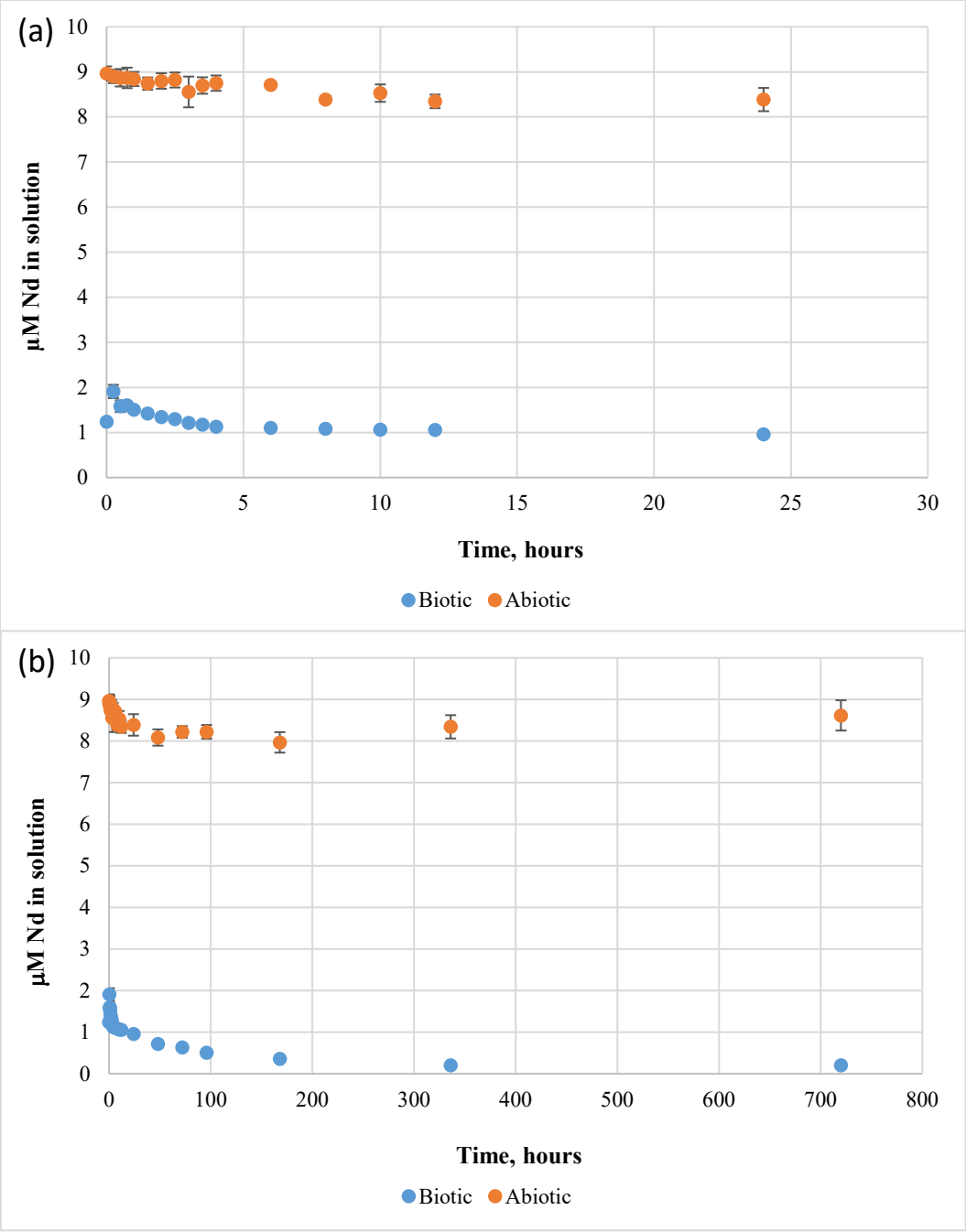


Figure 7. Neodymium concentration in *Thalassobacillus* sp. biotic samples and abiotic controls a) during the first 24 hours and b) over one month. Error bars represent the standard deviation of triplicate samples.

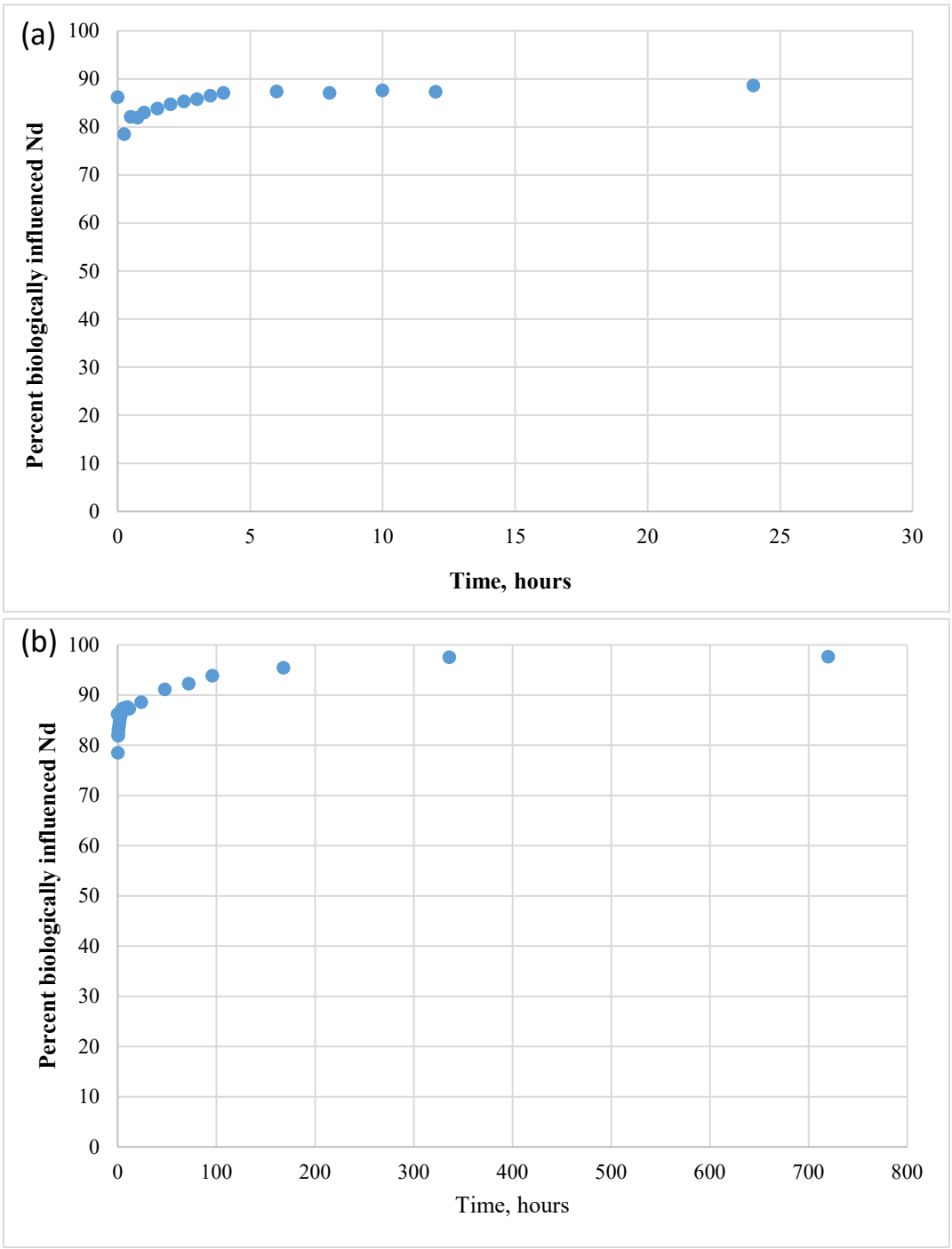


Figure 8. Percent biologically influenced neodymium in *Thalassobacillus* experiments a) during the first 24 hours and b) over one month.

3.2 Archaea

3.2.1 PB-1a

Archaeon PB-1a is the most halophilic of the WIPP archaeal isolates, with an optimum NaCl concentration for growth of 25% (4.28 M). It has not yet been identified taxonomically, but it was verified as an archaeon by amplification of its 16S ribosomal DNA using archaeal primers. This archaeon behaved similarly to *Halobacterium* sp. Neodymium loss from solution was slower and took over 48 hours before it was completely removed from solution. Approximately 10% of Nd was lost from the abiotic samples over time (Figures 9-10).

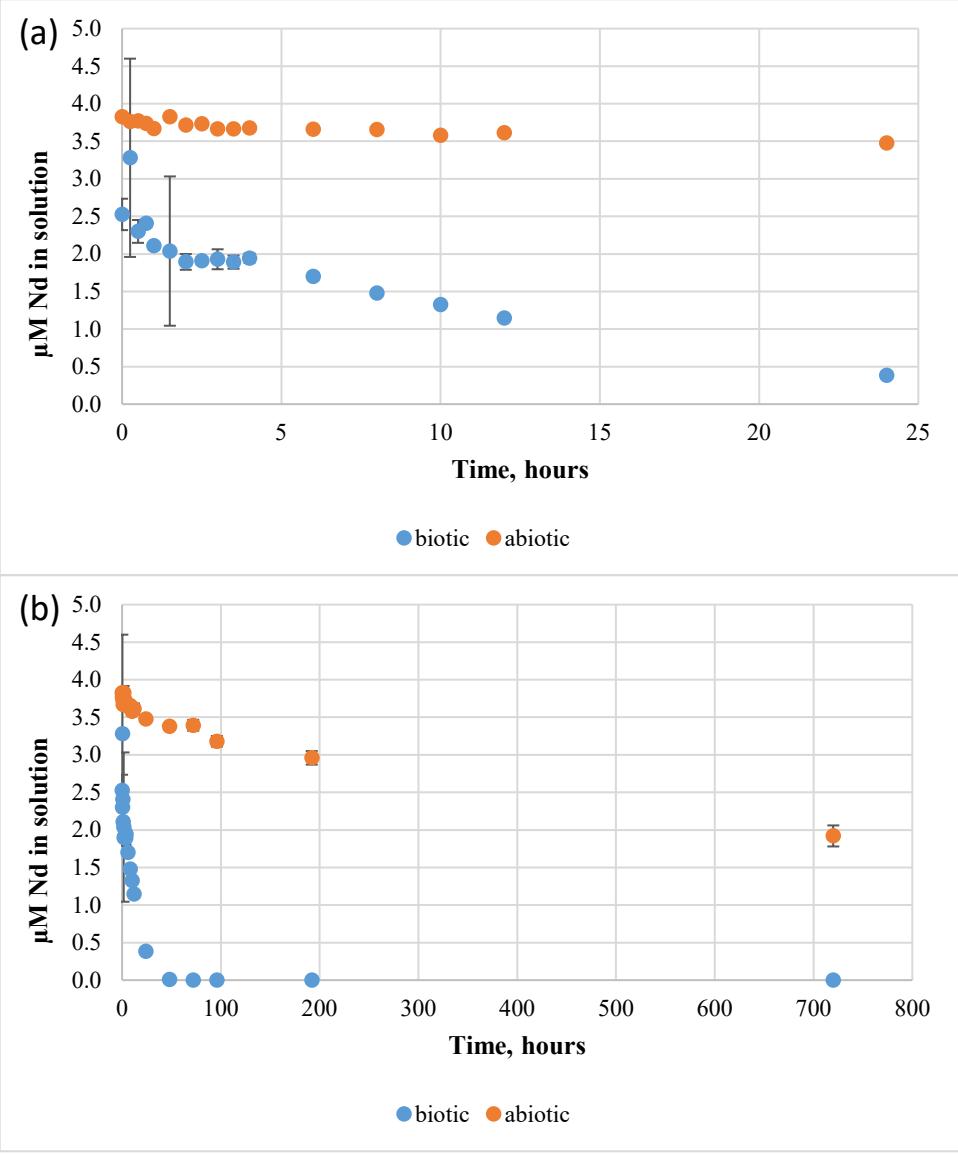


Figure 9. Neodymium concentrations in PB-1a biotic samples and abiotic controls a) during the first 24 hours and b) over one month. Error bars represent the standard deviation of triplicate samples.

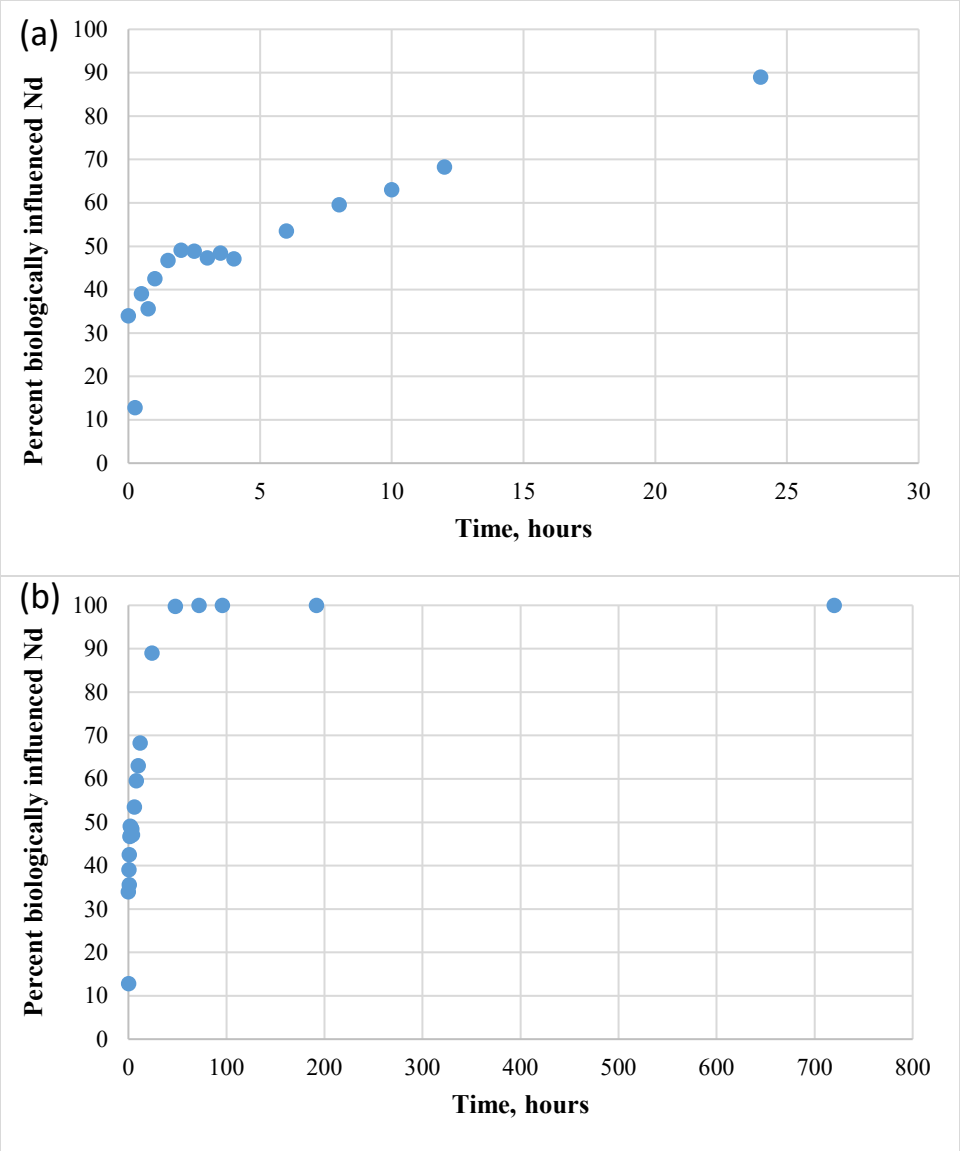


Figure 10. Percent biologically influenced neodymium in PB-1a experiments a) during the first 24 hours and b) over one month.

3.2.3 *Halobacterium* sp. (putatively *noricense*)

This organism was tested previously, and results of those experiments appear in report LCO-ACP-23 but are presented again here for comparison (Figures 11-12). To summarize, neodymium loss from solution was gradual but complete over the first 24 hours, but only at higher biomass concentrations (5.03E+09 cells/ml). By one month, approximately 50% of neodymium was also lost from suspensions containing lower biomass concentrations (2.02E+08 cells/ml).

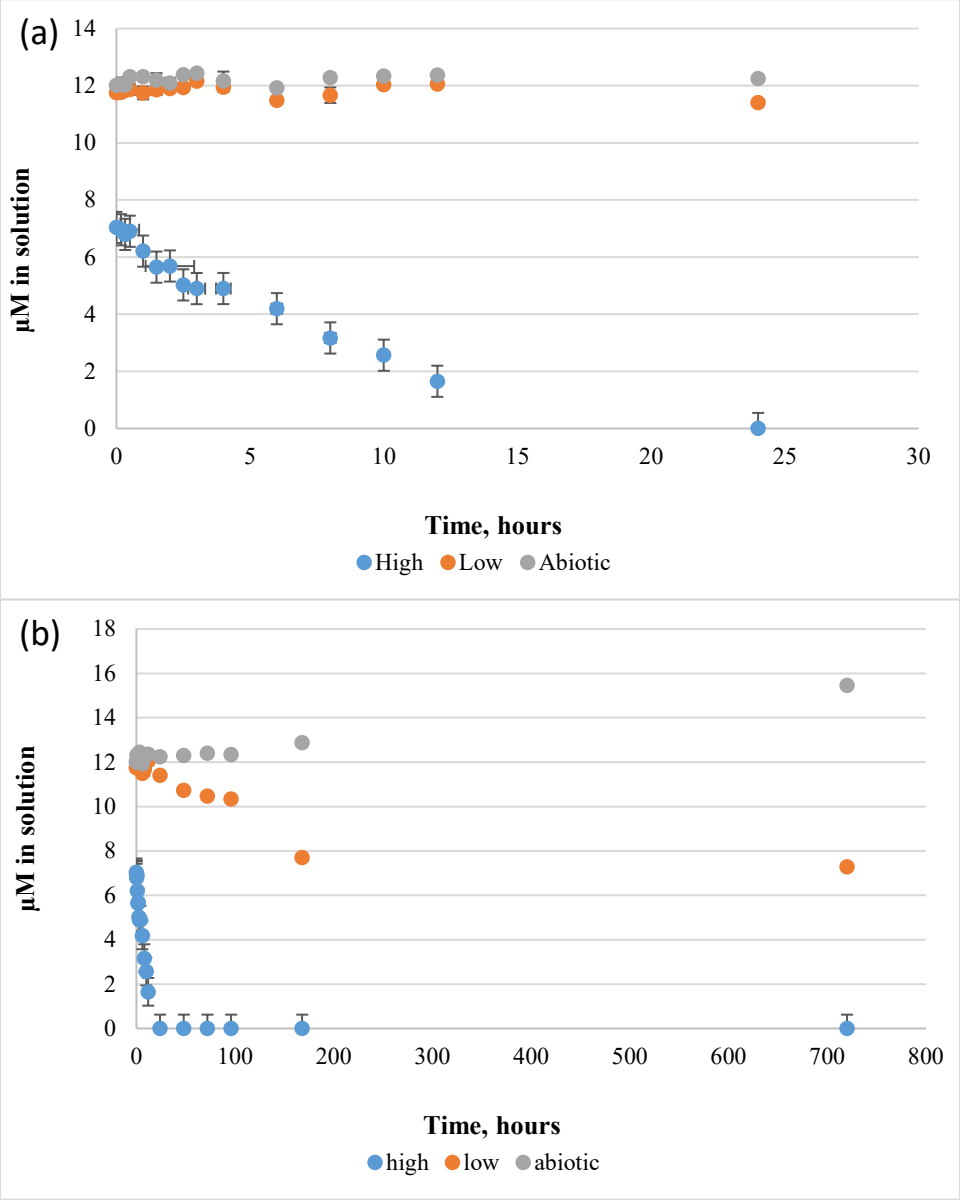


Figure 11. Neodymium concentration in *Halobacterium* sp. biotic samples and abiotic controls a) during the first 24 hours and b) over one month. Error bars represent the standard deviation of triplicate samples. High biomass ~ 5.03E+09 cells/ml; low biomass ~2.02E+08 cells/ml.

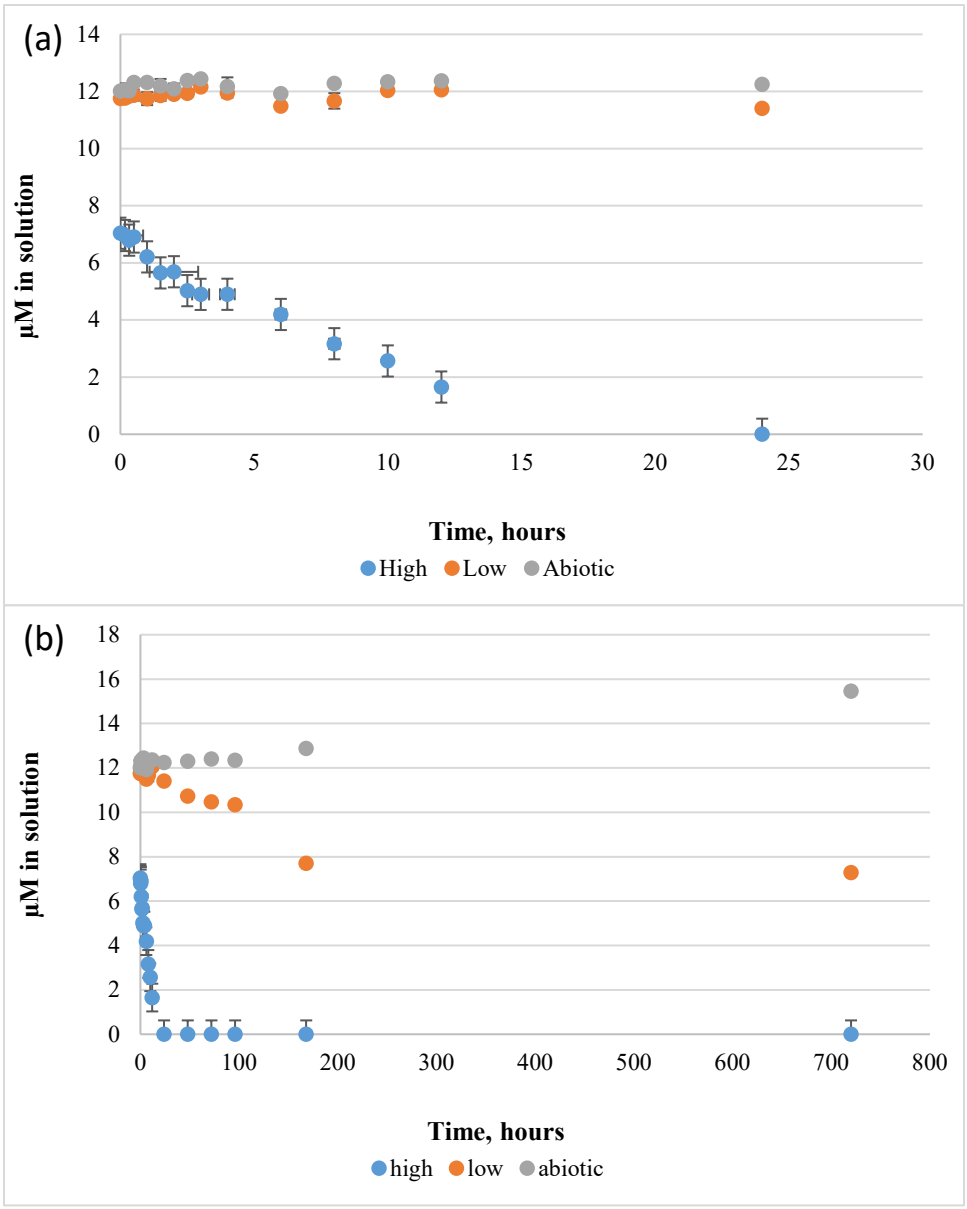


Figure 12. Percent biologically influenced neodymium in *Halobacterium* sp. experiments a) during the first 24 hours and b) over one month. High biomass ~ 5.03E+09 cells/ml; low biomass ~2.02E+08 cells/ml.

3.3 Halophiles in WIPP Brines with Nd

Experiments were extended to WIPP brines to determine how the test matrix affects Nd bioassociation. To date, three organisms have been tested: *Halobacterium* sp. (see previous report), isolate PB-1a, and *Chromohalobacter* sp. Anoxic brines were prepared at 90% of full strength. All experiments were conducted in the nitrogen-filled glove box.

3.3.1 *Chromohalobacter* sp.

There was very little loss of Nd from GWB, from either biotic or abiotic samples, throughout the course of the experiment (Figures 13 and 15). In contrast, significant loss from ERDA in the biotic samples was immediate and reached near 100% at approximately 5 weeks (Figures 14 and 16). There was also ~40% decrease in Nd in the ERDA abiotic samples by 5 weeks.

There was a gradual decrease in colony-forming unit counts in both brines within the first week, and a significant decrease by week 2. At the final time point, there were no viable cells in the ERDA samples and ~10^6 CFU/ml in GWB (Figure 17).

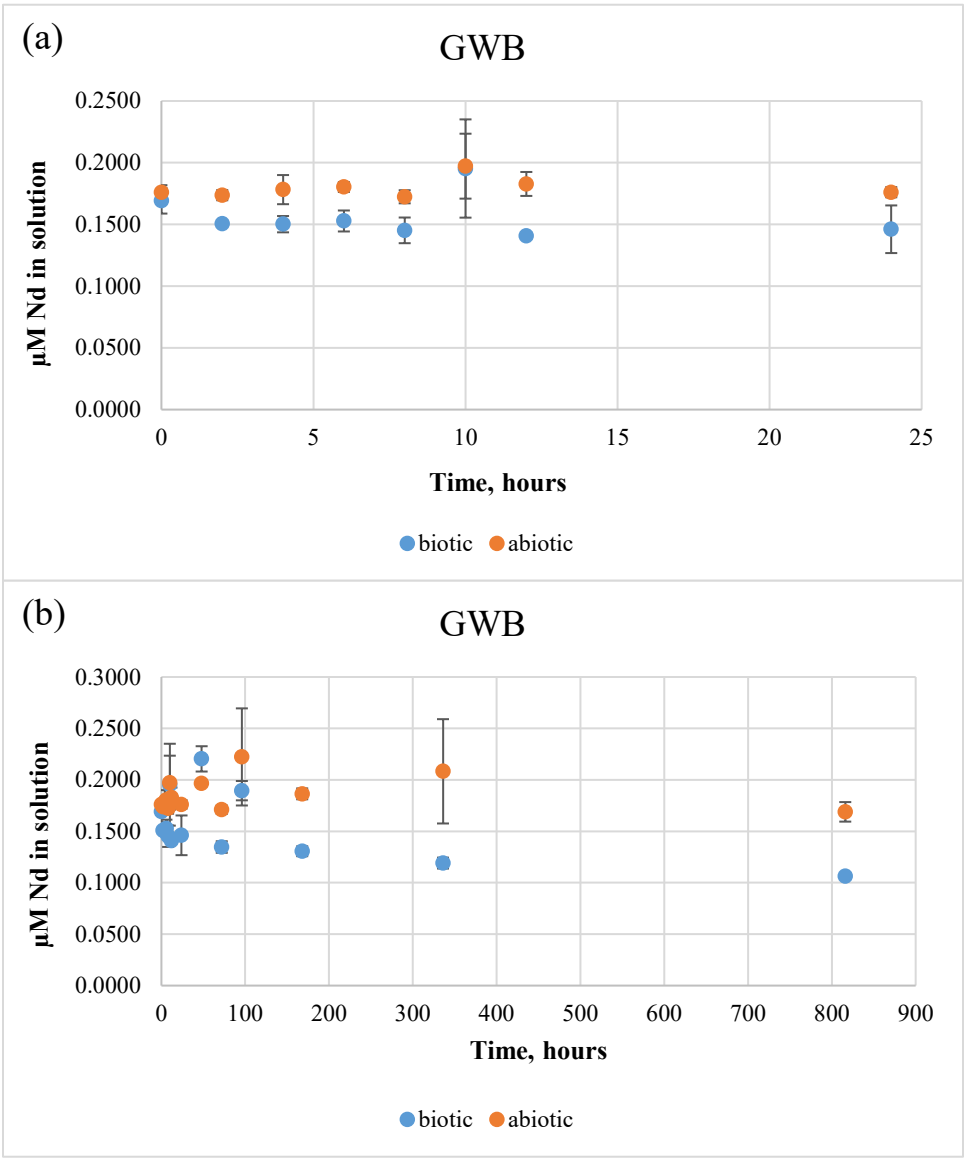


Figure 13. Neodymium concentration in *Chromohalobacter* sp. biotic samples and abiotic controls in GWB a) during the first 24 hours and b) over ~ 5 weeks. Error bars represent the standard deviation of triplicate samples.

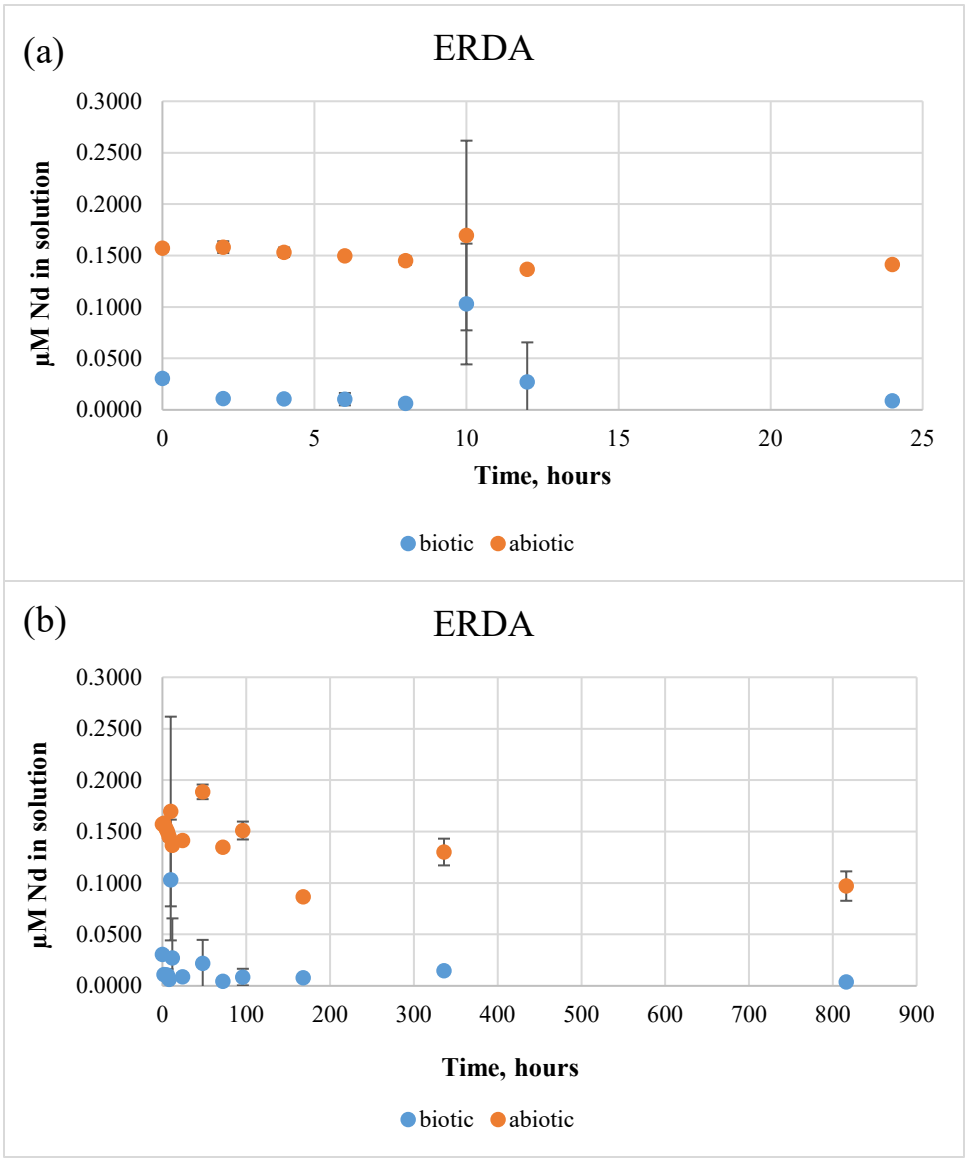


Figure 14. Neodymium concentration in *Chromohalobacter* sp. biotic samples and abiotic controls in ERDA a) during the first 24 hours and b) over ~ 5 weeks. Error bars represent the standard deviation of triplicate samples.

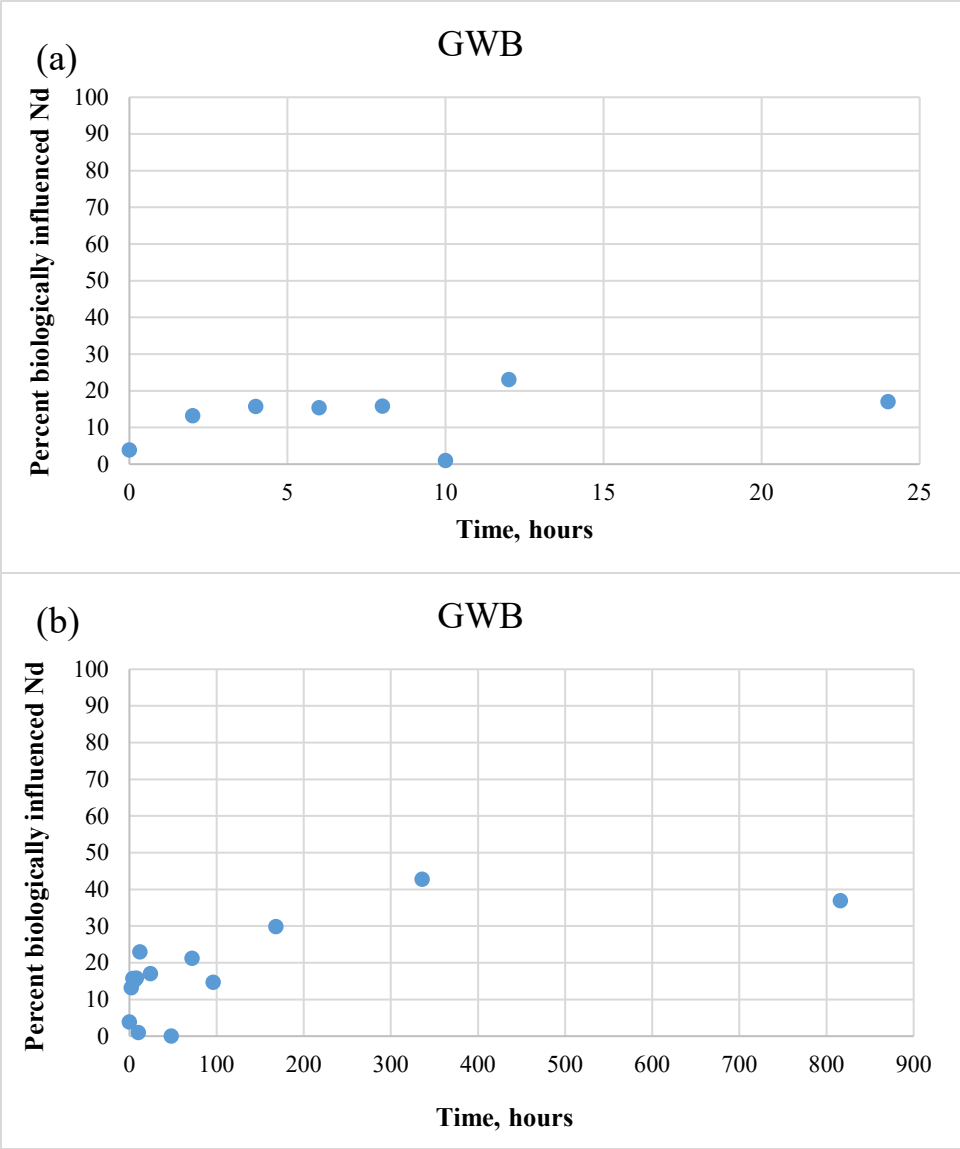


Figure 15. Percent of biologically influenced neodymium in *Chromohalobacter* sp. experiments in GWB a) during the first 24 hours and b) over ~ 5 weeks.

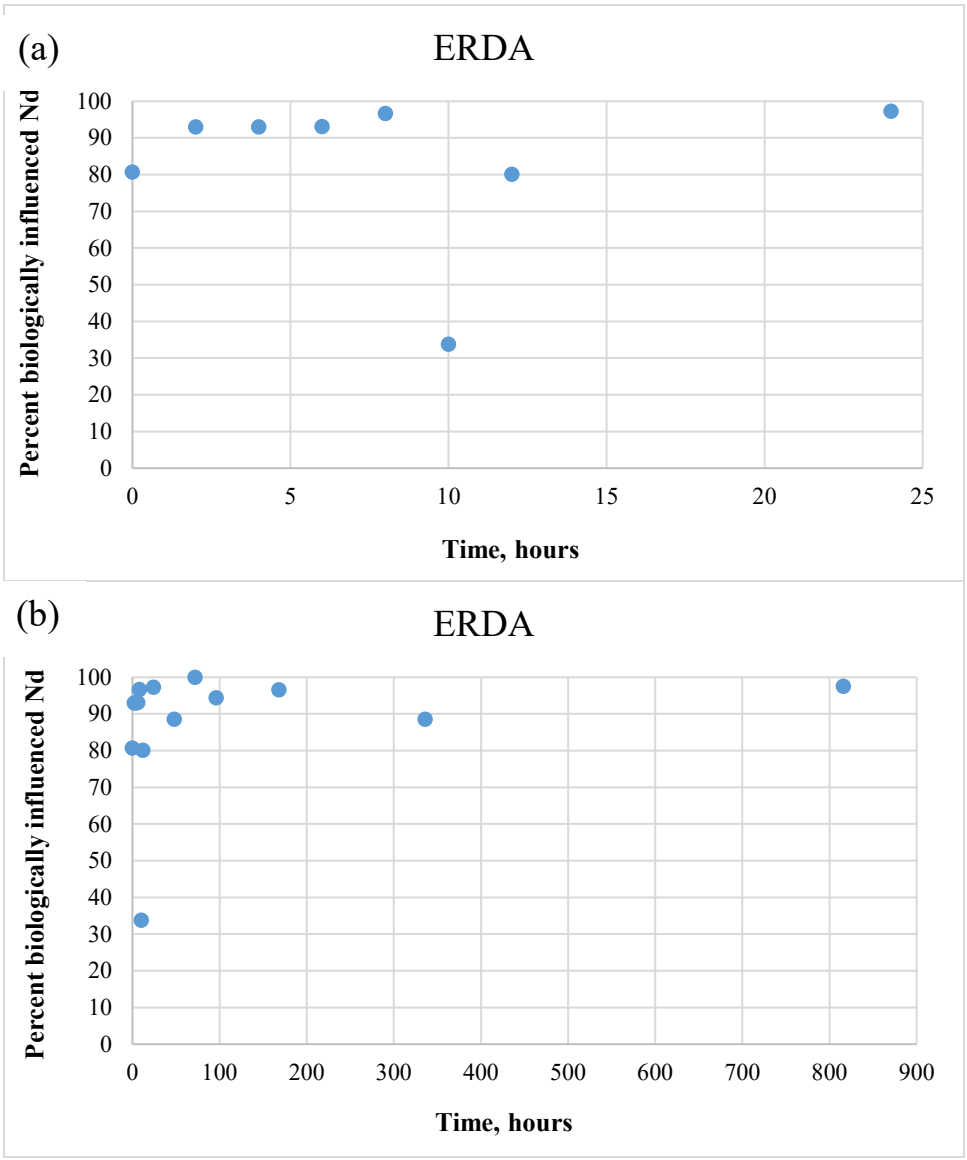


Figure 16. Percent of biologically influenced neodymium in *Chromohalobacter* sp. experiments in ERDA a) during the first 24 hours and b) over ~ 5 weeks.

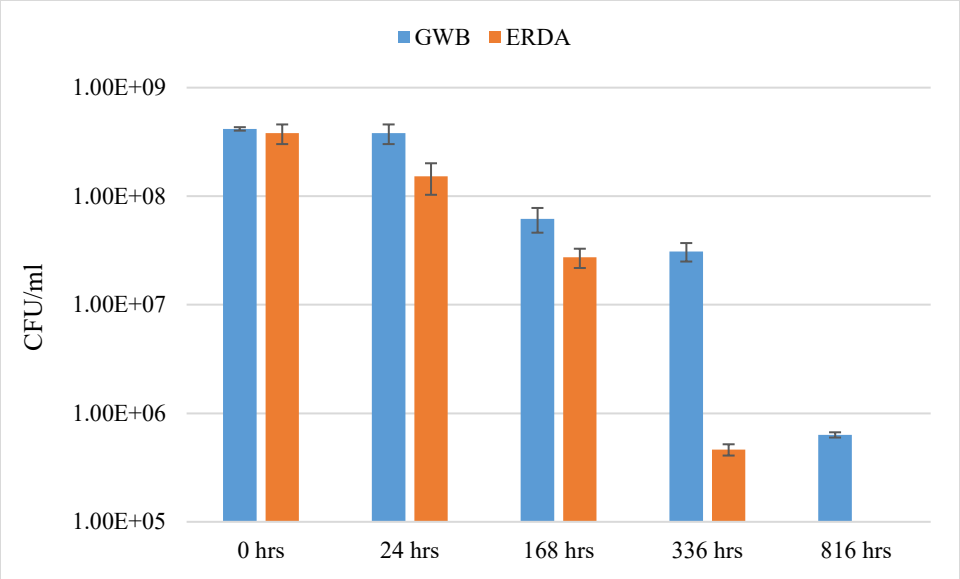


Figure 17. *Chromohalobacter* sp. CFU counts over time in GWB and ERDA experiments.

3.3.2 PB-1a

Results for this isolate were very similar to those of *Chromohalobacter* sp. There was little to no loss of Nd from GWB in either biotic or abiotic samples over time (Figures 18 and 20). In contrast, Nd was lost rapidly from biotic ERDA samples, reaching ~70% apparent sorption by 48 hours, but it did not change for the remainder of the experiment (Figures 19-21). Cell numbers remained constant throughout (Figure 22).

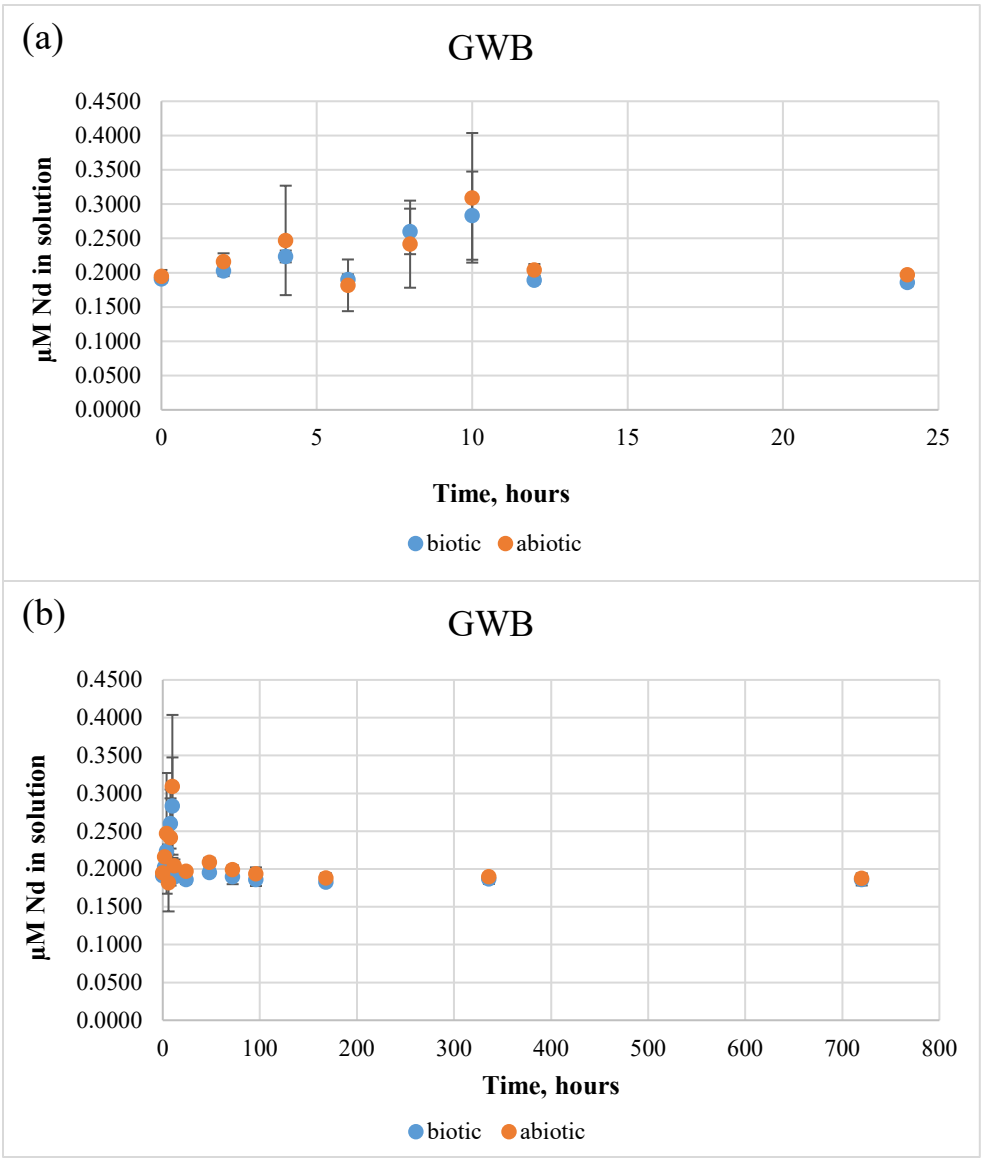


Figure 18. Neodymium concentration in PB-1a biotic samples and abiotic controls in GWB a) during the first 24 hours and b) over one month. Error bars represent the standard deviation of triplicate samples.

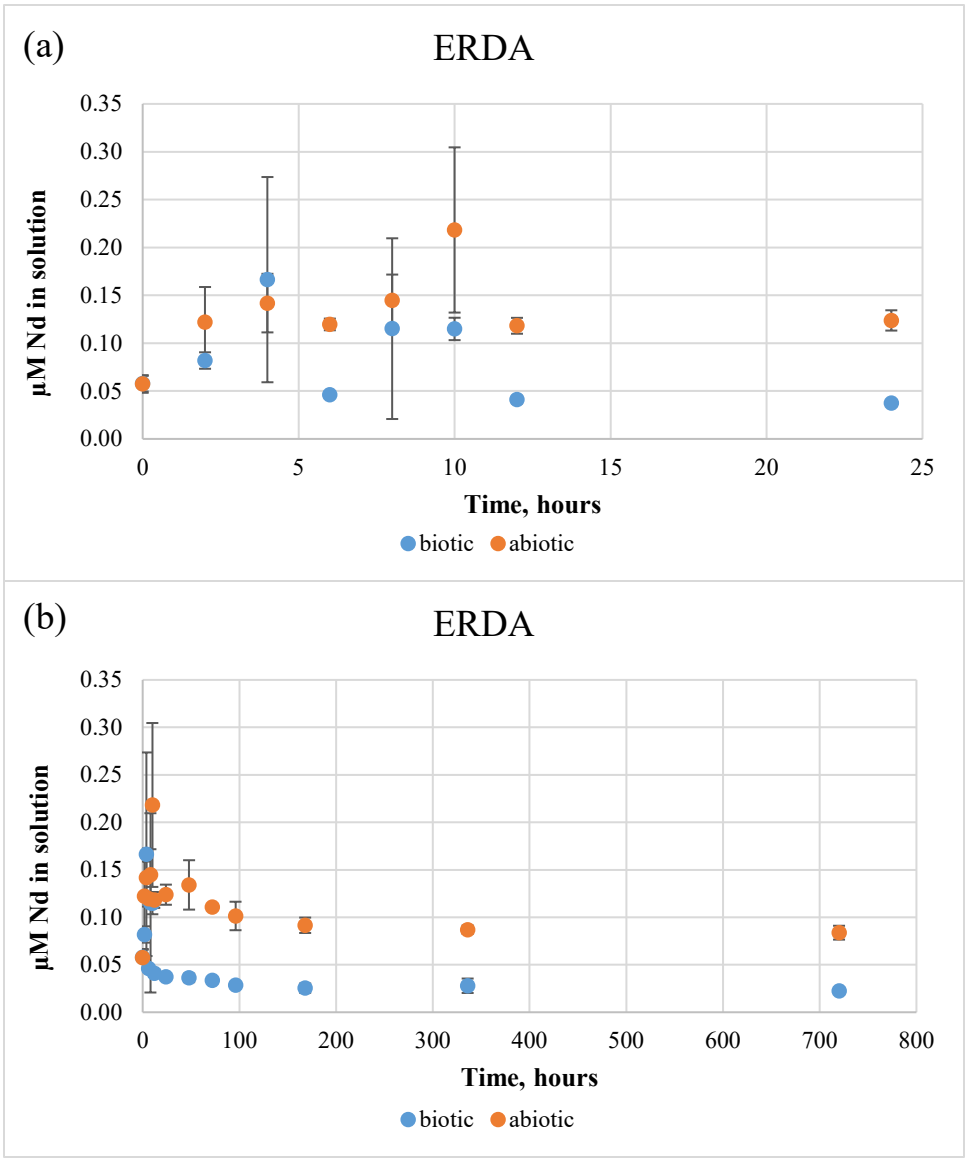


Figure 19. Neodymium concentration in PB-1a biotic samples and abiotic controls in ERDA a) during the first 24 hours and b) over one month. Error bars represent the standard deviation of triplicate samples.

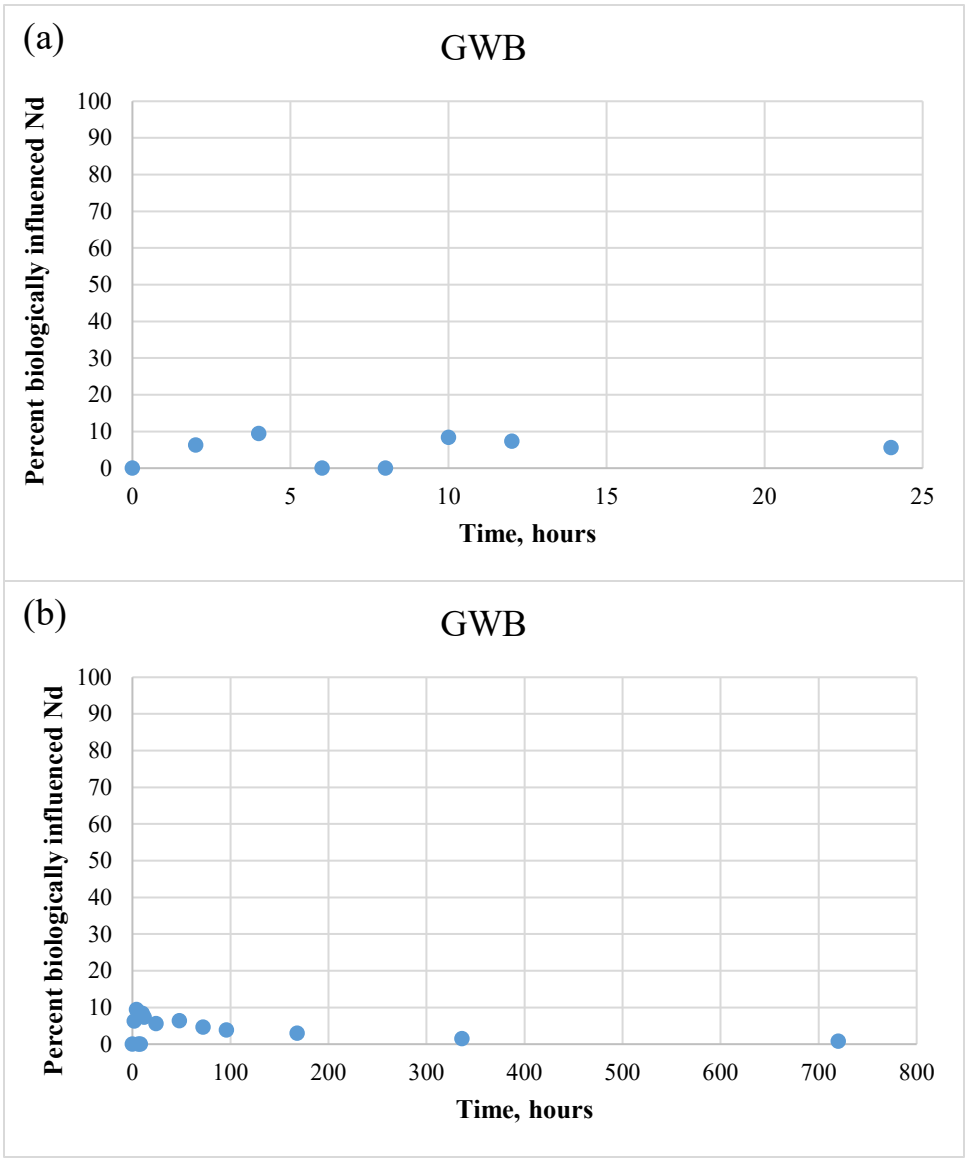


Figure 20. Percent of biologically influenced neodymium in PB-1a experiments in GWB a) during the first 24 hours and b) over one month.

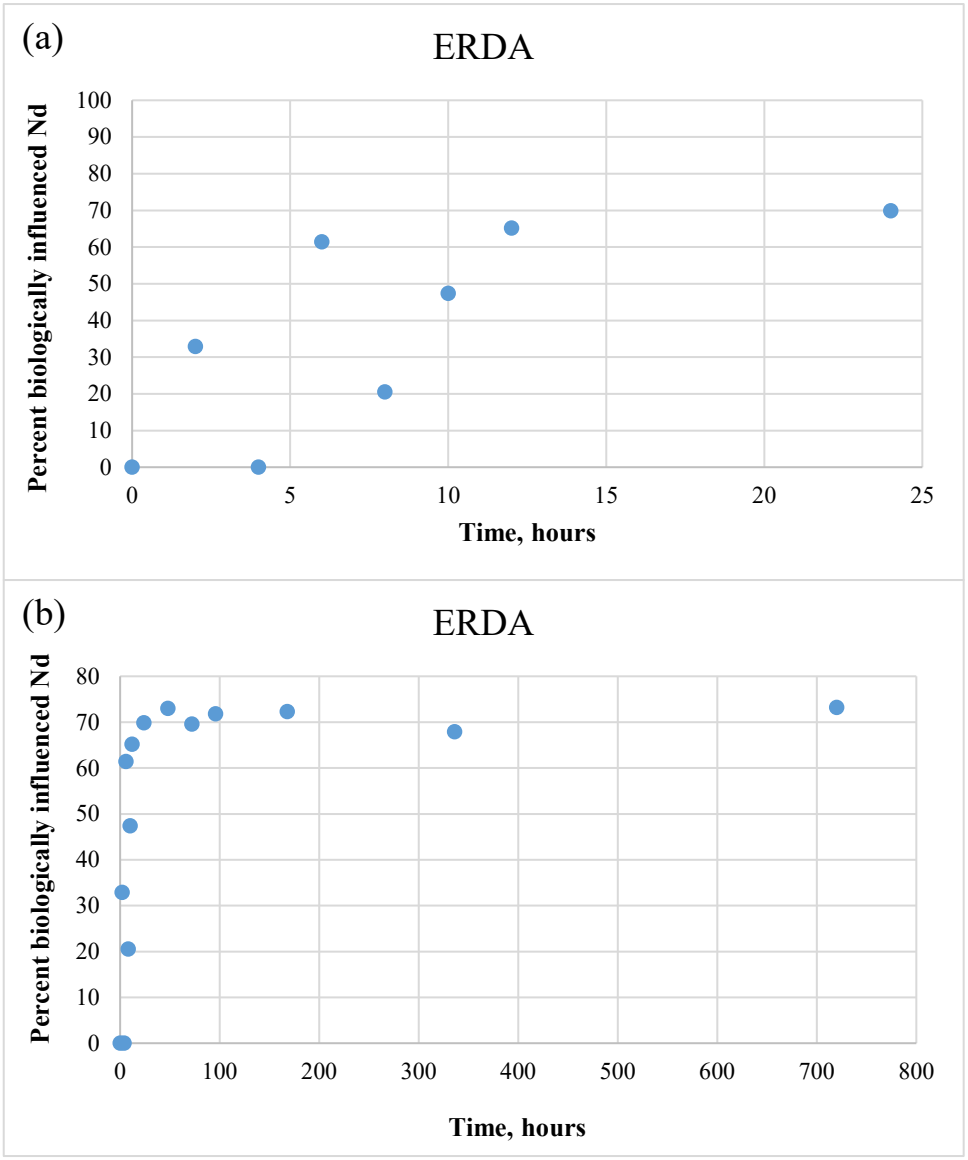


Figure 21. Percent of biologically influenced neodymium in PB-1a experiments in ERDA a) during the first 24 hours and b) over one month.

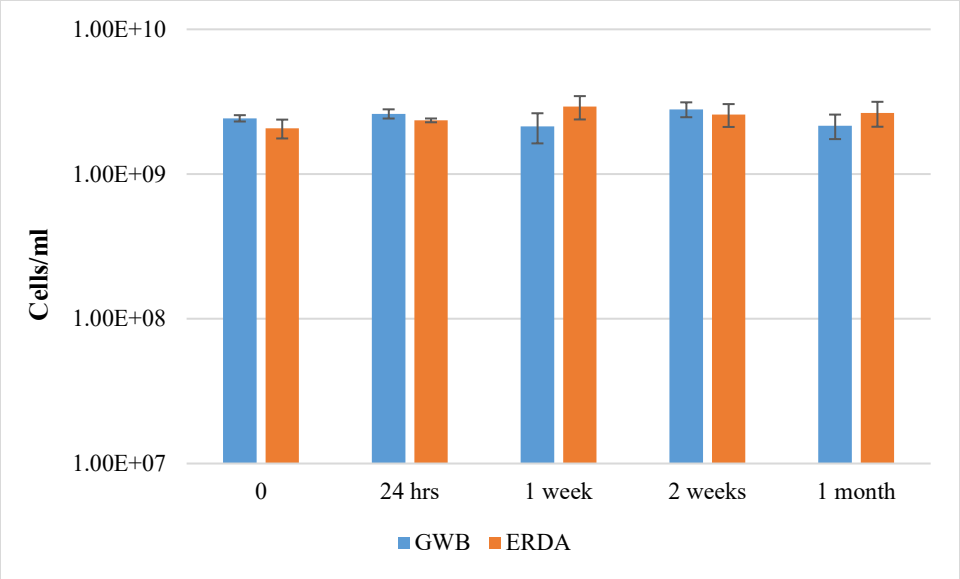


Figure 22. Cell counts of isolate PB-1a over time in WIPP brines with Nd.

3.3.3 *Halobacterium* sp.

Results for *Halobacterium* sp. in WIPP brines are also similar to the other two tested organisms (Swanson, 2019). There was almost no influence of organisms on Nd solubility in GWB (Figures 23 and 25). In ERDA, the percent of influence increased more slowly with time than for isolate PB-1a or *Chromohalobacter* sp. (Figures 24 and 26).

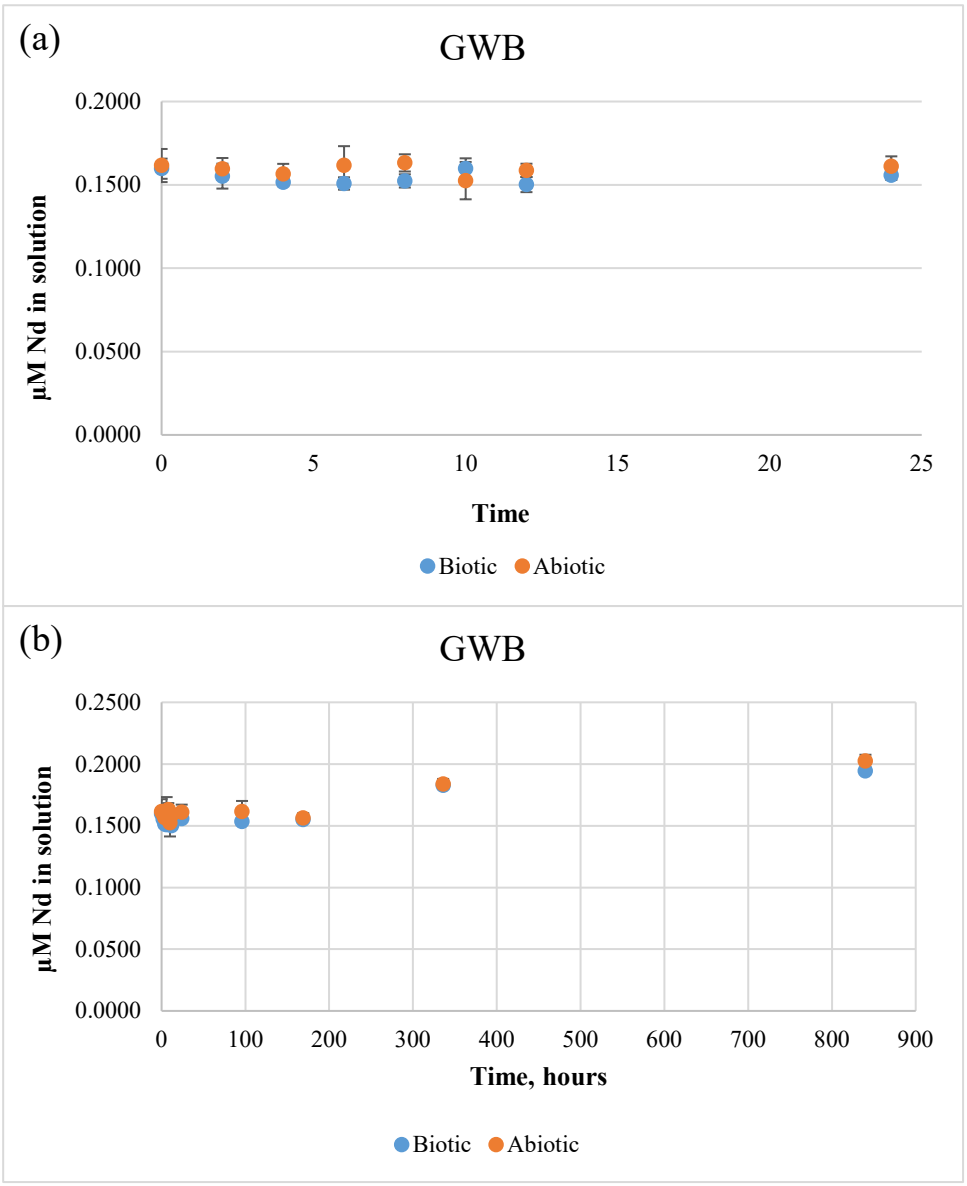


Figure 23. Neodymium concentration in *Halobacterium* sp. biotic samples and abiotic controls in GWB a) during the first 24 hours and b) over one month. Error bars represent the standard deviation of triplicate samples.

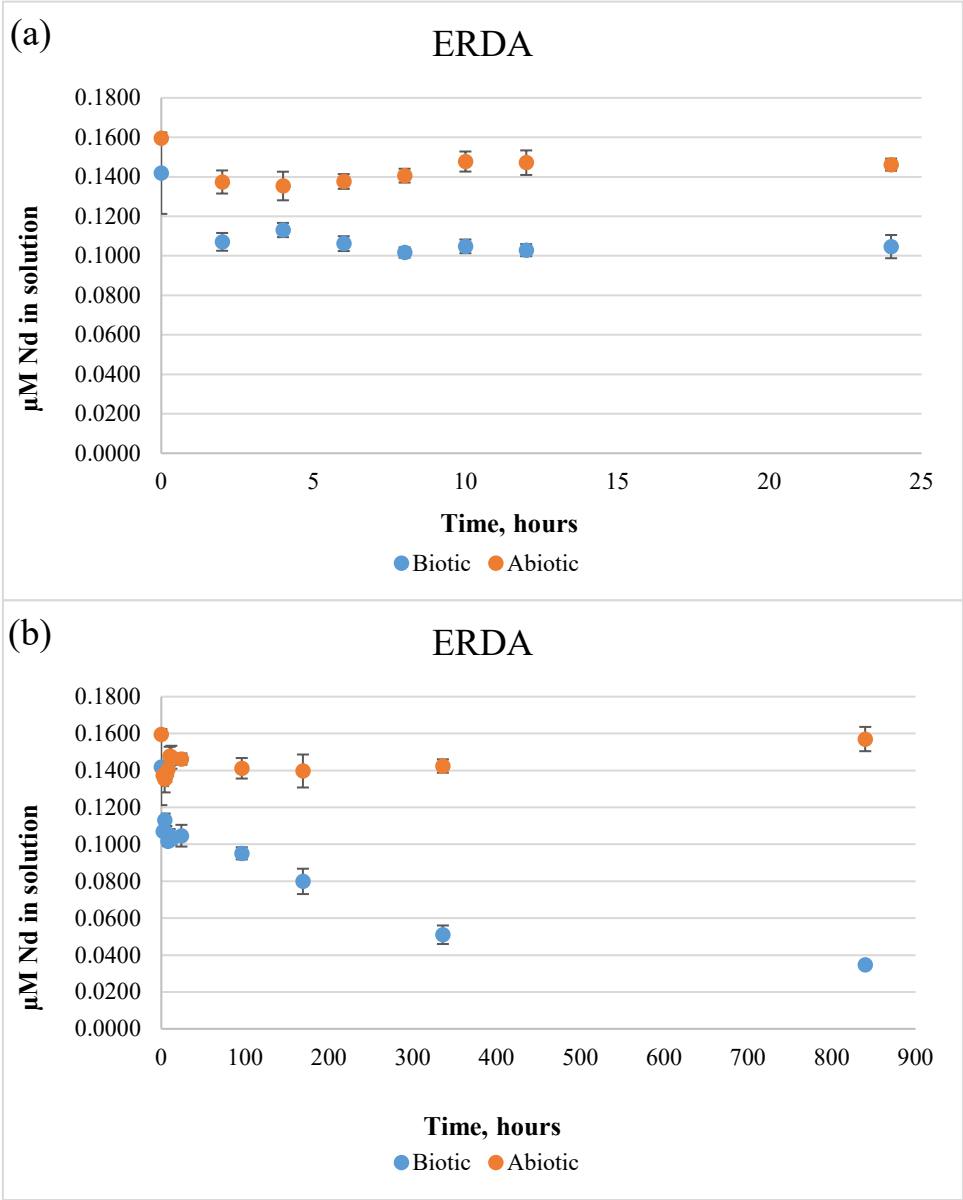


Figure 24. Neodymium concentration in *Halobacterium* sp. biotic samples and abiotic controls in ERDA a) during the first 24 hours and b) over one month. Error bars represent the standard deviation of triplicate samples.

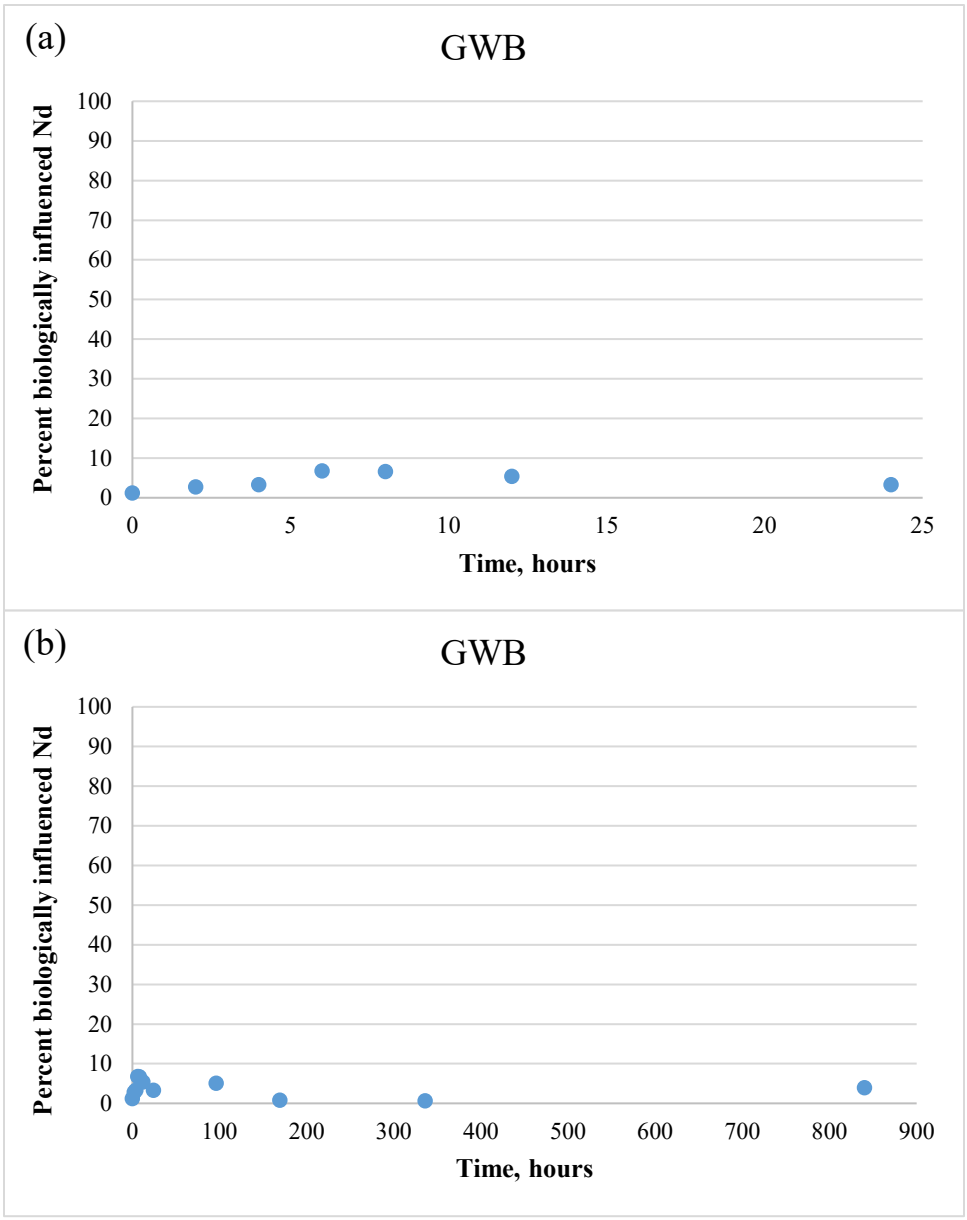


Figure 25. Percent of biologically influenced neodymium in *Halobacterium* sp. experiments in GWB a) during the first 24 hours and b) over ~ 5 weeks.

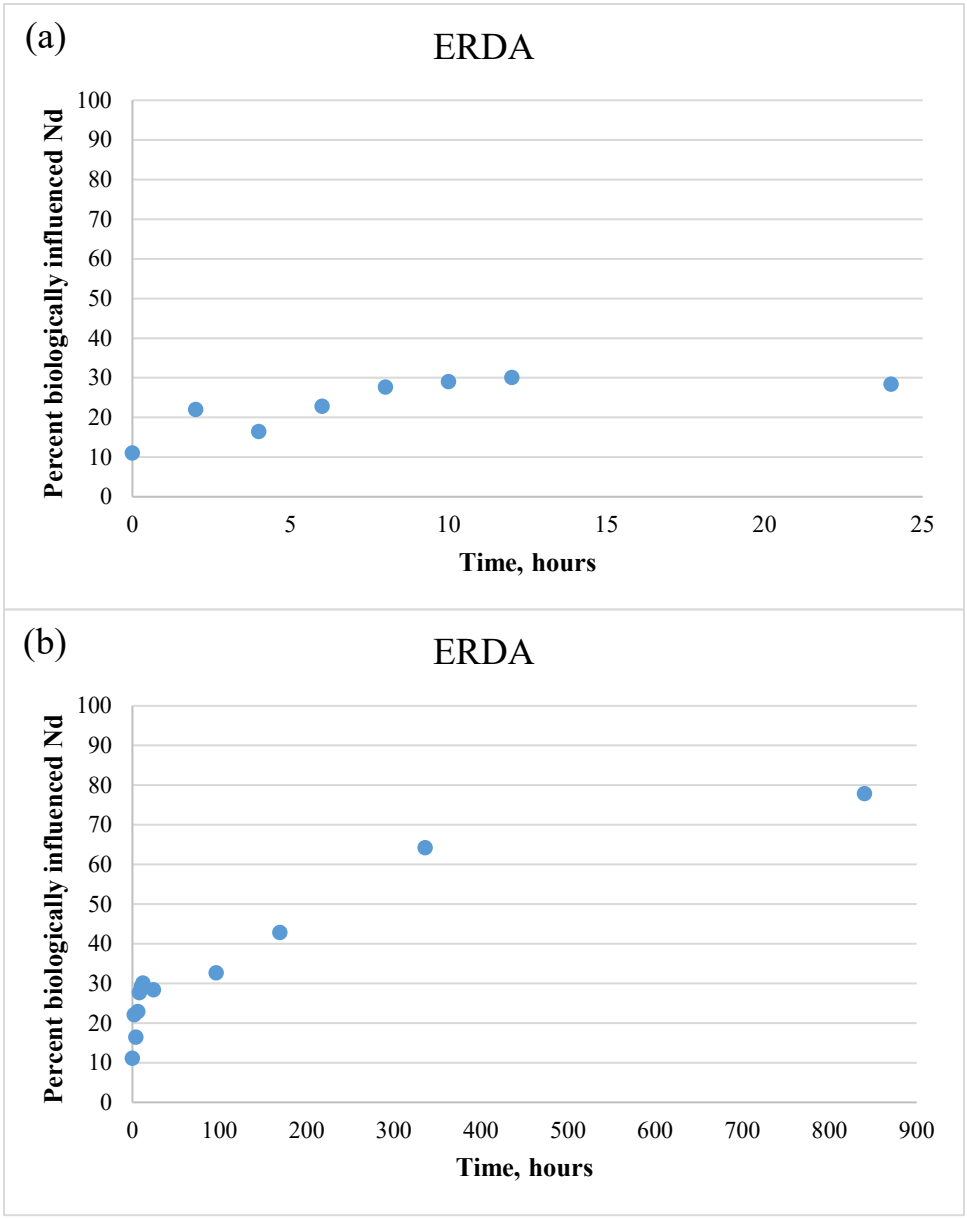


Figure 26. Percent of biologically influenced neodymium in *Halobacterium* sp. experiments in ERDA a) during the first 24 hours and b) over ~ 5 weeks.

4.0 DISCUSSION AND CONCLUSIONS

4.1 Comparison between organisms

Experiments in simplified NaCl were carried out with 7 prokaryotes—2 Archaea and 5 Bacteria (Figure 27). All Archaea were of the Phylum Euryarchaeota, Class Halobacteria. The Bacteria belonged to 3 different phyla: Proteobacteria (1), Actinobacteria (1), and Firmicutes (3). Of the Firmicutes, two were bacilli that were tested in spore form (one from previous experiments). Three organisms are Gram-negative, and four are Gram-positive. Four are pigmented, but their pigments are not the same. EPS production and the presence of a glycoprotein surface layer (S-layer) were not determined experimentally, but studies have found that two are known to produce EPS—*Salinicoccus* sp., *Chromohalobacter* sp.—and three are known to have S-layers—*Halobacterium* sp., isolate PB-1a, and *Bacillus* sp. (Biswas et al., 2014; Sidhu and Olsen, 1997). There does not appear to be any correlation between apparent bioassociation and each individual characteristic, but it is possible that a trend would emerge with a larger sample size (Table 2).

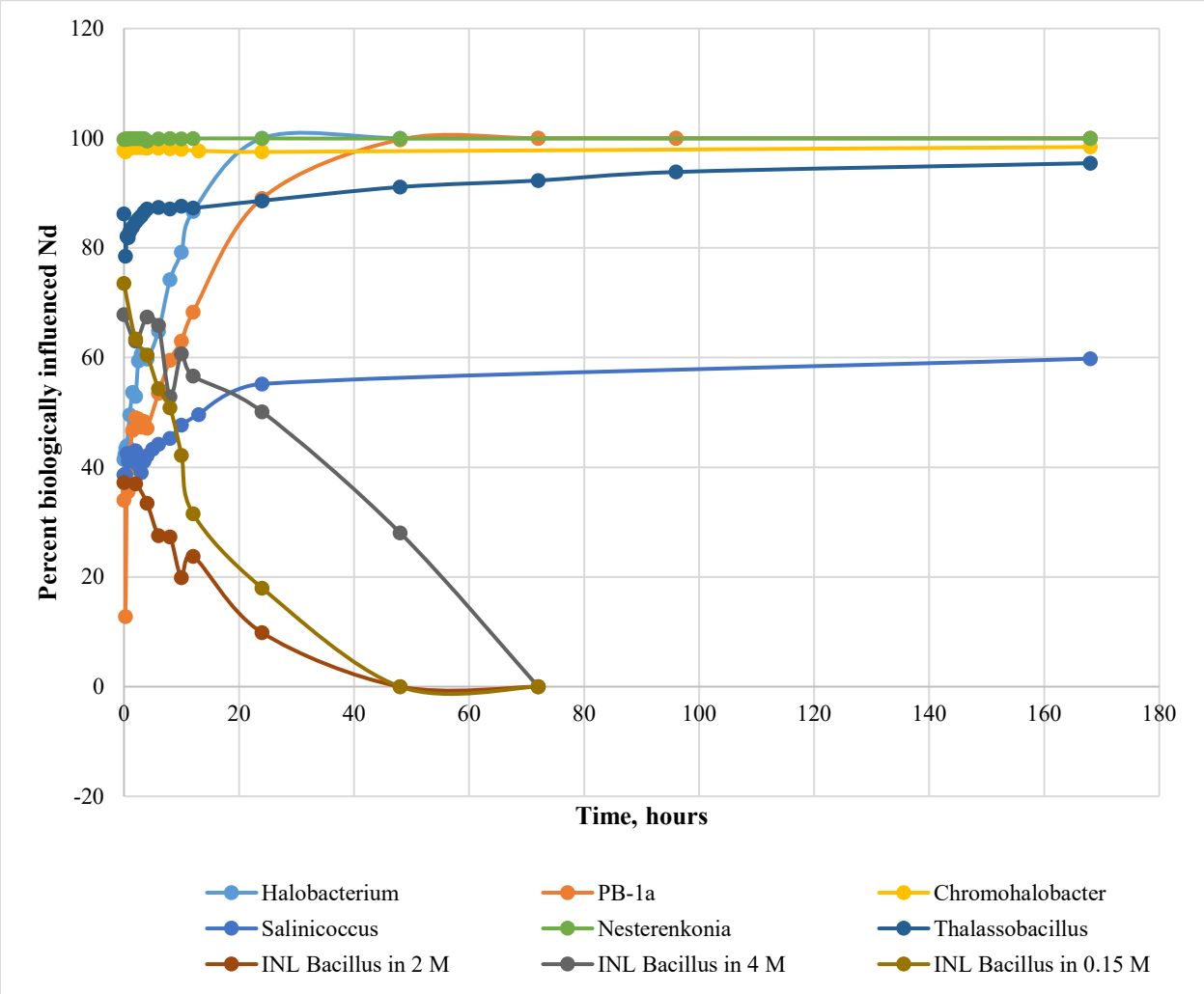


Figure 27. Comparison of all tested organisms in simple NaCl matrix over the course of one week.

The only two findings of note, based on organism type, are for *Salinicoccus* sp. and the DPA-producing spores of *Bacillus* sp.: 1) cell clumping might have reduced the surface area available for sorption in the case of *Salinicoccus* sp. and 2) the release of DPA by *Bacillus* spores led to resolubilization of Nd over time.

Table 2. Test organism characteristics that can affect surface sorption.

Organism	Gram Stain Reaction	S-Layer	EPS	Vegetative vs. Spore	Pigment	Test % NaCl (optimum NaCl)	Percent biological influence	
							t = 0	t = final
Halobacterium sp.	−	+	−	V	+	20	41.4	100
Isolate PB-1a	−	+	−	V	+	25	34.0	100
Chromohalobacter sp.	−	−	+	V	−	15	97.8	99.5
Salinicoccus sp.	+	−	+	V	+	10	38.6	97.2
Nesterenkonia sp.	+	−	−	V	+	1.95	99.8	100
Thalassobacillus sp.	+	−	−	S	−	4.45	86.2	97.6
Waste *Bacillus* sp.	+	+	−	S	−	0.85	98.4	92.7

4.2 Differences in biomass concentration

Not all organisms were tested for biomass dependence. For those that were (*Halobacterium* sp. and waste *Bacillus* sp.), a significant increase in influence was observed at higher biomass concentrations. This agrees with the literature and is logical based on the increased number of available surface sites with increasing biomass.

4.3 Differences between NaCl concentrations

A weak negative correlation can be seen between the initial percentage of biological influence and the test matrix NaCl concentration (Figure 28; note that background NaCl concentrations are based on each organism's optimum for growth). This type of correlation was also observed with *Chromohalobacter* sp. in neptunium-perchlorate solutions (Ams et al., 2013). However, this correlation disappears completely over time, suggesting that in the long term, Nd disposition is controlled by a mechanism other than surface complexation.

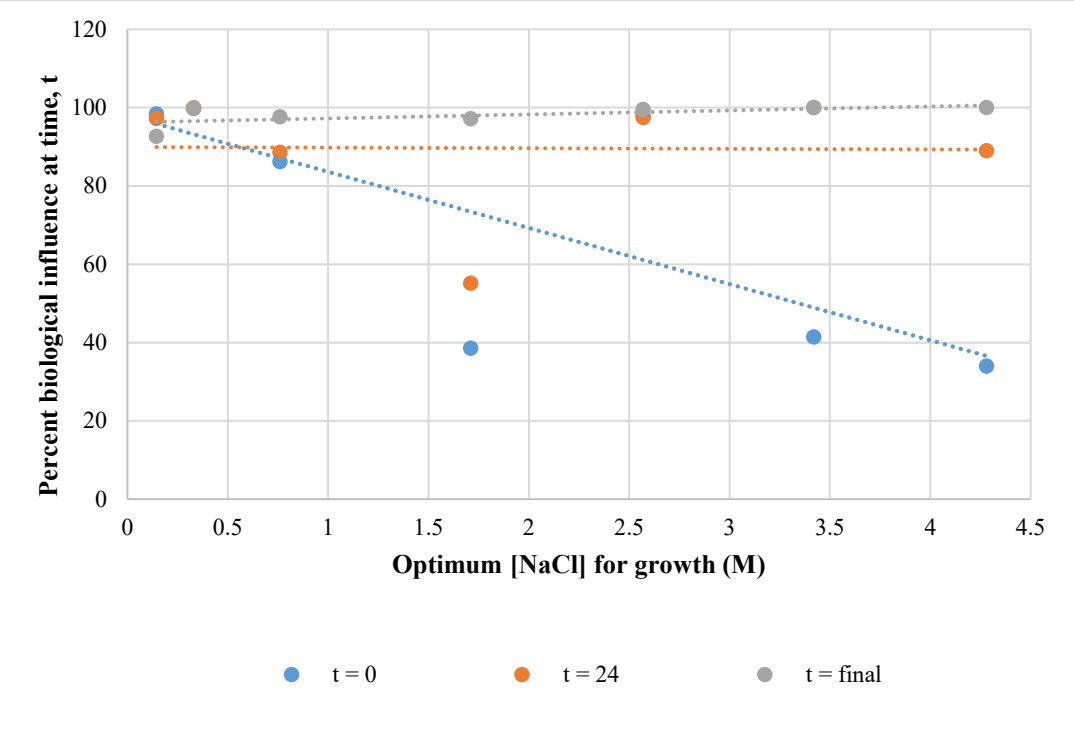


Figure 28. Correlation between the percent of biological influence and test [NaCl] at three time points. Each point within a series represents a different test organism.

4.4 Differences between simple NaCl and WIPP brines

Thus far, three of the seven isolates have been tested in WIPP brines—*Halobacterium* sp., isolate PB-1a, and *Chromohalobacter* sp. Findings were similar for all three organisms. There was little to no influence of the organism on Nd concentration in GWB, but Nd was lost from solution in ERDA, especially when organisms were present (Figure 29).

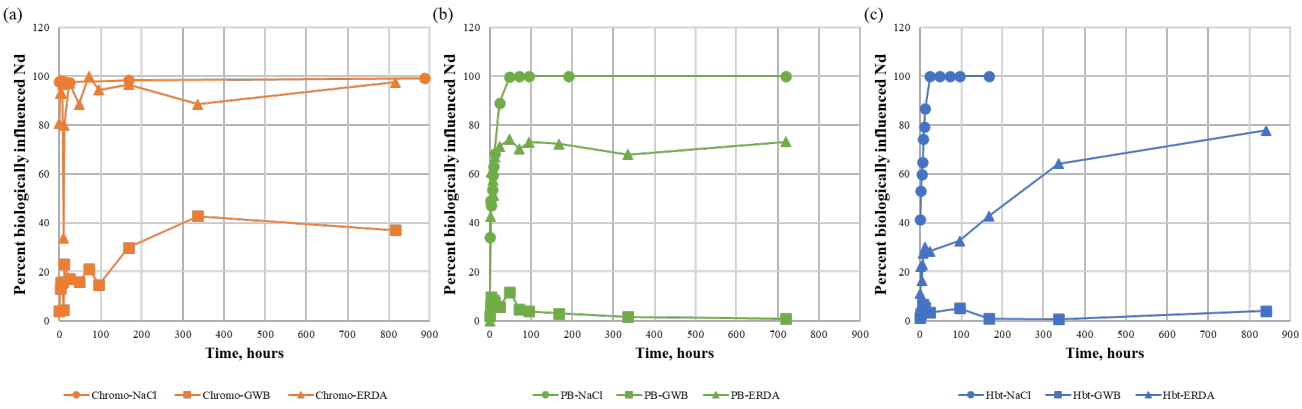


Figure 29. Comparison of the three tested isolates—a) *Chromohalobacter* sp., b) isolate PB-1a, and c) *Halobacterium* sp.—in all three matrices (simple NaCl, GWB, ERDA).

Because of the complex nature of the brine-microorganism suspensions, there is more than one possible explanation for our observations, and it is even more likely that a combination of these explanations provides the answer.

4.4.1 Experimental starting neodymium concentrations exceeded solubility

The test concentrations of Nd (0.2 μM) were chosen based on the solubility of Nd in each brine at its appropriate pC_{H+} value and on the detection limit for ICP-MS after the necessary sample dilution. Neodymium solubility is slightly lower in ERDA than in GWB (1.7×10^{-7} M versus 3×10^{-7}; Borkowski, 2009), and is also slightly less than the added target. This might explain the continued loss of Nd in abiotic solutions (for PB-1a and *Chromohalobacter* sp. experiments), except that Nd loss did not occur in the *Halobacterium* sp. abiotic controls in ERDA. Nevertheless, this loss does not affect bioassociation calculations.

4.4.2 Neodymium speciation

Experiments in simple NaCl solutions were conducted at pH ~ 6 to minimize the effects of hydrolysis and carbonate. In those experiments, the majority of Nd was present as Nd^{3+} and $NdCl^{2+}$ (M. Richmann, EQ3/6 calculations; Migdisov and Williams-Jones, 2002). Both charged species could be involved in surface complexation. In experiments in WIPP brines, the pH was not adjusted. At brine pC_{H+} values and in the absence of carbonate, Nd is expected to exist as the hydrolysis species $Nd(OH)_2^+$, and to a lesser extent $Nd(OH)^{2+}$ and $Nd(OH)_3(aq)$ (Borkowski, 2009; Neck et al., 2009). These species are also charged, suggesting that surface complexation is possible. However, this does not explain the differences in apparent sorption between ERDA and GWB.

4.4.3 Loss of S-layers

The S-layer helps to maintain cell structure in haloarchaea. Magnesium stabilizes S-layers, and changes in Mg^{2+} concentration can lead to S-layer loss and subsequent changes in cell morphology (Horikoshi et al., 1993). The requirement for Mg^{2+} varies between organisms, but a *Haloarcula* species was found to change morphology below 41 mM. The test organisms for these experiments are usually grown in broth containing 98 mM of Mg^{2+}. Ninety-percent ERDA contains 17 mM Mg^{2+}, and 90% GWB contains 900 mM, suggesting that S-layer loss was possible in ERDA but unlikely in GWB.

EDTA concentrations as low as 10 mM can also lead to S-layer shedding due to chelation of S-layer Mg^{2+} ions (Cohen et al., 1991; Dyall-Smith, 2009). While WIPP inventory does not contain this much EDTA, it is unknown what effect a lower concentration could have.

In these experiments, loss of the S-layer should have led to a decrease in surface sites, and a change in cell morphology to small coccoid cells would have resulted in less surface area overall, ultimately in both cases resulting in less apparent sorption. Therefore, this is unlikely to be the sole reason for the observed findings for the haloarchaea.

4.4.4 Borate influence

Borate can complex with Nd and increase its solubility slightly under certain conditions, such as at higher ionic strength and in the presence of high concentrations of Mg^{2+} (Borkowski, 2009; Hinz et al., 2015). However, under these experimental conditions, borate complexation should not outcompete hydrolysis or even complexation with cell surface functional groups (e.g., carboxylate and phosphate).

4.4.5 Magnesium concentration

High concentrations of Mg will outcompete Nd for the limited number of available cell surface sites. Preliminary investigations with *Halobacterium* sp. found that bioassociation decreased with increasing Mg^{2+}, but there were too many other variables in the study to draw a definite conclusion (Swanson, 2018).

4.4.6 Biologically induced formation of Nd-containing precipitates

Biologically induced mineral precipitation is a well-known phenomenon, and many reviews on the mechanisms of this process exist in the literature (e.g., Frankel and Bazylinski, 2003; Hoffmann et al., 2021). Microbial cell surfaces offer numerous potential nucleation sites, while microbial activity (even if minimal) can often alter solution characteristics, such as pH, localized ion concentrations, and organic releases that can promote mineralization. In some cases, cell surface characteristics (e.g., EPS) enhance these conditions. Furthermore, in high ionic strength matrices, it can be easier to reach the saturation index for some ions. Biologically-induced precipitation can result in loss of Nd from solution but would appear as apparent sorption in these experiments.

Some haloarchaea have been found to have a high density of carboxyl sites at their surface that catalyze dolomite precipitation under ideal Mg:Ca ratios, even under non-growth conditions (Kenward et al., 2013). Members of the *Chromohalobacter* and *Nesterenkonia* genera can precipitate different carbonate species, depending upon growth conditions (Rivadeneyra et al., 2000, 2006), and some can incorporate strontium into the mineral phase (Sánchez-Román et al., 2011), providing a possible radionuclide sink. Bader et al. (2018) found that resting cells of *Halobacterium noricense* DSM 15987 sequestered europium and curium through the release of phosphate species, one of which was a solid phase precipitate.

For some organisms, the S-layer also serves to protect cells from environmental toxicants, such as heavy metals (Chandramohan et al., 2019). If mineralization occurs on an S-layer, it can be shed and then precipitates (Schultze-Lam et al., 1996).

4.5 Impacts on colloid enhancement parameters for CRA

These recent experiments have shown that it might be easier to account for and predict long-term biological influence based on test matrix than on organism. This is quite beneficial to PA, because it suggests that chemistry, more than biology, is the controlling factor for biocolloid measurements in the long-term. This work will continue to focus on determining the mechanisms that best explain these new data (e.g., magnesium effects, biomineralization).

However, a full mechanistic understanding is not necessary for generating parameters, and the data presented in this report can be used to update PROPMIC and CAPMIC values for the upcoming CRA.

The impact of these new data on PROPMIC and CAPMIC calculations for the CRA will be discussed in an upcoming parameter update report. In summary, values for the +3 oxidation state will increase slightly from the previous CRA, as a result of the findings described herein.

5.0 QUALITY ASSURANCE, DATA TRACEABILITY AND DOCUMENTATION

All of the data presented in this report were generated as Quality Level 1 data, in accordance with the WIPP Quality Assurance Program Document. Experiments were performed under the test plan, "Microbial Interactions in the Waste Isolation Pilot Plant" (LCO-ACP-12). Descriptions of the experiments can be found in the scientific notebooks, "Sorption of An(III) and An(IV) Actinides or Analogues to Halophilic Microorganisms under WIPP-Relevant Conditions" (TIP-012-3/4 and 3/5). Preliminary supporting information can be found in the developmental scientific notebooks "Microbiology for the WIPP" (BIO 1 and 2). The following data packages were generated from these experiments:

- Neodymium-*Chromohalobacter* sp. Association Kinetics Experiment (ACP-2211-07-09-01)
- Neodymium-PB-1a sp. Association Kinetics Experiment (ACP-2209-02-09-02)
- Neodymium-*Salinicoccus* sp. Association Kinetics Experiment (ACP-2209-02-09-01)
- Neodymium-*Nesterenkonia* sp. Association Kinetics Experiment (ACP-2211-04-09-01)
- Neodymium-*Thalassobacillus* sp. Spores Association Kinetics Experiment (ACP-2211-07-09-10)
- Neodymium-*Chromohalobacter* sp. Association Kinetics Experiments in WIPP Brines (ACP-2211-07-09-07)
- Neodymium-PB-1a sp. Association Kinetics Experiments in WIPP Brines (ACP-2211-07-09-04)

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