Abstract

Three spore-forming bacilli were isolated from a radioactive waste drum bound for the Waste Isolation Pilot Plant (WIPP). These organisms were most likely in spore form in situ, induced by the stresses of desiccation and possible radionuclide toxicity. Many microorganisms have been tested for their potential influence on radionuclide migration via biosorption or other uptake processes. In this study, the potential for one of the three spore-forming waste-indigenous organisms to adsorb the radionuclide analog neodymium—an analog for the 3 oxidation state—was determined. Analysis showed moderate-low sorption under high salt conditions onto the spore, followed by progressive and eventually complete desorption over time, suggesting that bacteria in spore form may play a significant role in radionuclide transport.

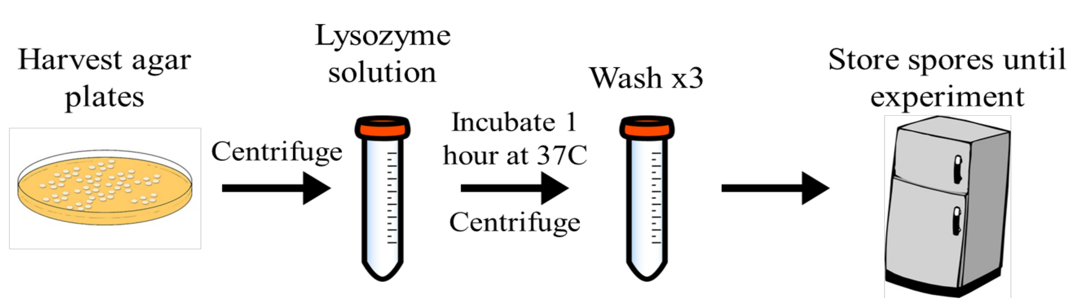
Introduction

The Waste Isolation Pilot Plant (WIPP) is the only geologic repository for permanent disposal of transuranic (TRU) waste generated as a byproduct of the nation's nuclear defense program. Culture results of microorganisms isolated from a radioactive waste drum bound for the WIPP, showed the presence of the phylum *Firmicutes*, which includes *Bacillus* species [1]. Bacilli form spores that are difficult to lyse, and thrive even in extreme conditions such as desiccation, UV irradiation, and high salt. These microorganisms were prevalent in cultures of soil samples affected by the Chernobyl incident, which exhibited increased UV and radiation resistance [2]. Unfortunately, there are no literature references available regarding *Bacillus* sp. spores and their ability to biosorb radionuclides. However, these spores have been shown to bind several heavy metals, including copper, cadmium, zinc, and nickel, in which the soluble ions bind to the negative sites on the spore surface [3].

Methods & Materials

Spore Purification

Organism: *Bacillus* species putatively identified as *megaterium* by 16S ribosomal DNA sequencing



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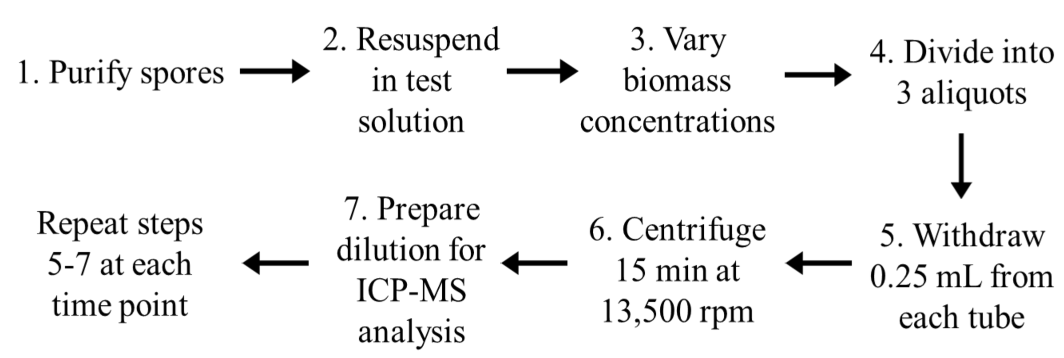
Adsorption Kinetics Experiment



Experimental conditions: pH at 6 (± 0.2); [Nd] of 1 μM

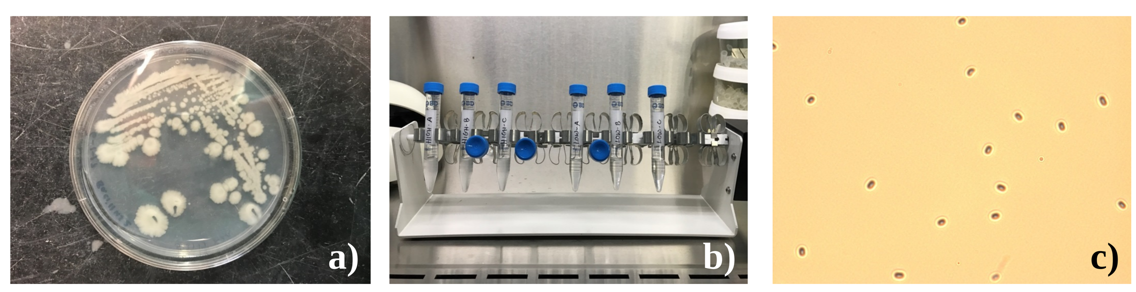


Figure 1: a) Cultured *Bacillus megaterium* on agar plate; **b)** Batches on rotator during adsorption kinetics experiments; **c)** *Bacillus megaterium* spores after completion of the spore purification procedure, showing the absence of vegetative cells

Results

Adsorption Kinetics Experiments

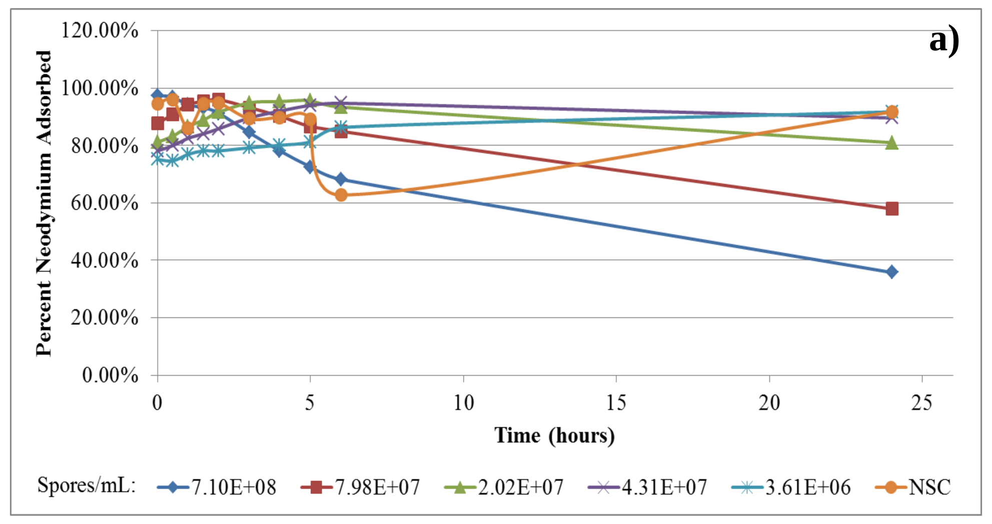


Figure 2: a) Trial 1 included a range of biomasses determined, measured over a period of 72 hours; spores were treated with lysozyme and SDS detergent and washed with high purity water; abiotic control adsorbed onto the tube wall, which resulted in method adjustment: batch solvent was switched from HPW to NaCl

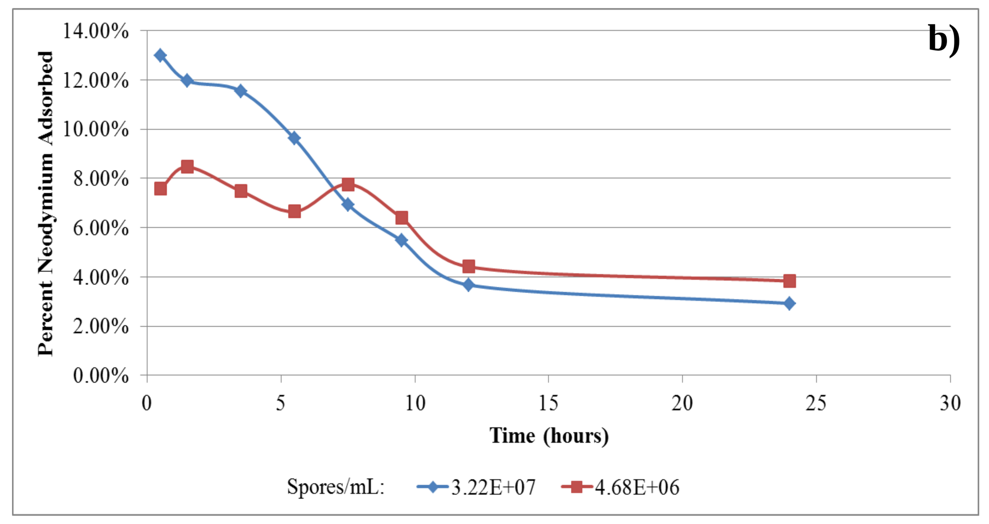


Figure 2: b) Trial 2 included two varying biomasses, measured over a period of 24 hours, with sampling every two hours; spores prior to experiment were treated with lysozyme and SDS detergent, and washed in a 0.145 M NaCl solution; spores were resuspended in a 0.145 M NaCl-Nd solution; SDS solution was suspected to greatly reduce sorption of radionuclide which resulted in method adjustment

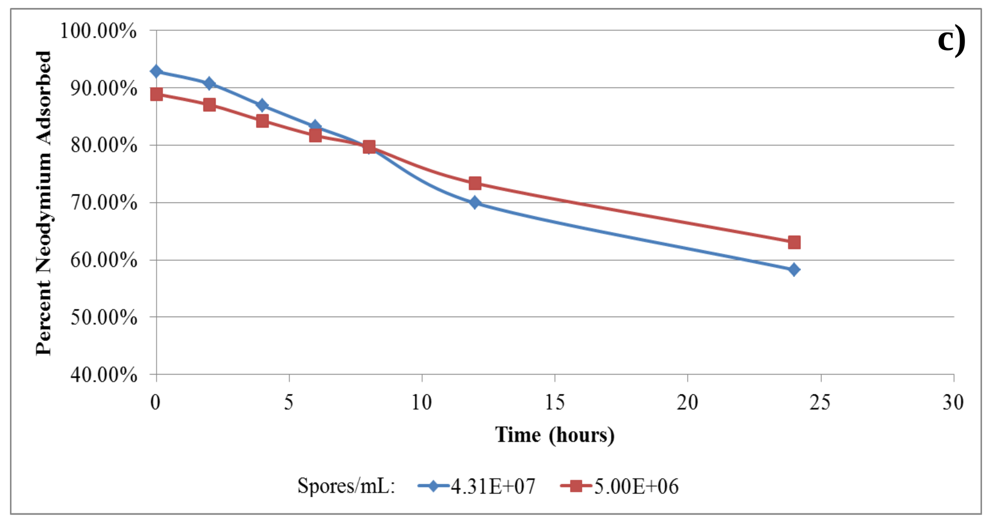


Figure 2: c) Trial 3 included two varying biomasses, measured over a period of 24 hours, with sampling initially every two hours; spores prior to experiment were treated with a lysozyme solution and washed with 0.145 M NaCl; spores were resuspended in a 0.145 M NaCl-Nd solution; results show high initial sorption followed by progressive desorption over time, with complete desorption after 1 week

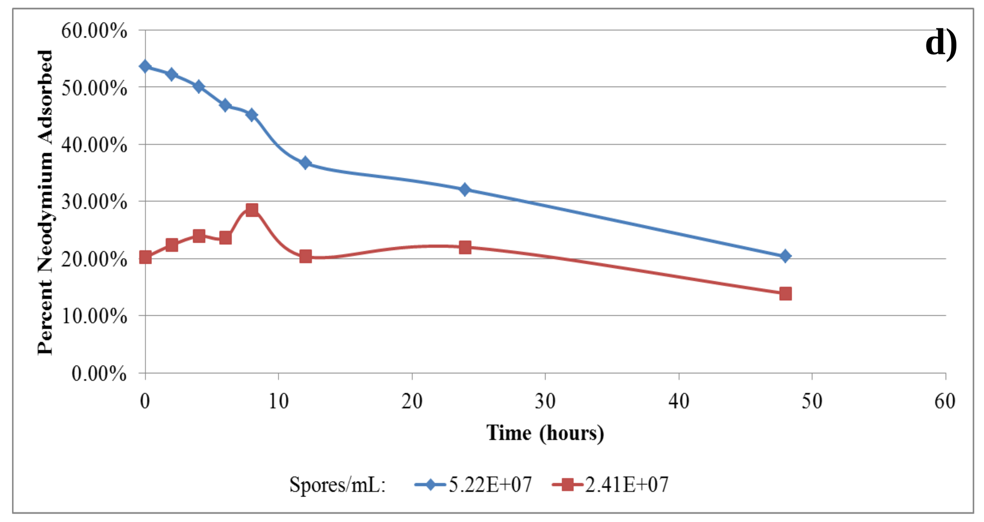


Figure 2: d) Trial 4 included two varying biomasses, measured over a period of 122 hours, with sampling initially every two hours; spores prior to experiment were treated with a lysozyme solution and washed with 0.2 M NaCl; spores were resuspended in a 2 M NaCl-Nd solution; results show moderate-low initial sorption followed by complete desorption after 72 hours

Monitoring Optical Density, Spore Counts, and pH

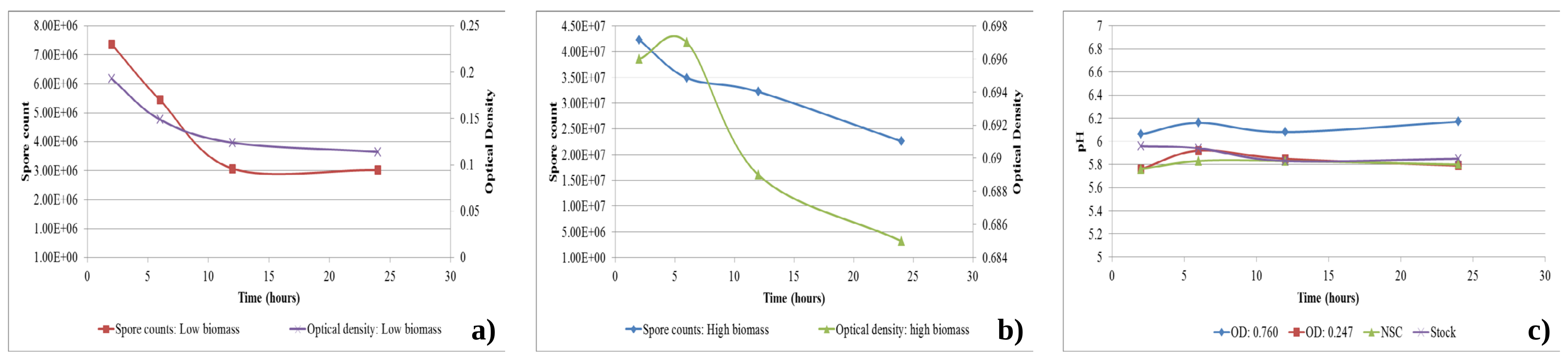


Figure 3: Spore counts and optical density were monitored throughout the course of trials 3 and 4 for the **a)** low biomass samples and the **b)** high biomass samples; above is trial 3; decreasing spore numbers and OD values were seen during both trials, however, an insignificant amount was observed in order to explain the desorption phenomenon; **c)** pH was monitored throughout the course of the third experiment in order to explain any potential desorption observed; overall, pH remained constant; these measurements were taken for trials 3, and 4; above is trial 3, this trend was observed for trial 4 as well, although pH was slightly lower (5.4-5.8)

Species Accounting for 99% or More of Aqueous Nd

Species	Condition A	Condition B
Nd^{3+}	95.78%	96.77%
$NdCl^{2+}$	2.88%	2.53%
$Nd(OH)^{2+}$	0.86%	-----
Subtotal	99.52%	99.30%

Table 1: a) pH 6.0 ± 0.2; 0.15 M NaCl; **b)** pH 5.6 ± 0.2; 2 M NaCl; These data were derived from the YPF revision 2 (2006) database using the EQ3/6 modeling package, equilibrated to atmospheric CO_2/O_2

Ongoing Viability Study

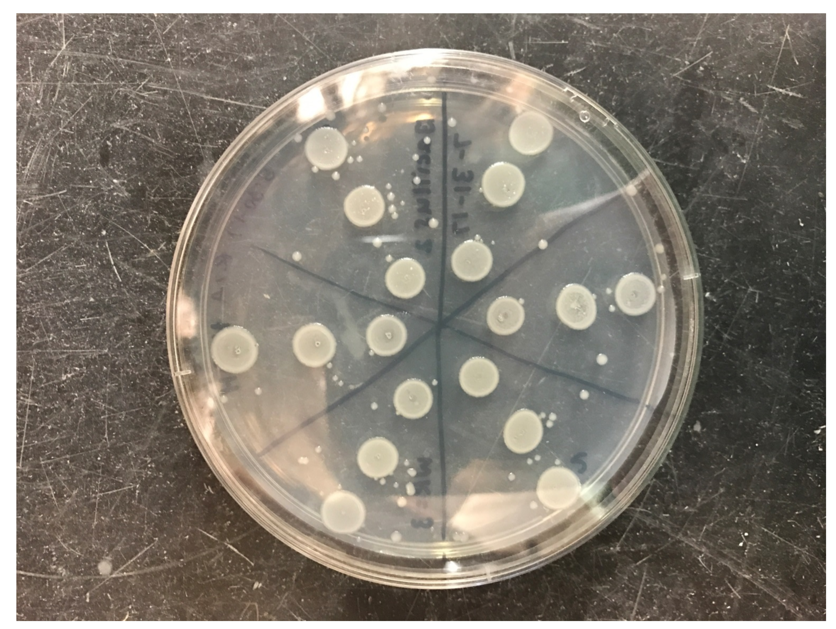


Figure 5: Spore viability in varying salt concentrations is being tested; spores immersed in up to 5 M NaCl solution still show signs of growth after 3 weeks

Discussion and Future Work

After numerous rounds of method validation, experimentation showed that *Bacillus megaterium* spores are capable of high sorption of the radionuclide analog, neodymium under low salt conditions, and moderate-low sorption under high salt conditions. However, initial sorption was consistently followed by progressive desorption over time, with complete desorption after 72 hours under high salt conditions.

A speciation calculation was conducted, showing that over 95% of the neodymium in solution, in both experimental conditions, was in the form of Nd^{3+}. This indicates that precipitation of Nd is not the cause of the observed sorption. Low sorption in the negative control proves this as well, since precipitation would have appeared as apparent sorption even if no spores were present. Prior to the second experiment, the spores were treated with an SDS detergent solution during the spore purification procedure. This was suspected to disrupt the spore's S layer [6], potentially reducing the amount of sorption observed. Initially, residual SDS was thought to be responsible for the desorption phenomenon observed; however, since desorption was observed when SDS was not present during purification, it can be concluded that residual SDS is not responsible for desorption.

It was speculated that under low salt conditions, germination was the cause of the desorption observed. The presence of chloride ions has been found to cause a spore to germinate [3], which may have caused the metal to desorb. However, even under high salt conditions, which are unfavorable for germination, desorption was still observed over time. Initial sorption was greatly reduced when compared to the sorption observed under low salt conditions—54% initial sorption in high salt as opposed to 93% initial sorption in low salt—which may have been due to spore clumping or uneven spore distribution at high ionic strength conditions. This still does not explain the observed desorption over time.

These data show that under WIPP-relevant conditions, the spores are only capable of adsorbing moderate to low amounts of Nd, depending on the amount of biomass present. It also shows that initial sorption is almost immediately followed by progressive desorption over time, with complete desorption after approximately 72 hours. This indicates that the spores most likely would not pose a threat in a breaching situation, since complete desorption occurs so rapidly.

Experimentation is currently underway testing a spore's long term viability in varying salt concentrations. The spores still show signs of growth after three weeks, indicating that they are substantially resistant to high salt conditions; however, radionuclide sorption and migration still seems unlikely. Future work will include experimentation to determine the cause of the desorption over time. It is hypothesized that the spores are exuding an organic complexant into solution due to potential germination, which will be the focus of future exploration.

References

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