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Microbial Influence on Radionuclide Transport through the Culebra

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History of Revisions

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ACRONYMS AND ABBREVIATIONS

DNA	deoxyribonucleic acid
Eh	measurement of redox potential
g	gram
g/L	grams per liter
IC	ion chromatography
ICP-MS	inductively-coupled plasma mass spectrometry
kD	kilo-Dalton, unit of macromolecule size
L	liter
M	molar; moles/liter
ml	milliliter
mM	millimolar
nm	nanometer
PA	performance assessment
pH	negative log of the hydrogen ion activity
SNL-10	Sandia National Laboratories, well 10
TEA	terminal electron acceptor
U	uranium
UO_2	uraninite
U(IV)	uranium in the +4 oxidation state
U(VI)	uranium in the +6 oxidation state
UV-Vis	ultra-violet visible (wavelength of light detected by spectrophotometry)
WIPP	Waste Isolation Pilot Plant
WQSP-6	Water Quality Sampling Program, well 6
XRD	X-ray diffraction

EXECUTIVE SUMMARY

The potential release of radionuclides into the Culebra dolomite formation is modeled extensively in the Waste Isolation Pilot Plant's Performance Assessment (WIPP PA) calculations. Uranium is of chief concern in these modeled scenarios, as it shows the greatest release. Because of this, a series of experiments was undertaken to determine if microbial activity in the Culebra formation could transform uranium, either directly or indirectly, to a state that could be immobilized.

Two WIPP groundwaters were sampled—Sandia National Laboratories Well 10 (SNL-10) and the Water Quality Sampling Program Well 6 (WQSP-6)—and enriched for iron- and sulfate-reducing microorganisms. These organisms were then inoculated into eight sample sets: filtered and amended SNL-10 ± crushed dolomite; simulated and amended SNL-10 ± crushed dolomite; filtered and amended WQSP-6 ± crushed dolomite; and simulated and amended WQSP-6 ± crushed dolomite. Acetate was provided as a carbon source and electron donor, and uranyl chloride was added as a potential electron acceptor. Uranium (U), acetate, and sulfate concentrations were measured at initial and final time points.

Uranium concentrations decreased in all samples over time by 40-77%. Loss of uranium was greatest in filtered groundwaters and in WQSP-6. A black precipitate was noted in all samples, especially in those with dolomite. This solid is hypothesized to be either sulfide from the reduction of sulfate or uraninite from the reduction of uranium or both. Although sulfate levels only slightly decreased, the sulfide generated would have been enough to cause the odor that was noted in all samples and enough to induce uranium reduction. Uraninite could not be verified by x-ray diffraction (XRD).

Oddly, acetate concentrations increased in samples without dolomite and decreased in all dolomite samples, including the negative controls. Neither scenario points toward acetate utilization coupled with sulfate or uranium reduction, and the reason for the changes in acetate concentration are currently under investigation.

Further analyses are required to confirm uranium oxidation state and/or mineralization and/or sorption to help determine the actual mechanism of uranium loss from solution and its potential for resolubilization. Regardless of mechanism, significant amounts of uranium were removed from both natural and simulated groundwaters in the presence of indigenous groundwater microorganisms. Should uranium be released into the Culebra, it is highly probable that it will be immobilized by these organisms, whether via reductive precipitation and/or biomineralization.

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1.0 INTRODUCTION

According to calculations for radionuclide transport through the Culebra, ^{234}Uranium is projected to account for the single largest release, by mass, in the event of an intrusion into the Waste Isolation Pilot Plant (WIPP) horizon. This occurs via a release point in the westernmost panels of the repository in full mining scenario simulations (Bethune, 2023). Should uranium be released into the Culebra, there are many factors that will affect its transport, one of which is microbial activity.

Many iron-reducing and sulfate-reducing microorganisms are capable of immobilizing oxidized uranium via reduction to its +4 state and/or incorporation into phosphate and carbonate minerals (Spear et al., 2000; Abdelouas et al., 2000; Wall and Krumholz, 2006; Newsome et al., 2014). Whether this is a result of cellular respiration using uranium as an electron acceptor, reduction without coupled cell growth, indirect reduction of U via reduced iron or sulfide, or merely a detoxification strategy is not relevant to Culebra transport scenarios, as long as the uranium has been immobilized. Iron- and sulfate-reducing bacteria have been detected in Culebra groundwaters via deoxyribonucleic acid (DNA) sequencing analysis and isolated through enrichment incubations (Swanson et al., 2013).

Uranium solubility, speciation, and redox state in natural groundwaters are dependent on the groundwater composition (especially the presence of complexing ligands, such as carbonate or sulfate), ionic strength, pH, and reducing capacity (Eh). Culebra groundwaters vary in these aspects depending on their location around the land withdrawal boundary (Lofton, 2021; WIPP ASER, 2022). All the groundwaters sampled to date by the Actinide Chemistry team have been high in sulfate, and because these waters are situated in a fractured dolomite formation, concentrations of carbonate can also be elevated. Almost all enrichment incubations of these groundwaters have resulted in biogenic sulfide formation and when iron is added, iron-sulfide precipitates. Even transitional enrichment incubations, where the atmosphere is sealed while oxic, result in a sulfide precipitate. This suggests that the organisms present in the Culebra could also reduce uranium, if not directly, then indirectly via sulfide formation (Lee et al., 2013; Bargar et al., 2013; Pidchenko et al., 2023).

The experiments described in this report were designed to determine whether Culebra-indigenous microorganisms are capable of immobilizing uranium through some form of biotransformation—i.e., reductive precipitation or biomineralization. While biosorption of uranium onto cell surfaces was not the focus of this work, it is possible that sorption can contribute to uranium loss from solution and even subsequent mineralization (Kelly et al., 2001; Bernier-Latmani et al., 2010).

2.0 SUMMARY OF EXPERIMENTS

Details of groundwater sampling efforts and experimental set-up can be found in the appropriate notebooks referenced at the end of this report. The following is a summary.

2.1 Well sampling

Culebra wells SNL-10 and WQSP-6 were chosen for sampling based on the projected radionuclide flow path from a release point in the westernmost panels of the WIPP (Figure 1 below; Bethune, 2023, Figures 1 and 14).

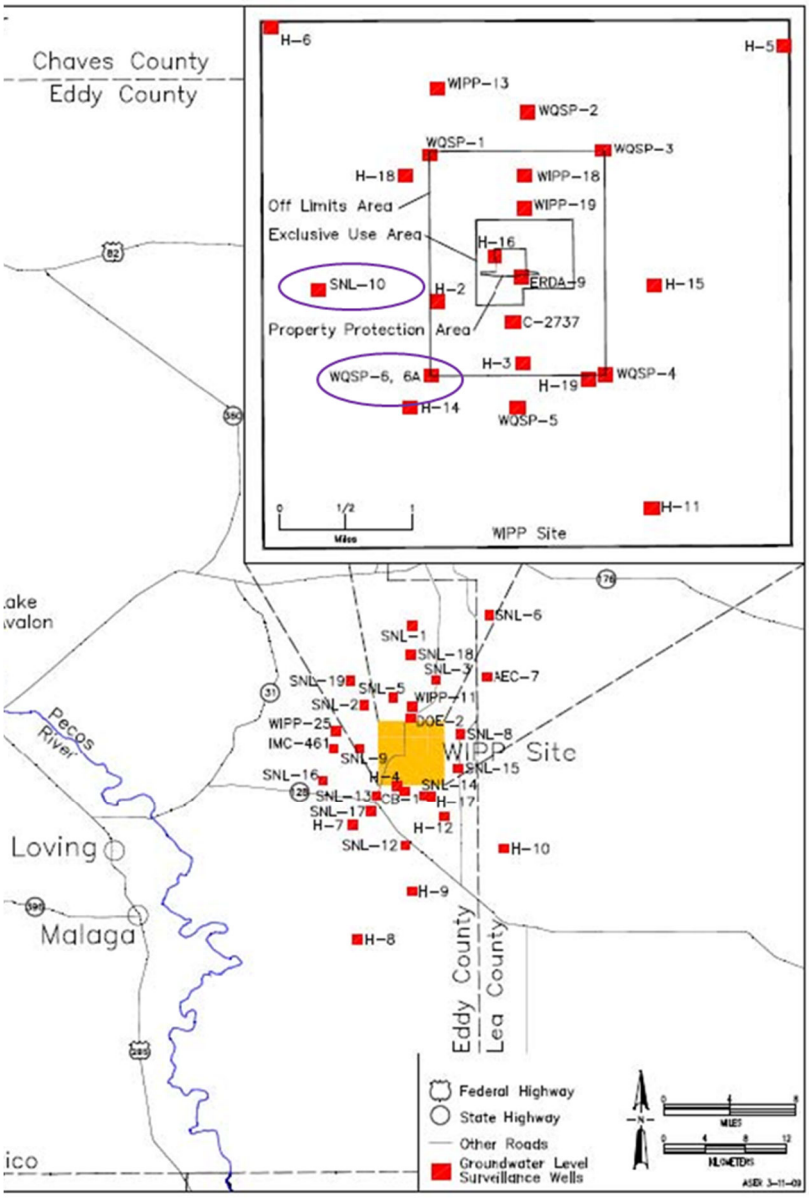

Figure 1. Location of wells sampled for uranium experiments.

Well WQSP-6 was sampled on day 2 of the scheduled WIPP annual sampling, after the well had stabilized. Water was obtained from the tap (using well-dedicated tubing from the well to the tap) under a stream of filter-sterilized nitrogen, directly into sterile 500 ml bottles until no headspace was left. The water was clear, but a strong sulfide smell was noted during sampling.

The bottles were placed on ice during transport back to the lab. Once in the lab, half of the bottles were brought directly into the anaerobic chamber for setting up enrichment incubations.

Well SNL-10 was sampled by SNL by deploying snap-samplers for a 24-hour period at depth. Samplers were retrieved the next day, placed on ice and returned to the lab. Half of the samplers were brought directly into the anaerobic chamber for setting up enrichment incubations. Numerous dark particulates were noted in the samplers, along with a strong sulfide smell.

2.2 Enrichment incubation set up

Iron- and sulfate-reducing enrichments were set up by adding amendments as concentrated stocks to the basal groundwater (Table 1). Incubations were set up in duplicate with an autoclave-killed control.

Table 1. Enrichment culture set-up.

Medium Component	Fe-reducing 1 (IR-1)	Fe-reducing 2 (IR-2)	Sulfate-reducing (SR)
Supplement Mix	0.15 ml	0.15 ml	1 ml
Organic 1	0.15 ml	---	1 ml
Organic 2	---	0.15 ml	---
Phosphate buffer	0.15 ml	0.15 ml	1 ml
Terminal Electron Acceptor (TEA) Fe-NTA Sulfate	0.15 ml	0.15 ml	 none added*
MOPS	0.015 ml	0.015 ml	0.1 ml
Reductant	---	---	1 ml
Vitamins	0.15 ml	0.15 ml	1 ml
Trace Elements	0.15 ml	0.15 ml	1 ml
Headspace	†N_2:H_2:CO_2	N_2:H_2:CO_2	N_2:H_2:CO_2
#Matrix balance	14.085 ml	14.085 ml	93.9 ml

Supplement mix: yeast extract, casamino acids, proteose peptone (final concentration 0.1g/L each); ammonium chloride (final concentration 5 mM)
Organic 1: mix of acetate, lactate, and pyruvate (3 mM each, final concentration)
Organic 2: acetate only (10 mM final concentration)
Phosphate buffer: final concentration 3 mM
Fe-NTA: iron(III)-nitrilotriacetic acid, final concentration 5 mM
*sufficient sulfate present in groundwater for TEA
MOPS (3-morpholinopropane-1-sulfonic acid): organic buffer, final concentration 0.5 mM
Reductant: cysteine-sulfide (final concentration 2 mM)
†90%:4%:6% (as certified from Plains Welding)
#one set each using SNL-10 and WQSP-6 groundwaters

Incubations were monitored daily for any changes in appearance, such as turbidity (indicating possible cell growth), color change (indicating possible iron reduction), and dark particulate matter (indicating possible sulfide formation from sulfate or iron reduction). When signs of growth were evident, subcultures were plated onto anoxically prepared marine agar to isolate organisms from these incubations. Subcultures from the incubations utilizing acetate as the carbon source/electron donor were maintained over time on anaerobic marine agar until the uranium experiments could be started.

2.3 Uranium experiments set-up

The compositions of each sampled groundwater were analyzed by inductively-coupled plasma-mass spectrometry (ICP-MS) and ion chromatography (IC), and from these results, simulated versions of SNL-10 and WQSP-6 were derived (Appendix 1). Both the simulated groundwaters and 0.2 micron-filtered groundwaters were used as diluents for the uranium reduction experiments. These incubations were set up to achieve final concentrations of 10 mM acetate, 10 mM sodium bicarbonate, 0.5 mM uranyl chloride, and growth supplements at 0.1 g/L. Bicarbonate was added to adjust the pH of the media to match that of the groundwaters, to account for the presence of dolomite carbonate in the Culebra, and to provide a ligand for uranium, other than acetate which was intended as the electron donor and carbon source. Organisms were washed 3-5 times with sterile normal saline (~0.85%) and then inoculated (3% by volume) into the amended groundwaters.

In general, anaerobic cultures are maintained in glass tubes or serum bottles sealed with butyl rubber, gas-tight stoppers, and are sampled via needle introduction. Due to safety concerns, media were dispensed into 30 ml polypropylene bottles sealed within secondary jars. Broth cultures can maintain anaerobicity in this way.

Samples were withdrawn at two timepoints for analysis: t = 0 and t = final (2 weeks). Both 100 kD-filtered/static and unfiltered/mixed samples were withdrawn and analyzed by ICP-MS for uranium and by IC for acetate and sulfate. Filtered/static samples were meant to measure uranium, acetate, and sulfate in solution. Unfiltered/mixed samples were diluted in 2 M nitric acid to reoxidize any U(IV) and are considered measurements of total uranium (Ganesh et al., 1997). Microscopic cell counts were performed on mixed, unfiltered samples. Absorbance spectra for uranium were obtained on the Ultraviolet-Visible (UV-Vis) spectrometer before inoculation and at the final time point. If solid precipitates were observed, these were collected for analysis by XRD, if sufficient mass was present.

A subset of experiments was also set up using the same bottles and medium but with 0.5 g of crushed dolomite added. This was intended to establish if the presence of dolomite had any effect on uranium disposition, such as sorption, mineralization, or enhanced dissolution. Uranium, acetate, and sulfate concentrations were not measured at the initial time point. Values for the samples without dolomite were used to approximate the initial values. At the final time point for these samples, an aliquot of the solids was removed, dried, ground, and analyzed by XRD. Cell counts were not monitored for this set due to time constraints and dolomite interference.

The overall sample matrix is shown in Table 2.

Table 2. Sample matrix for uranium studies.

Designation	Sample	Matrix
1	S10-A	SNL-10 filtered groundwater medium
2	S10-B	
3	S10-Neg	
4	S10-Sim-A	SNL-10 simulated groundwater medium
5	S10-Sim-B	
6	S10-Sim-Neg	
7	S10-D-A	SNL-10 filtered groundwater medium with crushed dolomite
8	S10-D-B	
9	S10-D-Neg	
10	S10-Sim-D-A	SNL-10 simulated groundwater medium with crushed dolomite
11	S10-Sim-D-B	
12	S10-Sim-D-Neg	
13	W6-A	WQSP-6 filtered groundwater medium
14	W6-B	
15	W6-Neg	
16	W6-Sim-A	WQSP-6 simulated groundwater medium
17	W6-Sim-B	
18	W6-Sim-Neg	
19	W6-D-A	WQSP-6 filtered groundwater medium with crushed dolomite
20	W6-D-B	
21	W6-D-Neg	
22	W6-Sim-D-A	WQSP-6 simulated groundwater medium with crushed dolomite
23	W6-Sim-D-B	
24	W6-Sim-D-Neg	

3.0 RESULTS

3.1 Acetate and sulfate concentrations over time

Measured concentrations of acetate and sulfate are presented in Figures 2 and 3 for all samples at initial and final time points. Measurements were not made on initial dolomite samples, but since the added solutions were the same, these are presumably not significantly different at the start.

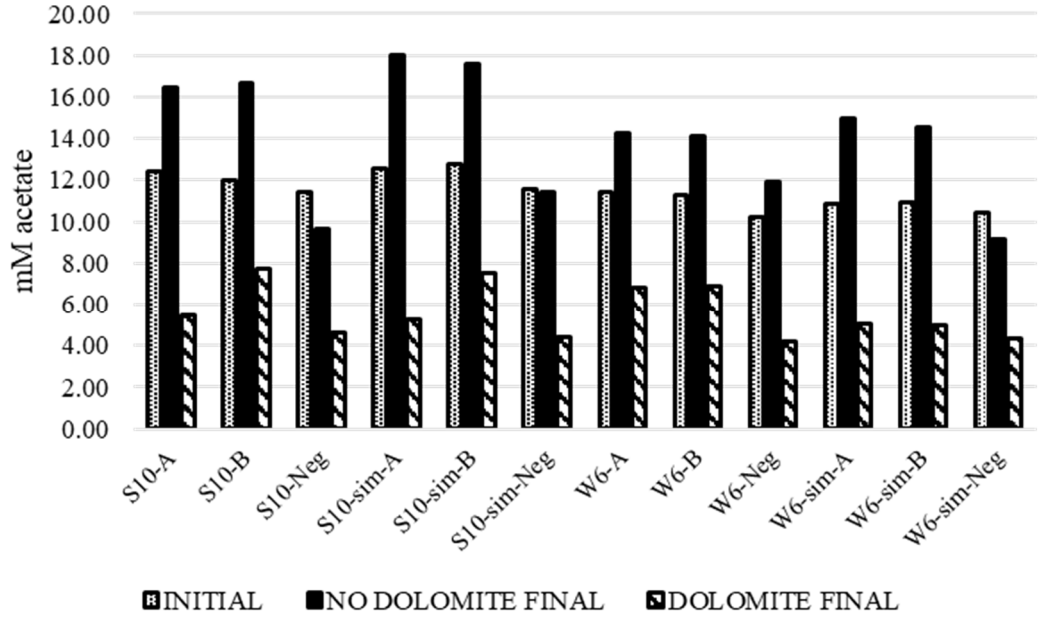

Figure 2. Acetate concentrations in filtered and simulated groundwater incubations at initial and final time points.

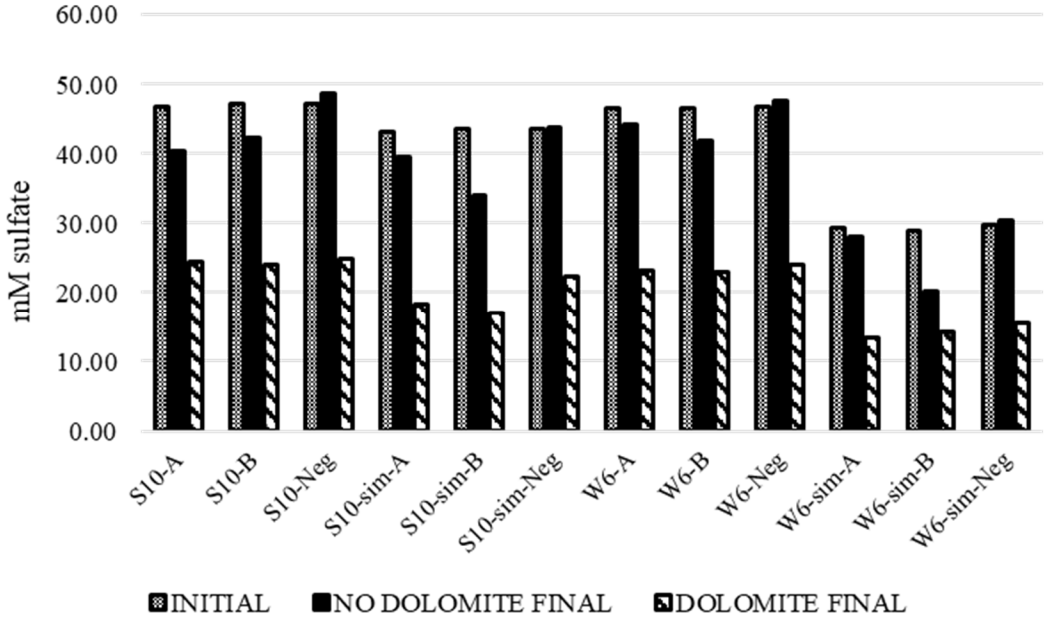

Figure 3. Sulfate concentrations in filtered and simulated groundwater incubations at initial and final time points.

3.1.1 No dolomite samples

Acetate concentrations in the biotic samples without dolomite appeared to increase by 3-5 mM to concentrations higher than what was originally added to the incubations (Figure 2). Sulfate concentrations in the biotic samples without dolomite decreased between 4-30% over time (up to 10 mM loss; Figure 3). Because the abiotic concentrations of acetate match the actual targeted concentration, the biotic measurements are presumed to be valid. The majority of sulfate measurements fall within the instrument uncertainty (10%), with the exception of SNL-10-A, SNL-10-Sim-B, and WQSP-6-Sim-B. However, given the distinct odor of sulfide in the samples, it is believed that these concentrations are also valid. The changes in sulfate concentration appear minor, but the starting concentrations were also very high, so it is feasible that these decreases are meaningful.

3.1.2 Dolomite samples

Acetate concentrations in the dolomite samples decreased between 4 to 7 mM in both biotic samples and negative controls (Figure 2), and sulfate concentrations decreased between 14-27 mM (48-60% change; Figure 3). The dolomite samples also contain more black precipitation, arguing for sulfate reduction in these samples. However, there appears to be no meaningful difference between biotic and abiotic final samples in the presence of dolomite, except possibly in SNL-10 simulated groundwater incubations, so the argument for biotic sulfate reduction is tenuous. Microscopy was performed on random negative controls after the fact, and no cells were observed.

3.2 Uranium concentrations over time

Uranium concentrations measured by ICP-MS at initial and final time points are presented in Figure 4. As with acetate and sulfate, no initial measurements were performed on dolomite samples, but concentrations are presumably not significantly different.

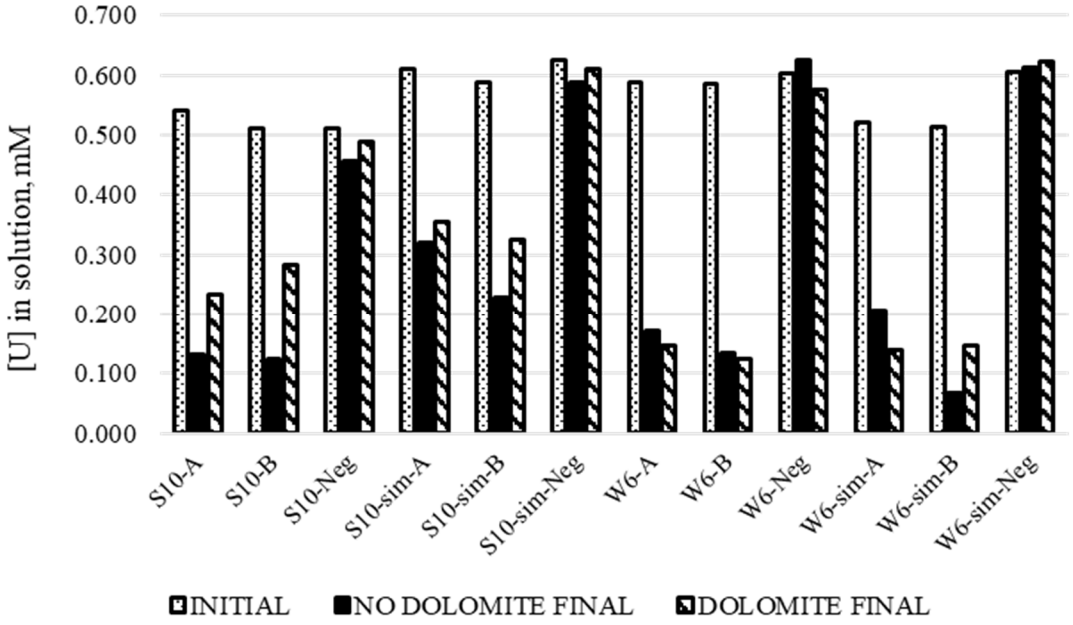

Figure 4. Uranium in solution at initial and final time points

3.2.1 No dolomite samples

Uranium concentrations in solution decreased by about 76% in the SNL-10 filtered groundwater samples and ~55% in the simulated groundwater samples. In contrast, 72-77% of uranium was lost in all WQSP-6 samples over the course of two weeks (Figure 4).

3.2.2 Dolomite samples

In samples with dolomite, uranium concentrations in solution decreased by about 51% in the SNL-10 filtered groundwater samples, ~43% in the SNL-10 simulated groundwaters, and 72-75% in the WQSP-6 samples over the course of two weeks (Figure 4).

The presence of dolomite does not appear to affect uranium removal from WQSP samples. In contrast, in SNL-10, less uranium was removed from dolomite samples than in samples with no dolomite: approximately 25% less from the filtered samples and ~12% less from the simulated groundwater samples.

3.3 UV-VIS spectra

Spectra performed on all initial samples show peaks for the U-carbonate species at ~463, 449, 435, 423, 413, and sometimes 404 nm (Figure 5; Appendix 2, Figures A1-A7; Migdisov et al., 2024). Final spectra show the same peaks in most samples, but with diminished amplitude as compared to the initial spectra, that would correspond to the decrease in concentration measured by ICP-MS. In some samples, the peaks have almost completely gone (Figures A5 and A6). Only one example is shown here (Figure 5); all remaining spectra are shown in the Appendix.

3.3.1 SNL-10 samples

All final biotic spectra from the no-dolomite samples show a decrease in U-carbonate peak amplitude compared to their initial spectra, while the before and after spectra for the abiotic controls are the same (Figures 5 and A1). Additionally, the final biotic spectra had elevated background absorbances, whether they were from filtered or simulated groundwater samples. This background was not observed in the negative controls, which suggests that it could be due to the presence of organic material, but this has not been demonstrated. Spectra measured in the filtered samples have much lower absorbance values than the simulated groundwater samples, but the disappearance of U from the simulated groundwater spectra is much more pronounced.

Initial spectra for the dolomite-containing samples were not measured. The U-carbonate peaks are present and distinct in all final samples, but the absorbances are low. There was no difference between biotic and abiotic spectra for the filtered, dolomite-containing samples (Figure A2), but peaks in the biotic, simulated, dolomite-containing samples were slightly smaller than those in the abiotic sample (Figure A3).

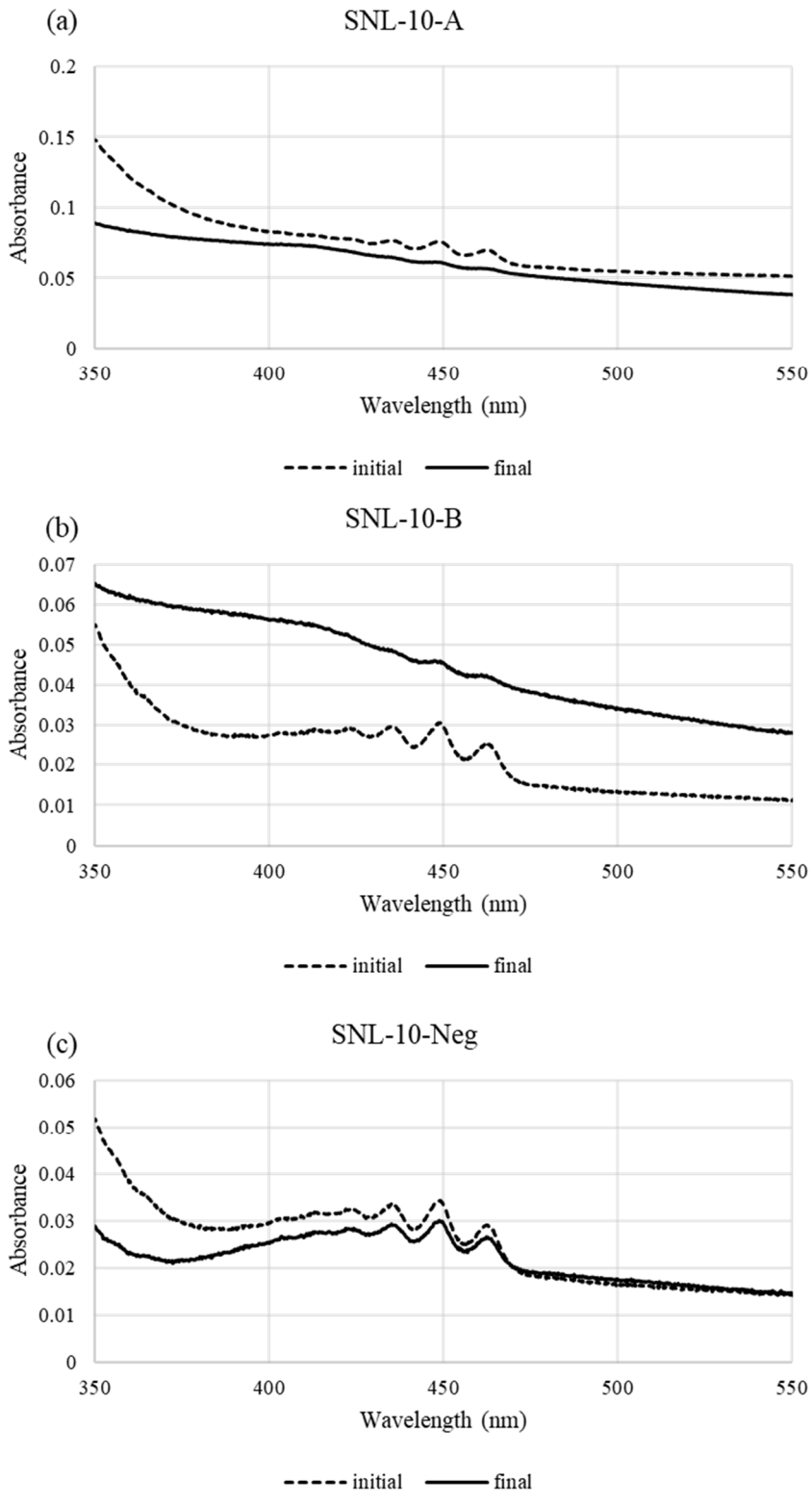

Figure 5. UV-Vis spectra for samples 1-3 (a-c; SNL-10 filtered and amended groundwater incubations) at initial and final (2-week) time points.

3.3.2 WQSP-6

In all WQSP-6 samples, the U-carbonate peaks are almost completely lost from the final spectra (Figures A4-A7). The final spectra do not exhibit the same elevated background as in SNL-10, with the exception of WQSP-6-Sim-A.

3.4 Cell numbers over time

It was not possible to track cell numbers over time in all samples. WQSP-6 samples (WQSP-6-A, WQSP-6-B, WQSP-6-Sim-A) did not take up the nucleic acid stain effectively, presumably due to some sort of chemical interference. The initial count obtained for WQSP-6-Sim-B was assigned to these samples for the sake of comparison. Alternatively, they can be marked as below the detection limit (approximately 5E+04 cells/ml). Dolomite samples were not tracked for counts.

Cell numbers in SNL-10 samples increased by less than an order of magnitude. Numbers in WQSP-6 increased by approximately an order of magnitude. These changes in cell numbers do not indicate significant growth, even for anaerobic microorganisms.

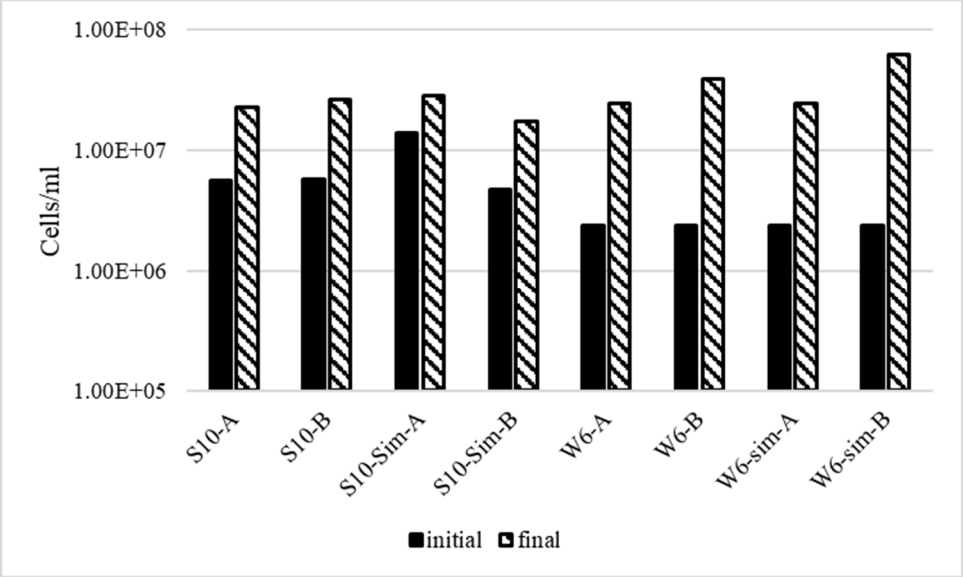

Figure 6. Cell counts at initial and final time points for all samples.

Cell morphologies showed short and long rods in both SNL-10 and WQSP-6 samples. Additionally, vibrious cells were frequently observed in WQSP-6 samples but only occasionally in SNL-10.

3.5 Precipitated solids

In all biotic samples, a black precipitate was observed at approximately one week of incubation and was especially evident in the dolomite-containing samples (Figures 7 and 8). More was visible to the naked eye in the filtered groundwater incubations than in the simulated

groundwater incubations, and more was visible in the SNL samples than in WQSP. Additionally, two of the negative controls also contained the black precipitate (SNL-10-Sim-D and WQSP-6-D, to a lesser degree).

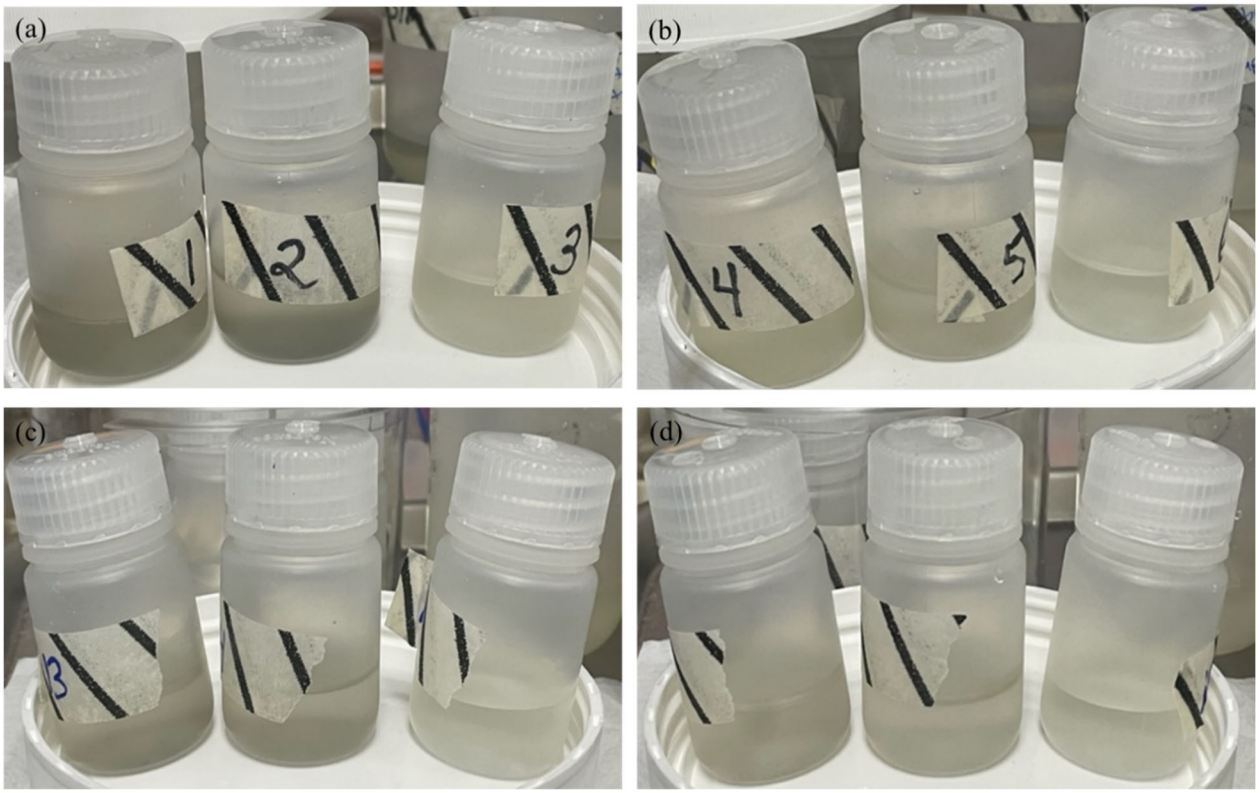

Figure 7. Groundwater incubations without dolomite: (a) SNL-10 filtered, (b) SNL-10 simulated, (c) WQSP-6 filtered, (d) WQSP-6 simulated.

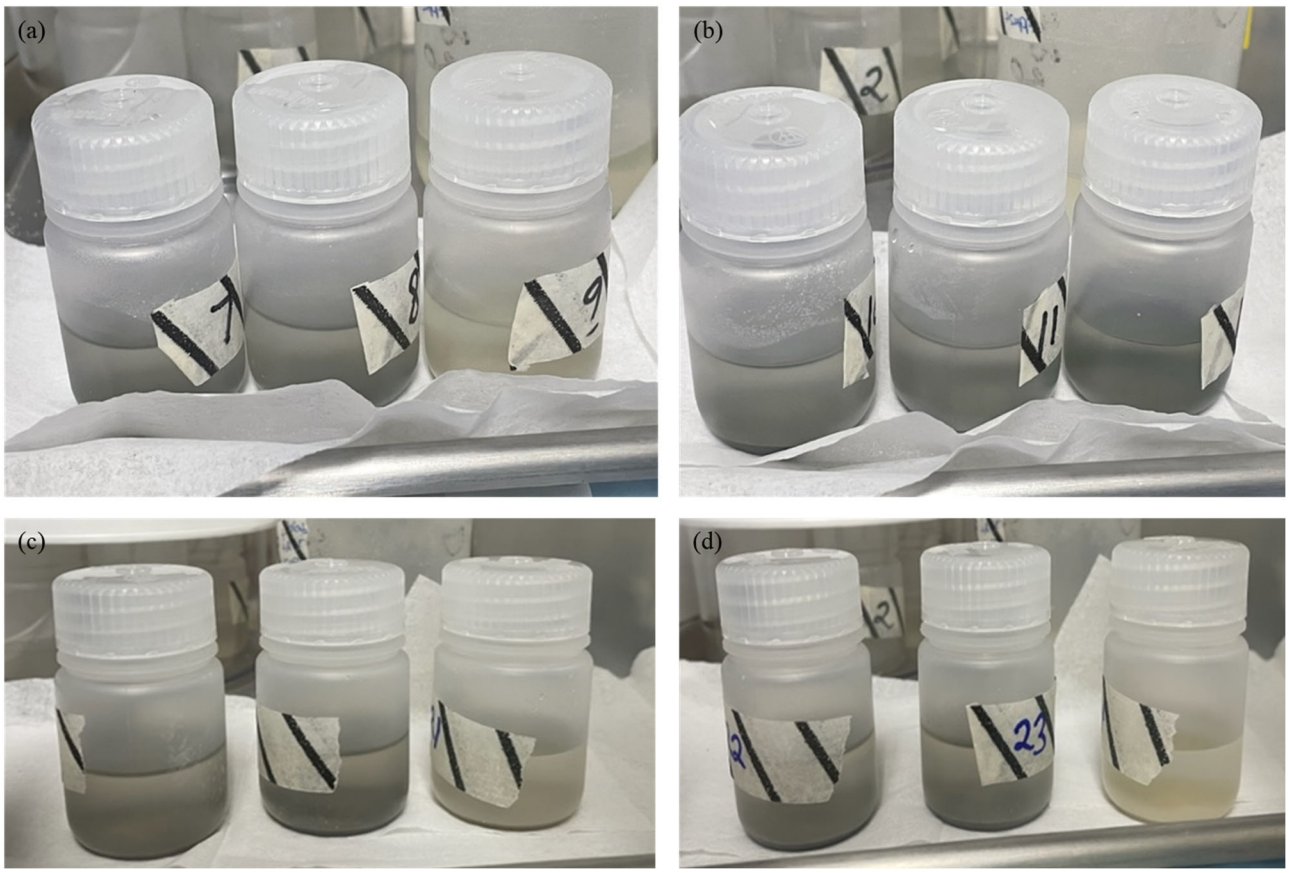

Figure 8. Groundwater incubations containing dolomite: (a) SNL-10 filtered, (b) SNL-10 simulated, (c) WQSP-6 filtered, (d) WQSP-6 simulated.

The black precipitation could be either sulfide from sulfate reduction or biogenic uranium solids, such as uraninite (UO_2) or both. Given the decrease in sulfate concentrations in most samples, however minor, and the accompanying odor, it is likely that some of the precipitation is sulfide. However, the presence of uraninite cannot be ruled out.

X-ray diffraction (XRD) was employed to identify the precipitated solids. However, most of the spectra were very noisy, and spectra on samples with dolomite contained overwhelming dolomite peaks (Figure 9). It is possible that uraninite or uranium-containing solids were present, but spectra were too inconclusive and will not be discussed here.

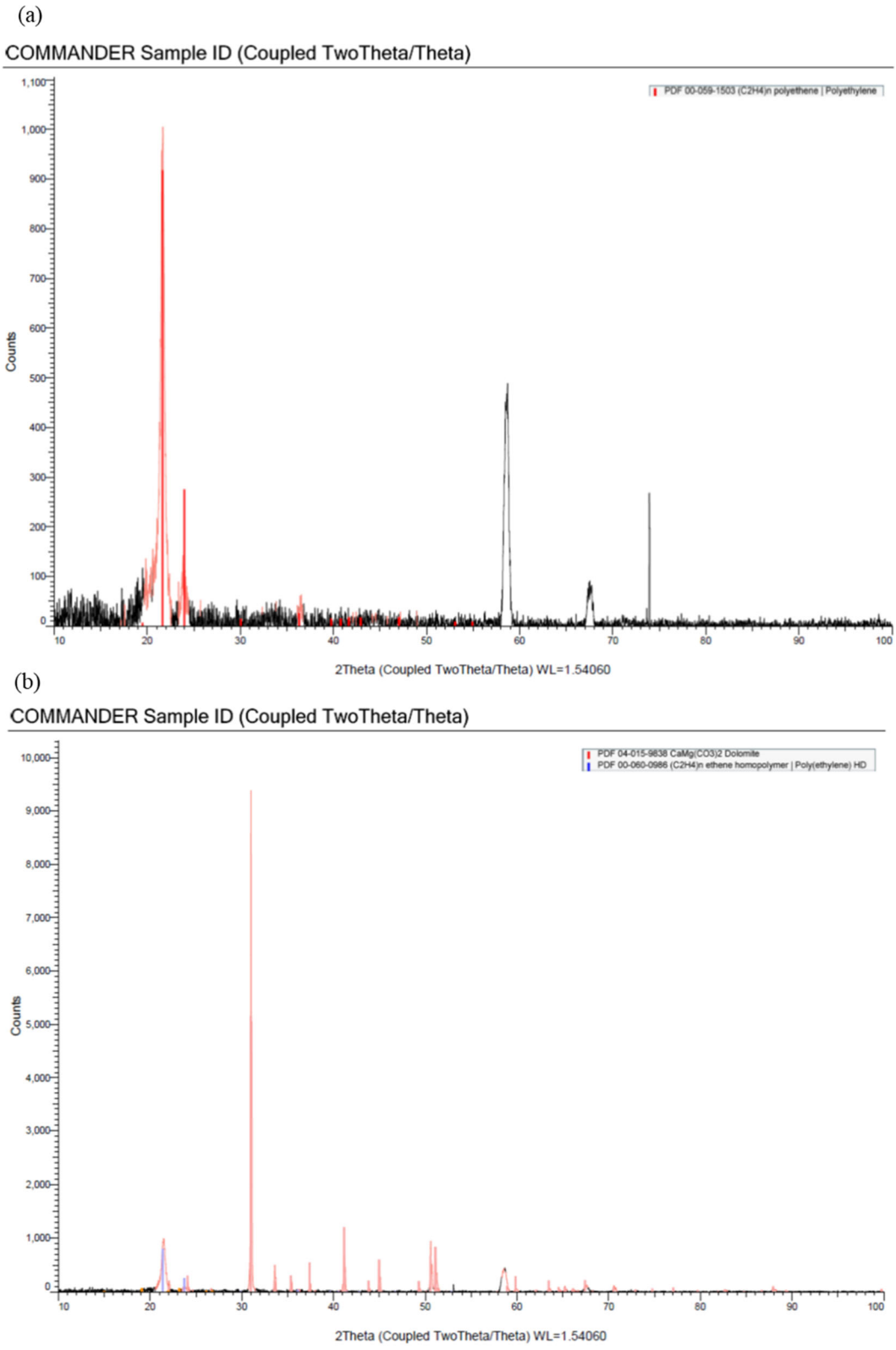

Figure 9. XRD spectra for SNL-10-Sim Rep B (a) without dolomite and (b) with dolomite.

4.0 DISCUSSION

In natural groundwaters, uranium mobility is dependent upon the pH and Eh of the groundwaters and the presence of complexing ligands, either dissolved or on solid surfaces. Possible interactions of uranium with the groundwaters and simulants in these experiments included: 1) complexation with dissolved sulfate, carbonate or acetate; 2) complexation with phosphate released by microorganisms; 3) incorporation into carbonate minerals, such as calcite but also including dolomite; and 4) sorption onto available surfaces, such as biomass or dolomite, with possible subsequent mineralization. UV-Vis spectra confirmed U-carbonate complexes in all initial samples, and these were likely to be in the +6 state. Peaks diminished over time in almost all samples, as did the uranium concentrations measured by ICP-MS.

It was hypothesized that the iron- and sulfate-reducing microbial cultures would be able to reduce uranium in the incubations, either directly concomitant with acetate oxidation or indirectly via sulfate reduction, or alternatively, that they would induce U mineralization, via phosphate release. Although there are more supporting analyses to be done and anomalies to be investigated, the results presented herein show uranium removal from solution in the presence of microorganisms, in either natural or simulated groundwaters, and in the presence or absence of dolomite. At this time, a definitive mechanism cannot be identified, and it would not be unusual for there to be interplay between different mechanisms. These are discussed below.

4.1 Bioreduction

Most microbiology studies on uranium reduction consider all uranium precipitated from solution to be U(IV). However, given the pH and composition of the Culebra groundwaters, especially the presence of carbonate and calcium, which are thought to inhibit uranium reduction (Brooks et al., 2003; Wan et al., 2005; Hua et al., 2006), we do not make this assumption.

As is, the data cannot support respiration on uranium as the cause for uranium reductive precipitation, since there was no apparent utilization of acetate, the electron donor and carbon source. Although some sulfate was reduced, the data also cannot support respiration by sulfate reduction at the expense of acetate oxidation. It is possible that the concentration of uranium in these experiments was toxic, but this was not tested (Francis et al., 2002). Nevertheless, uranium was removed from solution, and enzymatic U(VI) reduction can occur regardless of whether organisms are growing and respiring (Lovley and Phillips, 1992; Lovley et al., 1993; Francis et al., 1994).

In addition to uranium reduction in a non-growth scenario, it is also likely that indirect reduction of U(VI) occurred as a result of sulfide. Presumably, the sulfide was biotically generated, given that higher temperatures are required for abiotic sulfidogenesis. However, it is unclear if the sulfide was generated during these incubations or if it was already present. Both are likely.

To distinguish U(IV) from U(VI), some have used nitric acid to oxidize all uranium and give a total concentration from which the concentration of U(IV) could be calculated: $[U]_{total} = [U(VI)] + [U(IV)]$ (Ganesh et al., 1997). Because of the possibility that uranium sorption onto cells or

dolomite occurred, we do not assume that all uranium lost from solution is U(IV). Operationally, total uranium was defined as $[U]_{total} = [U]_{in\ 100\ kD\ filtrate} + [U]_{reduced,\ precipitated,\ sorbed}$ and the contribution of each to the mass balance was determined (Figure 12).

Figure 12 shows that the presence of dolomite inhibits uranium removal from most SNL-10 samples. However, dolomite did not affect uranium behavior in WQSP-6 nearly as much as in SNL-10, although a slight difference was noted between filtered and simulated WQSP samples.

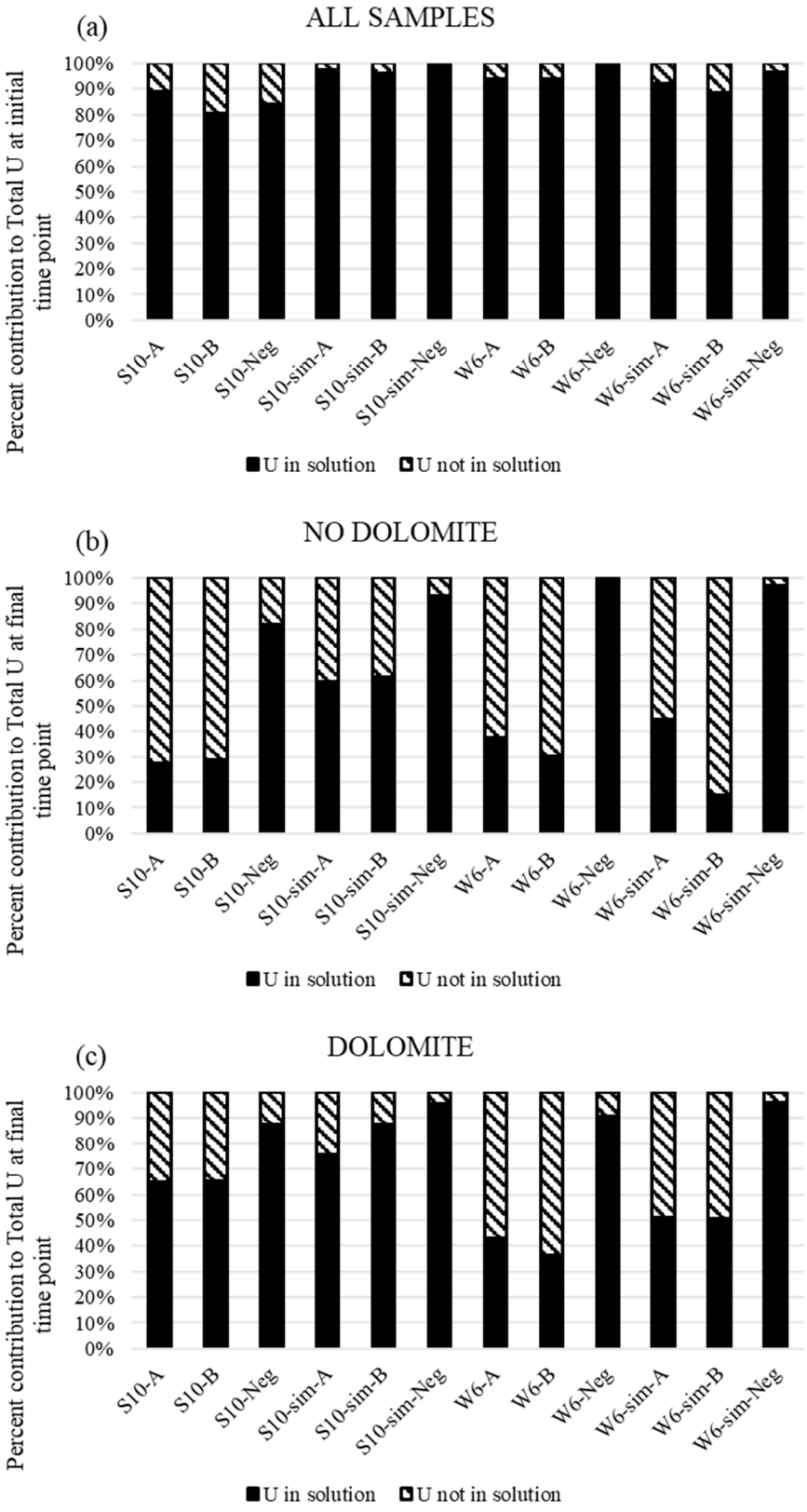

Figure 12. Uranium mass balance in all samples and comparison to initial measurements.

4.2 Sorption

Uranium sorption onto either cell surfaces or onto dolomite is another possibility for uranium disposition in these experiments. Dolomite has been screened for its ability to remove numerous heavy metals, as well as uranium (Irani et al., 2011; Khoshraftar et al., 2023; Yin et al., 2023). Sorption onto dolomite is a pH-dependent process for most metal cations, with greater removal at higher pH when the overall charge on the sorbent (i.e., dolomite) is negative. In contrast, uranium removal was found to decrease with increasing pH, due to the more complex speciation at slightly alkaline pH and in the presence of carbonates (Diwan et al., 2020). In studies with dolomite and humic substances, the presence of certain functional groups on fulvic acid was hypothesized to bridge dolomite and uranium to result in overall removal (Yin et al., 2023). Nevertheless, Figure 12 shows little effect of dolomite.

Microbial biomass is another possible sorbent for uranium. Uranium can complex with functional groups on cell surfaces and biosorption has repeatedly been proposed as a remediation strategy, especially if subsequent mineralization and precipitation occurs (Wall and Krumholz, 2006; Newsome et al., 2014). Although the biomass concentrations were relatively low in these experiments, it is possible that limited biosorption occurred.

4.3 Induced precipitation and/or mineralization

Uranium can complex with phosphate groups on cell surfaces, providing a nucleation site for mineral growth and stabilizing the U(IV) precipitates (Boyanov et al., 2011; Bargar et al., 2013). Alternatively, organisms can release phosphate into the surrounding medium to form a sparingly soluble complex with uranium and remove it from their environs. This would be especially true if uranium was exerting a toxic effect on the organisms.

Uranium can exchange with either calcium or magnesium in the dolomite lattice (Steffanowski and Banning, 2017). However, natural weathering can cause its release again, if the surrounding medium is undersaturated with respect to uranium. Uranium incorporation into other carbonate minerals, such as calcite, also occurs under microbial influence. In anoxic groundwaters from the Äspö underground laboratories, uranium was reduced and incorporated into calcite, when iron-sulfides were present (Pidchenko et al., 2023).

4.4 Condition-specific mechanisms for uranium removal

Studies have shown that different mechanisms used by different organisms under different conditions can result in different solubility-controlling uranium phases. These include U(IV)-phosphate precipitates when media are amended with phosphates, U(IV)-carbonate, uraninite, nanoparticulate uraninite, amorphous uraninite and non-uraninite U(IV) (Bernier-Latmani et al., 2010; Boyanov et al., 2011; Bargar et al., 2013; Xie et al., 2018). In some cases, this is a two-step process—sorption onto biomass or mineral followed by reduction—while in other cases reduction is direct or indirect in solution (Wersin et al., 1994). Given that the Culebra groundwaters contain little to no measurable phosphate, phosphate-controlled removal would be organism-dependent (Lofton, 2021; WIPP ASER, 2023).

For these experiments, we hypothesize different uranium removal mechanisms that depend on sample conditions, specifically the presence of certain organisms and dolomite. It is not uncommon for more than one redox pathway to exist in natural systems. However, without corroborating evidence from advanced analytical techniques, this would be speculative.

In all samples, amino acids were added as nutritional supplements (e.g., found in yeast extract and casamino acids). Fermentation of certain amino acids can generate acetate, as well as other products such as hydrogen, H_2 (Barker, 1981; Wildenauer and Winter, 1986). However, the concentrations of these supplements were low and only meant to provide organisms with required growth factors. Thus, although possible, fermentation seems unlikely in these samples.

Acetate can also be produced lithotrophically with H_2/CO_2. For this to occur, a source of molecular hydrogen is needed. Fermentation, as noted above, could provide this source. Hydrogen can then be used as an electron donor for either sulfate or uranium reduction by sulfate reducers. This would explain the increase in acetate concentrations in the samples without dolomite, the lack of significant sulfate reduction (sulfide reduces uranium in turn) and the lack of significant cell proliferation. Again however, fermentation is an uncertainty.

At best, we can say uranium reduction occurred but was decoupled from growth, or that limited sulfate reduction (or biogenic sulfide already present in the filtered groundwaters) led to the indirect reduction of uranium. It is also possible that uranium adsorbed onto cells or that uranium complexed with acetate sorbed onto dolomite.

4.5 Future work

4.5.1 Determine the mechanism(s) of uranium loss

The goal of this work was to show that microorganisms present in the Culebra could remove uranium from the groundwater. Although this has been successfully shown, we cannot provide a definitive mechanism for its removal at this time. Future analyses will help to determine which of the possible mechanisms discussed in this report is responsible.

Advanced analytical techniques are needed to determine the oxidation state and speciation of uranium in these samples (e.g., X-ray absorption spectroscopy, extended X-ray absorption fine structure spectroscopy). These analyses will confirm whether loss from solution was due to reductive precipitation, whether direct, indirect, or abiotic. Further, scanning electron microscopy with energy-dispersive X-ray diffraction (SEM-EDS) analyses can help determine if uranium is localized at cell surfaces or with precipitated solids. Finally, genomic analyses will be conducted from which metabolic pathways can sometimes be inferred.

4.5.2 Potential for reoxidation of uranium

Reoxidation and remobilization of uranium is a significant problem at contaminated sites, especially when other oxidants, such as oxygen or nitrates, are present and when high carbonate concentrations stabilize the mobile U(VI) oxidation state (Wan et al., 2005). If uranium is complexed with phosphate, this is less of an issue. Some have argued that the presence of sulfate

reducers and biogenic iron-sulfide minerals, such as mackinawite, can lead to uranium reduction but can also lead to its release into solution again (Lee et al., 2013). In contrast, more studies have shown that the presence of mackinawite not only promotes the formation of uraninite but also helps stabilize it by scavenging oxygen and providing a physical barrier to reoxidation (Bi et al., 2013; Bargar et al., 2013). The Äspö study confirmed U(IV) stabilization and immobilization and additionally showed that even very low micromolar concentrations of iron-sulfides prevented uranium reoxidation (Pidchenko et al., 2023).

The Culebra groundwaters have little to no measurable nitrates (Lofton, 2021; WIPP ASER, 2023), suggesting that uranium reoxidation coupled to nitrate reduction should not occur. Iron concentrations are higher in these tested groundwaters than in the Äspö waters, suggesting that reduced uranium should be stable. Phosphate control on solid phase uranium will depend on microbial activity.

5.0 QUALITY ASSURANCE, DATA TRACEABILITY AND DOCUMENTATION

The majority of data presented in this report were generated as Quality Level 1 data, in accordance with the CBFO Quality Assurance Program Document. Experiments were performed under the test plan, “Microbial Interactions in the Waste Isolation Pilot Plant” (LCO-ACP-12). Detailed descriptions of the experiments can be found in the scientific notebooks, “Characterization of the Microorganisms Indigenous to the WIPP” (ACP-12 Task 1/1) and “Microbially-Induced Oxidation/Reduction of Actinides and Other WIPP-Relevant Metals” (ACP-12-3-3/1).

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Appendix 1: Compositions of Filtered Groundwaters and Simulated Groundwaters

ICP-MS and IC MEASUREMENTS OF GROUNDWATER COMPOSITIONS: FILTERED AND SIMULATED			
Groundwater	**Analyte**	**measured concentration, mM**	**actual concentration in simulant, mM**
SNL-10	Cl^-	35.2 ± 0.54	47.1 ± 13.8
	Br^-	0.23 ± 0.004	NA
	SO_4^{2-}	44.0 ± 0.88	52.3 ± 4.31
	B	0.65 ± 0.01	NA
	Na^+	85.2 ± 1.19	94.8 ± 2.72
	Mg^{2+}	6.69 ± 0.13	6.26 ± 0.06
	K^+	1.37 ± 0.12	2.04 ± 0.29
	Ca^{2+}	12.1 ± 0.37	11.1 ± 0.84
WQSP-6	Cl^-	158 ± 1.14	177 ± 19.0
	Br^-	0.07 ± 0.002	NA
	SO_4^{2-}	53.3 ± 0.73	49.5 ± 6.01
	B	1.67 ± 0.04	1.87 ± 0.01
	Na^+	185 ± 1.91	178 ± 0.003
	Mg^{2+}	8.86 ± 0.05	9.23 ± 0.19
	K^+	4.03 ± 0.06	4.53 ±0.03
	Ca^{2+}	17.7 ± 0.12	23.6 ± 0.92

NA = not added

Appendix 2: UV-Vis Spectra

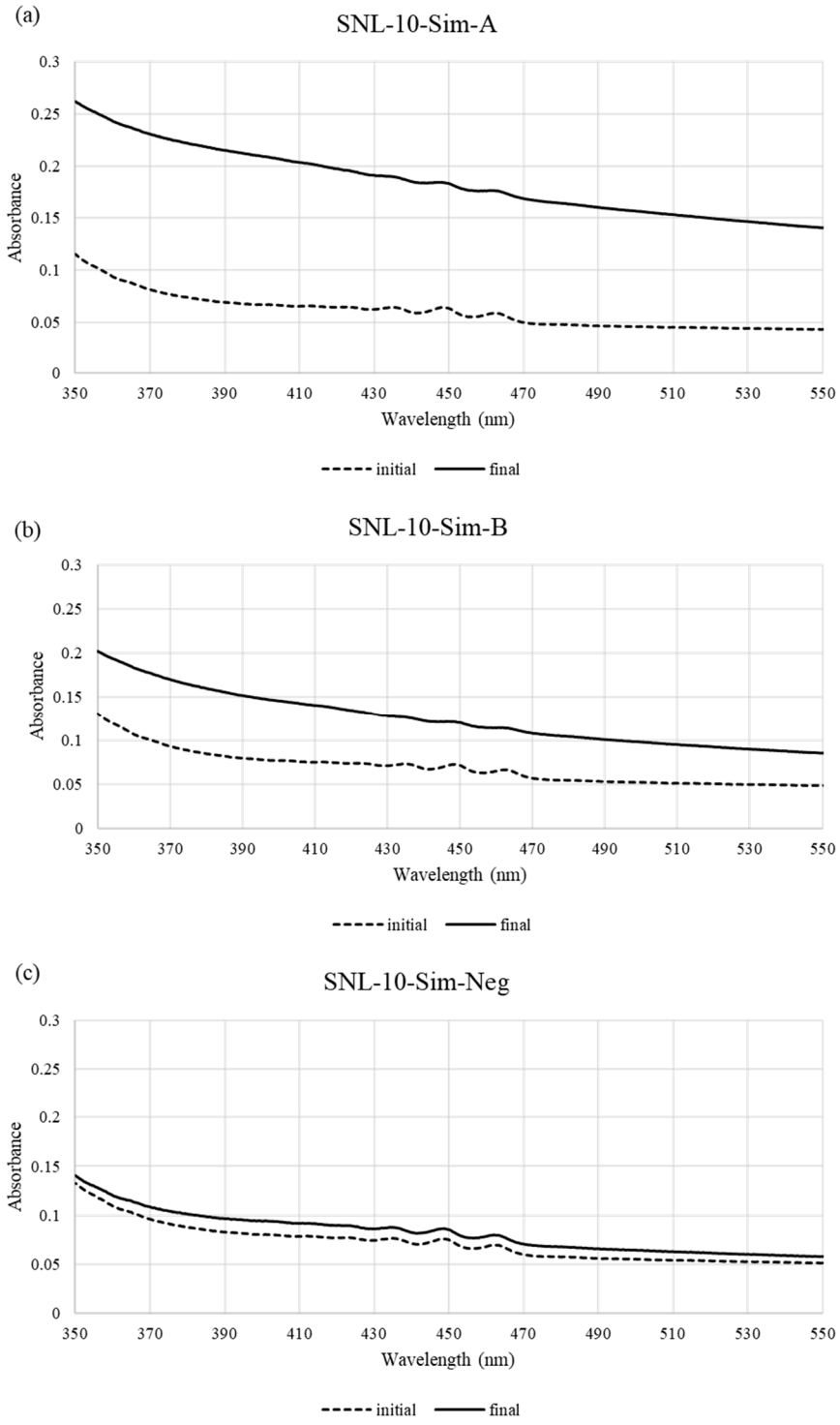

Figure A1. UV-Vis spectra for samples 4-6 (a-c; SNL-10 simulated and amended groundwater incubations) at initial and final time points.

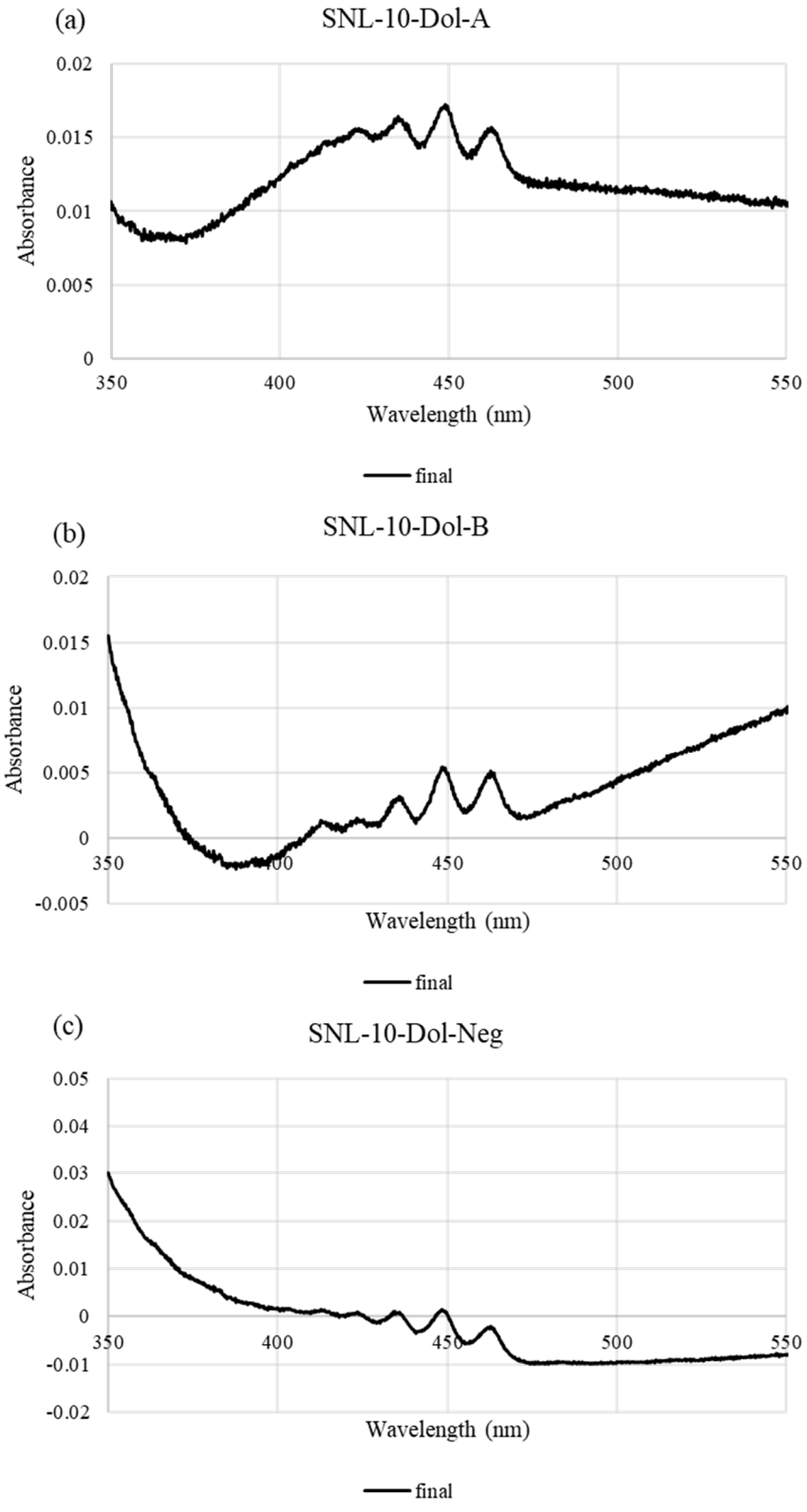

Figure A2. UV-Vis spectra of samples 7-9 (a-c; SNL-10 filtered and amended groundwater with crushed dolomite.

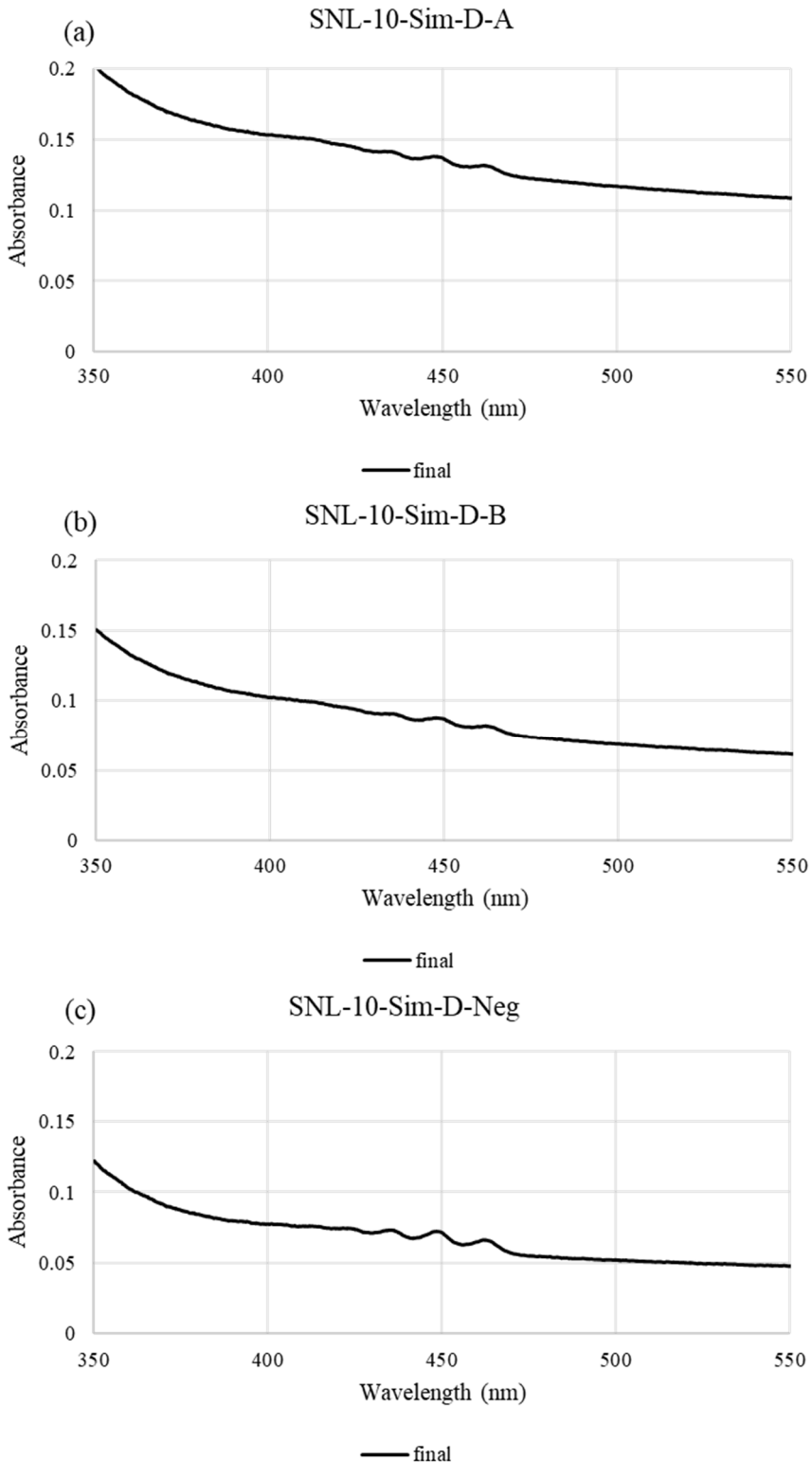

Figure A3. UV-Vis spectra of samples 10-12 (a-c; simulated and amended SNL-10 groundwater with crushed dolomite.

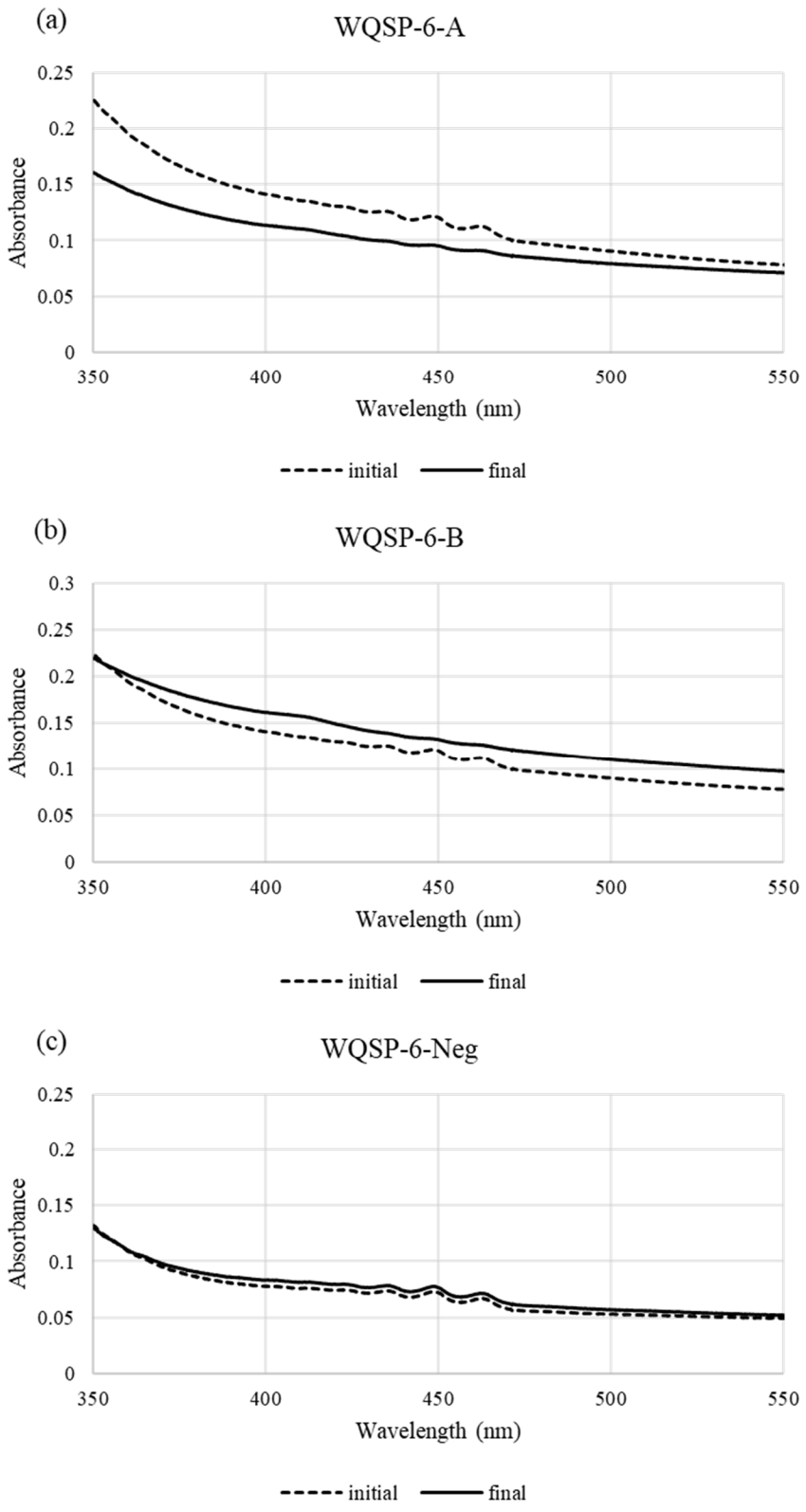

Figure A4. UV-Vis spectra in filtered WQSP-6 groundwater samples: (a) Rep A, (b) Rep B, (c) negative control.

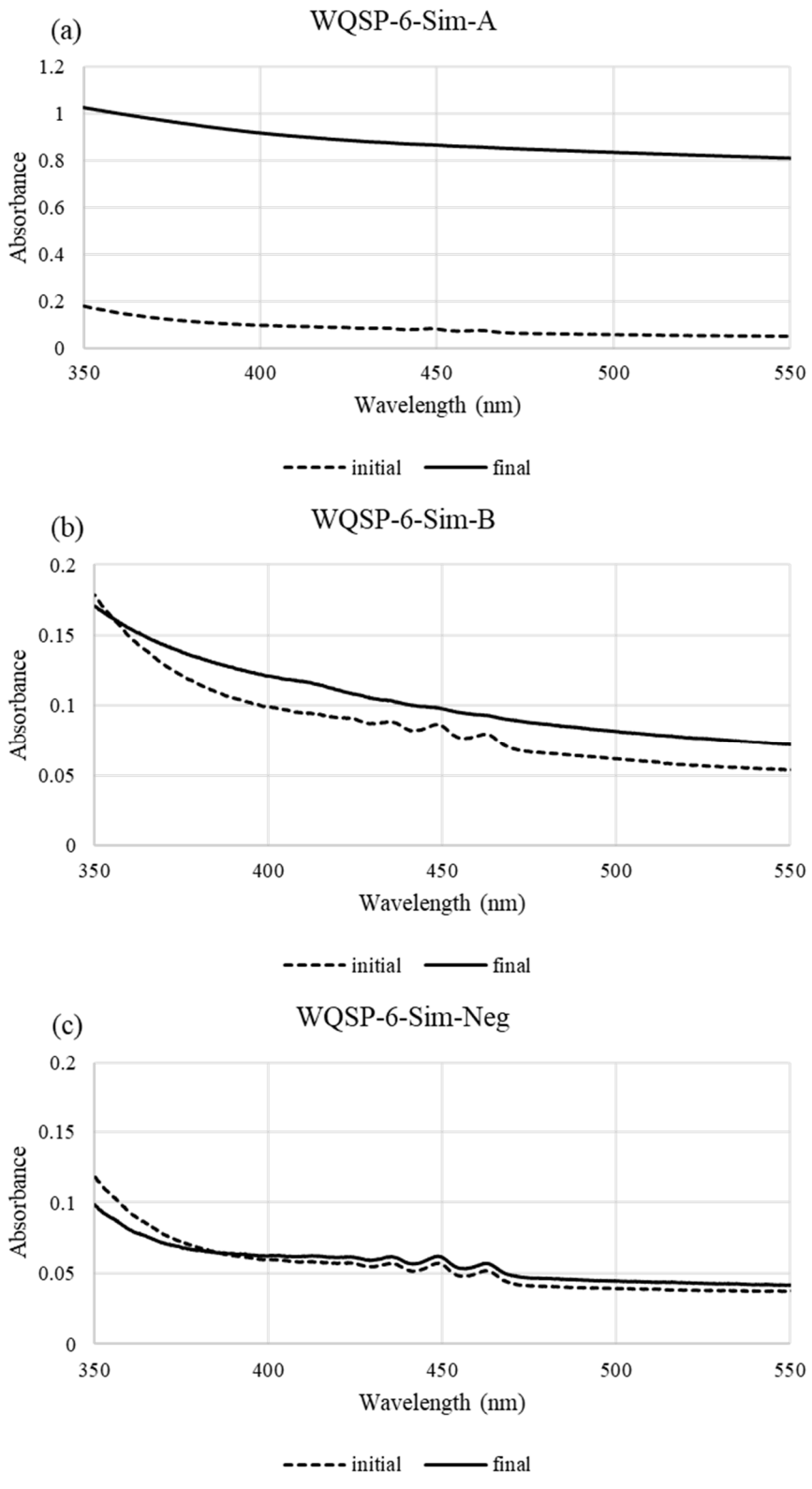

Figure A5. UV-Vis spectra in simulated WQSP-6 groundwater samples: (a) Rep A, (b) Rep B, (c) negative control.

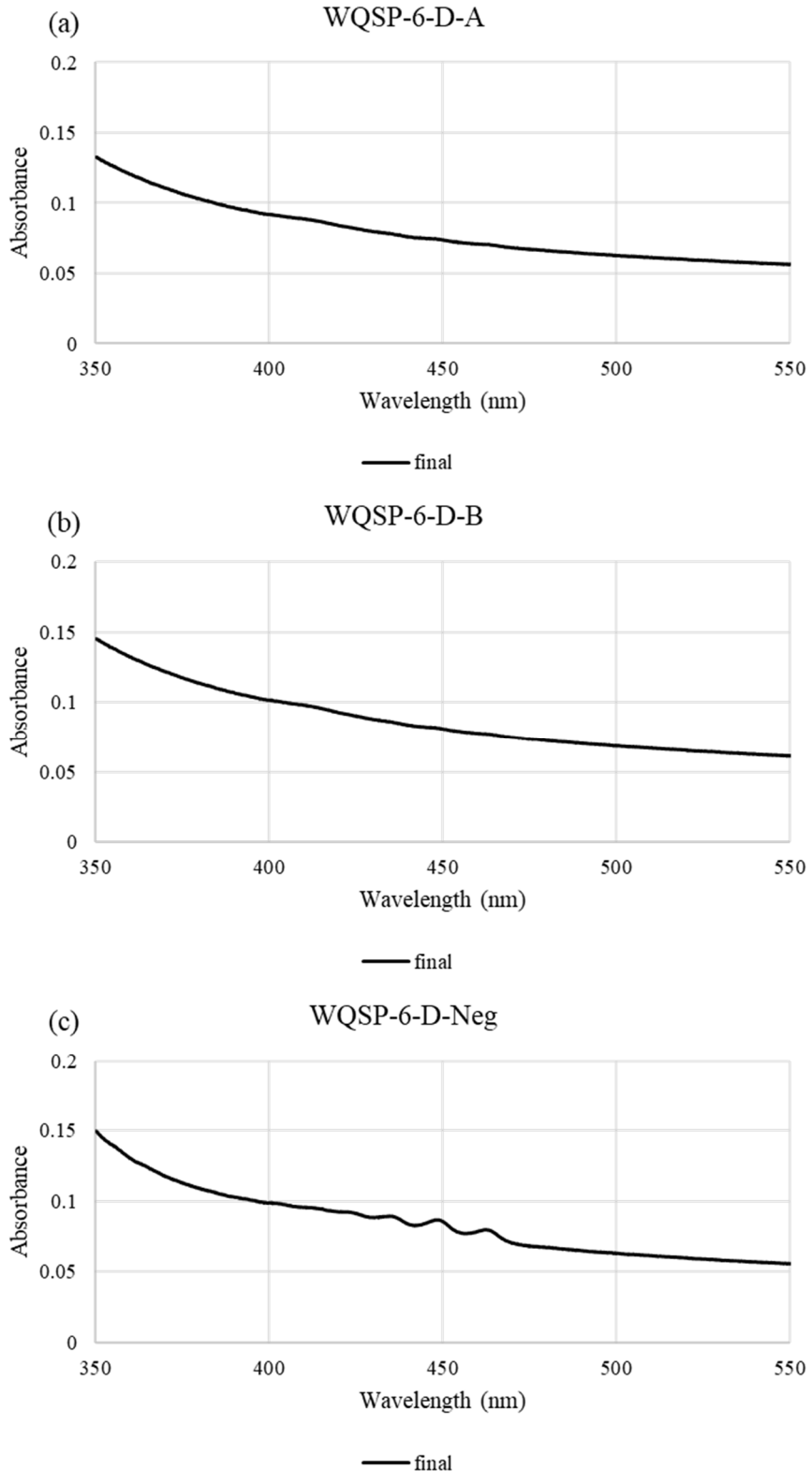

Figure A6. UV-Vis spectra of samples 19-21 (a-c; WQSP-6 filtered and amended groundwater with crushed dolomite.

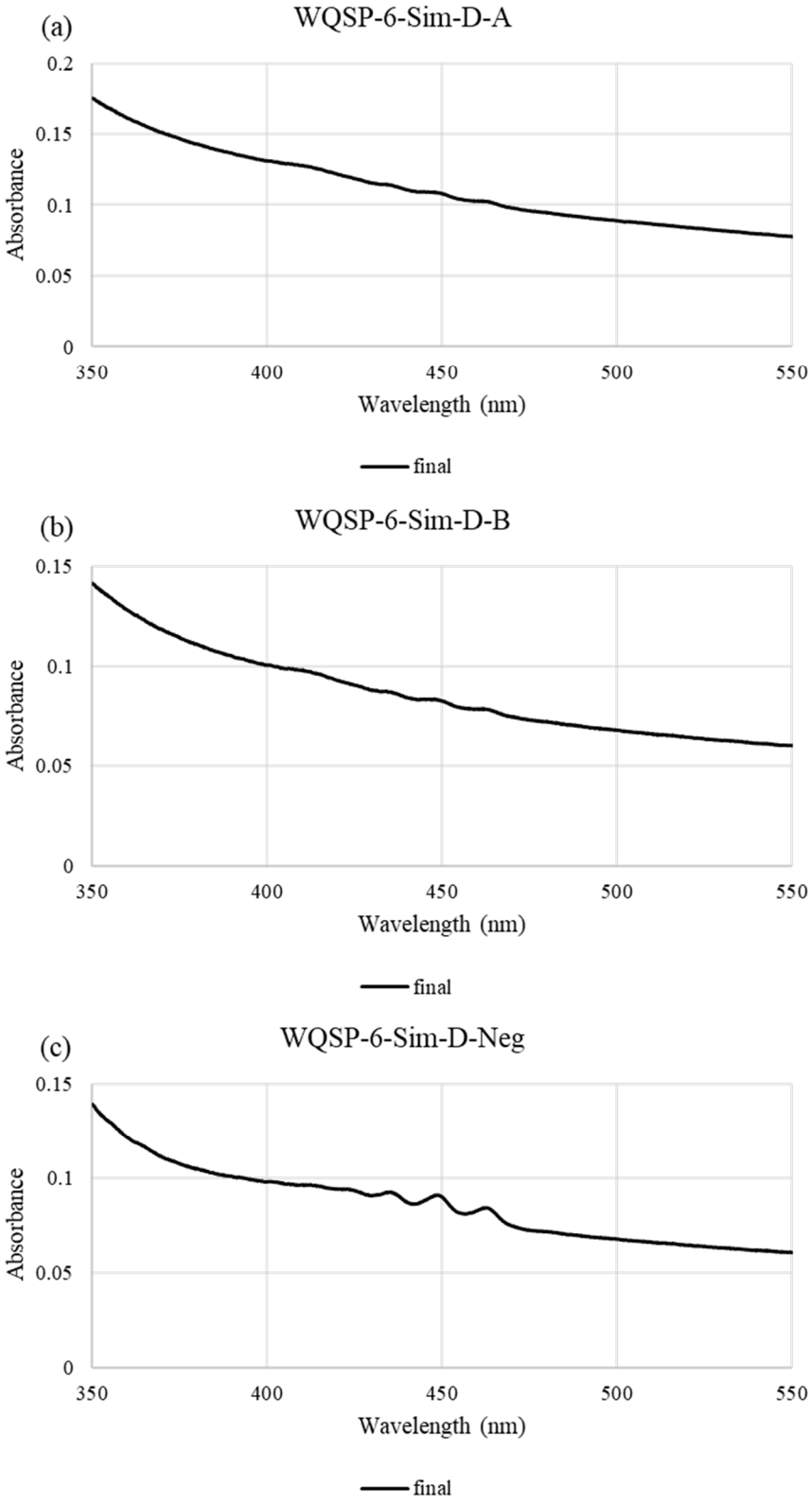

Figure A7. UV-Vis spectra of samples 22-24 (a-c; WQSP-6 simulated and amended groundwater with crushed dolomite.