

## Background

The Old Rifle Mill Processing site at Rifle, CO, contains uranium contaminated groundwater.

Uranium contamination is one of the most common problems at Department of Energy legacy sites.

Anaerobic bacteria and their metabolic byproducts can catalyze the reduction of uranium.

Reduced uranium [U(IV)], which is found in naturally reduced zones, is insoluble and less mobile than oxidized uranium [U(VI)].

Understanding the oxidation/reduction pathways will allow us to better predict the fate and transport of uranium.



## Motivation & Question

Metal reducing and sulfate reducing bacteria release sulfide [S<sup>2-</sup>] and ferrous [Fe<sup>2+</sup>] ions as products of their metabolic activities.

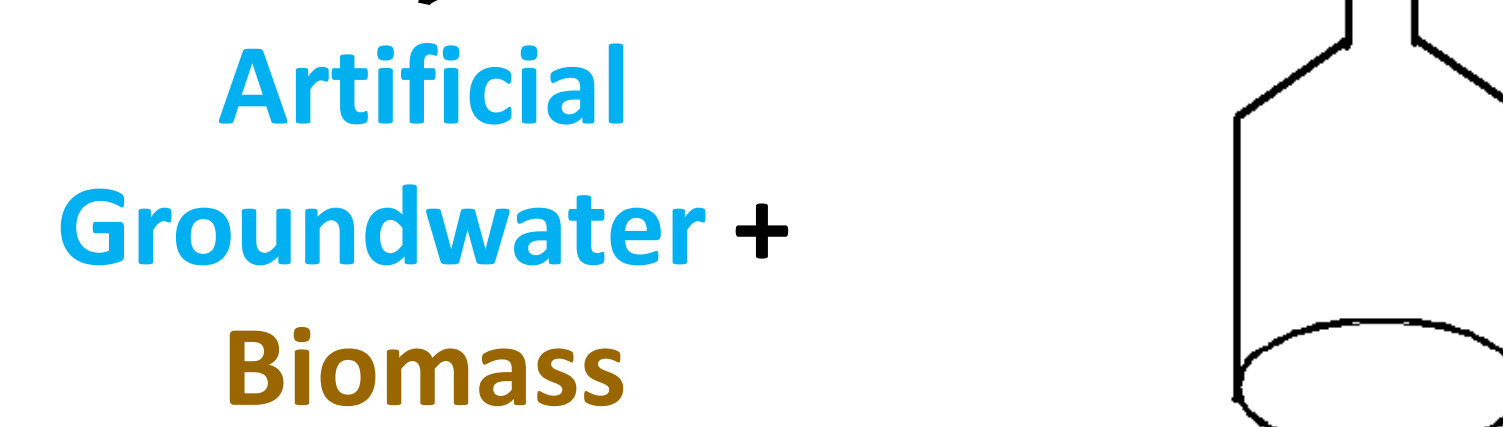
These ions can reduce uranium [U(VI)], but bicarbonate ions [HCO<sub>3</sub><sup>-</sup>] slows down the reaction in the environment.

Despite the presence of bicarbonate we want to know in the absence of microbes, if plant biomass in the environment enhances U(VI) reduction by Fe<sup>2+</sup> and S<sup>2-</sup>?

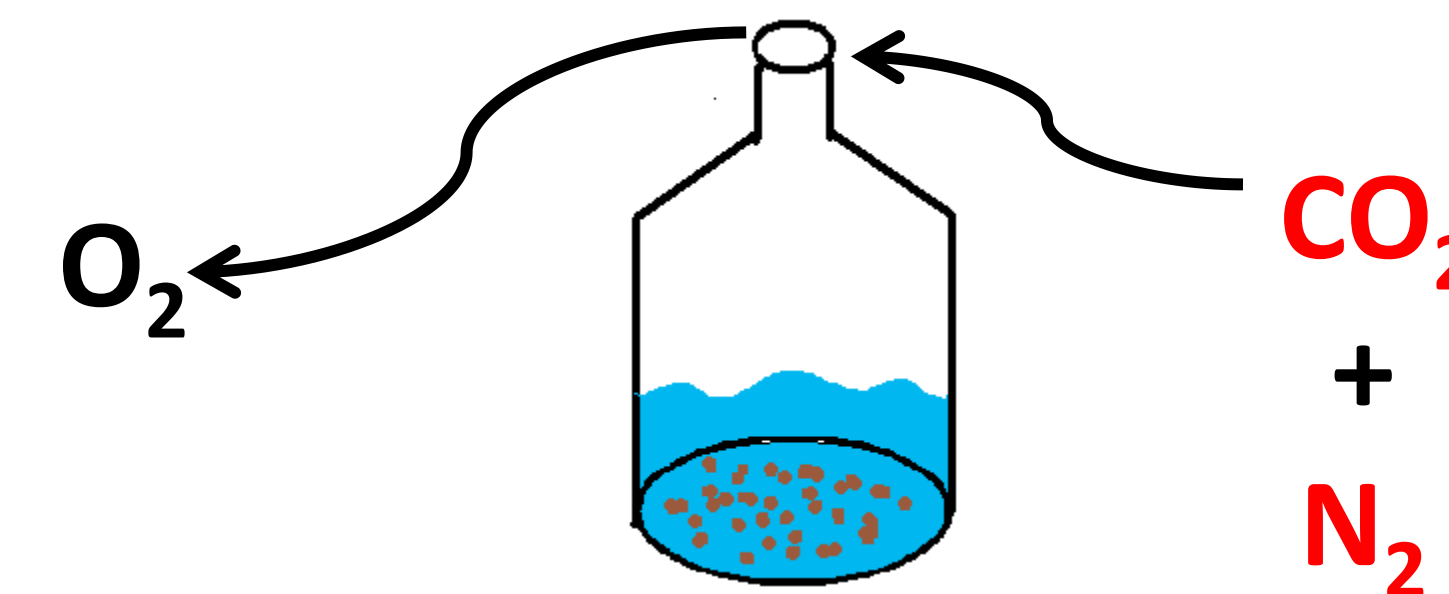
## Methods

### Creating Aquifer Settings & Conditions in Vials

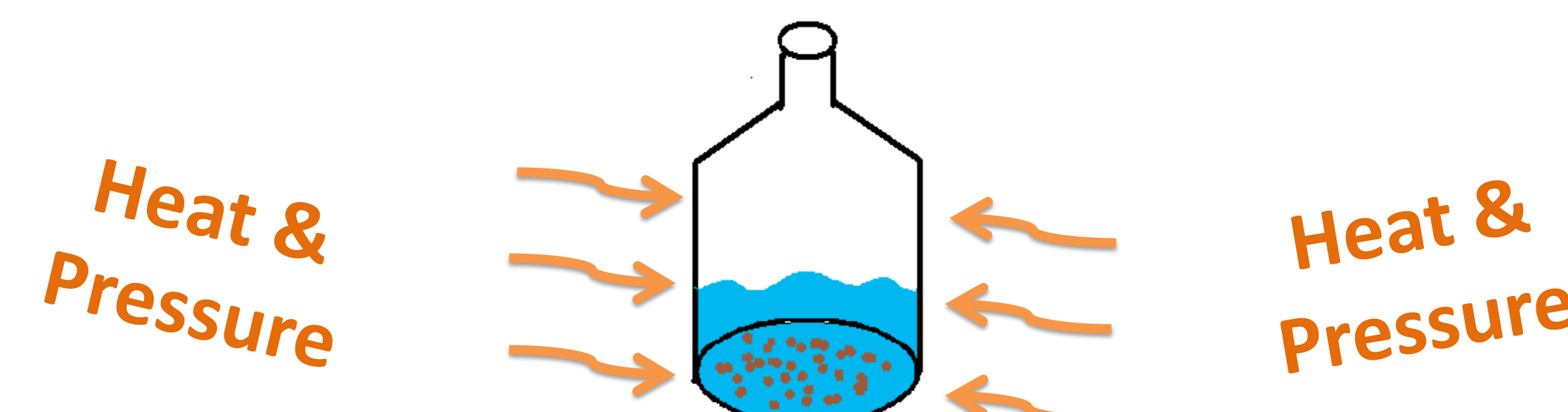
1) Artificial Groundwater & Blended Roots (Biomass) from Rifle, CO were added to each vial



2) Each vial was sparged with a mixture of carbon dioxide and nitrogen gas displacing oxygen to create an anaerobic environment



3) To kill bacteria, some vials were autoclaved with heat and pressure (130°C, 103kPa) for 30 minutes.



4) Reagents (uranium, sulfide and iron(II)) were added to the vials in different combinations and concentrations then were incubated on a shaker for 7-11 days to react.



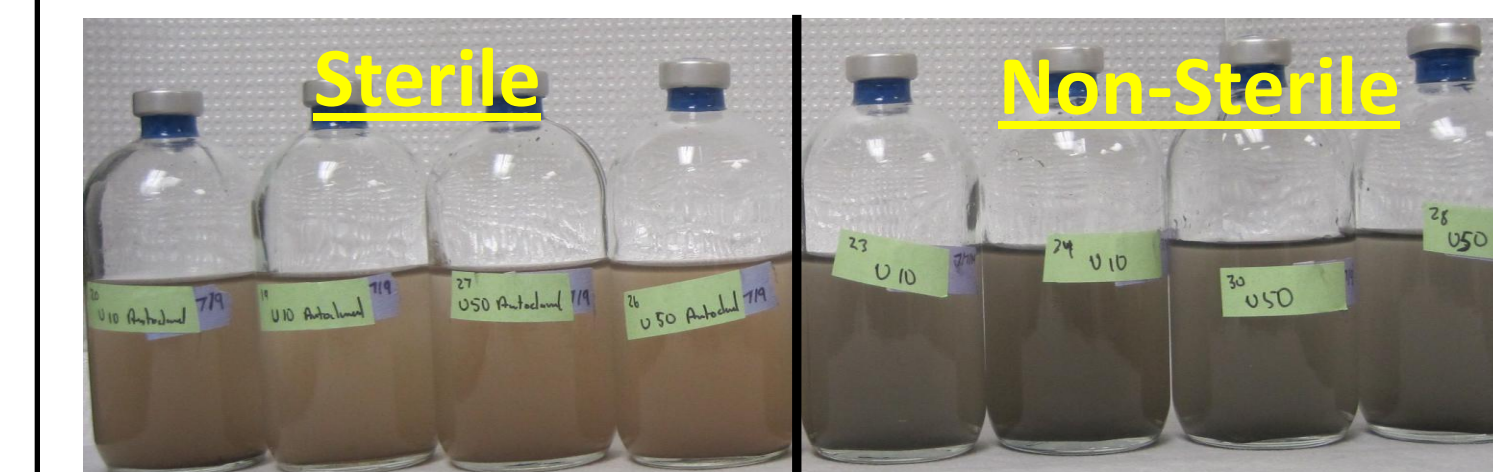
Biomass samples were collected using vacuum filtration. X-ray absorption spectroscopy (XAS) was then used to determine the oxidation state of uranium in the solid phase.



Aqueous samples were removed from each vial. Uranium concentration was measured using inductively coupled plasma mass spectrometry (ICP-MS)

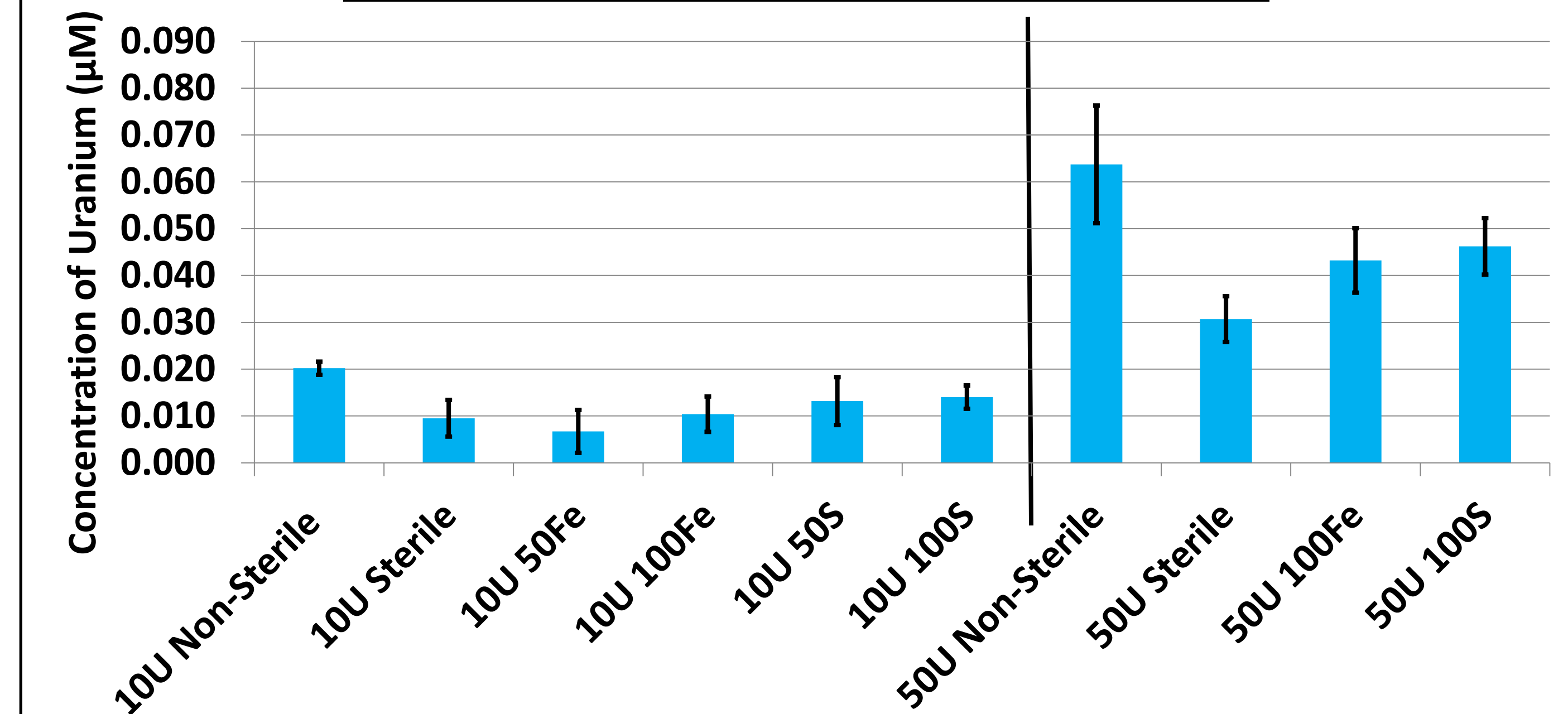


## Data and Results



At the end of the incubation period, vials that were not autoclaved and contained Uranium turned black in color.

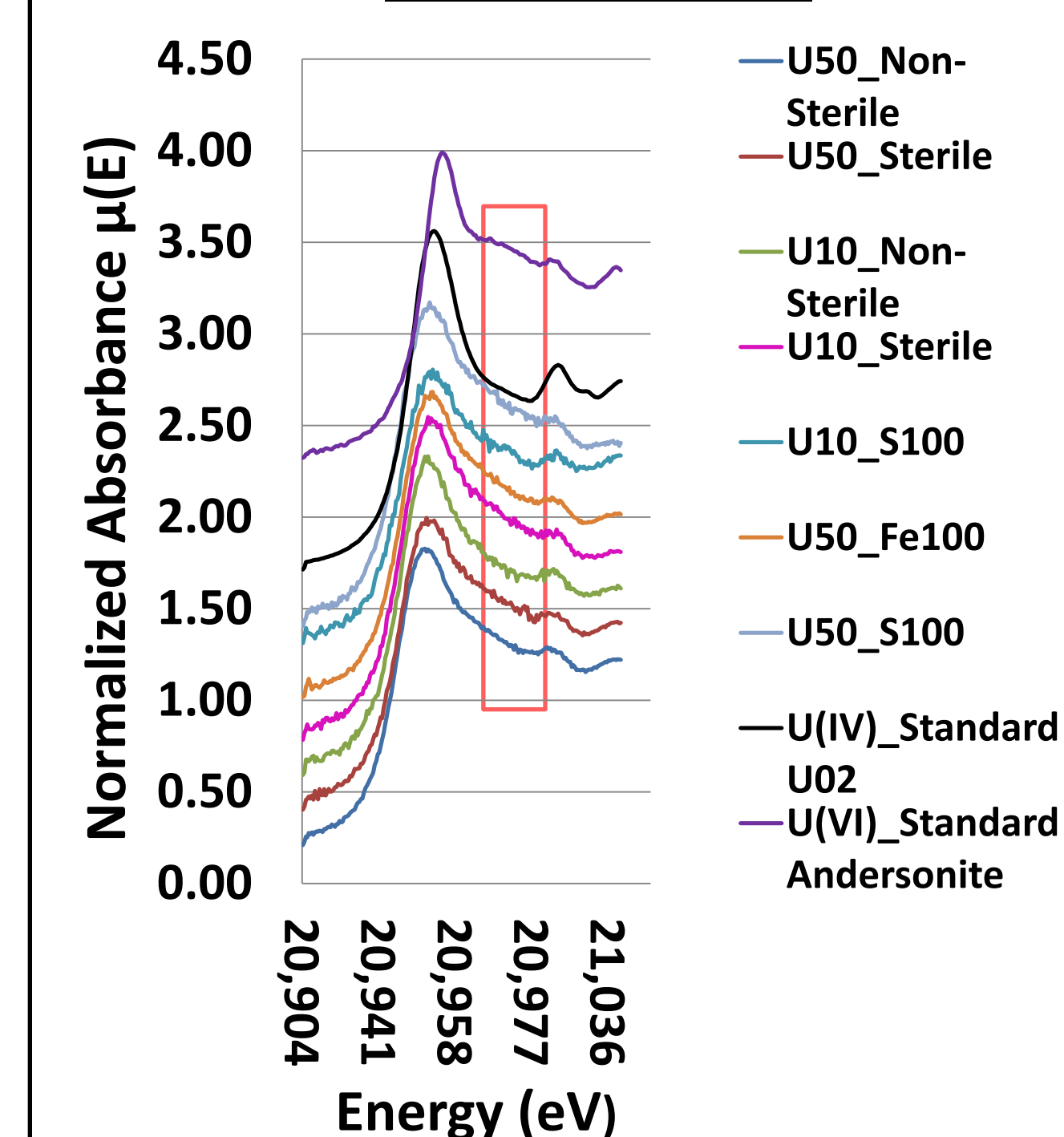
### Decrease of Uranium in Aqueous Solution



### Reagents Added to Vials (µM)

Aqueous samples were removed during the initial and final days of incubation. Overall more than 97.5 % of U initially added to the suspensions was sorbed by the biomass by the end of the incubation period. Results of the ICP-MS showed that the concentration of U decreased between the initial and final sampling times, as shown in the plot above. There was a greater change in the non-sterile vials. There is no difference between the vials that contain iron or sulfide. A one way ANOVA of the vials with 50 µM of U show a significant difference among the group having a p-value of 7.58 x10<sup>-2</sup> and vials with 10 µM of U show a significant difference among the group 1.46 x10<sup>-5</sup>. Sterile groups show no significant difference as their values overlap for vials with both 10 and 50 µM of U.

### XAS-Uranium



Uranium L-II edge XAS was used to assess whether U(VI) was reduced during the reaction. U(VI) spectra have a shoulder at 20,964eV, whereas U(IV) spectra do not. XAS show that U(VI) is still present and sorbed by the biomass. This can be seen by comparing the boxed region of the andersonite [U(VI) standard] and uraninite [U(IV) standard] spectra. The area highlighted by the boxed shoulder region shows that there is a mixture of both U(VI) and U(IV) in all of the biomass sampled. Non-sterile samples seem to have a steeper slope at the boxed region suggesting they have more U(IV).

## Conclusions and Future Directions

•ICP-MS data suggests that the products of microbial respiration Fe<sup>2+</sup> and S<sup>2-</sup> in a sterile solution does not enhance the removal of aqueous uranium in biomass suspension. Sterile samples with 10 µM U added show no difference amongst each other, however among the sterile samples with 50 µM U added, sulfide shows a greater change in U.

•The greater change in adsorption of uranium in the non-sterile vials relative to the sterile vials suggests that microbial activity may alter the organic composition of the biomass. Microbes consume and decompose the organic matter to survive and propagate.

•To confirm the removal of bacteria aqueous samples will be cultured, then extracted and sequenced of microbial DNA to compare to reducing microbes found at Rifle.

•To determine the biomass' effect, the experiment will be repeated in the absence of biomass.

## Acknowledgements

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