Functional Diversity in Oxic Sediments from the Hanford 200 West Area and Implications for Remediation – 15425

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ABSTRACT

Past plutonium enrichment and recovery processes at the Hanford site have resulted in sediments and groundwater contaminated with various mixtures of carbon tetrachloride, uranium, chromium, technetium-99, iodine-129, nitrate and tritium. Initial efforts to understand functional dynamics of the microbial community from sediment and groundwater samples recovered from beneath the 200-UP-1 Operable Unit will provide valuable insight that could be used in developing remedial strategies.

Core samples were taken from three depths (10, 30 and 50 ft.) below the water table for two extraction wells being drilled in the 200-UP-1 OU. Physical, geochemical and contaminant properties were analyzed on these Ringold formation sediments. Aerobic and anaerobic enrichments using a number of carbon and electron sources were performed. Metagenomes were developed targeting the16S rRNA gene to determine phylogenetic diversity. Functional diversity was determined using quantitative polymerase chain reaction (qPCR) and focused on aerobic heterotrophs, chemilithotrophs, nitrate reducers, iron reducers and sulfate reducers. Enrichments were also performed to establish stable communities exposed to contaminants such as iodine-129, technetium-99, nitrate and uranium.

Preliminary results indicate that cultivable microorganisms in these oxic sediments were primarily represented by aerobes, fermenters and denitrifying communities; however some iron reducing bacteria were present also. These results indicate that while oxic conditions predominate in these sediments samples, that facultative anaerobes with the functional capacity to biotransform radionuclide contaminants in the aquifer exist and would likely grow if carbon was added to the aquifer. Implications for findings related to biological remedies for groundwater beneath the 200 West Area will be discussed.

INTRODUCTION

Production of plutonium for nuclear weapons has led to an estimated release of 450 billion gallons of radioactive and hazardous waste to the subsurface at the Hanford site. Release of this waste has caused extensive vadose zone contamination as well as a groundwater plume that covers 150 square miles beneath the site. Groundwater contamination beneath the 200W Area represents a complex mixture of inorganic, radionuclide, and organic contaminants, primarily carbon tetrachloride, iodine-129, technetium-99, uranium, and nitrate (Figure 1). Average concentrations of contaminants in the groundwater are shown in Table 1. Major waste streams contributing to groundwater contamination in the 200-UP-1 Operable Unit (OU) were associated with plutonium separation and uranium recovery activities at the S and U Plant facilities where liquid effluent from these processes was disposed to the ground via ponds, cribs, ditches and trenches. Groundwater contamination in the 200-UP-1 OU also resulted from unplanned releases from single-shell tanks in Waste Management Area (WMA) S-SX and WMA U.

Local geology consists of approximately 554 feet of sediments consisting of the Hanford and Ringold formations, which are comprised of sand and gravel with some silt layers. Basalt of the Columbia River Basalt Group represents the lower level of the aquifer in the area. The water table in the 200-UP-1 OU averages approximately 270 ft below ground surface.

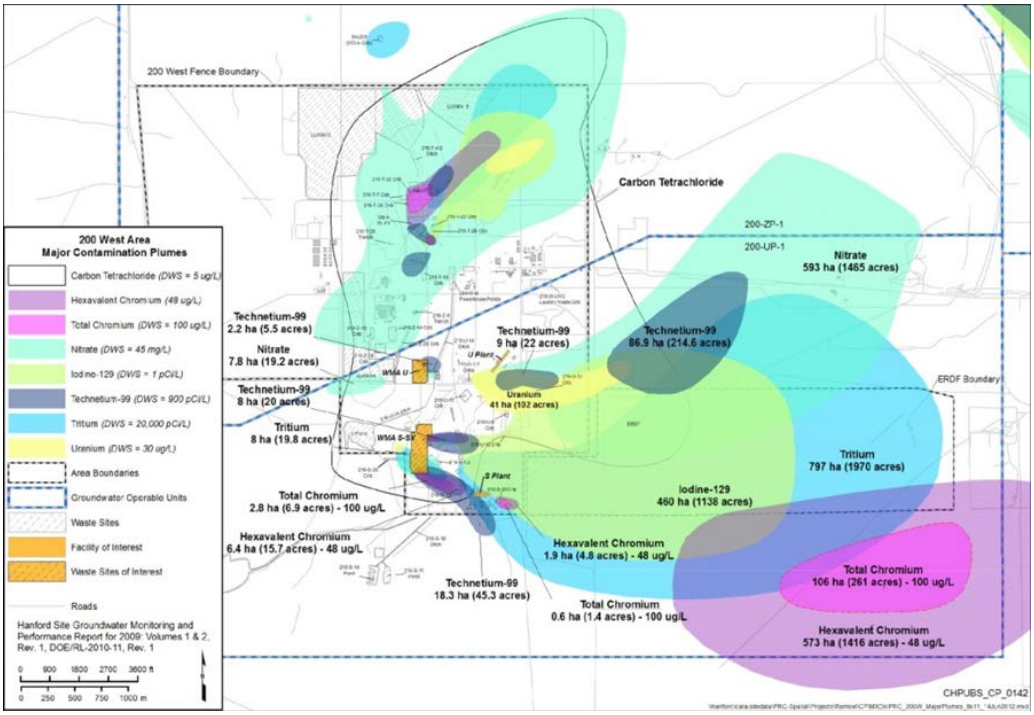

Figure 1. Schematic showing co-mingled contaminant plumes in groundwater beneath the 200-UP-1 and 200-ZP-1 Operable Units at the Hanford Site.

Table 1. Average concentrations of contaminants of concern in groundwater beneath the 200W Area at Hanford.

COC	Units	Concentration	Federal DWS
I-129	**pCi/L**	**3.5**	**1**
Tc-99	**pCi/L**	**4,150**	**900**
Tritium	**pCi/L**	**51,150**	**20,000**
Uranium	**µg/L**	**206**	**30**
Nitrate	**mg/L**	**133**	**45**
Total Chromium	**µg/L**	**99**	**100**
Hexavalent Chromium	**µg/L**	**52**	**No DWS**
Carbon tetrachloride	**µg/L**	**189**	**5**

The differing chemical nature of contaminants, along with the hydrologic and biogeochemical heterogeneity at the site, makes selection of *in situ* remedial options difficult. For this reason, pump and treat or hydraulic containment are currently the selected remedies for most contaminants listed in Table 1. When considering *in situ* remedial options, evaluation is typically performed on a contaminant-by-contaminant basis with no consideration of the competing interactions and reactions between co-mingled contaminants. However, it has thus far been demonstrated that contaminants such as nitrate can have detrimental effects on the performance of *in situ* stabilization approaches for uranium and technetium because amendments added for radionuclide reduction are used for nitrate reduction.

Therefore, it is of increasing importance to understand co-contaminant behavior in groundwater to remediate the remaining complex, recalcitrant co-mingled groundwater plumes and vadose zone source terms.

Work presented here represents the initial phases of characterizing hydrology and biogeochemical parameters associated with co-mingled contaminant plumes making up the 200-UP-1 OU. For the project as a whole, aquifer sediments will be characterized related to make up and function of the microbial community, contaminant extraction and desorption, and hydraulic conductivity and porosity. Work described will focus on characterization of the microbial community from sediments obtained during drilling of the first of two uranium extraction wells.

MATERIALS AND METHODS

Experiments were performed on split-spoon core samples obtained during drilling of a uranium extraction well in the 200-UP-1 OU (Figure 2). The water table at the site is located at a depth of approximately 270 ft below ground surface (BGS). Core samples were taken at 280, 300 and 320 ft BGS. Samples of groundwater were also taken at each of the sampling depths. Each sample was analyzed for geochemistry and was tested to determine the diversity and possible function of the microbial community related to contaminants of interest.

Figure 2. Split-spoon core samples containing aquifer sediments from 200-UP-1 OU.

Split-spoon and Water Sampling

Uranium extraction wells were drilled using an air rotary rig, followed by use of a cable tool to obtain the split spoon samples for analysis. Four six inch samples were taken consecutively and then put on ice and shipped to the laboratory.

Geochemical Analysis

Moisture Content

Gravimetric water contents of the sediment samples were determined using a PNNL procedure based on the American Society for Testing and Materials procedure "Standard Test Method for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass" (ASTM D2216-10 [ASTM 2010]). One representative sub-sample of 20 to 70 g of sediment was placed in pre-tared containers, weighed, and dried in an oven at 105 °C until constant weight was achieved. After at least 24 hours, the containers were removed from the oven, cooled, and weighed. At least two weighings were performed to ensure that all moisture was removed. All weighings were performed using a calibrated balance. A calibrated weight set was used to verify balance performance before weighing the samples. The gravimetric water content was computed as the percentage change in soil weight before and after oven drying.

1:1 Sediment:Water Extracts

The 1:1 sediment:deionized-water extracts were prepared by adding a weight of deionized water to approximately 60 to 150 g of sediment. The sum of the existing moisture (pore water) and the deionized water was fixed at the mass of the dry sediment. An appropriate amount of deionized water was added to screw cap jars containing the sediment samples. The jars were sealed and briefly shaken by hand, then placed on a mechanical orbital shaker for one hour. The supernatant was carefully decanted, filtered (passed through 0.45 µm membranes) and analyzed for conductivity, pH, anions, inductively coupled plasma mass spectrometry (ICP-MS), inductively coupled plasma optical emission spectrometry (ICP-OES), and alkalinity.

pH and Conductivity

Aliquots of the 1:1 sediment:water extract supernatants and groundwater were used for pH and conductivity measurements. The pH of the extracts was measured with a solid-state pH electrode and a pH meter calibrated with NIST traceable buffers (4, 7, 10 and/or 13). Electrical conductivity was measured and compared to NIST traceable potassium chloride standards with a range of 0.001 M to 1.0 M.

Anions

The 1:1 sediment:water extracts were analyzed for anions using a Dionex ICS 2500 ion chromatograph (IC). Fluoride, chloride, nitrite, bromide, nitrate, phosphate, and sulfate were separated on a Dionex AS17C column with a gradient elution of 1 mM to 35 mM potassium hydroxide and measured using a conductivity detector. This methodology is based on U.S. Environmental Protection Agency (EPA) Method 300.0A (EPA 1984) with the exception of using the gradient elution of potassium hydroxide.

Cations and Trace Metals

Major cation analysis was performed using a PerkinElmer 8300DV ICP-OES unit using high-purity calibration standards to generate calibration curves and verify continuing calibration during the analytical run. Multiple dilutions were made of each 1:1 water extract to investigate and correct for matrix interferences. This method is similar to EPA Method 6010B (EPA 2000b). The second instrument used to analyze technetium-99 and uranium-238 was a PerkinElmer ELAN DRC-II ICP-MS using a PNNL procedure similar to EPA Method 6020 (EPA 2000c).

Alkalinity

The alkalinity of the 1:1 sediment:water extracts and groundwater was measured using standard titration. This method uses 0.02N sulfuric acid to titrate to a pH endpoint of 4.5. The alkalinity procedure is equivalent to the U.S. Geological Survey (USGS) National Field Manual (USGS 2012) method.

Microbial Analysis

Cell numbers – Most probable number analysis (MPN)

Soil samples were treated with phosphate buffer and then diluted in media to determine relative numbers of bacteria present at each soil depth, using MPN technique. A gram of soil was added to 9 ml of phosphate buffer and then diluted in a 96-well microplate containing the media to enrich for microbes of interest. Microbes of interest included total heterotrophic bacteria, a number of anaerobic and

facultative anaerobic physiologies, including nitrate reducers, fermenters, iron reducers and sulfate reducers. In addition, aerobic ammonia and sulfur oxidizing bacteria were enumerated using this technique. Depending on the type of microbe being enumerated, plates were incubated over a period lasting a few days to a few months. Following incubation, the optical density (OD_{600}) was determined spectrophotometrically and bacterial numbers calculated from these readings.

Microbial diversity – 16S rRNA metagenomes

DNA was extracted from sediment samples using a MoBio Powersoil DNA Isolation Kit, and quantified using a NanoDrop spectrophotometer. DNA barcodes and linkers were added using PCR and then the resulting amplicons were sequenced at the Institute for Genomics and Systems Biology Next Generation Sequencing Core Facility at Argonne National Laboratory using an Illumina MiSeq instrument. Demultiplexing, quality filtering, and operational Taxonomic unit (OTU) picking were performed using the Quantitative Insights Into Microbial Ecology (QIIME) toolkit v. 1.8.0.

Enrichment and enumeration of mixed contaminant degrading microbial communities

In an initial experiment, aerobic and anaerobic enrichments were set up to determine the ability of aquifer bacteria to grow in R2A medium supplemented with mixtures of contaminants found in 200-UP-1 OU groundwater. Experiments were set up in 96-well microplates where glucose in R2A was used as a carbon source for growth and iodate, nitrate, and humic acid were added as potential electron acceptors. Anaerobic experiments were performed in GasPac containers to maintain anaerobic conditions. Samples were incubated for seven days prior to measuring OD_{600} on a spectrophotometer. A second experiment is being set up to enrich for bacteria able to grow in the presence of nitrate, uranium, technetium and iodine. In addition, sediment from each depth will be assayed with different carbon sources to determine the effects on differences in microbial community and associated contaminant biotransformation. Soil from samples taken from 280, 300 and 320 ft BGS were used as inoculum for each experiment.

PRELIMINARY RESULTS AND DISCUSSION

Mineralogy

In general, mineralogy of the samples changed with depth from high amounts of gravel with the void space filled with silt and sand in the sample from 280 ft BGS, to samples containing roughly half gravel and half sand and silt at 320 ft BGS. The color of the sediments at 280 ft BGS was a dark grayish brown, and as sampling depth increased, became an olive brown or olive gray color. Sediments at 280 and 300 ft BGS reacted strongly with HCl indicating the presence of carbonate minerals, while the sediment from 320 ft BGS reacted weakly showing lower amounts of carbonate minerals.

Microbiology

Functional group numbers

MPN experiments were performed to determine numbers of different functional groups of bacteria in sediments from a borehole being drilled to install uranium extraction wells. Cell density determined from this initial experiment can be seen in Table 2. Results from these experiments showed that while a functionally diverse group of bacteria exist in the sediments, that numbers able to grow under the conditions tested were relatively low. The only number that is surprising from this experiment is the

number of aerobic heterotrophs (Tryptic Soy Broth, Aerobic), but since higher numbers have been shown using other growth media, these results may be low because microbes from this environment don't grow well on TSB. Likewise, since these microbes come from a low carbon or an oligotrophic growth environment, a rich growth medium may have shocked the bacteria present.**Table 2.** Most probable number results for different function groups of bacteria enriched sediments taken from 280 ft BGS.

Enrichment	Cell Density (Cells/ml)
TSB Aerobic	9.3×10^3
TSB Anaerobic	2.3×10^3
Nitrate-reducing	3.6×10^2
Iron-reducing	9.2×10^2
Fermentative	3.6×10^2
Sulfate-reducing	7.4×10^2

Bacteria grown using alternate electron acceptors such as nitrate, ferric iron, sulfur or those growing under fermentative conditions would be expected to be low in this environment, since these sediments are considered oxic. These results are positive regarding the potential for biotransformation as a remedial option, since many bacteria in these functional groups have been shown to catalyze the reduction of waste constituents, such as carbon tetrachloride, nitrate, technetium and uranium.

Phylogenetic Diversity Additional insights into bioremediation potential at the site can be found by looking at the diversity of bacteria found in sediments and groundwater from the 200 Area. Figure 3 shows the phylogenetic diversity from middle and lower Ringold sediments. Diversity in these

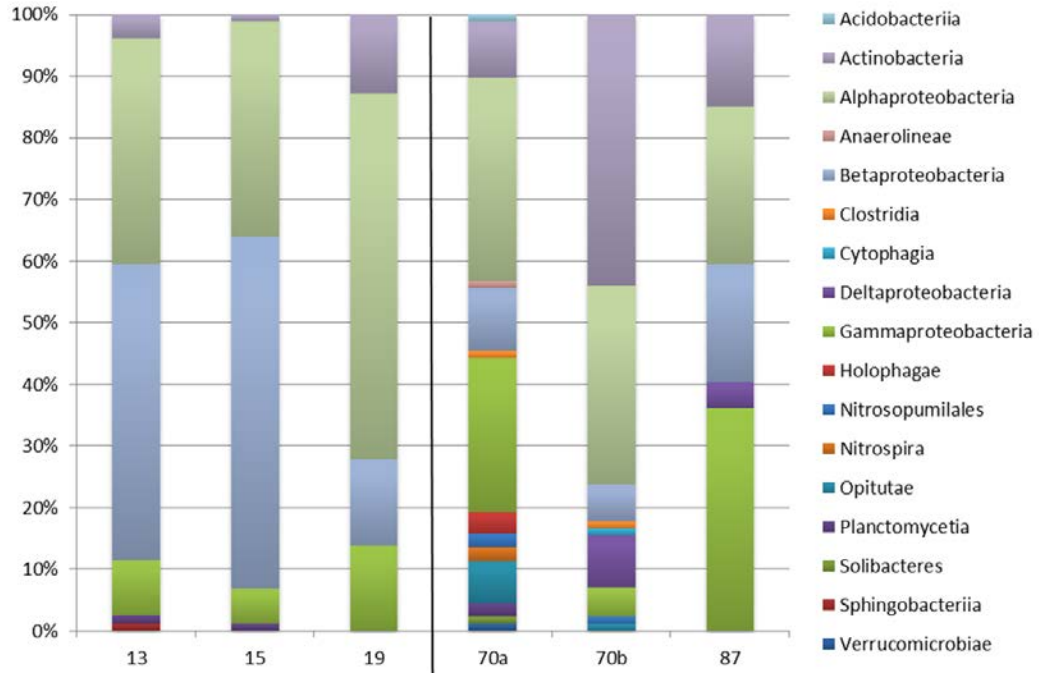

Figure 3. Bacterial diversity found in aquifer sediments obtained from the 200 Area aquifer.

experiments is dominated by bacteria from the phyla *Proteobacteria* and *Actinobacteria*. These results corroborate results from the functional analysis, since many bacteria know to biotransform metal and radionuclide contamination are found in the phylum *Proteobacteria*. Bacteria in this phylum include *Shewanella*, *Geobacter*, and *Anaeromyxobacter*, species found to reduce uranium and technetium, as well as nitrate. In addition, *Cellulomonas* species ES6 which has been shown to reduce hexavalent chromium and uranium is a member of the *Actinobacteria* phylum. Likewise, *Pseudomonas* and *Agrobacterium* are Proteobacterial species and can reduce nitrate, carbon tetrachloride and iodate found in the Hanford groundwater.

Consortia of bacteria are currently being grown under Hanford contaminant plume relevant conditions, in an effort to begin capitalizing on the diversity of bacteria found in the Hanford groundwater. Once these consortia are stable, microcosm and column experiments looking at biotransformation of mixed contaminants will be performed.

Growth in mixtures of Hanford contaminants

Initial experiments to look at the effect of contaminant mixtures on growth of microbes from sediments obtained as split-spoon samples from boreholes in the 200-UP-1 OU were set up. Sediment samples from 280, 300 and 320 ft BGS were incubated in the presence of contaminants such as nitrate and iodine, as well as natural organic matter (NOM) supplied as humic acid (HA). Incubation was performed at room temperature for 7 days. Figure 4 shows bacterial numbers found under aerobic (A) and anaerobic (B) conditions, and the mixtures of contaminants used.

Results indicate that bacteria from the different layers respond differently to the mixtures of contaminants. When growing in the presence of oxygen, bacteria from the 280 ft sampling interval appeared to be inhibited by the addition of humic acid when growing in the presence of iodate. Microbes from 300 and 320 ft BGS did not appear to be inhibited under these same exposure conditions, in fact microbes from 320 ft BGS appeared to grow better when humic acid was present. In the presence of iodide, microbes from 280 and 300 ft BGS appeared to be severely inhibited by the addition of humic acid, while the microbes from 320 ft BGS were not affected.

When tested under anaerobic conditions (Figure 4B), results were quite different than those seen under aerobic conditions. Bacteria from the 320 ft BGS sample interval appeared to grow the best under the various conditions tested. In general, bacteria grew better in the presence of iodate than iodide. Under anaerobic conditions, iodate, nitrate and humic acid can act as potential electron acceptors. As additional electron acceptors were added when iodate was present, more growth was noted for bacteria from the 320 ft BGS sediment sample as shown by the increase in cell density with each addition. Under the conditions tested, nitrate appeared to be a better electron acceptor for growth than humic acid. While the increase of bacteria in the samples appeared to be additive and correlated to addition of electron acceptors, it is not clear whether the same bacteria are growing to higher cell density or different bacteria are growing adding to the measured cell density. Growth of bacteria from sediment samples taken from the 280 ft BGS sample interval improved with the addition of humic acid and nitrate compared to iodate alone, but no difference was noted in cell density generated regardless of whether nitrate or humic acid was the electron acceptor. Bacteria from the 300 ft BGS sample interval grew the least when tested with iodate.

Less growth was noted when the bacteria were grown in the presence of iodide, but similar trends were noted in the 320 ft BGS sample when nitrate and humic acid were added. Unlike iodate, iodide would

not act as an alternate electron acceptor for the bacteria, which may justify the decrease in observed cell density.

These results indicate that there are bacteria present in the 200-UP-1 OU that may be capable of transforming the mixed contaminants in the groundwater. Additional experiments will be set up and transformation of the contaminants will be monitored.

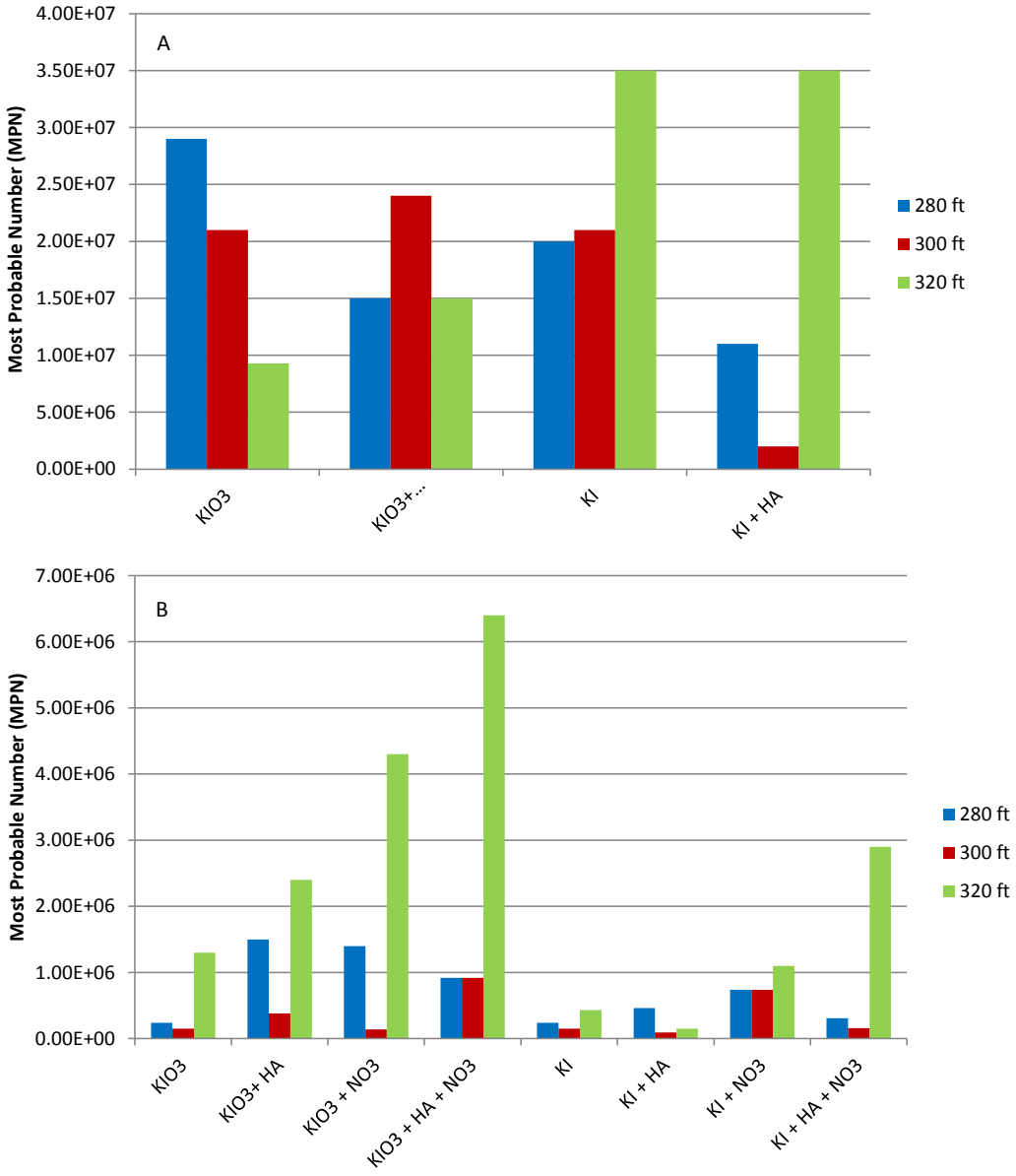

Figure 4. Comparison of aerobic (A) and anaerobic (B) bacterial counts from UP-1 operable unit soil samples enriched for 7 days in ½ R2A substituted with 0.2 µM KIO_3^-, 0.2 µM KI^-, 10 mg/mL humic acid, and 10 mM NO_3^-.

CONCLUSIONS

Preliminary results from these experiments shows that a phylogenetically and functionally diverse microbial community exists in contaminated aquifer sediments in the 200-UP-1 OU. This is important because it indicates potential for biotransformation for destruction and possible immobilization of contaminants.

Future experiments will be performed to continue to characterize these sediments for microbial potential, but will also be studied to determine uranium, technetium and iodine desorption and extraction profiles, as well as hydraulic conductivity and porosity. Additional studies will be performed to further characterize the microbial community as well as look at complex interaction between microbial populations and mixtures of contaminants.

REFERENCES

1. U.S. DOE. 2013. 200-UP-1 Groundwater Operable Unit Remedial Design/Remedial Action Work Plan. DOE/RL-2013-07.
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