Evaluating the Potential for Microbial Iodine Immobilization: Humic Acid Bioremediation – 14354

Brady Lee*, Hope Lee*, Danielle Saunders*, Kent Parker*
* Pacific Northwest National Laboratory

ABSTRACT

A dilute and laterally extensive iodine plume in groundwater beneath the 200 Area of the Hanford Site represents a complex remediation problem for the U.S. Department of Energy. Radioactive iodine (I-129) is found in two separate plumes in the 200 Area, covering 1,500 acres with I-129 concentrations of ~3.5 pCi/L. Dissolved organic matter and microbial activity may affect iodine speciation, which in turn will affect iodine mobility. The objective of the research was to determine the effect of humic additions on iodine speciation in the presence of aquifer microbial communities, and determine whether this may be a feasible approach for *in situ* treatment and immobilization of I-129. Traps constructed from polyvinyl chloride pipe were filled with Ringold sand or control materials (i.e., glass beads) as media for microbial attachment, and were incubated in groundwater monitoring wells affected by the iodine plume at the 200 Area. Traps were retrieved from three monitoring well locations after 50 days and an additional three monitoring wells after 150 days of incubation in the screened interval within the well. Sample substrates were analyzed directly for microbial diversity and activity; the remaining materials were used to conduct subsequent laboratory studies. The microbial community was analyzed using a range of molecular and physiological methods, including enrichment for iodide oxidizing bacteria and DNA extraction, to determine total bacterial diversity (community fingerprint) and quantify iodide oxidizing bacteria. In addition, substrate from the traps was used to run batch experiments, which received water containing potassium iodide (KI), potassium iodate, KI and humic acid, or potassium iodate and humic acid. Carbon was supplied to the system by adding 1% R2A (a common microbial growth medium). Iodine speciation will be performed on liquid and solid material from the batch experiments to determine ratios of inorganic and organic iodine species. Results from the experiments will be important in determining iodine biogeochemistry in iodine impacted groundwater and the effect of humic acid materials on speciation of iodine. Understanding iodine biogeochemistry will provide insight into mobility of I-129 in the Hanford subsurface.

INTRODUCTION

The U.S. Department of Energy (DOE) Office of Environmental Management (EM) manages the largest groundwater and soil cleanup in the world. Although DOE EM has made substantial progress in its cleanup mission, significant challenges remain. The difficult subsurface remediation problems that remain are some of the most complex encountered, including large quantities of residual contamination and extensive and challenging groundwater contamination. One particularly challenging issue is a dilute and laterally extensive plume of iodine in the Hanford 200 Area groundwater [1, 2]. DOE is implementing selected groundwater remedies in the 200-UP-1 Groundwater Operable Unit for cleanup or control of plumes of carbon tetrachloride, uranium, nitrate, chromium, iodine-129 (I-129), technetium-99 (Tc-99), and tritium.

Contamination in 200-UP-1 was associated with plutonium-separation and uranium recovery operations. The S and U Plant chemical separation and recovery processes produced chemical waste streams from process condensate, cooling water, and laboratory waste discharged to waste facilities. Mobile contaminants from these wastes migrated through the vadose zone soil and reached the groundwater, contaminating soil along the migration path and the groundwater. Figure 1 shows the mixed contaminant

plumes comingled in groundwater underlying the 200 West Area, and includes I-129.

Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) required remedial investigations (RIs) were conducted to assess potential risks to human health and the environment from contamination in the area. Initial phases of the RIs were used to characterize the nature and extent of chemical and radiological contamination and associated hydrogeologic conditions. The RIs determined that the contaminants dispersed in the groundwater and that remedial action alternatives are warranted.

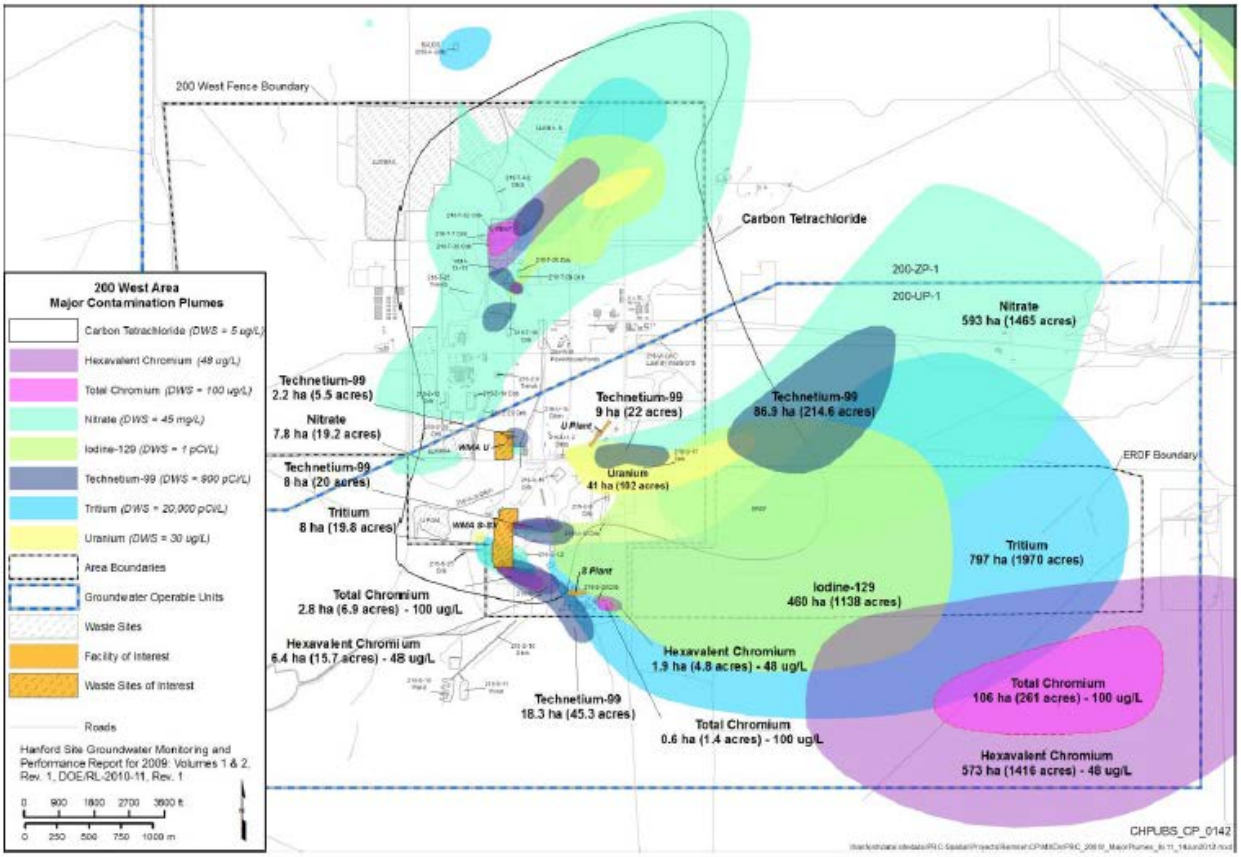

Figure 1. Major contaminant plumes in groundwater below 200 West Area on the Hanford Site (from [2]).

Pump and treat as an interim remedial action for TC-99 in groundwater was initiated in 1997 for 200-UP-1. Groundwater was extracted downgradient from the ditches, cribs, ponds and trenches in the U Plant area, treated at the Effluent Treatment Facility to remove contaminants, and then injected back into the aquifer outside the area of impact. Remedial action objectives were achieved in 2011, after treatment of 886 million liters of groundwater, removing 220 kg of uranium and 127 g Tc-99. Carbon tetrachloride and nitrate were also removed from the groundwater.

Hydraulic containment is the current planned remedial action for I-129 in the groundwater beneath 200-UP-1. While treatment technologies exist for achieving drinking water standards (DWS) for many of the contaminants in the 200-UP-1 groundwater, treatment technologies for I-129 are insufficient to reach the 1 pCi/L DWS. Treatment is complicated by the geochemistry of I-129 and total iodine in the site groundwater, which appears to be driven by the alkaline, oxygenic conditions present in groundwater

across the Hanford Site [3]. Speciation of iodine in the 200 Area groundwater shows that iodate is the prevalent form of iodine, representing an average of 70.6% of the iodine present (Zhang et al. 2013). Iodide and organo-iodine are present at 3.6% and 25.8%, respectively. Iodate representing the primary species of iodine is significant because, based on chemical thermodynamics, the dominant species should be iodide.

Studies have shown that dissolved organic carbon in groundwater will complex with iodate to form organo-iodine, which may affect overall mobility of iodine in groundwater [4, 5, 6]. In addition, microbes have been isolated that can oxidize iodide and reduce iodate, depending on redox conditions in the groundwater [7, 8, 9, 10]. Understanding the microbial component will be important in determining the mechanism of formation of the predominant iodine species at Hanford (iodate), which may be microbial, since thermodynamic determinations indicate iodide should be the predominant species. These studies will be important since little work has been done to evaluate the potential mechanisms for I-129 fate and transport at the Hanford Site. Based on current knowledge, I-129 speciation at the 200 West Area is similar to other DOE sites with similar co-contaminants, such as Savannah River National Laboratory. The objective of the research was to determine the effect of humic additions on iodine speciation in the presence of aquifer microbial communities, and determine whether this may be a feasible approach for *in situ* treatment and immobilization of I-129.

METHODS

Laboratory-scale research was performed to determine the effect of humic acid addition on iodine speciation with and without microbes indigenous to 200 Area groundwater. Research was accomplished by providing natural and synthetic substrates for attachment of groundwater microbes, followed by batch and column experiments to determine how humic acids influence iodate speciation and whether microbial communities also have a role in this process.

Microbial Recovery

Microbial traps were constructed using polyvinyl chloride (PVC) pipe (Figure 2). Each section of PVC pipe (3.5”L x 2”D) was perforated along the length with 1/8” slots to allow groundwater contact with the attachment media. Three types of attachment media were used in an effort to recover microbes from groundwater, glass beads, Middle Ringold sediments, and Lower Ringold sediments. Ringold sediments are thick, sedimentary geological sequences; the Lower Ringold is represented by silt and clay, while the Middle Ringold is oxidized sediments and coarse rock [11]. To prevent fine material from escaping from the column, Spectra/Mesh polypropylene filter sheets were placed over the internal surface of the traps prior to loading. Once the attachment media was added, caps were placed on the ends of the tubes and secured with screws.

Figure 2. Loaded microbial traps daisy-chained for deployment into monitoring well.

Traps were then lowered into the screened intervals of monitoring wells (~250 feet bgs) used to sample groundwater in the 200 Area. Monitoring well locations were selected in areas of the plume that would provide background, low, and high I-129 concentrations (Table 1). Traps were incubated in the wells for ~50 and 150 days to determine whether there was a temporal effect on establishment of microbes on the attachment media. Traps were removed from the well and transported on ice to the laboratory, where substrate materials were homogenized and subsamples were taken for iodine speciation, molecular analysis of the microbial community, and humic acid testing.

Iodine Speciation

Table 1. Well information from trap placement in iodine plume. Well ID numbers, trap time in well, and relative I-129 levels for experiment.

Deployment Date	Retrieval Date	Duration of Deployment (days)	Well ID	Level of I-129	Well ID	Screened Formation	Screen Interval Depth
4/5/2013	08/30/2013	146	299-W22-87	Background	C4977	Middle Ringold	250-285’
4/9/2013	09/06/2013	150	699-36-70B	Low (Perimeter)	C4399	Middle Ringold	264.2-299.1’
4/5/2013	08/29/2013	145	699-36-70A	High (In Plume)	A9901	Middle Ringold	257.5-287.7’
4/5/2013	5/23/2013	48	299-W14-19	Background	C3957	Middle Ringold	223.5-258.5'
4/5/2013	5/22/2013	47	299-W14-15	Low (Perimeter)	C3114	Middle Ringold	219.8-254.6’
4/9/2013	5/28/2013	49	299-W14-13	High (In Plume)	B8549	Middle Ringold	216.6-251.7’

Samples were sent to the Laboratory for Environmental and Oceanographic Research at Texas A&M University at Galveston for iodine speciation. Each of the three attachment media was homogenized and dried at 60 °C. Iodide, iodate and organo-iodine of both iodine isotopes (I-127 and I-129) were determined using gas chromatography mass spectrometry analysis [12, 13]. Aqueous iodide concentrations were determined after derivation with 4-iodo-N,N-dimethylanaline; iodate was determined by measuring the difference in iodide concentration after reduction with $Na_2S_2O_5$. Total iodine, organic and inorganic, was determined after conversion to iodate by combustion, followed by $Na_2S_2O_5$ reduction. Organo-iodine was calculated from difference between total iodine versus iodide and iodate species (inorganic iodine).

Molecular Characterization

DNA was extracted from samples of the packing material using a method modified from [14]. Samples were mixed with cetyl trimethyl ammonium bromide (CTAB) buffer (10% CTAB, 240 mM potassium phosphate, 300 mM NaCl) and an equal volume of phenol:chloroform:isoamyl alchohol (25:24:1) and agitated to lyse cells. Tubes were centrifuged, the aqueous phase was mixed with chloroform:isoamyl alcohol (24:1), and the tubes were centrifuged again. The aqueous phase was removed and mixed with 30% polyethylene glycol, incubated, and then centrifuged. DNA was ethanol precipitated and then dissolved in nuclease-free water.

The presence of Eubacteria and Archaea on the trap material was determined by amplification of the DNA with the Universal Eubacterial primers 8F and 907R, and the Archaeal primers 4F and 915R. Polymerase chain reaction (PCR) was performed using an Eppendorf Mastercycler ProS. Amplified DNA was then visualized using agarose gel electrophoresis.

Iodine Speciation: Humic Acid Effects

Batch experiments were set up to determine the effect of humic acid on iodine speciation in the presence and absence of bacteria. Iodine speciation was monitored in solution as well as on the surface of attachment media. Batch experiments were performed in 250 ml Erlenmeyer flasks that contained 10 g of attachment media from traps incubated in the monitoring wells, or for controls, the same substrates were

sterilized (not exposed to groundwater) and then added to flasks. Media from the traps was resuspended in millipure water and exposed to either 1) iodide or iodate, or 2) iodide or iodate plus 1% R2A and humic acid. Iodide and iodate was supplied at a concentration of 10 nM as potassium iodide or potassium iodate. Humic acid was supplied at a concentration of 0.1 g/L of water. Since dissolved organic carbon is low in the aquifer, R2A was added to supply carbon to stimulate biological processes in the experiments.

RESULTS AND DISCUSSION

Traps containing media for attachment of aquifer microorganisms were placed in monitoring wells within the iodine plume in the 200 Area on the Hanford Site. The primary purpose of these experiments was to allow attachment of bacteria that had been exposed to iodine and may be able to transform iodine, whether in the form of iodide or iodate. Due to thermodynamic stability, iodide was hypothesized to be the dominant iodine species in the Hanford groundwater; however, analysis demonstrated iodate was the predominant species in the groundwater [13]. While the alkaline and non-reducing conditions of the aquifer may help maintain the abundance of iodate, microbes may also be important in the oxidation of iodide to iodate. This finding is extremely significant since there are currently no proposed mechanisms for the majority of the iodine being in the form of iodate in the groundwater at the Hanford 200 West Area. To date there is little to no understanding of the fate or transport of I-129 in the subsurface; the current speciation and forms of I-129 presently in groundwater at the Hanford Site cannot be explained completely based on current knowledge of the geochemical or biological actions on iodine.

Iodine Speciation in Traps

The iodine speciation data in Table 2, total iodine, iodide and iodate, does not represent groundwater concentrations; rather, it represents iodine adsorbed to the surface of the attachment media/substrate. I-129 species in the trap media were below the detection limit for the analysis (2 pCi/L); therefore, all data reported is for I-127. Most of the iodine present was in the non-extractable form and is hypothesized to be iodine trapped in intra-granular spaces, bound to calcite or organo-iodine [3]. Since the amount of organic material in the Hanford aquifer is low, it is most likely that iodine is bound to calcite or trapped in intra-granular spaces, accounting for this non-extractable fraction. Of the ion exchangeable forms, iodate was the predominant species found on the solid material. The glass beads showed the least amount of total iodine adsorption. The most interesting result from this experiment was that total iodine in the rock fraction analyzed increased between days 50 and 150. Material extracted on approximately day 50 from the high and low iodine portions of the plume (13-14-R and 15-13-R) contained total iodine concentration of 0.04 and 0.61 µg/g, respectively. Rocky material from traps taken after nearly 150 days in the same concentration ranges (70B-24-R and 70A-21-R) contained 2.69 and 3.36 µg/g total iodine. There did not appear to be differences in the total amount of iodine absorbed over time in the other fractions tested (glass beads, clay, and sediment).

Bacterial attachment

A qualitative estimation of the biomass attached to the incubated materials was determined from the DNA extracted from bacteria attached to the different media types. Quantitation of bacteria on the attachment media was not determined because the goal of this phase of the research was to determine species diversity and the presence of bacteria that may be capable of iodine transformation. Amplifiable DNA was extracted from the traps that had been incubated in the wells for 50 days and 150 days, indicating that aquifer microorganisms were able to colonize the material that was added to the traps.

Table 2. Speciation of iodine found in trap samples that had been incubated in monitoring wells.

PNNL Biotrap samples	Total I-127 (µg/g)	Exchangeable - I-127 (µg/g)	(% TI)	Exchangeable - IO3-127 (µg/g)	t (% TI)	Non-extractable-I-127 (µg/g)	(% TI)
13-3-GB	0.01	1.39E-03	12.09	0.00E+00	0.00	0.01	87.91
13-14-S	0.25	1.72E-03	0.69	1.23E-02	4.93	0.24	94.38
13-14-R	0.04	7.08E-05	0.19	3.45E-03	9.08	0.03	90.73
15-4-GB	0.18	2.78E-04	0.16	5.04E-03	2.84	0.17	97.01
15-13-S	0.34	2.05E-03	0.60	1.82E-02	5.33	0.32	94.07
15-13-R	0.61	0.00E+00	0.00	3.30E-02	5.39	0.58	94.61
15-34-C	0.24	2.89E-03	1.19	6.34E-02	26.04	0.18	72.78
19-7-GC	0.01	3.21E-04	3.41	4.32E-04	4.60	0.01	91.99
19-15-S	0.39	1.07E-02	2.77	2.87E-02	7.42	0.35	89.82
19-15-R	0.01	2.35E-03	16.64	8.10E-03	57.41	0.00	25.95
19-35-C	0.30	2.50E-03	0.83	2.00E-02	6.65	0.28	92.52
70A-10-GB	0.01	0.00E+00	0.00	1.03E-03	9.53	0.01	90.47
70A-21-S	0.21	8.63E-04	0.42	6.55E-03	3.17	0.20	96.42
70A-21-R	3.36	0.00E+00	0.00	6.94E-03	0.21	3.35	99.79
70A-30-C	0.24	7.55E-04	0.31	2.42E-02	9.89	0.22	89.81
87-6-GB	0.06	2.92E-05	0.05	4.72E-04	0.76	0.06	99.20
87-17-S	0.40	3.66E-04	0.09	4.23E-03	1.06	0.40	98.85
87-17-R	0.10	0.00E+00	0.00	1.42E-03	1.37	0.10	98.63
87-33-C	0.18	4.35E-04	0.25	9.32E-03	5.27	0.17	94.48
70B-12-GB	0.03	2.50E-04	0.77	4.00E-03	12.32	0.03	86.91
70B-24-S	0.45	9.43E-04	0.21	9.37E-03	2.10	0.44	97.69
70B-24-R	2.69	0.00E+00	0.00	2.00E-02	0.74	2.67	99.26
70B-31-C	0.22	9.28E-04	0.43	2.29E-02	10.58	0.19	88.99

DNA was used to make clone libraries and to perform PCR for iodine oxidizing bacteria. Clone libraries will allow determination of the potential for bacterial transformation of iodine, which will affect speciation and mobility of the iodine in the groundwater.

Humic Acid Augmentation

Batch experiments were run using the different attachment media from the traps. Each of the three media types was added to water that had been amended with 10 nM potassium iodide or potassium iodate. To determine the effect of organic material and bacteria on speciation of iodine, a second set of experiments was set up similarly, but these flasks also received R2A as a nutrient source and humic acid as a source of organic material. Low humic acid concentrations were used in an effort to achieve a process that is sustainable in the field. Adding a carbon source that stimulates microbial growth may also affect speciation of iodine in the experiments. These studies could provide valuable data on the effect of carbon additions to the subsurface. These types of amendments are often overdesigned and lead to downgradient issues within groundwater plumes. Evaluating how small concentrations of carbon may affect immobilization of iodine is therefore critical for implementing effective and sustainable future remediation strategies.

Experiments were set up and microbial growth, humic content, and iodine speciation was analyzed. After one week of incubation, the flasks were sacrificed and the liquid separated from the solids, and iodine species was determined. Iodine speciation in solution and on the surface of the attachment media was determined to see whether changes in speciation between iodide, iodate, and organo-iodine affect sorption of the iodine to solid surfaces. It is expected that the Middle and Lower Ringold material will be more reactive to adsorption than the glass beads, ands likely more representative of *in situ* conditions. The glass beads were included in these studies to ensure retrieval of microbial biomass from the impacted groundwater plume, as they have been historically used in molecular and microbial studies. Glass beads will also allow microbial attachment without biases caused by reactive surfaces on the Middle and Lower Ringold materials.

It is hypothesized that humic acid addition affects speciation through the formation of organo-iodine species, and associated mobility of iodine in the Hanford subsurface. In addition, humic material may stimulate microbial activity that may affect iodine speciation. Microbes have been shown to degrade humic material, which produces aromatics that can stimulate microbial oxidation/reduction of iodine. Likewise, aromatic monomers produced by this activity may complex with iodine forming organo-iodine species. These experiments will lead to future experiments using columns as well as more in-depth analysis of how speciation affects specific adsorption to aquifer solids, and how speciation is affected by microbial transformation of iodine.

CONCLUSIONS

PVC traps filled with attachment media representing Hanford geological media and a synthetic attachment media (glass beads) were incubated in iodine contaminated groundwater. Material that was exposed to the groundwater for nearly five months yielded more DNA than traps incubated for less than two months. Ion-extractable iodine species present in the traps appeared to be dominated by iodate, which is consistent with species found in the groundwater. Much of the iodine in the traps, however, was not ion extractable, and thus was likely inaccessible or bound to calcite in the groundwater.

This on-going effort will provide the critically needed understanding of the impacts of microbial activity on the fate of iodine including speciation and sorption with Hanford sediments. This knowledge will enable resolving critical data gaps that currently limit the ability to accurately predict the mobility and enable

development and deployment of *in situ* remediation strategies for iodine through evaluation and demonstration of biological immobilization/degradation.

REFERENCES

1. U.S. Department of Energy (2009). Remedial Investigation/Feasibility Study for the 200-UP-1 Groundwater Operable Unit. DOE/RL-2009-122.

2. U.S. Department of Energy (2010). Proposed Plan for Remediation of the 200-UP-1 Groundwater Operable Unit. DOE/RL-2010-005.

3. Santschi, P. H., C. Xu, S. Zhang, Y.-F. Ho, H.-P. Li, K. A. Schwehr, D. I. Kaplan (2012). Laboratory report on iodine (^{129}I and ^{127}I) speciation, transformation and mobility in Hanford groundwater, suspended particles and sediments. SRNL-STI-2012-00592.

4. Shimamoto, Y. S., Y. Takahashi, Y. Terada. (2011). "Formation of Organic Iodine Supplied as Iodide in a Soil−Water System in Chiba, Japan." Environ. Sci. Technol. **45**(6): 2086-2092.

5. Xu, C., E. J. Miller, S. Zhang, H.-P. Li, Y.-F. Ho, K. A. Schwehr, D. I. Kaplan, S. Otosaka, K. A. Roberts, R. Brinkmeyer, C. M. Yeager and P. H. Santschi (2011). "Sequestration and Remobilization of Radioiodine (129I) by Soil Organic Matter and Possible Consequences of the Remedial Action at Savannah River Site." Environ. Sci. Technol. **45**(23): 9975-9983.

6. Yamaguchi, N., M. Nakano, R. Takamatsu and H. Tanida (2010). "Inorganic iodine incorporation into soil organic matter: evidence from iodine K-edge X-ray absorption near-edge structure." J. Environ. Radioactivity **101**(6): 451-457.

7. Zhao, D., C.-P. Lim, K. Miyanaga and Y. Tanji (2013). "Iodine from bacterial iodide oxidization by Roseovarius spp. inhibits the growth of other bacteria." Appl. Microbiol. Biotechnol. **97**(5): 2173-2182.

8. Arakawa, Y., Y. Akiyama, H. Furukawa, W. Suda and S. Amachi (2012). "Growth Stimulation of Iodide-Oxidizing α-Proteobacteria in Iodide-Rich Environments." Microbial Ecol. **63**(3): 522-531.

9. Amachi, S., Y. Muramatsu, Y. Akiyama, K. Miyazaki, S. Yoshiki, S. Hanada, Y. Kamagata, T. Ban-nai, H. Shinoyama and T. Fujii (2005). "Isolation of Iodide-Oxidizing Bacteria from Iodide-Rich Natural Gas Brines and Seawaters." Microbial Ecol. **49**(4): 547-557.

10. Fuse, H., H. Inoue, K. Murakami, O. Takimura and Y. Yamaoka (2003). "Production of free and organic iodine by Roseovarius spp." FEMS Microbiol. Lett. **229**(2): 189-194.

11. U.S. Department of Energy (2010). Hanford Site Groundwater Monitoring and Performance Report for 2009: Volumes 1 & 2. DOE/RL-2010-11.

12. Zhang, S., K. A. Schwehr, Y. F. Ho, C. Xu, K. A. Roberts, D. I. Kaplan, R. Brinkmeyer, C. M. Yeager and P. H. Santschi (2010). "A Novel Approach for the Simultaneous Determination of Iodide, Iodate and Organo-Iodide for 127I and 129I in Environmental Samples Using Gas Chromatography−Mass Spectrometry." Environ. Sci. Technol. **44**(23): 9042-9048.

13. Zhang, S., X. Chen, D. Creeley, Y.-F. Ho, H.-P. Li, R. Grandbois, K. A. Schwehr, D. I. Kaplan, C. M. Yeager, D. Wellmen, P. H. Santschi. (2013). "Iodine-129 and Iodine-127 Speciation in Groundwater at the Hanford Site, U.S.: Iodate Incorporation into Calcite." Environ. Sci. Technol. **47**(17): 9635-9642.

14. Druhan, J. L., M. Bill, H. Lim, C. Wu, M. E. Conrad, K. H. Williams, D. J. DePaolo and E. L. Brodie (2014). "A large column analog experiment of stable isotope variations during reactive transport: II. Carbon mass balance, microbial community structure and predation." Geochim. Cosmochim. Acta **124**(0): 394-409.

ACKNOWLEDGEMENTS

The authors wish to express appreciation to Dr. Peter Santschi and others in the Laboratory for Environmental and Oceanographic Research at Texas A&M University at Galveston for iodine speciation in attachment media from the traps. Funding for this work was provided by the U.S. Department of Energy Office of Environmental Management and Richland Operations Office. The Pacific Northwest National Laboratory is operated by Battelle Memorial Institute for the Department of Energy (DOE) under Contract DE-AC05-76RL01830.