Humic Acid and Microbial Redox Influence on Iodine Speciation at the 200 West Hanford Site, WA – 15282

Joshua Ellis *, Erin Moser **, Elsa Cordova *, Brady Lee *, M.Hope Lee *
* Pacific Northwest National Laboratory (USA)
** University of Michigan (USA)

ABSTRACT

Radioactive iodine-129 (^{129}I), a by-product of nuclear fission, is one of the key risk-drivers due to its long half-life, mobility, and hazardous potential to humans through bioaccumulation in the thyroid gland. Understanding the mechanisms and contributors to iodine speciation is important in order to develop effective remediation strategies for contaminated areas. The effects of microbial communities and humic acid on iodine speciation were explored using Lower Ringold sediment from the Hanford Site 200 West Area which contained varying concentrations of anthropogenic ^{129}I contamination: background, low, and high in conjunction with varying growth media constituents. The sediment was used in a series of batch studies with two commercially available humic acid stock solutions, including one that was deployed at the Savannah River Site for remediation. Various assays, molecular techniques, and microbial isolations were subsequently performed. Several isolates obtained from these batch studies have been shown to reduce over 80% of iodate present in growth media when nitrate was present. No iodate reduction was observed in the absence of nitrate. Analyses are underway to quantify the effect of humic acid and microbial interaction on iodine speciation along with characterization of microbial isolates. However, results have already demonstrated the coupled reduction of iodate and nitrate from Lower Ringold sediment microbial isolates, offering potential remediation strategies for bioremediation of ^{129}I. Further understanding of how microbial and humic acid interactions speciate ^{129}I will enable development and deployment of engineered strategies for iodine remediation at Hanford, as well as other contaminated sites such as SRNL.

INTRODUCTION

Little is known about the biogeochemical cycling and speciation of radioactive iodine (^{129}I) due to the complexity of microbial influences and interactions with organics in the environment. ^{129}I is of environmental concern due to its long half-life (1.6×10^7 years), toxicity, and mobility in the environment [1]. While iodine is a necessary micronutrient for thyroid hormone production in humans, one of its many radioisotopes, ^{129}I, a by-product of nuclear fission, is thought to have potential toxicity through bioaccumulation in the food chain and in the thyroid gland of humans, leading to thyroid cancer [1, 2]. Treatment in groundwater is complicated by the biogeochemistry 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]. Currently, effective remediation strategies to mitigate ^{129}I in the groundwater to below federal drinking water standards (<1 pCi/L) is unidentified [4].

The 200 West Area of the Hanford Site, WA (USA), contains two separate plumes covering 1,500 acres where ^{129}I concentrations are ~3.5 pCi/L [5]. Speciation analysis shows that iodate comprises 70.6% of the iodine present, and organo-iodide and iodide comprise 25.8% and 3.6% respectively [4]. Iodate, iodine in 5+ oxidation state, is a thermodynamically stable species of iodine. However iodide, iodine in the 1- oxidation state [1], has been shown to be the dominant iodine species in many marine surface waters [6, 7], contrariwise to the findings in the Hanford Site groundwater [4]. Microbial redox activity within these iodine plumes may affect iodine speciation, consequently affecting iodine mobility within the subsurface and the potential options available for bioremediation strategies. Furthermore, there is potential that organic acids such as humic materials present in sediment could catalyze these redox reactions, lending themselves

as carbon sources to microbes in subsurface environments as well as participating in abiotic redox reactions. Humic acids have been well studied for their ability to participate in redox reactions with elements in soil [8], and have been shown to act as an electron shuttle between microorganisms and oxidized minerals [8, 9].

If the biogeochemical cycling and mobility of iodine is facilitated by humic acids and microbial influences, then exploration of these relationships has the potential to provide effective ^{129}I bioremediation strategies. The purpose of this study was to explore the influence of humic acids and microbial populations on iodine speciation.

MATERIALS AND METHODS

Batch Experiments

Lower Ringold sediment collected in traps incubated in monitoring wells was used in two batch microcosms implemented with two week incubation periods. One batch study contained sediment incubated in traps for 50 days while the other used sediment incubated for 150 days. Experiments were conducted at room temperate and were shaken at 125 rpm. The sediment was exposed to high levels of 129-I (average of 27.25 pCi/L) in the 200 West Hanford site plume, and was added in a 10% weight to volume ratio per flask (4g/40ml). Controls were set up with sediment collected that was not incubated in situ and thus had no iodine exposure. Stock solutions of potassium iodate and potassium iodide were prepared at a final concentration of 50 µg/mL. Commercially available stock humic acid was added to four of the solutions at concentrations of 10 mg/L. Additionally, 1/10 R2A growth medium was added to two solutions (containing humic acid) to stimulate microbial growth. R2A medium contained (liter^{-1}): enzymatic digest of casein (0.25g), proteose peptone (0.25g), acid hydrolysate of casein (0.5g), yeast extract (0.5g), dextrose (0.5g), solube starch (0.5g), K_2HPO_4 (0.3g), $MgSO_4 \cdot 7H_2O$ (0.05g), and $C_3H_3NaO_3$ (0.3g). Humic material from SRNL was also used in this study to compare varying humic material (Table 1). Initially two samples were taken: one 10 ml liquid sample of each flask, taken immediately upon integration of sediment and solution, was filtered and sent off for iodine speciation at ORNL; and 1ml of unfiltered liquid was combined with 50% glycerol are stored at -80 °C for archiving. Flasks were incubated on a shaker table at 23 °C (room temperature) for two weeks. At two weeks, 10 ml samples were again removed from flasks and filtered for iodine speciation analysis.

Table 1. Batch experimental design for Lower Ringold sediments incubated in high levels of I-129.

	Control	High I-129 exposed samples	
		50 day	150 day
Iodide	1	1	1
Iodate	1	1	1
Iodide + HA	1	1	1
Iodate + HA	1	1	1
Iodide + 1/10 R2A + HA	1	1	1
Iodate + 1/10 R2A + HA	1	1	1
Iodide + SV	1	1	NC
Iodate + SV	1	1	NC
Iodide + HA + SV	1	1	NC
Iodate + HA + SV	1	1	NC
Iodide + 1/10 R2A + HA + SV	1	1	NC
Iodate + 1/10 R2A + HA + SV	1	1	NC

* HA: humic acid; SV: Savannah River humic acid; NC: not completed

Microbial Isolation

Several bacterial strains were isolated by plating enriched soil material on 1/10 R2A supplemented with 10 mg/L humic acid, 10 mM sodium nitrate, 0.2 µM potassium iodate, and 900 µg/mL cyclohexamide. Incubations were carried out at 23°C and individual colonies were subsequently streaked for isolation four times to ensure isolation of individual bacterial strains.

16s rRNA Sequencing and Phylogenetic Analysis

16S rRNA was amplified by colony PCR using the bacterial consensus primers 8F and 1492R. Colony PCR was performed with an initial denaturation at 95°C for 5 min (to lyse cells and extract DNA) followed by 30 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 2 min, followed by a final 10 min extension at 72°C. The PCR mixture, generated using *Taq* PCR Core Kit (www.qiagen.com), contained a small amount of bacterial colony material (template), 1 µL of each primer (25mM), 5 µL of 10x PCR buffer, 1 µL of BSA (15mg/mL), 1 µL of dNTP's (10mM each), 0.25 µL *Taq* DNA polymerase (5U/ µL), in a final reaction volume of 50 µL. PCR products were purified with Qiagen's QIAquick PCR Purification Kit according to the manufactures instructions and sequenced by a 3130XL DNA sequencer using primers 8F, 341F, 907R, and 1492R, and assembled accordingly using BioEdit. The obtained 16S rRNA sequence was subjected to BLAST search to determine 16S rRNA similarities with sequences deposited into GenBank. The retrieved sequences were aligned by using the ClustalW function within MEGA 6. A phylogenetic tree was constructed based on the distance matrix data obtained with the Nearest-Neighbor-Interchange heuristic method. Robustness of the tree topology was evaluated by bootstrap resampling analysis with 1000 bootstraps and applying maximum-likelihood analysis using MEGA 6 [10].

Iodate Reduction and Analytical Techniques

All incubations were carried out at 25°C in the absence of light throughout this study. 1/2R2A with 200µM iodate was used to subculture isolates. The growth medium used for iodate reduction was a minimal medium and contained (liter^{-1}): KH_2PO_4 (0.14g), $MgCl_2 \cdot 6H_2O$ (0.20g), $CaCl_2 \cdot 2H_2O$ (0.15g), $Na_2SO_4 \cdot 10H_2O$ (0.14), $NaHCO_3$ (0.5g), ATCC vitamin supplement (1.0 ml), ATCC trace mineral supplement (1.0 ml), bacto-tryptone (1.0g) , NaCl (1.5g), 10 mM $NaNO_3$, and 200 µM KIO_3. The pH was adjusted to 8.0 prior to autoclaving. To detect iodate reducing capabilities, isolates were grown aerobically overnight, harvested during log phase, washed twice with Phosphate Buffered Saline (PBS) and diluted to an OD_{600} of 0.2 (corresponding to 0.14 mg protein ml^{-1}) and inoculated at 1%. Iodate reduction was conduction under anaerobic conditions and were supplemented with 10 mM lactate as electron donor and sparged with O_2 free N_2 for 10 min after inoculation to generate anoxic conditions.

Total iodine from batch experiments was conducted as previously described [11]. Iodate concentrations were determined colorimetrically according to the method by Amachi et al. (2007). Briefly, 40 µl of 2% (wt/vol) sulfamic acid and 20 µl of 2N HCl were added to 400 µl of clarified supernatant, mixed by vortexing, and incubated for 5 min at room temperature. Subsequently, 400 µl of 300mM potassium iodide was added to yield triiodide (I_3^-), followed by 400 µl of 0.1% (wt/vol) soluble starch to yield a purple iodine-starch complex, which was immediately measured at 525 nm [12]. Nitrate concentrations were determined by measuring the OD_{220} of clarified cellular supernatant as previously described [13, 14].

RESULTS AND DISCUSSION

In general, when analyzing total iodine data from Figure 1 and Figure 2, it is not conclusive how humic material affects iodine. Very little differences in total iodine were shown between time initial and time final,

particularly when compared to control samples. However, there is a general trend where total iodine appears to decrease when microbial populations are enriched in the presence of R2A medium. Currently, iodine speciation is underway which will allow us to understandi the affect humics and microbial physiotypes have on the speciation of total iodine. However, analysis of total iodine at this time does not allow us to determine conclusive effects of microbes and humic material on iodine species. Data from iodine speciation will determine what redox reactions occur under the experimental conditions.

Twenty-nine bacterial species have been isolated from Lower Ringold sediments incubated in the high ^{129}I contaminated plume. Analysis of pure culture isolate DNA did not show multiple 16 rRNA gene sequences indicating successful isolation of a single species Figure 3 shows the phylogenetic relationship of our isolates to other characterized organisms in the NCBI BLAST database. The majority of organisms isolated where highly related to *Pseudomonas* sp., however there were isolates similar to *Arthrobacter*, and *Variovorax*, among others (Figure 3).

Several isolates were analyzed for their ability to reduce iodate. Previous findings within our lab group suggest that iodate reduction occurs in a coupled reduction with nitrate, where periplasmic bound nitrate reductases reduce both iodate and nitrate in a coupled manner. There are few reports describing the bacterial reduction of iodate. Anaerobic reduction of iodate by *S. onedensis* MR-4 [15], and washed cells of *D. desulfuricans* and *S. putrefaciens* [1] have been previously described. Tsunogai and Sase (1969) initially described the importance of nitrate reducing bacteria in iodate reduction, and originally discovered multiple aerobic bacteria that could reduce iodate in the presence of nitrate [16]. Amachi et al. (2007) described an iodate-reductase in the periplasm of *Pseudomonas stutzeri* SCT, which was induced by the presence of iodate [17]. Here, several strains were found to reduce iodate under anaerobic conditions and in the presence of nitrate (Table 2).

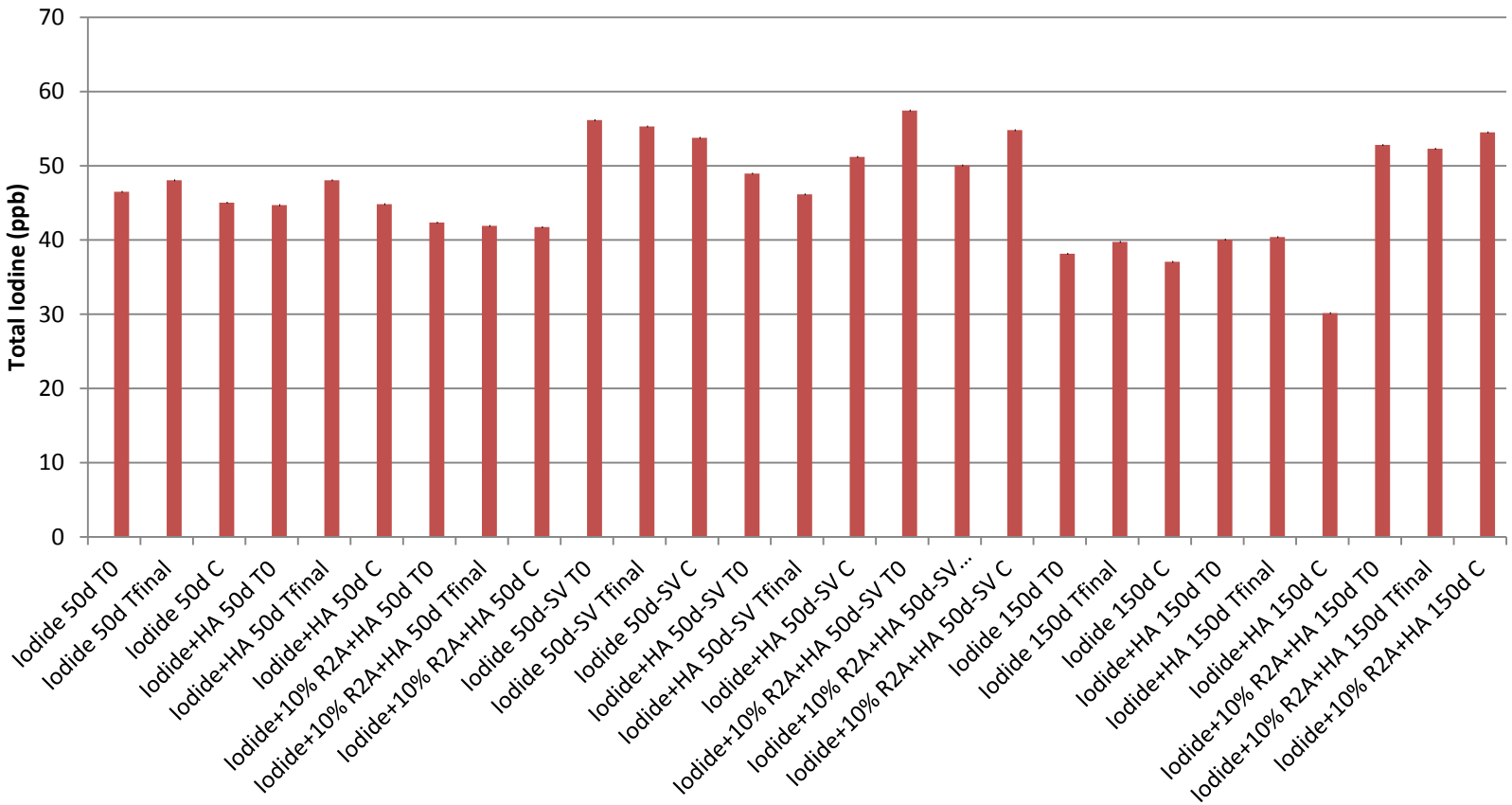

Figure 1. Total iodine (µg/L) for iodide batch experiments. HA: humic acid; SV: Savannah River humic acid.

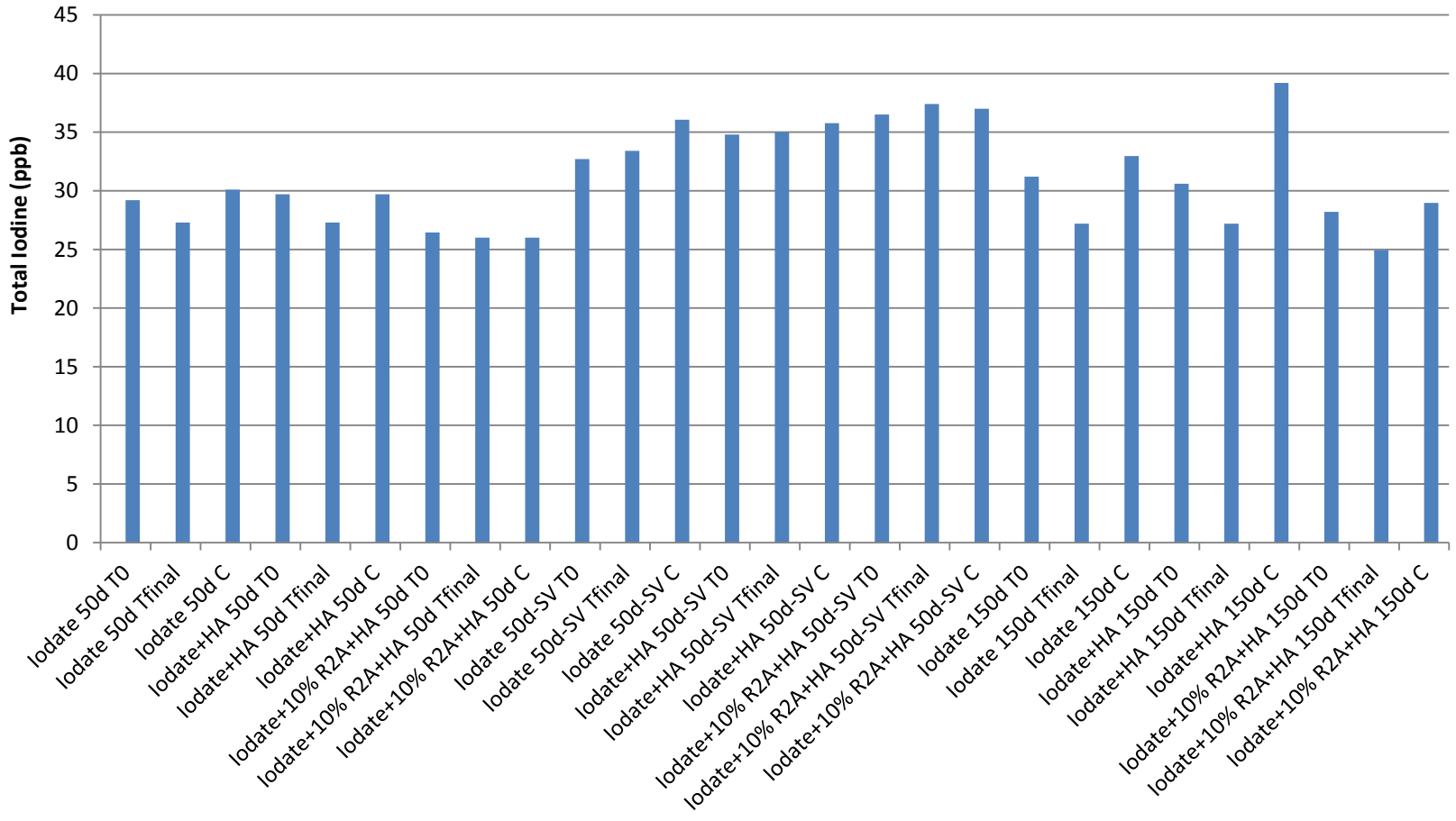

Figure 2. Total iodine (µg/L) for iodate batch experiments. HA: humic acid; SV: Savannah River humic acid.

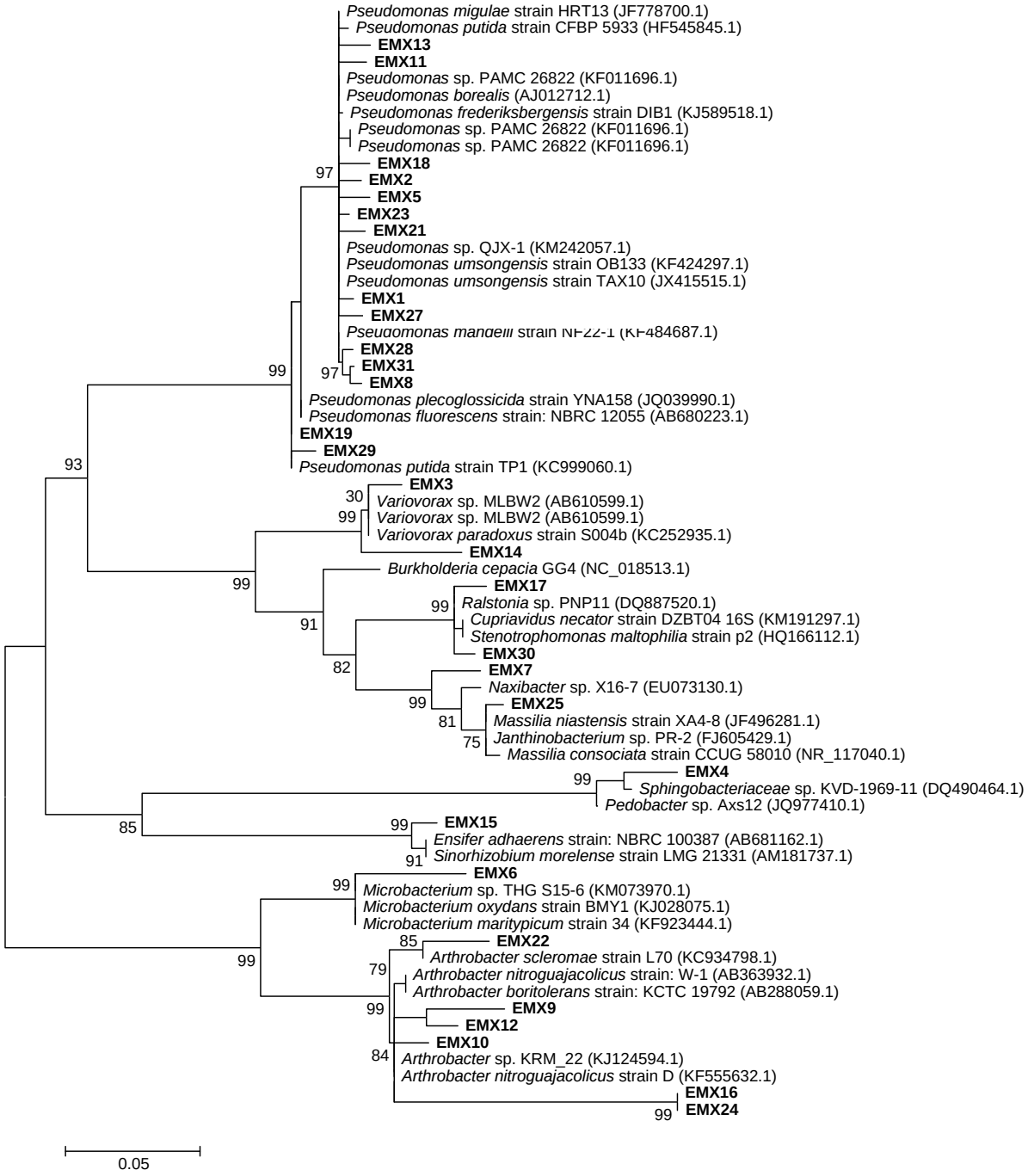

Figure 3. Phylogenetic tree illustrating the evolutionary position of isolates obtained from Lower Ringold sediments enriched in 1/10 R2A media supplemented with iodine and humic acids. Sequences are based on partial 16S rRNA sequencing. Scale bar corresponds to 5 substitutions per 100 nucleotides. Accession numbers are shown in parentheses.

Table 2. Iodate and nitrate reduction analysis by isolated microbes from Lower Ringold sediments.

Isolate	Affiliated Genera	Percentage of KIO_3^- reduced	Percentage of NO_3^- reduced
Cell free control		0	0
5	*Pseudomonas*	9.58	85.57
7	*Naxibacter*	63.33	87.11
10	*Arthrobacter*	62.08	83.51
13	*Pseudomonas*	60.21	85.57
17	*Ralstonia*	61.88	85.57
18	*Pseudomonas*	60.21	83.51
21	*Pseudomonas*	6.88	85.57
23	*Pseudomonas*	58.75	84.54
27	*Pseudomonas*	59.17	84.02
31	*Pseudomonas*	57.92	84.54

CONCLUSIONS

Based on the initial batch studies using Lower Ringold sediments along with a variety of supplementations show that total iodine was not influenced by the presence of humics, however iodine speciation may have been affected (analyses in progress). Humic materials could serve as a carbon source for microbes in subsurface environments and potentially harnessed for reduction power in the process of nitrate/iodate reduction. Results of iodate/nitrate assays depicted several isolates capable of reducing iodate and nitrate. These data could be significant in developing strategies for in situ and ex situ bioremediation strategies.

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