Journal of Hazardous Materials 350 (2018) 19–26

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

Iodate and nitrate transformation by *Agrobacterium/Rhizobium* related strain DVZ35 isolated from contaminated Hanford groundwater

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ARTICLE INFO

Keywords:
Hanford
Iodate reduction
Agrobacterium
Radiobacter
Biotransformation

ABSTRACT

Nitrate and radioiodine (^{129}I) contamination is widespread in groundwater underneath the Central Plateau of the Hanford Site. ^{129}I, a byproduct of nuclear fission, is of concern due to a 15.7 million year half-life, and toxicity. The Hanford 200 West Area contains plumes covering 4.3 km^2 with average ^{129}I concentrations of 3.5 pCi/L. Iodate accounts for 70.6% of the iodine present and organo-iodine and iodide make up 25.8% and 3.6%, respectively. Nitrate plumes encompassing the ^{129}I plumes have a surface area of 16 km^2 averaging 130 mg/L. A nitrate and iodate reducing bacterium closely related to *Agrobacterium*, strain DVZ35, was isolated from sediment incubated in a ^{129}I plume. Iodate removal efficiency was 36.3% in transition cultures, and 47.8% in anaerobic cultures. Nitrate (10 mM) was also reduced in the microcosm. When nitrate was spiked into the microcosms, iodate removal efficiency was 84.0% and 69.2% in transition and anaerobic cultures, respectively. Iodate reduction was lacking when nitrate was absent from the growth medium. These data indicate there is simultaneous reduction of nitrate and iodate by DVZ35, and iodate is reduced to iodide. Results provide the scientific basis for combined nitrogen and iodine cycling throughout the Hanford Site.

1. Introduction

From 1944 to 1987, plutonium production and processing at the Hanford Site (Washington State, USA) generated liquid waste containing a variety of hazardous constituents including carbon tetrachloride, nitrate, and chromium, and radionuclides, such as uranium, technetium-99 (^{99}Tc) and iodine-129 (^{129}I). These waste streams were disposed of in single-shell tanks, as well as pits, cribs, and trenches. Leaking tanks and direct disposal into the subsurface has resulted in the majority of comingled contaminants found in Hanford groundwater [1]. Nitrate and radioiodine account for some of the larger plumes in groundwater underlying the Central Plateau at the site where concentrations regularly exceed drinking water standards (DWS) (Table 1). Radioiodine is of particular environmental interest, due to a long half-life, mobility in groundwater and toxicity [2]. Nitrate is a common co-contaminant with ^{129}I and is typically present at mg/L concentrations, while the ^{129}I is in the μg/L concentration range. For these reasons, effects of nitrate on iodine transformation must be understood.

Groundwater monitoring wells east of Waste Management Areas T and TX-TY within the 200-ZP-1 Operable Unit (OU) have shown ^{129}I concentrations exceeding the 1 pCi/L DWS; in some wells ^{129}I activity was greater than 20 pCi/L. Interestingly, significant stable iodine (^{127}I) is also found in groundwater from this area, and concentrations are nearly 300 times higher than ^{129}I [3]. Speciation from the ^{127}I analysis showed that based on data from 7 monitoring wells, iodate comprises 70.6% (range 45.2–84.3%) of the iodine present, and organo-iodine (range 14.6–39.8%) and iodide (range 0.5–15%) comprise 25.8% and 3.6%, respectively. Radioiodine is thought to have a similar species distribution, but when the analyses were performed, due to detection limits for the method, only iodate ($^{129}IO_3^-$) was detected. Iodate, iodine in 5^+ oxidation state, is a thermodynamically stable species of iodine. However, iodide-iodine in the 1^- oxidation state [2] has been shown to be the dominant iodine species in many groundwater and marine surface waters [4,5], contrary to the findings in the Hanford Site groundwater [1].

While microbial activity is known to affect nitrate in subsurface environments through assimilatory and dissimilatory denitrification processes, very few accounts of iodate reduction have been published. Iodate can be used as an alternate electron acceptor for growth of microorganisms, and is converted to iodide under anaerobic or microaerobic conditions. Amachi et al. [6] demonstrated the dissimilatory iodate reduction by *Pseudomonas* sp. SCT, isolated from marine

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https://doi.org/10.1016/j.jhazmat.2018.02.006
Received 20 August 2017; Received in revised form 17 January 2018; Accepted 3 February 2018
Available online 06 February 2018
0304-3894/

Table 1
Nitrate and ^{129}I concentrations in groundwater across Hanford's Central Plateau.

Groundwater Operable Unit	Nitrate (mg/L)	^{129}I (pCi/L/μg/L)	^{127}I(μg/L)
200-ZP-1	2740	22.5/0.14	30.1
200-UP-1	409	11.2/0.07	11.2
200-BP-5	1,700	6.8/0.04	7.7
200-PO-1	158	9.98/0.06	ND[a]
Drinking Water Standard	45	1.0/0.006	

[a] [3], ND – ^{127}I concentration has not been determined for 200-PO-1.

sediment slurry, in the presence of nitrate under anaerobic conditions. During these experiments, cells pre-grown without iodate did not reduce it, nor could they reduce iodate aerobically [6]. The iodate-reducing enzyme, which was called an iodate reductase, was found in the periplasmic space, which is located between the inner and outer membranes of this bacterium.

Tsunogai and Sase [7] reported several marine laboratory strains of nitrate-reducing bacteria were able to reduce iodate aerobically, concluding that iodate is reduced in a coupled mechanism by nitrate reductases. Other studies have shown microbial reduction of iodate with anaerobic cell suspensions of *Desulfovibrio desulfuricans* and marine bacterium *Shewanella putrefaciens* [2]. In addition to these two bacterial species being able to directly reduce iodate to iodine, soluble ferrous iron, sulfide, and iron monosulfide produced by the metabolism of these microbes, were shown to abiotically reduce iodate to iodide. Likewise, *S. putrefaciens* strain MR-4 was shown to reduce iodate to iodide [8]. Reduction of IO_3^- has also been demonstrated by the perchlorate respiring bacterium strain GR1 [9]. An enzyme that was isolated from the periplasm of this bacterium was shown to reduce perchlorate, chlorate, nitrate, iodate, and bromate. In marine environments, phototrophs, such as diatoms and algae, have been shown to reduce iodate, these organisms are not likely to be present in groundwater in the deep aquifers beneath the Central Plateau at Hanford [10–13].

Comingled contaminant plumes in heterogeneous subsurface environments such as those at the Hanford Site represent a remedial challenge due to the competing processes driven by microbial and chemical thermodynamics. We hypothesize that microbes indigenous to the Hanford subsurface are capable of transforming both nitrate and iodate. In this study, a bacterial isolate closely related to *Agrobacterium* and *Radiobacter* species was enriched from Hanford groundwater, characterized physiologically and studied for the ability to reduce both nitrate and iodate under anaerobic and during transition from microaerobic to anaerobic conditions.

2. Materials and methods

2.1. Enrichment of sediment-trap material

Sediment-traps constructed from polyvinyl chloride pipe were filled with Unit 5 Upper Coarse sediments from the Ringold Formation as substrate for microbial attachment. Traps were incubated for 50 days in a groundwater monitoring well (299-W14-13) within a comingled nitrate and ^{129}I plume within the 200 ZP-1 Operable Unit of the 200 West Area (Fig. 1). Sediment-traps were deployed at a depth interval of 242 ft below ground surface in groundwater where nitrate and ^{129}I concentrations of 2740 mg/L and 22.5 pCi/L, respectively, have been recorded. Following retrieval from the monitoring well, substrate from the traps was enriched under oxic conditions at 25 °C (room temperature) in 50-ml 1/2R2A media supplemented with 7.23 mM potassium iodate at pH 8.0, and shaken at 125 rpm. 1/2R2A medium contained (liter^{-1}): Proteose peptone (0.25 g), casamino acids (0.25 g), yeast extract (0.25 g), dextrose (0.25 g), soluble starch (0.25 g), K_2HPO_4 (0.15 g), $MgSO_4{\cdot}7H_2O$ (25 mg), and $C_3H_3NaO_3$ (0.15 g). Cycloheximide was added at a concentration of 90 μg/ml in an effort to inhibit fungal growth on the initial enrichment media. Enrichments were cultivated for 4 weeks and subsequently streaked 3 times to ensure bacterial isolation. Elevated iodate was used for enrichment to assure iodine exposure in the culture medium using non-inhibitory species.

2.2. Cultivation

All incubations were carried out at 25 °C in the absence of light throughout this study. 1/2R2A media with 200 μM iodate was used to subculture DVZ35. The growth medium used for iodate reduction was a minimal medium and contained (liter^{-1}): KH_2PO_4 (0.14 g), $MgCl_2{\cdot}6H_2O$ (0.20 g), $CaCl_2{\cdot}2H_2O$ (0.15 g), $Na_2SO_4{\cdot}10H_2O$ (0.14 g), $NaHCO_3$ (0.5 g), ATCC vitamin supplement (1.0 ml), ATCC trace mineral supplement (1.0 ml), bacto-tryptone (1.0 g), NaCl (1.5 g), 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%. Transition cultures (i.e., cultures started under micro-aerobic conditions and transitioning to anaerobic conditions) were supplemented with 25 mM lactate as the electron donor, and the culture headspace was flushed with oxygen free nitrogen gas for 3 min after inoculation to remove residual oxygen. Anaerobic cultures were supplemented with 10 mM lactate as electron donor and the media was sparged with O_2 free N_2 for 10 min after inoculation to generate anoxic growth conditions. More lactate was added to the transition cultures to assure that sufficient electron donor would be available following removal of oxygen from the test vials. Negative controls for all experiments contained sparged media, DVZ35 inoculum, 0.2 mM iodate, and 10 mM nitrate, but no lactate was added.

Iodate reduction experiments were also conducted where nitrate was consistently spiked into the growth media to maintain 10 mM nitrate once it was depleted by approximately 50% to determine if further iodate reduction ensued. Experiments were conducted with 25 mM lactate for transition and 10 mM lactate for anaerobic conditions as the electron acceptor for nitrate and iodate reduction.

Experimental conditions used for testing iodate reduction by DVZ35 were repeated using two other species of *Agrobacterium* (currently designated as *Rhizobium*), *Agrobacterium radiobacter* ATCC 19358 (isolated from saprobic soil) and *Rhizobium radiobacter* ATCC 23308 (isolated from crown gall of apple seedling). These experiments were performed to determine whether other species similar to DVZ35 exhibit iodate reduction capabilities.

2.3. Growth characterization

Temperature and pH growth optima was determined by inoculating 1% mid-log phase growth into aerobic ½ R2A over a temperature range of 4 °C–37 °C and a pH range of 3–11. Growth was determined spectrophotometrically by determining the optical density at a wavelength of 600 nm (OD_{600}). Substrate spectrum was analyzed by transferring 1.0% mid-log phase growth into minimal medium containing lactate (10 mM), acetate (10 mM), glucose (1%), H_2 (10% H_2: 10% CO_2:90% N_2), succinate (10 mM), cellobiose (0.2%), cellulose (0.2%), xylan (0.75%), xylose (1%), or ethanol (10 mM). All substrate growth experiments contained 10 mM nitrate and 0.2 mM iodate to provide a representative comparison. The ability of DVZ35 to respire on a variety of electron acceptors was analyzed by transferring 1.0% mid-log phase growth into minimal medium. To determine the ability of DVZ35 to respire on certain compounds, 25 mM lactate was used throughout as the electron donor. Nitrate (10 mM), nitrate:iodate (10 mM:200 μM), iodate (200 μM), iron(III), fumarate (10 mM), chromium (10 mM), and sulfate (10 mM) were all analyzed. Electron acceptor experiments were conducted under anoxic conditions, and media with no electron acceptor added was used as the negative control. Due to the varying