Research in Microbiology 166 (2015) 419–427

www.elsevier.com/locate/resmic

Original article

The effect of uranium on bacterial viability and cell surface morphology using atomic force microscopy in the presence of bicarbonate ions

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Received 8 October 2014; accepted 16 March 2015
Available online 2 April 2015

Abstract

Past disposal practices at nuclear production facilities have led to the release of liquid waste into the environment creating multiple radionuclide plumes. Microorganisms are known for the ability to interact with radionuclides and impact their mobility in soils and sediments. Gram-positive *Arthrobacter* sp. are one of the most common bacterial groups in soils and are found in large numbers in subsurface environments contaminated with radionuclides. This study experimentally analyzed changes on the bacteria surface at the nanoscale level after uranium exposure and evaluated the effect of aqueous bicarbonate ions on U(VI) toxicity of a low uranium-tolerant *Arthrobacter oxydans* strain G968 by investigating changes in adhesion forces and cell dimensions via atomic force microscopy (AFM). Experiments were extended to assess cell viability by the Live/Dead BacLight Bacterial Viability Kit (Molecular Probes) and quantitatively illustrate the effect of uranium exposure in the presence of varying concentrations of bicarbonate ions. AFM and viability studies showed that samples containing bicarbonate were able to withstand uranium toxicity and remained viable. Samples containing no bicarbonate exhibited deformed surfaces and a low height profile, which, in conjunction with viability studies, indicated that the cells were not viable.

Keywords: *Arthrobacter* sp.; Aqueous bicarbonate; Toxicity; Atomic force microscopy; Live/dead analysis

1. Introduction

Uranium is one of the most abundant actinide elements found in the environment and is a key contaminant of concern at many US Department of Energy sites, serving a leading role in the nation's defense for over 50 years. Uranium contamination of soil and groundwater is of great environmental concern due to the toxicological properties of the uranyl species. The behavior of uranium and its mobility in the subsurface is affected by various factors such as chemistry of the pore water, groundwater and soil minerals, presence of complex-forming ligands and micro-organisms that thrive under these conditions. Uranium exists in a number of valence states, but under oxidizing conditions, it dominates as a highly soluble and stable uranyl ion, UO_2^{2+}. In neutral or basic pH conditions, uranium undergoes hydrolysis in aqueous solutions and can readily complex with a wide variety of ligands such as carbonate and phosphate. These complexation reactions often result in the formation of mobile aqueous species or precipitation of U-bearing minerals.

Dissolved inorganic carbon present in soil and groundwater is one of the primary factors controlling uranium aqueous speciation. In oxidized conditions at a pH > 4, uranyl ions interact with carbonate, creating strong neutral and negative soluble anionic complexes such as $UO_2CO_3^0$, $UO_2(CO_3)_2^{2-}$, and $UO_2(CO_3)_3^{4-}$ that dominate aqueous speciation of U(VI) [1].

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http://dx.doi.org/10.1016/j.resmic.2015.03.003
0923-2508/

In Ca-rich soil, calcium-uranyl-carbonate complexes, $Ca_2UO_2(CO_3)^0_3(aq)$ and $CaUO_2(CO_3)^{2-}_3$, became the predominant forms of dissolved U(VI) in circumneutral and alkaline pH conditions [2,3]. These uranyl carbonate and calcium uranyl carbonate complexes were identified in contaminated pore water at the Hanford Site, Washington State and have been shown to inhibit the microbial reduction of U(VI) under specific conditions [2,4].

Microbial impact on processes that govern the fate and transport of contaminants in soils and sediments is well established. According to several studies, *Arthrobacter* sp. are considered to be ubiquitous and predominant members of culturable soil microbial communities; they are found in large numbers in Hanford soil as well as other subsurface environments contaminated with heavy metals and radioactive materials [5–9]. *Arthrobacter* sp. are aerobic, chemo-organotrophic, Gram-positive bacteria characterized by a rod-to-coccus morphology change as they enter the stationary phase. These microorganisms are able to survive and reproduce in oligotrophic conditions in the presence of minimal concentrations of organic content [7,8]. Microbial interaction with toxic metals and radionuclides in aqueous environments is well known and several studies have given insight into uranium tolerance of these microorganisms [10,11]. In a previous assessment, *Arthrobacter oxydans* strain G975 was found to be the fastest-growing and the most uranium-tolerant strain among the studied microorganisms obtained from the Subsurface Microbial Culture Collection (SMCC) [12]. In contrast with G975, *A. oxydans* strain G968 was found to have low resistance to U(VI) toxicity. The alteration in surface morphology for strain G968, with a reduction in cell size and distorted surfaces, was noted at 0.5 mg/L of U(VI); in comparison, strain G975 showed signs of cell inhibition at the much higher concentration of 19 mg/L of U(VI) [13]. Inhibition of bacterial activity was associated with the binding of U(VI) to the cell envelope. The passive binding of uranyl to cell surfaces reduces the fluidity of the cell membrane, limiting nutrient uptake [14]. However, uranyl–carbonate complexes formed in the solution in the presence of bicarbonate do not strongly interact with the negatively charged bacterial surface, which in turn can mitigate U(VI) toxicity on the cells [13,14].

Uranium-bacteria interactions were rigorously studied to understand the microbial effects that influence the mobility of U(VI) in groundwater, as well as bacterial viability in the presence of U(VI) [15,16]; however, the mitigation effect of bicarbonate on the viability of cells exposed to U(VI) has not yet been evaluated. The main focus of this investigation was to qualitatively and quantitatively characterize changes on the bacteria surface at the nanoscale level after uranium exposure and evaluate the effect of aqueous bicarbonate on U(VI) toxicity of *A. oxydans* strain G968 by analyzing changes in adhesion forces and cell dimensions via profile plots. In addition, cell viability was assessed by the Live/Dead BacLight Bacterial Viability Kit (Molecular Probes) to quantitatively illustrate the effect of varying concentrations of bicarbonate ions on viability of bacterial cells exposed to uranium. Culturability of *A. oxydans* strain G968 was also determined following exposure to uranium.

2. Materials and methods

2.1. Cell growth and atomic force microscopy (AFM) analysis on bacteria–uranium interactions

2.1.1. Arthrobacter strain growth culture conditions

A. oxydans strain G968 previously isolated from Hanford Site soil was obtained from the Subsurface Microbial Culture Collection (SMCC) maintained at Alabama State University. Detailed descriptions of site geology, sample collection procedures, isolation and identification are described in detail in [5,7]. Bacterial cells of *A. oxydans* strain G968 were grown in a 5% peptone, tryptone, yeast and glucose (PTYG) liquid culture media, consisting of 0.25 g/L peptone, 0.25 g/L tryptone, 0.5 g/L yeast extract, 0.5 g/L glucose, 0.6 g/L $MgSO_4 \cdot 7H_2O$ and 0.07 g/L $CaCl_2 \cdot 2H_2O$. The media was prepared in deionized water (DIW) (Barnstead NANOpure Diamond Life Science (UV/UF), Thermo Scientific), autoclaved at 121 °C and 15 psi for 15 min, then allowed to cool before being used. Cells of *A. oxydans* strain G968 were aerobically grown in stoppered 50 mL polypropylene centrifuge tubes amended with 10 mL of 5% PTYG media and kept in an incubator/shaker at 29 °C. Bacterial cells were harvested in the late logarithmic phase of growth, washed and experiments were initiated by adding log 7 cells/mL of a bacterial culture to the sterile synthetic groundwater (SGW) solution amended with uranium (Table 1). Prior to inoculation, cell stock suspensions were vortexed for 10 s and counted using an INCYTO C-Chip disposable hemocytometer to determine the number of cells/mL in the suspension, needed to calculate the desired cell concentration in the tubes. Cell densities were also calculated in all samples at the end of the experiment after 24 h exposure to U(VI).

2.1.2. Sample preparation for AFM imaging

The carbon and phosphorus-free synthetic groundwater (SGW) solution prepared in deionized water contained 5.22 mg/L of KCl and 520.58 mg/L of hydroxyethyl piperazine ethanesulfonic acid (HEPES) with the pH of the solution measured as 7.2. The SGW solution was autoclaved at 121 °C, 15 psi for 15 min and allowed to cool to about 30 °C. Sterile SGW was equally distributed between three 250 mL bottles and aseptically amended to contain 0 mM, 5 mM and 10 mM of $KHCO_3$ (Table 1). Each of the three individual bicarbonate SGW solutions was filter-sterilized (0.2 μm) and kept refrigerated until the time of use. The composition of sterile bicarbonate and uranium amended SGW is presented in Table 1.

After 24 h, the 5 mL aliquot of bacterial samples kept at 29 °C in an incubator/shaker were centrifuged and pellets were washed three times with deionized water to remove U(VI) and media residuals and 10 μL of a concentrated sample was immobilized onto the 3-aminopropyltrimethoxysilane-coated silicon wafer AFM substrates. Bacterial cells must firmly adhere to a silicon wafer substrate so that the sample is