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The effect of bacterial growth phase and culture concentration on U(VI) removal from aqueous solution

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ABSTRACT

Bacteria play a key role in controlling the mobility of contaminants, such as uranium (U), in the environment. Uranium could be sourced from disposed radioactive waste, derived either from surface disposal trenches for Low Level Waste (LLW) that, because of the waste type and disposal concept, would typically present acidic conditions or from the geological disposal of LLW or Intermediate Level Waste (ILW) that, because of the waste type and the disposal concept, would typically present alkaline conditions. In disposed radioactive waste, there could be variable amounts of cellulosic material. Bacterial cells may be living in a range of different growth phases, depending on the growth conditions and nutrients available at the time any waste-derived U migrated to the cells. A key knowledge gap to date has been the lack of a mechanistic understanding of how bacterial growth phases (exponential, stationary, and death phase) affect the ability of bacteria to remove U(VI) from solution. To address this, we first characterised the cells using potentiometric titrations to detect any differences in proton binding to proton active sites on *Pseudomonas putida* cells at each growth phase under aerobic conditions, or under anaerobic conditions favourable to U(IV) reoxidation. We then conducted batch U(VI) removal experiments with bacteria at each phase suspended in 1 and 10 ppm U aqueous solutions with the pH adjusted from 2 to 12 as well as with culture concentrations from 0.01 to 10 g/L, to identify the minimal concentration of bacteria in solution necessary to affect U removal. We found that, in death phase, *P. putida* cells exhibited double the concentration of proton active sites than bacteria grown to exponential and stationary phase. However, we did not see a difference in the extent of U(VI) removal, from a 10 ppm U solution, between the different growth phases as a function of pH (2 to 12). Culture concentration affected U removal between pH 2–8, where U removal decreased with a decreasing concentration of cells in solution. When the pH was 10–12, ≤55% of U precipitated abiotically. The presence of bacteria in solution (0.01–10 g/L), regardless of growth phase, increased the precipitation of U from ≤55% up to 70–90%, accumulating inside the cells and on the cell walls as ~0.2 μm uranyl phosphate precipitates. These precipitates were also found at low pH with the exception of cells at exponential growth phase. This study demonstrates that growth phase affects the proton-active site concentration but not the extent of U bound to *P. putida* cells and that growth phase dictates the form of U removed from solution. Since the pH of trench-disposed LLW is controlled by the degradation of cellulosic waste, leading to acidic conditions (pH 4–6), bacterial concentrations would be expected to highly affect the extent of U removed from solution. The cement in grouted ILW and LLW, for geologic disposal, will allow for the development of extremely high pH values in solution (pH 9–13), where even the smallest concentrations of bacteria were able to significantly increase the removal of U from solution under aerobic conditions, or under anaerobic conditions favourable to U (IV) reoxidation.

1. Introduction

Radionuclides can be released into the environment via the degradation of radioactive waste disposal containers and the evolution of their associated wasteform. The release of radionuclides, such as uranium (U), from low- and intermediate-level waste (LLW and ILW, respectively) containers will occur under different pH environments, depending on the means and location of disposal. Historically, disposal

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of LLW was made to trenches at the Low Level Waste Repository (LLWR) in the UK, where the combination of the corrosion of metal containers and the degradation of cellulosic wastes has led to mildly acidic pH conditions (Cummings and Raaz, 2011). Current and future disposal of grouted LLW and plans for future geologic disposal of ILW, which will be grouted and backfilled with cement, would allow for the development of very alkaline solution (pH 9–13; Francis et al., 2004; Cummings and Raaz, 2011). LLW and ILW may be in an aerobic environment during on-site storage, the operation phase, and the early stages of post-closure, and therefore it is important to understand how U will behave under both pH regimes under aerobic conditions. Additionally, under geologic disposal conditions which contain bicarbonate, along with Fe or Mn, U(IV) may be reoxidized to U(VI) in anaerobic solutions (Wan et al., 2005). Outside the geochemically disturbed near-field zone there will be sharp geochemical gradient to the pre-existing natural conditions of the host rock, and thereby a need to understand the behaviour of radionuclides across of range of geochemical conditions.

Bacteria are ubiquitous in the environment, persisting in rock bodies beneath the earth's surface (Pedersen and Ekendahl, 1990), and would therefore be inevitably found in the near-field environment of radioactive waste disposal sites as well as within the sites as introduced via geologic disposal facility (GDF) construction, operation, and from the waste itself. Due to the variability of waste materials disposed of in LLW and ILW, the concentration of cellulosic material available to be solubilised would also be variable (Bourbon and Toulhoat, 1996). Aqueous organic matter dissolved from the waste would be a nutrient source for the bacteria, acting as a carbon source for bacterial growth and therefore bacterial growth phase may vary throughout a disposal site. Depending on the influx of nutrients to the near-field environment of the GDF, bacterial populations may be living in a variety of growth phases when waste-derived U migrates from the disposal site and interacts with the evolving natural system. The growth phases that the bacteria may be undergoing are variable: exponential growth, stationary phase, death phase, where stationary or death phase may persist in aqueous environments for long periods of time if unperturbed. Microorganisms undergo varying physiological processes resulting in various exudates and cell wall protein expression as a function of changing growth phase. Currently there is little information available concerning how the various growth phases may affect the ability of bacteria to adsorb or precipitate uranium across the pH ranges relevant to radioactive waste disposal.

In bacteria-free systems, U would tend to adsorb to rocks and minerals below pH 9. Above pH 9, U would tend to precipitate abiotically, as a sodium uranium mineral, in solutions rich in NaCl (e.g. Bots et al., 2014; Kenney et al., 2017), but it is unclear what role bacteria will play in such higher pH environments as could be associated with some radioactive waste disposal concepts. Previous studies have shown that bacteria undergo varying physiological processes during growth, which can result in various exudates and cell wall protein expression as a function of changing growth phases (e.g. Gad et al., 2004; Azam et al., 1999; Janssen et al., 2000; Maassen et al., 2004; Lalonde et al., 2008; Rolfe et al., 2012; Liu et al., 2015; Snider et al., 2016; Liu et al., 2016). Limited information is available concerning how bacterial surface properties change as a function of growth phase, and the information that is available shows that the site density of functional groups on the cell surface or associated with bound capsular extracellular polymeric substance (EPS) may be higher at exponential phase or death phase, depending on whether the cells are Gram-positive or Gram-negative. Daughney et al. (2001) performed surface complexation modelling on the cells of *Bacillus subtilis*, a Gram-positive bacterium, during exponential and stationary phase to determine the acidity constants of the sites available for binding and the concentration of those sites on the bacterial cell surface. They found that exponential phase bacteria had higher acidity constants and site concentrations than those at stationary phase. The exponential phase bacteria in that study, having more sites available for metal-binding, removed more Cd and Fe from solution than the stationary phase. Liu et al. (2015) studied the effect of growth phase on the surface properties of *Synechococcus* cyanobacterium, a Gram-negative bacterium, and found little differences between acidity constants derived from modelling the titrations of the cells, but did find that cells in death phase had significantly higher concentrations of EPS associated with the cell surface that was produced at death phase. An increase in EPS produced during bacterial growth may lead to increased sites available for immobilising metals, such as U, however several studies have not seen a difference in the proton active sites when comparing cells with and without their EPS removed (Ueshima et al., 2008; Kenney and Fein, 2011). Increased EPS may also increase the propensity for bacteria to adhere to a rock surface and thereby reduce its environmental mobility (Hong et al., 2013).

The adsorption of aqueous U(VI) onto bacterial cells has been examined in detail on bacteria in stationary growth phase and at pH values < 9 (Fowle et al., 2000; Gorman-Lewis et al., 2005; Sheng and Fein, 2013; Alessi et al., 2014). Bacteria under those conditions remove nearly 100% of U at circumneutral pH values and > 20% removal at pH values as low as pH 1.5 (Fowle et al., 2000; Gorman-Lewis et al., 2005). Gorman-Lewis et al. (2005) also noticed that U adsorption decreased with decreasing concentration of cells in solution. At higher pH values associated with cementitious LLW and ILW disposal (pH > 9), we predict that U would precipitate abiotically, as has been seen by Bots et al., 2014 and Kenney et al., 2017, but it is unclear whether bacteria would enhance or inhibit precipitation at those pH values.

The aim of this study was to understand how growth phase and culture concentration affect the ability of bacterial cells to remove uranium from solution. To achieve this, we first studied how growth phase affected the surface properties of the bacterium *Pseudomonas putida*, a microbe found both in soils and in the subsurface. This was done by using surface complexation modelling of potentiometric titrations to determine the acidity constants and site concentration of functional groups on the cells surface and to identify any changes as a function of growth phase. This was complemented with Fourier Transform infrared (FT-IR) spectroscopy on the cells from different growth phases with varying pH, to identify proton active functional groups available for binding. We then conducted batch U removal experiments as a function of pH, growth phase, and culture concentration. Cells incubated with and without U were analysed using FT-IR spectroscopy to elucidate which functional groups were responsible for U removal from solution. In order to confirm if the U-bacteria complexes observed using FT-IR spectroscopy formed due to adsorption or precipitation and to identify the composition of the precipitates, transmission electron microscopy (TEM) combined with energy dispersive x-ray spectroscopy (EDX) were used to generate spatially resolved elemental maps of the mineral precipitates within the cells.

2. Material and methods

2.1. Bacterial growth

Cells of *P. putida* were donated by Dr. Thomas Bell in the Department of Life Sciences at Imperial College London, and were cultured aerobically at 37 °C in 10 mL of Luria-Bertani medium and incubated for 24 h. The biomass was transferred to 1 L of the same growth medium and incubated for enough time to bring them to the desired growth phase (exponential, stationary, and death phase). Cells were harvested at 8 h for exponential phase, 24 h for stationary phase, and 96 h for death phase. Each phase was monitored using optical density at 540 nm using a UV–vis spectrometer, with bacteria-free medium as a background to gauge growth.

After incubation, the biomass was separated from the growth media via centrifugation at 3300 × *g* and washed (Kenney and Fein, 2011). Briefly, cells were rinsed 5 times with a clean 0.1 M NaCl electrolyte solution to remove all growth media from the cells so any possible