Response of Arthrobacter Isolates from Hanford Site Soil to Uranium Contamination

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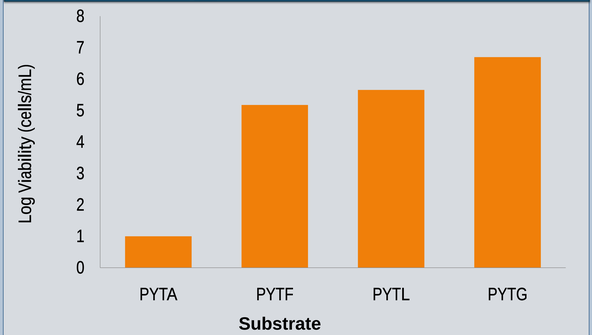
Abstract

Uranium is one of the most prevalent radiological groundwater and soil contaminants at the U.S. Department of Energy (DOE) Hanford Site, USA. Remediation strategies, such as injections of a soluble sodium tripolyphosphate amendment into the contaminated groundwater in order to sequester uranium through the formation of insoluble uranyl phosphate minerals, may result in providing readily available nutrients for various microorganisms that thrive under oligotrophic conditions, such as aqueous orthophosphate; this may lead to an increase in their growth. Increased microbial activity influencing meta-autunite stability, is an important geochemical factor affecting the uranium dissolution and transport under the specific environmental conditions present in the Hanford subsurface. However, the role of bacteria in phosphate remediation technology and the interactions between uranyl phosphates and the microbes are unknown. The lack of knowledge of the long-term stability of the sequestered uranium in the subsurface that may undergo subsequent remobilization, severely limits the design of remediation strategies for uranium-contaminated sites. *Arthrobacter* sp., a genus of gram-positive aerobic bacteria, can survive for elongated periods under adverse environmental conditions and account for about 25% of the microbial population in Hanford soil. Five *Arthrobacter* strains isolated from the Hanford subsurface, were obtained from the Subsurface Microbial Culture Collection (SMCC) (Florida State University). The interaction studies consisted of acclimating the microbial strains in various substrates; subsequent pre-screening tests using direct cell count and electrical cell substrate impedance sensing (ECIS) methods helped identify the most rapidly growing and uranium-resistant strain of *Arthrobacter* sp. to be used in the following autunite mineral leaching experiments. The results obtained at specific uranium concentrations, have shown that G975 & G954 are the fastest growing strains. The uranium tolerance assessment experiments have helped identify G975 as the most resistant *Arthrobacter* strain. These experiments have indeed provided a first insight into the essential defining parameters, which are needed in support of the ensuing microbial meta-autunite dissolution experiments mimicking the oligotrophic Hanford environment.

Substrate Analysis

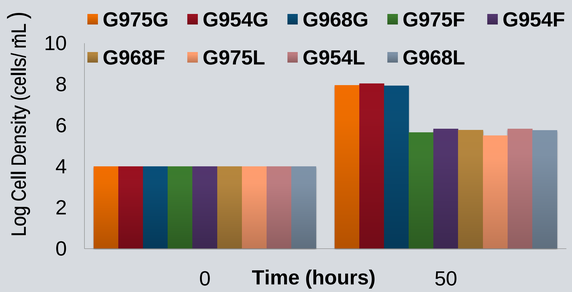


This is carried out by measuring cfu/mL grown on petri dishes in the various substrates using dilution factors 1:10. Result was obtained from two data sets very similar in values.

Materials and Methods

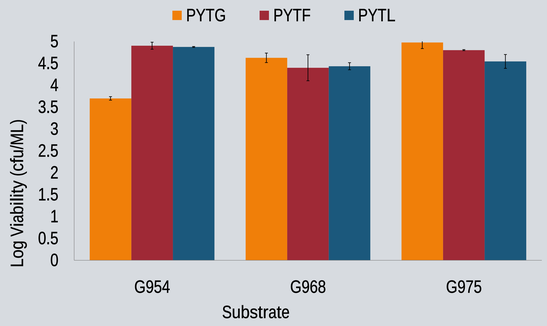
- Three working strains of *Arthrobacter* sp: *Arthrobacter globiformis* (G954), and *Arthrobacter oxydans* (G968 & G975).
- Contamination of the remaining strains by *Streptomycetes.*
- Cell culture: complex media, PYTG (5 g/L peptone, 5 g/L tryptone, 10 g/L yeast extract, 10 g/L glucose, 0.6 g/L $MgSO_4.7H_2O$, 0.07 g/L $CaCl_2.2H_2O$) or PYTL (10 g/L lactose), PYTF (10 g/L formate) and 15 g/L agar. Studies carried out in 5% liquid culture media and petri dishes with agar.
- Cells grown to reach confluence in 50 mL sterile polypropylene tubes, with a foam stopper to simulate aerobic conditions in 10 mL of media at 29˚C in the shaker/incubator.
- Cell density (cells/mL) calculated with hemocytometer and viability determined by cfu (colony forming units) on petri dishes.
- Uranium tolerance studies: incubation of microorganisms with various concentration (10-100 ppm) of uranyl ions; total cell count performed by hemocytometer and viability determined by cfu.
- Impedance Sensing (ECIS) studies used to study bacterial cell growth (rate) and uranium resistance.

Microbial Growth as a Function of Cell Density



An assessment of growth of strains (G975, G954, G968) No/Nt in various substrates (PYTG, PYTL, PYTF) is done by means of calculating total cell densities.

Uranium Tolerance of *Arthrobacter* (Viability)



The studies were done with U(VI) (30 ppm) and the highest viability was shown by G975.

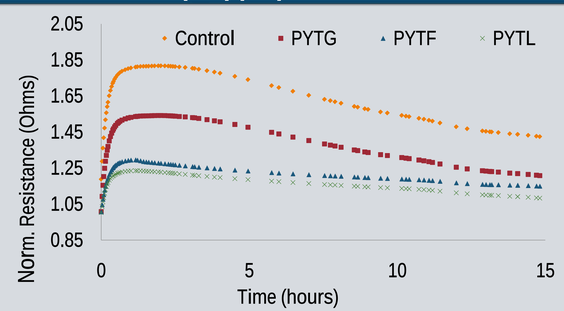
Objectives

Conduct prescreening tests with the *Arthrobacter* sp. isolates from Hanford soil to determine:

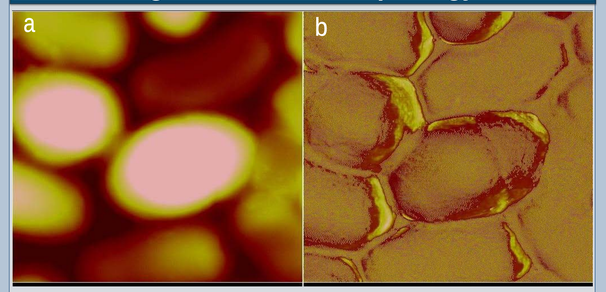
- Bacterial cell density
- Growth both in the absence and in the presence of uranium
- The most uranium-resistant strain

These results are to be used in the subsequent microbial leaching experiments with autunite mineral.

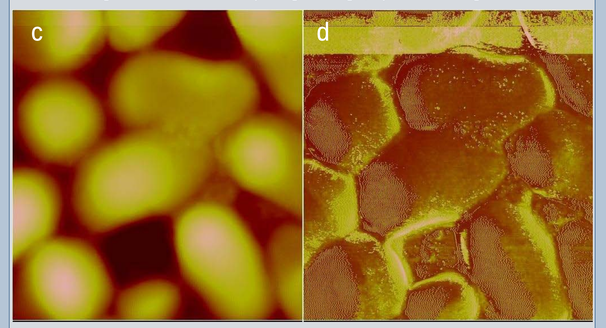
ECIS Uranium (30 ppm) Tolerance



G968 Changes in Cellular Morphology

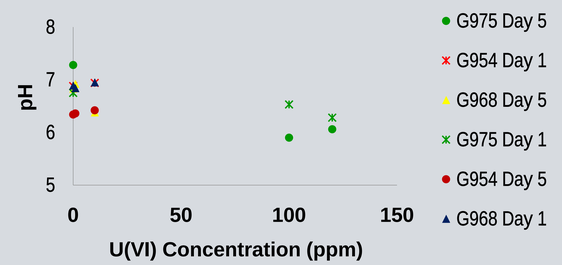


AFM images of control cells a) Height image, b) Phase image



Post growth in 1ppm of U(VI): c) Height image, d) Phase image.

pH Changes in Bacterial Broth



pH changes in bacterial broth solutions spiked with U(VI) amended with 0.01 M HEPES buffer. Buffer will be used in the subsequent leaching experiments.

Conclusions

- G975 & G954 grown on PYTG display highest rate of growth followed by G968.
- Glucose is found to be the most preferred substrate, while Acetate is the least. Glucose is followed by Lactose & Formate which do not display a significant difference in terms of microbial cell growth and viability. This study enabled us to identify the most suitable non-fermentable substrate(s) for the ensuing autunite leaching studies.
- Uranium tolerance of the cells was assessed by ECIS, cfu as well as total cell count.
- G975 is the most resistant strain, growing in the presence of 80 ppm U(VI) (data not shown).
- G954 &G968 show cell viability up to a uranyl concentration of 30 ppm.
- AFM studies of control samples & those grown in the presence of U(VI) have shown crystal deposition on the cellular surface. This is assumed to be precipitation of uranium on the cell wall of the *Arthrobacter* sp.

Future Work

- These experiments have given us preliminary insight about the growth & uranium tolerance of the *Arthrobacter* sp. Assessment of the crystal deposition on the cell wall of the bacteria needs to be done by EDS analysis to confirm the presence of uranium.
- The uranium tolerance mechanism of *Arthrobacter* needs to be understood in order to perform microbial leaching experiments in the future. SPR would be used to elucidate this mechanism.
- Microbiological studies need to be undertaken in order to understand the protein expression or inhibition mechanisms that the bacteria might display in the presence of uranium.

Acknowledgments

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