MARINE CYANOBACTERIA AS SUITABLE CANDIDATES FOR URANIUM RECOVERY FROM AQUATIC ENVIRONMENT

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Abstract

Biorecovery/bioremediation of uranium from natural environment and from nuclear wastes is an important program of research in our division. We investigated the interactions of uranium with two marine cyanobacteria - a unicellular cyanobacterium *Synechococcus elongatus* BDU 75042 and a filamentous cyanobacterium *Anabaena torulosa* at a pH of 7.8, where the soluble carbonate complexes of \( \text{UO}_2^{2+} \) i.e. \([\text{UO}_2(\text{CO}_3)_2]^{2-}\) or \([\text{UO}_2(\text{CO}_3)_3]^4-\) are the predominant anionic species. The marine, unicellular cyanobacterium, *S. elongatus* was found to remove 72% (53.5 mg U g\(^{-1}\) dry wt) of uranium from test solutions containing 100 \( \mu \)M uranyl carbonate within 1h. Light and scanning electron microscopy coupled with energy dispersive X-ray fluorescence (EDXRF) spectroscopy confirmed the uranyl adsorption by this organism. The filamentous, heterocystous cyanobacterium, *A. torulosa* was also found to bind uranium efficiently from aqueous solutions containing 100 \( \mu \)M uranyl carbonate at pH 7.8. The uranyl sequestration kinetics exhibited (a) an initial rapid phase, binding 48% uranium within 30min resulting in a loading of 56 mg U g\(^{-1}\) of dry wt, followed by (b) a slower phase, binding 65% uranium with resultant loading of 77.35 mg U g\(^{-1}\) in 24h. A detailed study revealed the involvement of acid soluble polyphosphates in uranium accumulation by this brackish water cyanobacterium. Also, long term experiments involving repeated exposure of *Synechococcus* biomass to fresh simulated sea water every third day, showed a loading of 2,960 \( \mu \)g U g\(^{-1}\) in 4 weeks.

Introduction

Increasing contamination of the environment by uranium on account of its mining and disposal of tailings, nuclear power/weapons production, nuclear testing or nuclear accidents, is a worldwide problem. The versatility of microbial systems to remove heavy metals and radionuclides from their immediate environment is well recognized. Microbial interactions with metals form an important part of the natural biogeochemical processes and have important consequences for human society. It, is therefore, vital to advance our understanding of the metal-microbe interactions in order to develop suitable bioremediation strategies for metal contaminated sites.

Cyanobacteria represent a morphologically diverse group of oxygenic, gram-negative photosynthetic prokaryotes, which are widely distributed in freshwater, marine and terrestrial environments. They can tolerate, accumulate and detoxify metal contaminants in aquatic environments, thereby affecting the mobility and bioavailability of metals. Their mass cultivation is economic and feasible which qualifies them as suitable bioremediation agents for the recovery and recycling of target metals. Cyanobacterial cell surface harbours functional groups like carboxyl, phosphoryl, hydroxyl, amine, which bind metal ions to form metal ligand surface complexes. This potential is available even when the cells are dead. Cyanobacterial cells have been shown to concentrate several metals within the cells.
Intracellularly, the metal ion sequestration is facilitated either by polyphosphate bodies or small, cysteine rich proteins, called metallothioneins.

Uranium exists primarily as U (VI) in oxic aqueous systems in the form of free divalent oxocomplex, \( \text{UO}_2^{2+} \) at \( \text{pH} \leq 5 \). The aqueous speciation of uranium undergoes major changes within a pH range of 5 to 7 because of complexation with carbonates and hydroxides. Its contamination in surface, ground or natural waters (ponds, lakes, sea water), resulting from activities like mining, storage of radioactive waste, nuclear energy production, is a subject of intense public concern. Uranium is known for its chemical toxicity rather than radiotoxicity and its contamination in surface or ground water poses health hazards. In microbial systems, no specific mechanism has been attributed for uranium toxicity.

We carried out studies on uranium sequestration from micromolar concentrations of uranyl carbonate solutions (pH 7.8) by two marine cyanobacteria, one being unicellular i.e. *Synechococcus elongatus* BDU/75042 and another being a filamentous, heterocystous, nitrogen fixing brackish water cyanobacterium, *Anabaena torulosa*.

**Uranium sequestration by unicellular cyanobacterium, *Synechococcus elongatus* BDU/75042**

*Synechococcus elongatus* cells exposed to 100\( \mu \text{M} \) or 23.8 mg L\(^{-1} \) U at pH 7.8 for 5h, bound 72% U resulting in a loading of 53.5 mg U g\(^{-1} \) dry wt\(^{-1} \). Such U loaded cells exhibited black deposits around the cell margins as compared to control untreated cells (Figs. 1a and b). Treatment of U loaded cells with 0.1N HCl showed loss of black deposits from the cell surface along with ~80% U desorption (Fig. 1c). Energy Dispersive X-ray fluorescence (EDXRF) spectroscopy of uranium loaded biomass revealed all components of UL X-rays (UL\(_{\alpha} \), UL\(_{\beta} \), UL\(_{\gamma} \), and UL\(_{\delta} \)) confirming the association of uranium with the cells (Fig. 1d). Further, studies revealed that the uranium was predominantly associated with the EPS and the FT-IR results confirmed that the carboxyl and amide groups harboured within the EPS, were found to be involved in uranyl binding. The uranyl binding efficiency of the heat killed or the non-viable *Synechococcus* cells was similar.

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**Fig. 1:** Uranium exposure to *S. elongatus* cells. The mid-exponential phase cells were incubated (a) under control conditions or (b) were exposed to 23.8 mg L\(^{-1} \) uranyl carbonate \([\text{UO}_2(\text{CO}_3)_2]^{2-}\) at pH 7.8 for 5h resulting in loading of 53.5 mg U g\(^{-1} \) dry wt and (c) subsequently washed with 0.1N HCl. Cells were observed using bright field microscopy in a Carl Zeiss Axioscop 40 microscope, with oil immersion objectives (magnification 1500X), the bars in figs 1a, b and c represent 5\( \mu \text{m} \). (d) EDXRF spectra of control (i) and U loaded (ii) (53.5 mg U g\(^{-1} \) dry wt) *S. elongatus* biomass.
to that of live cells, corroborating their extracellular localization.

**Uranium sequestration by filamentous cyanobacterium, *Anabaena torulosa***

Our recent studies on uranium binding by *A. torulosa* have revealed the involvement of acid soluble polyphosphate bodies in uranium accumulation at pH 7.8. On being challenged with 100μM uranyl carbonate for 24h at pH 7.8, under phosphate limited conditions, *A. torulosa* cells bound 65% (15.47 μg mL⁻¹) of input U (23.8μg mL⁻¹) resulting in a loading of 77.35 mg U g⁻¹ dry wt. The kinetics of uranyl binding by live *A. torulosa* cells revealed a rapid phase (lasting for 30 min) resulting in 48% binding or a loading of 56 mg U/g dry wt., followed by a slower phase extending up to 24h and resulting in 65% binding or loading of 77.35 mg U/g dry wt., of the initially added 100μM U. Cells challenged with 23.8 mg L⁻¹ U for 24h showed dense dark granular structures resembling poly phosphate bodies as compared to the unchallenged cells (Figs. 2a, b and d). Nearly 80% (12.37μg mL⁻¹) of bound uranium (15.47 μg mL⁻¹) could be released from 24h U challenged cells on desorption with 0.1N HCl. These 24h uranium challenged cells exhibited distinct ‘hole’ like structures upon acid desorption (Fig. 2c). Treatment of uranium loaded cells with 1N HCl at 100°C for 15 min resulted in complete extraction of total cell bound uranium and inorganic phosphate demonstrating co-localization of uranium with acid soluble polyphosphates.

**Uranium concentration from sea water using *Synechococcus elongatus* BDU/75042**

Sea water is an inexhaustible and green source of uranium with an estimated uranium content of 4.5 billion tonnes. However, the uranium concentration in sea is very low i.e. 13nM or 3μg L⁻¹. Uranium sequestration from simulated sea water containing 3μg L⁻¹ U by the marine cyanobacterium, *S. elongatus* was assessed over short (24h-5d) or long (38d) exposure time periods. The organism could remove 90-98% uranium resulting in a loading of 42 μg U g⁻¹ in 5d (Fig. 3a). When evaluated for its ability to sequester uranium from simulated sea water under continuous replenishment conditions, *Synechococcus* biomass immobilized in dialysis bags, demonstrated superiority over the other tested chemical and biological alternatives in terms of high uranium loading values (2960 μg g⁻¹) in 4 weeks, and tolerance to the high salinity of sea water (0.5M NaCl) (Fig. 3b). Nearly 85-90% of cell bound uranium could be desorbed using 0.1N HCl. The organism could sequester uranium (13,306 μg U g⁻¹) in 24h from aqueous solutions supplemented with 0.6 M NaCl and ~5 mg L⁻¹[UO₂(CO₃)₂]⁻ at pH 7.8 (simulated brine reject solutions).

**Conclusions**

Cyanobacteria represent an important component of aquatic environments like ponds, sea or oceans which receive direct or indirect metal contamination. Our recent
studies have identified two marine cyanobacteria with a high potential for uranyl sequestration from aqueous solutions above circumneutral pH. A fundamental understanding of mechanisms employed by cyanobacterial cells to resist/alleviate uranium toxicity will prove useful for development of strategies for either uranium recovery or remediation from aquatic environments.

Acknowledgements

The authors thank Prof. L. Uma and N. Thajuddin, NFMC, Tiruchirapally, India for providing Synechococcus elongatus BDU/75042, Dr. Daisy Joseph, Nuclear Physics Division, BARC, for extending technical help in EDXRF analyses and Dr. S. A. Kumar, Analytical Chemistry Division, BARC, for extending technical help in ICP-MS analyses of uranium in cyanobacterial biomass samples.

References