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Bioremediation of Uranium-Contaminated Water: Magnetic Bacteria as Potential Supporters

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Abstract

The ability of magnetotactic bacteria to remove dissolved uranium(VI) from U-contaminated water appears to be very effective, even in a broad pH range. High amounts (78–96%) of uranium were removed from a bacterial suspension in laboratory experiments after one day of exposure and bound in the cell wall of the bacteria showing a stable immobilization of uranium(VI). Our findings may initiate new remediation strategies on effective immobilization of uranium(VI). In combination with the magnetic properties of these bacteria, a simple technical water purification process can be realized not only for uranium(VI), but probably also for other metals.

Keywords: Mine water, uranium, magnetotactic bacteria, bioremediation

Introduction

Uranium mining activities and the processing of ores have left a legacy of environmental contamination. Radionuclides can migrate into surrounding aquifers and soils, thus representing a human health risk. Conventional technologies based on physicochemical treatments are traditionally used to remediate contaminated mine water. However, these approaches are cost-intensive and ineffective particularly for low uranium concentrations. Bioremediation, on the other hand, is a promising alternative to remove uranium from contaminated waters that is less expensive, easy to implement and effective at low uranium concentrations (Sánchez-Castro *et al.* 2021; Newman-Portela *et al.* 2024). Several mechanisms of interactions of microorganisms with radionuclides are known, like biosorption on functional groups of the cell surface (Lloyd and Macaskie, 2002), bioaccumulation, where the metal is taken up into the cell (Suzuki and Banfield, 2004), the enzymatic reduction of metals, which is called bioreduction (Beyenal *et al.*, 2004), and biomineralization, where radionuclides can precipitate with microbial generated ligands, e.g., phosphate, sulfide or carbonate (Merroun *et al.*, 2011). The investigated microorganisms included bacteria as well as fungi. The organisms can interact with uranium, transforming it into less soluble and toxic forms. Thus, bioremediation is considered a more sustainable and less invasive environmental remediation strategy than other traditional technologies. In a unique combination of analytical methods and transmission electron and fluorescence microscopy as well as various spectroscopic techniques, the ability of magnetotactic bacteria to remove uranium from contaminated waters was tested. Important indications were gained on possible binding sites in the bacterial cell walls. Findings from the present study suggest a promising method to support or outperform the physicochemical treatments. By utilizing the magnetic properties of magnetotactic bacteria (e.g. *Magnetospirillum magneticum* AMB-1), it appears to be possible to biologically remediate uranium-contaminated mine water.

Methods

For analytical, microscopic and spectroscopic studies, a cell suspension of *Magnetospirillum magneticum* AMB-1 was prepared in laboratory experiments, using sterile tap water at different pH values (3.5, 4.5, 5.5, 6.5 and 7.5), and combined with a 0.1 M stock

solution of $UO_2(NO_3)_2$ to adjust an initial uranium(VI) concentration of 0.1 mM. During the incubation on a rotary shaker at 30 °C, cell suspension was collected at distinct time points (5, 15, 30 and 45 min, as well as after 1, 2, 3, 4, 5, 6, 24 and 25 hours) and centrifuged at 13.000 x g for 1 min. The supernatants were sampled for inductively coupled plasma mass spectrometry (ICP-MS) measurements to determine the residual uranium(VI) concentration. For cryo-time resolved laser-induced fluorescence spectroscopy (cryo-TRLFS) the pellets were transferred into a copper holder. The cryo-TRLFS method was chosen due to the high sensitivity toward uranium(VI) complex formation in aqueous solutions (Moulin *et al.* 1995). In addition, important ligands of the cell wall, e.g., peptidoglycan, lipopolysaccharide, L-rhamnose, D-(+) galactose, D-(+) mannose were used as reference ligands and measured for comparison and interpretation of possible binding sites of uranium(VI) to *Magnetospirillum magneticum* AMB-1 cell walls (Krawczyk-Bärsch *et al.* 2022). The mixed cryo-TRLFS spectra were analysed by parallel factor (PARAFAC) analysis to extract single component spectra from the total emission spectra data sets (Drobot *et al.* 2015). In addition, during the uranium(VI) interaction experiments, samples were taken at the beginning of the experiments and after 1, 3, 6 and 24 hours to check the viability of *Magnetospirillum magneticum* AMB-1 cells depending on the uranium(VI) incubation time and on the pH. For this purpose, the cells were tested by live-dead staining (SYTO®9/propidium iodide). The cell viability was observed using a confocal laser scanning microscope (CLSM). Transmission electron microscopy (TEM) and energydispersive X-ray spectroscopy (EDXS) studies were performed to locate uranium in *Magnetospirillum magneticum* AMB-1 cells. For this study, ultrathin sections of *Magnetospirillum magneticum* AMB-1 cells were prepared and loaded with 0.1 mM uranium at pH 6.5 for 5 hours. Detailed sample preparation protocols are available in Krawczyk-Bärsch *et al.* (2022).

Results and discussion

The removal efficiency of dissolved uranium(VI) by *Magnetospirillum magneticum* AMB-1 cells is very effective and independent of the pH. High amounts of uranium(VI) were removed from the suspension at all pH values tested. As shown in Fig. 1, the removal of uranium(VI) takes place within the first hour, where at pH 6.5 almost 78% of the uranium(VI)

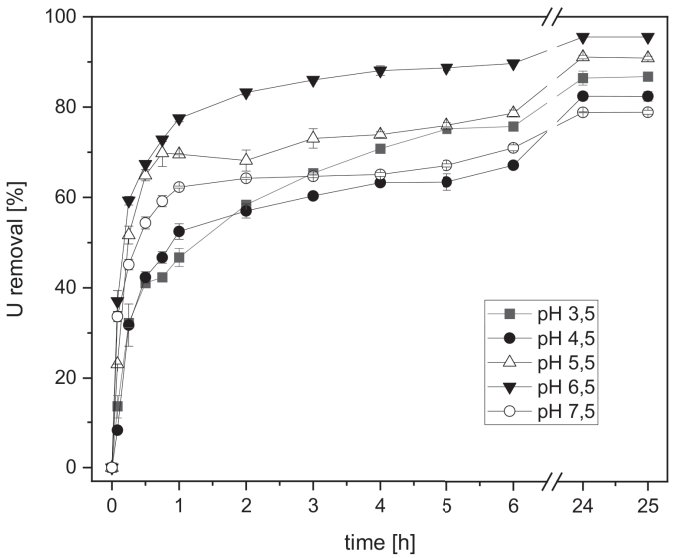

Figure 1 *Uranium(VI) removal from contaminated water by Magnetospirillum magneticum AMB-1 cells versus time in sterilized tap water at different pH and 30 °C; initial uranium(VI)conc. = 0.1 mM.*

is eliminated from the contaminated water. After 24 h, almost all of the initial uranium(VI) concentration (96%) is removed by *Magnetospirillum magneticum* AMB-1 cells. In contrast, at a pH of 3.5, only 46% U(VI) is bound in the first hour. Nevertheless, 86 % of the U(VI) was removed from the suspension at the end of the experiment, which even indicates efficient removal of U(VI) by *Magnetospirillum magneticum* AMB-1 cells at low pH.

As the cell viability test showed, the majority of the cells, with the exception of those treated at pH 3.5 as well as pH 7.5, were still alive after 24 hours, despite being exposed to high concentrations of uranium(VI). The bright-field TEM image in Fig. 2a shows a typical elongated *Magnetospirillum magneticum* AMB-1 cell with up to five crystals, which are visible as black dots. EDXS analysis clearly confirms that these dots exclusively consist of Fe. They are so-called magnetosomes consisting of magnetic mineral crystals, which were synthesized intracellularly as a special feature by magnetotactic bacteria and are responsible for the magnetic property of the bacteria (Balkwill *et al.* 1980). Studies have shown that they are not involved in the removal of uranium. Rather, analysis of the elemental distribution of uranium at pH 6.5 clearly indicated the predominant binding of uranium to the cell wall (Fig. 2b).

After the incubation of *Magnetospirillum magneticum* AMB-1 cells with 0.1 mM uranium(VI) at different pH values (3.5–7.5) and different contact times (0.5, 2, 5 and 24 h), the cells were used for cryo-TRLFS measurements. A subsequent analysis of all emission spectra from the TRLFS data set by PARAFAC extracted the single component spectra of five uranium(VI) species, which were probably formed during the uranium(VI) biosorption of *Magnetospirillum magneticum* AMB-1 cells. Since EDXS elemental distribution analysis clearly indicate that uranium(VI) is predominantly bound in the cell wall, important ligands were considered as possible complexants for uranium(VI) and used as reference spectra of potential ligands (peptidoglycan, lipopolysaccharide, L-rhamnose, D-(+) galactose, D-(+) mannose). The luminescence properties have shown that there is no correspondence with most ligands, with the exception of peptidoglycan. The results reveal that peptidoglycan, a key ligand in the cell wall, plays a crucial role in uranium biosoption. The formation of three characteristic species were determined over a wide pH range. The

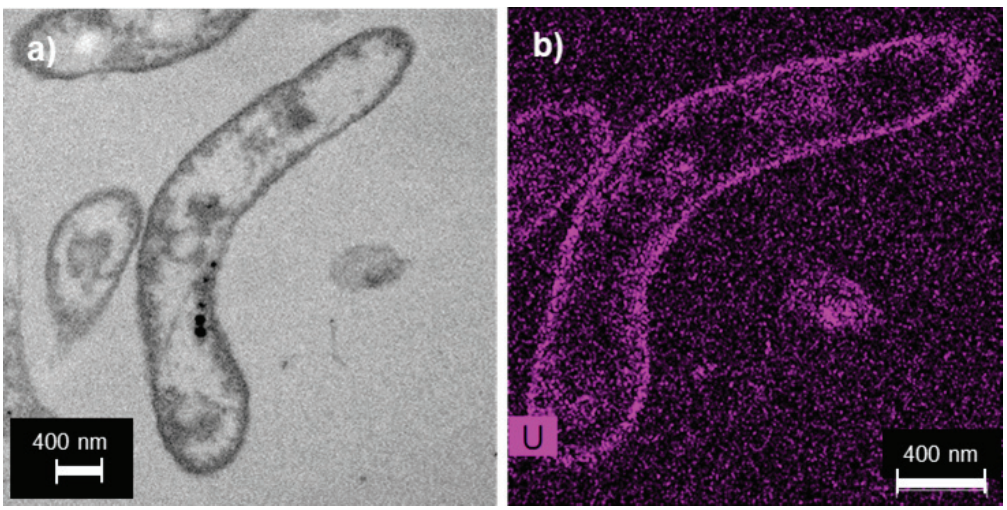

Figure 2 *Bright-field TEM image of a thin sectioned Magnetospirillum magneticum AMB-1 cell (a) with magnetosomes (black dots) together with EDXS-based element distribution analysis of U (b). Sterilized tap water, pH 6.5; initial uranium(VI) conc. = 0.1 mM; duration: 5 h at 30 °C.*

relative luminescence intensity distribution of the three uranium-peptidoglycan reference species versus pH show that uranium-peptidoglycan species (1) is mainly important in the acidic pH range and is rather negligible in the neutral and basic pH range. Species (2) dominates the bioassociation of uranium(VI) in the mentioned broad pH range, with a dominance at pH 5.5 being emphasized. Species (3), on the other hand, only gains significance in the basic pH range (s. Fig. 3).

Conclusions

With regard to the development of innovative bioremediation strategies of contaminated water, the presented studies show clearly that magnetotactic bacteria, such as *Magnetospirillum magneticum* AMB-1, are suitable candidates. They can survive as planktonic cells both in a wide pH range and with relatively high uranium(VI) concentrations of up to 0.1 mM, while effectively and almost completely immobilizing uranium(VI). Uranium is bound on the bacterial cell wall almost entirely, showing a stable immobilization of uranium. An outstanding feature however, is the formation of nanoscopic magnetic crystals within the cell of magnetotactic bacteria, which were proved by TEM/EDXS. Thus, in combination with its magnetic properties, magnetotactic bacteria offer many advantages for the development of various bioassociation technologies. The magnetic properties could be harnessed for straightforward magnetic separation of uranium-loaded bacteria from contaminated water. Consequently, a simple technical water purification process could be realized, not only for uranium(VI), but probably also for other metals with the objective of potential industrial applications in the field of microbiological purification of water.

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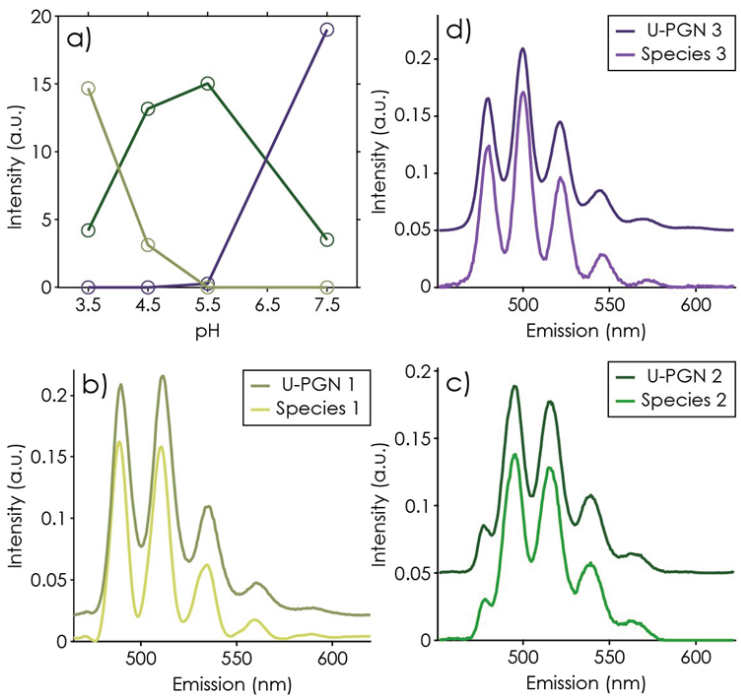

Figure 3 *a): Relative luminescence intensity distribution of three uranium-peptidoglycan (U-PGN) reference species versus pH. b, c, d): Main PARAFAC extracted species in comparison to the appropriate U-PGN reference spectra. Sterilized tap water, initial uranium(VI) conc. = 0.1 mM at 30 °C.*

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