Peptidoglycan as major binding motif for Uranium bioassociation on *Magnetospirillum magneticum* AMB-1 in contaminated waters

Krawczyk-Bärsch, E.; Ramtke, J.; Drobot, B.; Müller, K.; Steudtner, R.; Kluge, S.; Hübner, R.; Raff, J.;

Originally published:

June 2022

Journal of Hazardous Materials 437(2022), 129376

DOI: https://doi.org/10.1016/j.jhazmat.2022.129376

Perma-Link to Publication Repository of HZDR:

https://www.hzdr.de/publications/Publ-34534

Peptidoglycan as major binding motif for Uranium bioassociation on *Magnetospirillum magneticum* AMB-1 in contaminated waters

Evelyn Krawczyk-Bärsch[1*+], Justus Ramtke[1,3*], Björn Drobot[1], Katharina Müller[1], Robin Steudtner[1], Sindy Kluge[1], René Hübner[2], Johannes Raff[1]

[1] Helmholtz-Zentrum Dresden-Rossendorf, Institute of Resource Ecology, Bautzner Landstraße 400, 01328 Dresden, Germany
[2] Helmholtz-Zentrum Dresden-Rossendorf, Institute of Ion Beam Physics and Materials Research, Bautzner Landstraße 400, 01328 Dresden, Germany
[3] University of Applied Sciences Zittau/Görlitz, Faculty of Natural and Environmental Sciences, Theodor-Körner-Allee 8, 02763 Zittau, Germany

[*] The authors have contributed equally
[+] Corresponding author
E-mail address: E.Krawczyk-Baersch@hzdr.de (E. Krawczyk-Bärsch)
phone: +49-351-2602076; fax: +49-351-26012076

Abstract

The U(VI) bioassociation on *Magnetospirillum magneticum* AMB-1 cells was investigated using a multidisciplinary approach combining wet chemistry, microscopy, and spectroscopy methods to provide deeper insight into the interaction of U(VI) with bioligands of Gram-negative bacteria for a better molecular understanding. Our findings suggest that the cell wall plays a prominent role in the bioassociation of U(VI). In time-dependent bioassociation studies, up to 95 % of the initial U(VI) was removed from the suspension and probably bound on the cell wall within the first hours due to the high removal capacity of predominantly alive *Magnetospirillum magneticum* AMB-1 cells. PARAFAC analysis of TRLFS data highlights that peptidoglycan is the most important ligand involved, showing a stable immobilization of U(VI) over a wide pH range with the formation of three characteristic species. In addition, *in-situ* ATR FT-IR reveals the predominant strong binding to carboxylic functionalities. At higher pH polynuclear species seem to play an important role. This comprehensive molecular study may initiate in future new remediation strategies on effective immobilization of U(VI). In combination with the magnetic properties of the bacteria, a simple technical water purification process could be realized not only for U(VI), but probably also for other heavy metals.

1. Introduction

Weathering and leaching of geogenic deposits as well as their industrial use can lead to high local contamination of soils and waters with heavy metals and radionuclides. The resulting pollution of the environment is a worldwide problem with serious consequences for the environment and local populations. A particular challenge here is the remediation of radionuclide-polluted waters. Since chemical treatments are often associated with high costs and toxic waste, research into alternative ways of purifying water has been ongoing for several years. One possibility here is the development of customized nanomaterials for the removal of radionuclides. Examples include carbon nanotubes as potential carriers of pollutants (Schierz and Zänker, 2009), hydroxylated vanadium carbide $V_2C(OH)_2$ MXene nanosheets (Zhang et al., 2017), metal-organic frameworks with abundant functional groups and tailorable structure (Li, J. et al., 2018), porous microcubes composed of fine Fe_2O_3 nanoparticles (Li, X. et al., 2018), and MXenes, consisting of transition metal nitrides, carbides and carbonitrides (Yu et al., 2022), to name just a few. Nanomaterials could have great potential in water treatment. However, so far the practical application of these materials is still difficult due to the complicated product process and the high costs. Another possibility is the use of microorganisms for bioremediation purposes and the removal of heavy metals (Gadd, 2008; Lesmana et al., 2009; Young et al., 2009). Several mechanisms of interactions of microorganisms with radionuclides are known, like biosorption on functional groups of the cell surface (Lloyd and Macaskie, 2002; Kelly et al., 2002; Merroun et al., 2005; Ojeda et al., 2008), bioaccumulation, where the metal is taken up into the cell (Suzuki and Banfield, 2004; Brookshaw et al., 2012), the enzymatic reduction of metals, which is called bioreduction (Beyenal et al., 2004; Wu et al., 2006), and biomineralization, where radionuclides can precipitate with microbial generated ligands, *e.g.*, phosphate, sulphide or carbonate (Merroun et al., 2011; Macaskie et al., 2016). The investigated microorganisms included Gram-negative and Gram-positive bacteria as well as fungi.

The Gram-negative bacteria include, among others, magnetotactic bacteria (Bazylinski and Frankel, 2004). According to the current state of research, only a few studies on magnetotactic bacteria exist, although magnetotactic bacteria are widely distributed in aquatic environments, in sediments of freshwater, brackish, marine, and hypersaline habitats, where they make up an important part of the bacterial biomass (Lefèvre and Bazylinski, 2013). The abundance of the

magnetotactic bacteria has been determined by several researchers. Monteil et al. (2021) report about a large abundance up to 5.8×10^5 cells mL^{-1} of porewater in the sediments of Lake Pavin (France). In previous studies, the abundance was even found to be up to 10^7 cells mL^{-1} in sediments of aquatic systems (Spring et al., 1993; Flies et al., 2005; Jogler et al., 2009), so that already Spring et al., (1993) assigned a dominating role to these bacteria in the microbial ecology in his studies of Lake Chiemsee sediments. Magnetotactic bacteria belong to the family of *Rhodospirilliaceae* and are associated with the Alpha-, Gamma-, and Deltaproteobacteria classes of the Proteobacteria phylum and with the *Nitrospirae* phylum (Amann et al., 2007). They vary greatly in shape, existing in spiral, vibrio, rod, or even coccoid forms (Lefèvre and Bazylinski, 2013). The special feature of magnetotactic bacteria is that they synthesize intracellular magnetic mineral crystals, the so-called magnetosomes (Balkwill et al., 1980), which are protected by a lipid bilayer membrane about 3–4 nm thickness. The magnetic crystals can be made of iron oxide magnetite (Fe_3O_4) (Frankel et al., 1983) or iron sulfide greigite (Fe_3S_4) (Mann et al., 1990). Some cells are able to form both types of mineral (Bazylinski et al., 1993). The magnetosomes are responsible that magnetotactic bacteria orient in the presence of external Earth's magnetic field lines (Blakemore, 1975) and actively swim by the means of their flagellar rotation toward a preferred oxygen concentration (Frankel et al., 1997). They are either microaerophiles, anaerobes, or both and are generally found at the oxic-anoxic interface and the anoxic regions of the habitat (Bazylinski et al., 2013).

Due to the magnetic properties of the magnetosomes, potential applications in microbiology, biophysics, biochemistry, nanotechnology and also biomedicine are known, *e.g.*, immobilization of biomolecule, drug delivery, targeted and controlled delivery of anticancer agents for tumor therapy (Jacob et al., 2016; Afkhami et al., 2011; Mokrani et al., 2010). So far, a potential environmental application for magnetotactic bacteria in water purification processes was not yet implemented, although the use of the magnetic properties of these bacteria could play a major role in water treatment by removing heavy metal-loaded bacteria from water using simple technical means. Presumably it can be justified by the fact that so far only a few studies on the sorption of heavy metals by magnetotactic bacteria are known to date. In the past, the uptake of Pu by magnetotactic bacteria was shown by adding purifed Pu, consisting of mixed α (^{238}Pu, ^{239}Pu, ^{240}Pu) and β (^{241}Pu) emitters to a culture of magnetotactic bacteria (Bahaj et al., 1998). In a study from Bahaj et al. (1994) the effect of heavy metals (Al, Cd, Co, Cu, Fe, Mg, Mn, Pb, Zn) at different concentrations (l ppm, 10 ppm, 100 ppm) were measured on the motility of magnetotactic

bacteria. In somewhat recent studies more detailed data are given by Wang et al. (2011). The removal efficiency of Ag(I) and Cu(II) by *Magnetospirillum gryphiswaldense* MSR-1 strain was mentioned to be 91 % and 62 %, respectively when using a wet weight biomass of 10 g/L and an initial concentration of 80 mg/L Ag(I) and Cu(II) at a pH of 4.0 and 5.0, respectively. In studies with the magnetotactic bacterial isolate *Stenotrophomonas* sp. it was shown, that Au(III) was removed from the contaminated water with a high biosorption capacity of 506, 369 and 308 mg Au(III) per g dry weight biomass at the initial pH values of 2.0, 7.0 and 12.0, respectively (Song et al., 2008). In recent studies, *Pseudomonas aeruginosa* Kb1, which was found to synthesis intracellular magnetosomes, was able to remove 99.4 % and 70 % of Cd and Pb at initial concentration of 4 ppm (Kabary et al., 2017). Jayaraman et al. (2021) showed that the strains of *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa* and *Achromobacter xylosoxidans* are able to tolerate higher concentration of Mn, Zn, Cu and Cr ranging from 20, 30, 50, 70, 90 and 150 mg. The studies were performed on modified nutrient agar medium using $MnCl_2$, $ZnSO_4$, $CuSO_4 \cdot 5H_2O$ and $K_2Cr_2O_7$. Although these studies already indicate, that magnetotactic bacteria could be a promising biosorbent for heavy metals, detailed information about binding properties, molecular identification of the bioassociation process, and statements on the formed species are missing. In the presented study, these issues are addressed using a multidisciplinary approach that includes wet chemistry, microscopy, and spectroscopy. The highly sensitive *cryo*-time resolved laser-induced fluorescence spectroscopy was used in combination with parallel factor analysis. This new type of evaluation offers the advantage over the previous spectroscopic method to obtain individual spectra of metal-ligand species from a sum spectrum. *In-situ* attenuated total reflection Fourier transform infrared spectroscopy can furthermore contribute to the molecular identification of the bioassociation process. These information are helpful for a better understanding of the mechanisms involved in the interaction of magnetotactic bacteria with heavy metals and could contribute to the development of remediation strategies of contaminated waters.

Our studies focused on uranium (U) since, to our knowledge, there are no published studies to date showing the interaction of U with magnetotactic bacteria, although there should be great interest in it, since the existence of U in waters and sediments causes hazardousness to the environment and humans. The entry of U into the human body via the food chain, by inhalation of dusts, and through water can lead to serious illness or even death (Lloyd and Macaskie, 2002).

It is known from previous studies that U often binds to the cell wall of Gram-negative and Gram-positive bacteria by interacting with ligands in the cell wall such as phosphate, hydroxyl, carboxyl and amino groups (Lloyd and Macaskie, 2002; Kelly et al., 2002; Ojeda et al., 2008; Merroun et al., 2008; Krawczyk-Bärsch et al., 2018; Hufton et al., 2021). The cell wall of Gram-negative bacteria is mainly made up of a thin peptidoglycan layer, which is rich in carboxylate groups. A lipid/protein bilayer forms the outer membrane, including lipopolysaccharides with their phosphate groups (Bäuerlein, 2003). In our studies, peptidoglycan, lipopolysaccharide and several other ligands of the cell wall were used as reference and measured for possible binding sites of U to the cell wall.

2. Materials and methods

2.1. Cultivation

The strain of *Magnetospirillum magneticum* AMB-1 was kindly provided by the Molecular and Environmental Microbiology Department of the Institute of Biosciences and Biotechnologies from CEA Cadarache in France. The cells were grown in 200 mL flasks in 1.5 mM MagMin medium containing Wolfe´s mineral solution, Wolfe´s vitamin elixir and iron-malate after Komeili et al. (2004), modified by CEA Cadarache with the addition of iron malate (1:2000) and vitamin elixir (1:2000). Before the cells were transferred to the flasks, the sealed flasks, filled with 98 mL of MagMin medium (pH 6.9), were gassed with N_2/O_2 in a 98 % / 2 % ratio for 20 minutes to create a low oxygen medium with a low oxygen gas atmosphere. Subsequently, 50 µL vitamin solution and 50 µL iron malate solution were added to each flask. For inoculation of *Magnetospirillum magneticum* AMB-1 cells, 2 mL of an inoculum were added to the medium. During the incubation, the flasks were kept at 30 °C on a rotary shaker (Rotamax 120, Heidolph Instruments) at 80 rpm. The best growth conditions and the highest optical density at 600 nm of 0.4 was achieved using UV/Vis spectrometry (Specord 50 Plus, Analytik Jena, Germany) after an incubation of 24 h. Evidence that the bacteria synthesize magnetosomes was provided by placing a small magnet close to the flasks. After a few hours, an accumulation of bacteria formed (Supplementary Fig. 1).

2.2. U bioassociation experiments with varying biomass concentration

For each experiment cells were grown in 200 mL flasks in MagMin medium for 24 h at 30 °C on a rotary shaker at 80 rpm. To reach a defined biomass concentration, a different number of flasks was used, concentrated and the optical density at 600 nm (OD_{600nm}) was controlled. This resulted in a biomass concentration of 89.00, 77.04, 49.62 and 21.45 mg dry biomass (DBM) with an OD_{600nm} of 2.32, 1.64, 1.02 and 0.45, respectively. The cells were centrifuged at 7.870 x g for 10 minutes (Centrifuge Avanti J-20 CP, Beckman Coulter, Country?), suspended and washed twice in sterilized tap water at pH 6.5. The washed cells were suspended again for each experiment in 100 mL sterilized tap water at pH 6.5. Subsequently, 100 µL of a 0.1 M $UO_2(NO_3)_2$ stock solution were added to each experiment to reach a final U(VI) concentration of 0.1 mM. During the incubation on a rotary shaker (Rotamax 120, Heidolph Instruments) at 30 °C, 1 mL of each cell suspension was collected three times at distinct time points (5, 15, 30 and 45 min, as well as after 1, 2, 3, 4, 5, 6, 24 and 25 h). Each sample was centrifuged at 13.225 x g for 1 minute (Centrifuge 5415R, Eppendorf AG, Hamburg, Germany). The supernatants were sampled and acidified with HNO_3 for Inductively Coupled Plasma Mass Spectrometry (ICP-MS) measurements using an ELAN 9000 type ICP-MS spectrometer (Perkin Elmer, Überlingen, Germany) to determine the residual U(VI) concentration within the supernatant.

2.3. U bioassociation experiments with different pH

For the experiments a high biomass concentration was chosen (74.3−82.8 mg DBM) for each experiment, which was determined in prior by an OD_{600nm} in the range of 1.60−2.44. The cells were centrifuged, suspended and washed twice in sterilized tap water at defined pH related to each experiment (3.5, 4.5, 5.5, 6.5 and 7.5). The washed cells were suspended again for each experiment in 101 mL sterilized tap water at the defined pH and treated as described in the previous experiment. 1 mL was taken for OD_{600nm} measurements. To reach a final U(VI) concentration of 0.1 mM, 100 µL of a 0.1 M $UO_2(NO_3)_2$ stock solution was added to each experiment. 1 mL of each cell suspension was taken three times at distinct time points (5, 15, 30 and 45 min, 1, 2, 3, 4, 5, 6 and 24 h) during the incubation, centrifuged, acidified and used for ICP-MS measurements to

determine the U(VI) concentration. The cell pellets were dried for 25 h at 100°C in an oven (Memmert UE500, Schwabach, Germany) and the weight were determined. Together with the residual biomass, which was taken from the flask at the end of the experiments, the weight of the biomass was used for the calculation of the uranium removal capacity dependent on the dry biomass by normalizing the amount of removed U(VI) from the solution to the dry biomass.

2.4. Live-dead staining

During the U(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 U(VI) incubation time, but also on the pH. The cells were harvested by centrifugation at 13.225 x g (Centrifuge 5415R, Eppendorf AG, Hamburg, Germany) for 1 min., the supernatants discarded and the pellets resuspended in 200 µL of tap water. A volume of 0.3 µL of a Syto®9/propidium iodide mixture (vol/vol) (LIVE/DEAD™ *Bac*Light™ Bacterial Viability Kit, Invitrogen™) was added to the cell suspensions and incubated in the dark for 15 min. The green fluorescent dye SYTO®9 can diffuse through intact cell walls into the cells. The red fluorescent dye propidium iodide can only enter dead cells through their defect cell walls. The cells were washed again to remove the unbounded stains. The washed and stained cells were diluted in 330 µL 0.9% NaCl. The cell viability was observed using a confocal laser scanning microscope (Leica CLSM, Leica Microsystems, Wetzlar, Germany).

2.5. (Scanning) transmission electron microscopy ((S)TEM) and Energy-dispersive X-ray spectroscopy (EDXS)

For (S)TEM/EDXS studies, U(VI) interaction experiments were performed using cell suspensions with the same optical density at pH 6.5. An initial U(VI) concentration of 0.1 mM was adjusted and the sample was stored on a rotary shaker at 30°C. After 5 hours, the U(VI) incubation was stopped. At the end of the experiment, the cells were harvested by centrifugation for 10 minutes at 7.870 x g (Centrifuge5804R, Eppendorf AG, Hamburg, Germany) at 30 °C. The supernatant was

removed and the cell pellet was washed twice with sterilized tap water at the defined pH. Subsequently, the cells were fixed with 2.5 % (vol/vol) glutardialdehyde from a 25 % (vol/vol) stock solution (Serva, Heidelberg, Germany) and stored at 4 °C. A reference sample without U was treated the same way. The fixed samples were prepared for transmission electron microscopy studies at the Center for Regenerative Therapies Dresden (CRTD) of the Technical University Dresden (Germany). In particular, ultrathin sections of < 100 nm were placed on carbon-coated Cu grids (200 mesh, Plano GmbH, Wetzlar, Germany). Bright-field transmission electron microscopy (TEM) images were recorded with an image-C_s-corrected Titan 80-300 microscope (Field Electron and Ion Company (FEI), Eindhoven, The Netherlands) operated at an accelerating voltage of 300 kV. Furthermore, high-angle annular dark-field scanning transmission electron microscopy (HAADF-STEM) imaging and spectrum imaging analysis based on energy-dispersive X-ray spectroscopy (EDXS) were performed with a Talos F200X (Thermo Fischer Scientific) microscope equipped with a high-brightness X-FEG electron source and a Super-X EDX detector system at an accelerating voltage of 200 kV (FEI). Prior to (S)TEM analysis, the specimen mounted in a high-visibility low-background holder was placed for 2 s into a Model 1020 Plasma Cleaner (Fischione, Export, PA, USA) to remove potential contamination.

2.6. Cryo-time resolved laser-induced fluorescence spectroscopy (cryo-TRLFS)

The *cryo*-TRLFS method was chosen due to the high sensitivity toward U(VI) complex formation in aqueous solutions (Moulin et al. 1990, 1995). For our experiments cultures of *Magnetospirillum magneticum* AMB-1 with an optical density of 1.6 at 600 nm were taken after 24 h of incubation and washed twice using sterilized tap water at defined pHs (3.5, 4.5, 5.5, 6.5, 7.5). Each washed cell suspension at the defined pH was resuspended in sterilized tap water and transferred into 4 flasks. A 0.1 M stock solution of $UO_2(NO_3)_2$ was added to the flasks to adjust an initial U(VI) concentration of 0.1 mM in the cell suspension. The flasks were stored at 30 °C on a rotary shaker (Rotamax 120, Heidolph Instruments). After 0.5, 2, 5 and 24 h samples were taken and centrifuged for 5 min at 7870 x g (Centrifuge5804R, Eppendorf AG, Hamburg, Germany). A volume of 1 mL of each supernatant was used for determination of the U concentration by means of ICP-MS. For *cryo*-TRLFS measurements each pellet was washed twice in sterilized tap water at the defined pH, and transferred into a UV cuvette, shock frozen by N_2 and stored at −80 °C. In preparation of the

cryo-TRLFS measurements, the pellets were transferred just before into a cupper holder. At 153 K the U(VI) luminescence was measured after excitation with laser pulses at 266 nm (Minilite high-energy solid-state laser; Continuum) and average pulse energy of 300 µJ. The emission of the samples was recorded using an iHR550 spectrograph (HORIBA Jobin Yvon) and an ICCD camera (HORIBA Jobin Yvon) in the 370.0–670.0 nm wavelength ranges by averaging 100 laser pulses and using a gate time of 2000 µs. The measurements were performed from 0.1 µs to 12.503 µs to ensure that the lifetimes of all species present were detected. The specific separation of the sum spectra obtained from the measurements into individual spectra was performed using parallel factor analysis (PARAFAC). For more information see Drobot et al. (2015). Important ligands, *e.g.*, peptidoglycan (PGN) from *Bacillus subtilis* (Sigma Aldrich), lipopolysaccharide (LPS) from *Pseudomonas aeruginosa* (Sigma Aldrich), L-rhamnose (Sigma Aldrich), D-(+) galactose (Sigma Aldrich), D-(+) mannose (Sigma Aldrich) were used as reference ligands and measured for comparison and interpretation of possible binding sites of U(VI) to the cell wall. For this, each ligand was dissolved in 2 mL of sterile tap water at pH of 3.5, 4.5, 5.5, 6.5 and 7.5 with a ligand excess of 1:20. A defined volume of a 0.1 M $UO_2(NO_3)_2$ stock solution was added to reach a final U concentration of 0.1 mM. The samples were transferred into a UV cuvette, shock frozen by N_2, stored at −80°C and used for *cryo*-TRLFS measurements.

2.7. In-situ attenuated total reflection Fourier-transform infrared spectroscopy (ATR FT-IR)

For molecular identification of the U(VI) bioassociation process of *Magnetospirillum magneticum* AMB-1 cells, *in-situ* ATR FT-IR spectroscopy with a sub-minute time resolution was performed. On a Bruker Vertex 80/v vacuum spectrometer equipped with a Mercury Cadmium Telluride detector infrared spectra were measured from 4000 to 400 cm^{-1}. Spectral resolution was 4 cm^{-1}, and spectra were averaged over 256 scans. A horizontal diamond crystal with nine internal reflections (DURA SamplIR II, Smiths Inc.) was used. Further details on the experimental ATR FT-IR spectroscopic setup are compiled in Müller et al. (2012). In our experiments a suspension of *Magnetospirillum magneticum* AMB-1 cells after 24 hours of incubation was centrifuged at 7870 x g for 10 minutes, washed twice with autoclaved tap water (at pH 4.5 and 6.5) and resuspended in 100 µL tap water at the same pH. A thin cell film was prepared directly on the

surface of the ATR diamond crystal as stationary phase by pipetting twice 1 µL of the sample on the ATR crystal and subsequent drying with a gentle stream of N_2. The cell film was flushed using a flow cell with a blank solution (sterilized tap water, pH 4.5 and 6.5) with a flow rate of 0.1 mL min^{-1} for 60 min for conditioning. Subsequently, a 0.01 mM uranylchloride solution (in sterilized tap water, pH 4.5 and 6.5) was rinsed over the cell film. Difference spectra are calculated at several times during the U(VI) bioassociation process. After 120 min of bioassociation, equilibrium was reached and no further U(VI) accumulation or change of the cell film was observed within the spectral data. Then, the U(VI)-loaded cell film was flushed again with sterilized tap water for 60 min for controlling the possible reversibility of the sorbed species. Further details on the calculation of difference spectra are given in Müller et al. (2013).

3. Results

3.1. U(VI) bioassociation of Magnetospirillum magneticum AMB-1

The U(VI) bioassociation of *Magnetospirillum magneticum* AMB-1 cells was first determined by time-dependent experiments, using different amounts of biomass (89.00, 77.04, 49.62 and 21.45 mg DBM) and an initial U(VI) concentration of 0.1 mM. The experiments were performed at pH 6.5, each with an incubation time of 25 h. The results showed a fast increase of the U(VI) bioassociation during the first hour for all biomass concentrations (Fig. 1). Equilibrium conditions were already reached in the experiment with the highest biomass concentration after 1 h. After 24 h at the latest, a steady state has been established in all other experiments. The removal efficiency of U(VI) by *Magnetospirillum magneticum* AMB-1 cells appears to be very effective, independent of the biomass concentration. As shown in Fig. 1, between 85.7 and 95.5 % of the initial U(VI) was removed from the suspensions, except the experiment with the lowest biomass. Here, only 55.8 % of the U(VI) was bound.

In further U(VI) bioassociation experiments a defined biomass concentration, each in a range from 74.3−82.8 mg DBM was chosen at different pH (3.5, 4.5, 5.5, 6.5, 7.5). These experiments provide

evidence of a pH dependent bioassociation. It is shown in Fig. 2, that the most U is bound at pH 6.5. The U(VI) bioassociation, measured in mg U/g DBM, shows the highest value of 30.17 mg U / g DBM after 24 h. All other pH values included, 25.2–27.6 mg U were absorbed per 1g DBM. It is a fast bioassociation that takes place in the first hour. Already 77.5 % of the U is removed from the suspension at pH 6.5. After 24 h, at the end of our experiment, 95 % of the initial U concentration is bound to *Magnetospirillum magneticum* AMB-1 cells. A slower bioassociation at the beginning of the experiment could be detected for acidic pH. At pH 3.5 there are only 46.7 % U(VI) bound in the first hour. Nevertheless, at the end of the experiment 86.7 % of U(VI) were removed from the suspension, even indicating an efficient removal of U(VI) by *Magnetospirillum magneticum* AMB-1 cells at low pH.

3.2. Cell viability by live-dead staining

During the U(VI) removal experiments, which were performed with an initial U(VI) concentration of 0.1 mM at pH 3.5–7.5 the cells were tested for their cell viability by live-dead staining (Syto®9 and propidium iodide) using CLSM. The results show that high numbers of viable or living cells were detected after 1 h incubation, with the exception of pH 3.5, where individual agglomerates of cells were visible (Supplementary Fig. 2). Compared to samples after 3 h of incubation, the AMB-1 cells showed nearly the same number of viable cells. After 24 h the majority of cells were viable with the exception of the cells, which were handled in tap water at a pH of 3.5 and also at a pH of 7.5. At acidic and basic pH the cells have only a very limited viability after 24 h. Agglomeration of dead cells are already determined at the beginning of the experiment at pH 3.5.

3.3. Localization of U by (S)TEM/EDXS analysis

For (S)TEM/EDXS studies, ultrathin sections of *Magnetospirillum magneticum* AMB-1 cells loaded with 0.1 mM U for 5 h at pH 6.5 were prepared. The bright-field TEM image in Fig. 3a shows a typically elongated *Magnetospirillum magneticum* AMB-1 cell that has formed up to five magnetosomes, which are visible as black dots. EDXS analysis clearly confirms that they

exclusively consist of Fe (Fig. 3b). Furthermore, element distribution analysis of P (Fig. 3c) and U (Fig. 3d) clearly indicates that U is predominantly located in the cell wall.

3.4. Determination of U species from cryo-TRLFS using PARAFAC analysis

After the incubation of *Magnetospirillum magneticum* AMB-1 cells with 0.1 mM U(VI) at different pH (3.5–7.5) and different contact times (0.5, 2, 5 and 24 h), the cells were washed, centrifuged and 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 U(VI) species, which were probably formed during the U(VI) bioassociation of *Magnetospirillum magneticum* AMB-1 cells (Fig. 4b, c, d, f, g). The single component spectra are characterized by different emission bands. For the identification and assignment of the extracted spectra to possible binding sites of U on the cells, reference spectra of potential ligands loaded with U(VI) were measured. Since EDXS elemental distribution analysis clearly indicate that U(VI) is predominantly bound in the cell wall, important ligands, *e.g*., peptidoglycan (PGN), lipopolysaccharide (LPS), L-rhamnose, D-(+) galactose and D-(+) mannose were considered as possible complexants for U(VI) on *Magnetospirillum magneticum* AMB-1 cell walls. The luminescence properties have shown that there is no correspondence with the extracted single component spectra of U species formed on *Magnetospirillum magneticum* AMB-1 cells with LPS, L-rhamnose, D-(+) galactose and D-(+) mannose (Supplementary Fig. 3). However, the situation is completely different concerning PGN. In Fig. 4b, c, d three extracted single component spectra (species 1–3) are shown together with the appropriate reference spectra of U loaded PGN from *Bacillus subtilis*. Peak positions and ratios of the U associated species indicate a very good match with the three reference spectra of U-PGN (Tab. 1). Since the studies were performed in the pH range from 3.5 to 7.5, statements on the pH dependent formation of the U species with PGN are significant. As shown in the relative luminescence intensity distribution of the PARAFAC extracted species (1–5) versus pH and time (Fig. 4e), species (1) is mainly significant in the acidic pH range and is rather negligible in the neutral and basic pH range. Species (2) dominates the bioassociation of U(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. The detected properties of the species are also proved by studies performed on the U-PGN references of the relative luminescence intensity distribution

of the extracted U-PGN species. Fig. 4a shows the significance of the U-PGN species as a function of pH by PARAFAC analysis, which clarifies the distribution of the U-PGN species in the pH range from 3.5 to 7.5.

In addition to the PGN species two more species should be mentioned. Species (4) shows four characteristic maxima of the luminescence emission peaks (Fig. 4f) and a dominance in the pH range from 3.5–4.5 (Fig. 4e). The emission peaks are comparable to literature data of reference spectra. These are spectra of molecules containing carboxylic acids. A study on the interaction of UO_2^{2+} with malonate by using TRLFS and a metal concentration relevant for environmental aquatic systems in a 0.1M perchloric media at pH 4 shows comparable data (Brachmann et al., 2002). The position of the emission bands of $UO_2C_3H_2O_4^0{}_{(aq)}$ are in good agreement with those of species (4). Thus, we conclude the formation of a malonate-like species at acidic pH during the bioassociation of U(VI) on *Magnetospirillum magneticum* AMB-1 cells.

The PARAFAC extracted single component spectra of species (5) is characterized by a broad emission band. Similar spectra are known from the literature as an unidentified species (Bader et al., 2018). In *Magnetospirillum magneticum* AMB-1 cells this species exists mainly at pH ≥ 6.5. In this pH range it may be the dominant U species bound on *Magnetospirillum magneticum* AMB-1 cells and thus have to be considered as an important species in the bioassociation process at higher pH.

3.5. Analysis of U(VI) coordination by in-situ ATR FT-IR

In-situ ATR FT-IR spectroscopy allows to discriminate which functional groups are responsible for U(VI) complexation at microorganism interfaces, *e.g.*, carboxylate, phosphoryl, amino or even hydroxyl groups (Barkleit et al., 2008; Barkleit et al., 2011; Comarmond et al., 2016; Jiang et al., 2004; Li et al., 2010; Theodorakopoulos et al., 2015; Krawczyk-Bärsch et al., 2018; Hilpmann et al., 2022). The experimental procedure involves the preparation of a cell film as stationary phase directly on the ATR crystal, which is then rinsed with aqueous background and U(VI) solutions at identical pH and ionic strength. In the meantime the cell film is continuously monitored by

recording single beam spectra in the sub-minute range. The calculation of difference spectra extracts only changes of the cell film that are associated with U(VI) interaction, constant spectral parts are not given. The time-resolved ATR FT-IR spectra at pH 4.5 and 6.5 are comparatively presented in Fig. 6. First, the cell film is equilibrated to aqueous sample conditions, *e.g.*, pH 4.5 and 6.5 by flushing a blank solution. The red traces in Fig. 5 show only weak absorption bands during last 30 minutes of conditioning that can be attributed to cell interactions in contact with water. This is a measure of quality for the stability of the cell film and hence, prerequisite for the *in-situ* ATR FT-IR experiment. The black spectra are difference spectra calculated at different times of U(VI) accumulation, ranging from 5 to 120 minutes. At both pH conditions significant spectral changes are observed. The increasing intensities of the bands upon U(VI) contact time suggests metal accumulation at the cell surface. The bands below at 940 cm^{-1} are attributed to the asymmetric stretching vibrational mode of the uranyl(VI) moiety, $\nu_3(UO_2)$. Generally, the frequency of this mode is observed at 961 cm^{-1} for the fully hydrated ion in strong acidic aqueous solution (Quilès and Burneau, 2000). Upon complexation in solution and at biogeochemical interface it is shifted to lower frequencies (Müller et al., 2008; Kretzschmar et al., 2021; Hilpmann et al., 2022).

At pH 4.5, $\nu_3(UO_2)$ is observed at 925 cm^{-1}, characteristic for complexation to carboxylate groups (Fig. 5a). This is in accordance with the spectral signature, we observe at higher wavenumbers. The bands at 1453 cm^{-1} and 1535 cm^{-1} can be assigned to the symmetrical $\nu_s(COO^-)$ and asymmetrical $\nu_{as}(COO^-)$ stretching vibrations of the carboxylate group (Barkleit et al., 2008; Theodorakopoulos et al., 2015; Krawczyk-Bärsch et al., 2018). The spectral region, where the asymmetric $\nu_{as}(PO)$ stretching modes are observed, shows also intense peaks at 1197 and 1106 cm^{-1}, suggesting that also phosphoryl functional groups are involved in U(VI) binding. However, since the symmetry of the $\nu_3(UO_2)$ band hints strongly to the predominance of only one surface species and phosphoryl binding is characterized by a stronger shift of $\nu_3(UO_2)$ to 905 cm^{-1}, this binding motif can be ruled out. With regard to the time-resolved data, the $\nu_3(UO_2)$ band shows a fast bioassociation of U during the first minutes. After 60 min sorption still takes place, but to a less extent, possibly due to mostly occupied binding sites. These findings agree very well with the kinetic batch results (Fig. 1, 2).

At pH 6.5, the spectra offer some significant differences (Fig. 5b). First, it must be noted that the overall intensity is considerably reduced in comparison to lower pH. Second, the spectral signature has changed, the $\nu_3(UO_2)$ mode is now characterized as an asymmetric-shaped broad band. Here, a multiple species set can be derived from 2nd derivative analysis of the time-resolved data. At 15 min of U(VI) bioassociation, three local peak maxima at 923, 911 und 896 cm^{-1} are extracted (data not shown). With ongoing bioaccumulation, the $\nu_3(UO_2)$ mode is distinctly shifted to wavenumbers below 900 cm^{-1} and contributions from 923 and 911 cm^{-1} become less important. One may interpret this spectral data set as a combination of U(VI) bioassociated with carboxyl as well as phosphoryl functionalities, as this was observed for certain microorganisms (Bader et al., 2017; Krawczyk-Bärsch et al., 2018). The vibrational modes of the ligands $\nu_{as\,s}(COO^-)$ and $\nu_{as}(PO)$ would support this. However, the appearance of $\nu_3(UO_2)$ mode is different in comparison to these previous works, where we clearly observed only two contributions, (1) the coordination to carboxylic groups with local band maximum at 925 cm^{-1} and (2) to the phosphorylic groups at 905 cm^{-1}. Local band maxima below 900 cm^{-1} were not observed so far. Unfortunately, the information given by (S)TEM/EDXS elemental distribution analysis, where phosphorous compounds together with U are detected in the cell wall, do not bring further insight on the binding motif. Therefore, an alternative interpretation of the IR data set becomes necessary. In a previous study of the complexation of U(VI) with citrate, a hydroxy-tricarboxylic acid, we observed in the experimental IR data a similar shifting of the $\nu_3(UO_2)$ mode with increasing complex nuclearity and changing speciation from binary U(VI)-CA to ternary complexes involving also hydoxides (Kretzschmar et al., 2021). Spectral shifts were observed from 961 down to 879 cm^{-1} with changing absorption properties of the different complexes.

As a last step of the *in-situ* ATR FT-IR experiment, the U(VI) loaded cell film is again flushed with the blank solution at the respective pH (blue traces in Fig. 5). Thus, the reversibility of the bioassociation process is given by the difference spectra calculated at distinct times after changing aqueous solutions. Please note the differences in absolute intensities upon comparing the sorption (black) and flushing IR spectra (blue). At pH 4.5 the spectral features at both stages are very similar and the reversibility seems to be very low. That means, the coordination of U(VI) to carboxylic groups at pH 4.5 is very strong and only few quantities are removed from the cell walls by simply flushing the blank solution. Again, the situation is different at pH 6.5. From the set of coordinating species only those exhibiting the $\nu_3(UO_2)$ mode mostly red shifted to 899 cm^{-1} is reversible. From

comparing the relative intensities, it seems that the assigned polynuclear species is quantitatively removed from the cells upon flushing a blank solution at the same pH.

4. Discussion

The interaction of U(VI) with *Magnetospirillum magneticum* AMB-1 cells is characterized by a fast pH dependent bioassociation that takes place in the first hours of incubation due to the removal capacity of predominantly alive cells. Up to 95 % of the initial U(VI) concentration was bound to the cells, which tolerate a U(VI) concentration of 0.1 mM, but are not able to survive permanently at acidic and basic pH. Similar results, which were received under similar experimental conditions, were reported in Gerber et al. (2016). Batch sorption and flow cytometry studies using the live-dead staining approach confirmed that metabolically active cells of a Gram-negative *Acidovorax facilis* strain were able to remove nearly 100 % of the initial U(VI) concentration from the solution in the first hours of incubation with 0.05 mM and 0.1 mM U(VI) at pH 5. Also Hufton et al. (2021) mentioned the highest U(VI) binding to cell wall components in Gram-negative bacteria at pH 5.5. A high sorption capacity was also determined by the Gram-positive bacterium *Brachybacterium* sp. G1 (Hilpmann et al., 2022), which was explained by the high number of carboxylate groups within the PGN layer of the cell wall (Bäuerlein, 2003). In the previously performed infrared spectroscopic studies on *Brachybacterium* sp. G1 by Bader et al. (2018) it has already been shown that carboxylate groups of the cell wall were dominantly involved in the U(VI) bioassociation. It seems that high sorption capacities of U(VI) could be found both in both Gram-positive and in Gram-negative bacteria, although the structure of the cell walls differs. In Gram-positive bacteria the cell wall components are mainly PGN (Hufton et al., 2021), while only a thin, 4 nm thick, PGN layer is found in the cell walls of Gram-negative bacteria (Bäuerlein, 2003). In addition to the PGN layer the Gram-negative cell wall also contains an outer membrane, which consists of phospholipids and lipopolysaccharides, representing potential binding sites for the predominant positively charged UO_2^{2+} species. Involvement of phospholipids and lipopolysaccharides in the bioassociation would therefore be expected and has been demonstrated in previous studies on Gram-negative bacteria (Kelly et al., 2002; Merroun et al., 2005; Llorens et al., 2012; Luetke et al., 2012; Krawczyk-Bärsch et al., 2018). In contrast, in the presented work, it is clearly shown that phosphoryl sites could be excluded as major binding site for U(VI). The data obtained by ATR FT-

IR spectroscopy provide evidence for the formation of strong complexation of U(VI) with carboxylic functionalities at pH 4.5 during the first minutes of incubation. The spectroscopic proof of a fast U(VI) bioassociation agrees very well with the kinetic batch results. TRLFS studies in combination with PARAFAC confirm the complexation of U(VI) to carboxylate groups as described by ATR FT-IR. The comparison of three PARAFAC extracted single component spectra with the appropriate reference spectra of U(VI) loaded PGN from *Bacillus subtilis* showed an excellent match and emphasizes that PGN is the most important ligand in the bioassociation of U(VI) to *Magnetospirillum magneticum* AMB-1 cells. A pH dependent study indicates furthermore that the complexation of U(VI) with PGN even takes place in the investigated pH range from 3.5–7.5. In previous potentiometric titration and TRLFS studies of Barkleit et al. (2009) on the interaction of UO_2^{2+} with PGN reference samples, three different U-PGN complexes were identified as well. However, we must take into account that the previous studies were not conducted under the same conditions as our studies. All solutions in the previous studies were prepared with carbonate-free deionized water with an ionic strength of 0.1 M by adding stock solutions from $NaClO_4 \cdot H_2O$. Also, UO_2^{2+} was added as $UO_2(ClO_4)_2 \cdot 6H_2O$ and all samples were measured at room temperature. To obtain comparable data from the reference studies to our experiments with *Magnetospirillum magneticum* AMB-1 cells, tap water was used. High ionic strength was thus omitted in order to provide the bacteria with a natural environment in which they can survive. For the same reason, care was also taken to use the added UO_2^{2+} in form of $UO_2(NO_3)_2$ stock solution. The most important difference, however, is that in the studies of Barkleit et al. (2009) mixed spectra were presented, whereas in our investigations with the help of PARAFAC single spectra of U-PGN could be shown. Nevertheless, the results of Barkleit et al. (2009) are indicating possible complexations due to the observed luminescence properties. Following these studies, species U-PGN 1 was determined in our studies at acidic pH. According to the previous studies, it could be assigned to the 1:1 uranyl carboxyl PGN complex $R–COO–UO_2^+$ (glutamic acid or diaminopimelic acid), which appears between pH 3 and 5. U-PGN 2 could be assigned to the 1:2 uranyl carboxyl chelate complex $(R–COO)_2–UO_2$, which dominates around pH 5.6 (Barkleit et al. (2009). In the neutral pH range the U-PGN 3 species is dominating. This U-PGN species might be the uranyl chelate complex with the mixed coordination $(R–COO–UO_2^{(+)}–Ai–R)$ (Ai = NH_2 or O^-).

The interpretation of time-resolved ATR FT-IR spectroscopy data obtained at pH 6.5 indicates differences. Considerable shifts, when increasing U(VI) loading at the cell surface, hint to multiple

surface species. Initially, possibly mononuclear U(VI) species are predominating, whereas at pH 6.5, polynuclear species become more prominent. The interpretation of the TRLFS/PARAFAC data also reflect the transition from mononuclear to polynulear U(VI) carboxylic coordination. In Bader et al. (2018) similar spectra were described as a typical loss of fine structure of polynuclear species, which they assigned to the 3:5 U(VI) hydroxo complex ($(UO_2)_3(OH)_5^+$). Wollenberg et al. (2021) summarizes it more generally and assume that this polynuclear species could consist of ligands in which amino, hydroxyl or sulphonyl groups could be important. In *Magnetospirillum magneticum* AMB-1 cells they may become the dominant U(VI) species, which mainly exists at pH ≥ 6.5. In contrast to the data obtained at pH 4.5, ATR FT-IR spectroscopy data indicate that the polynuclear species seems to be not as strongly coordinated and presents a higher reversibility under the given experimental conditions. When using magnetotactic bacteria for remediation purposes, this higher reversibility at pH ≥ 6.5 will not be a disadvantage. In open systems there will always be an inflow and thus a continuous flow of a high amount of bacteria.

Even though magnetotactic bacteria show similar strong sorption capacities of heavy metals and radionuclides as other Gram-negative or Gram-positive bacteria, there are some advantages that make magnetotactic bacteria interesting for the use in bioremediation of contaminated waters: i) Magnetotactic bacteria prefer to live planktonically (Lefèvre et al., 2013) and thereby provide an ideally large surface area for interactions with metals. ii) They do not form biofilms, as is known from many other bacteria such as *Bacillus* spp. (Zhong et al., 2021) or *Shewanella* sp. (Cao et al., 2011). Biofilm consists of multilayered cell clusters embedded in a matrix of exopolymeric substance (EPS), which facilitate the adherence of microorganism. As shown in Zhong et al. (2021), the EPS of these biofilms might also play a key role in U(VI) sequestration where U(VI) could be bound through cellular phosphate, hydroxyl, carboxyl, and amide groups through enzymatic bioprecipitation (Wei et al., 2017). However, biofilms are less suitable for flow-through systems than planktonic bacteria due to the risk of clogging and sedimentation, and the need for a carrier matrix which results in larger amounts of waste during disposal. The removal of heavy metal-loaded biofilms thus seems difficult and cost intensive. Similar problems could also arise with filament networks, *e.g.*, fungal systems, although fungi are also known as very good biosorbents for heavy metals and radionuclides due to the immobilization in the mycosphere, the sorption to cell walls or the uptake into fungal cells (Baeza and Guillén, 2006; Harms et al., 2011; Vázquez-Campos et al., 2015; Wollenberg et al., 2021). iii) Magnetotactic bacteria are unique

574 species of bacteria due to the presence of intracellular magnetic nanoparticles. With simple and
575 cheap technical means, the heavy metal-loaded magnetotactic bacteria could thus be removed very
576 easily from water with the help of magnets. In a laboratory study by Tanaka et al. (2016) it was
577 shown that over 80 % of bacteria were successfully recovered within 10 hours by magnetic force.
578 Even in our small microcosm experiments we could already show that this is already possible with
579 a small magnet.
580
581
582 **5. Conclusions**
583
584 The results obtained from our multidisciplinary approach combining microscopy and different
585 spectroscopic techniques to study the U(VI) biassociation on magnetotactic bacteria extend our
586 knowledge about the interactions of U(VI) with bioligands in the cell wall of Gram-negative
587 bacteria. In contrast to the previously characterized binding of U(VI) to carboxyl and phosphoryl
588 groups of Gram-negative bacteria, carboxylic functionalities now play the dominant role in the
589 bioassociation of U(VI) by *Magnetospirillum magneticum* AMB-1 showing a stable
590 immobilization of U(VI) over a wide pH range. The results presented highlight the dominant role
591 of the ligand peptidoglycan as main sorbent of U(VI) on the cell wall of *Magnetospirillum*
592 *magneticum* AMB-1 cells. Three characteristic peptidoglycan species were identified by use of
593 respective references. Especially with regards to the development of innovative bioremediation
594 strategies of contaminated water, the presented studies show clearly that *Magnetospirillum*
595 *magneticum* AMB-1 cells are suitable candidates. They can survive as planktonic cells both in a
596 wide pH range and with relatively high U(VI) concentrations of up to 0.1 mM, while effectively
597 and almost completely immobilizing U(VI). In combination with its magnetic properties,
598 *Magnetospirillum magneticum* AMB-1 cells offer many advantages for the development of various
599 bioassociation technologies not only for U(VI), but probably also for many other heavy metals.
600
601
602 **Acknowledgments**
603 The authors are grateful to Damien Faivre and Sandra Prévéral (CEA Cardarache, France) for pro-
604 viding the strain of *Magnetospirillum magneticum* AMB-1. We thank Sabrina Beutner and Sylvia
605 Schöne (both HZDR, Germany) for ICP-MS analysis and DIC measurements. Karsten Heim´s

skilful work on *in-situ* ATR FT-IR spectroscopy is gratefully acknowledged. The Biotechnology Center of the Technical University Dresden (Germany) is thanked for the preparation of the (S)TEM samples. Furthermore, the use of the HZDR Ion Beam Center TEM facilities and the funding of TEM Talos by the German Federal Ministry of Education and Research (BMBF; grant No. 03SF0451) in the framework of HEMCP are acknowledged.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at:

References

Afkhami, F., Taherkhani, S., Mohammadi, M., Martel, S., 2011. Encapsulation of magnetotactic bacteria for targeted and controlled delivery of anticancer agents for tumor therapy. Engineering in Medicine and Biology Society, EMBC, 33rd Annual International Conference of the IEEE EMBS Boston, Massachusetts USA, p. 6668–6671.

Amann, R., Peplies, J., Schüler, D., 2007. Diversity and taxonomy of magnetotactic bacteria, p 25-36. In: Schüler D (ed), *Magnetoreception and magnetosomes in bacteria*. Springer, Berlin, Germany.

Bader, M., Müller, K., Foerstendorf, H., Drobot, B., Schmidt, M., Musat, N., Swanson, J.S., Reed, D.T., Stumpf, T., Cherkouk, A., 2017. Multistage bioassociation of uranium onto an extremely halophilic archaeon revealed by a unique combination of spectroscopic and microscopic techniques. *J. Hazard. Mater*., 327, 225−232.

Bader, M., Rossberg, A., Steudtner, R., Drobot, B., Großmann, K., Schmidt, M., Musat, N., Stumpf, T., Ikeda-Ohno, A., Cherkouk, A., 2018. Impact of Haloarchaea on Speciation of Uranium – Multispectroscopic Approach. *Environ. Sci. Technol*., 52, 12895−12904.

Baeza, A. and Guillén, J., 2006. Influence of the soil bioavailability of radionuclides on the transfer of uranium and thorium to mushrooms. *Appl. Radiat. Isot*. 64, 1020–1026.

Bäuerlein, E., 2003. Biomineralization of unicellular organisms: An unusual membrane biochemistry for production of inorganic nano- and microstructures. *Angew. Chem. Int. Ed*., 42:614–641.

Bahaj, A.S., James, P.A.B., Croudace, I.W., 1994. Metal uptake and separation using magnetotactic bacteria. Magnetics, *IEEE Trans. Magn*. 30, 4707–4709.

Bahaj, A.S., Croudace, I.W., James, P.A.B., Moeschler, F.D., Warwick, P.E., 1998. Continuous radionuclide recovery from wastewater using magnetotactic bacteria. *J. Magn. Magn. Mater*. 184, 241–244.

Balkwill, D.L., Maratea, D., Blakemore, R.P., 1980. Ultrastructure of a magnetic spirillum. *J. Bacteriol*. 141, 1399–1408.

Barkleit, A., Foerstendorf, H., Heim, K., Sachs, S., Bernhard, G., 2008. Complex formation of uranium(VI) with l-phenylalanine and 3-phenylpropionic acid studied by attenuated total reflection Fourier transform infrared spectroscopy. *Appl. Spectrosc*. 62 (7), 798–802.

Barkleit, A., Moll, H., Bernhard, G., 2009. Complexation of uranium(VI) with peptidoglycan. *Dalton Trans*., 5379–5385.

Barkleit, A., Foerstendorf, H., Li, B., Rossberg, A., Moll, H., Bernhard, G., 2011. Coordination of uranium(VI) with functional groups of bacterial lipopolysaccharide studies by EXAFS and FT-IR spectroscopy. *Dalton Trans*. 40, 9868–9876.

Bazylinski, D.A., Heywood, B.R., Mann, S., Frankel, R.B., 1993. Fe_30_4 and Fe_3S_4 in a bacterium. *Nature* 366, 218.

668 Bazylinski, D.A. and Frankel, R.B., 2004. Magnetosome formation in prokaryotes. *Nat. Rev.*
669 *Microbiol*. 2:217–230.
670
671 Bazylinski, D.A., Lefèvre, C.T., Schüler, D., 2013. Magnetotactic bacteria, p 453–494. In
672 Rosenberg E, DeLong EF, Lory S, Stackebrandt E, Thompson F (ed), The prokaryotes. Springer,
673 Berlin, Germany.
674
675 Beyenal, H., Sani, R.K., Peyton, B.M., Dohnalkova, A.C., Amonette, J.E., Lewandowski, Z., 2004.
676 Uranium immobilization by sulfate-reducing biofilms. *Environ. Sci. Technol*. 38, 2067–2074.
677
678 Blakemore, R., 1975. Magnetotactic bacteria. *Science* 190, 377–379.
679
680 Brachmann, A., Geipel, G., Bernhard, G., Nitsche, H., 2002. Study of uranyl(VI) malonate
681 complexation by time resolved laser-induced fluorescence spectroscopy (TRLFS). *Radiochim.*
682 *Acta* 90, 147–153.
683
684 Brookshaw, D.R., Pattrick, R.A.D., Lloyd, J.R., Vaughan, D.J., 1012. Microbial effects on mineral-
685 radionuclide interactions and radionuclide solid-phase capture processes. *Mineral. Mag*. 76, 777.
686
687 Cao, B., Shi, L., Brown, R.N., Xiong, Y., Fredrickson, J.K., Romine, M.F., Marshall, M.J., Lipton,
688 M.S., Beyenal, H., 2011. Extracellular polymeric substances from Shewanella sp. HRCR-1
689 biofilms: characterization by infrared spectroscopy and proteomics. *Environ. Microbiol.* 13(4),
690 1018–103.
691
692 Comarmond, M.J., Steudtner, R., Stockmann, M., Heim, K., Müller, K., Brendler, V., Foerstendorf,
693 H., 2016. The sorption processes of U(VI) onto SiO_2 in the presence of phosphate: from binary
694 surface species to precipitation. *Environ. Sci. Technol.* 50 (21), 11610–11618.
695
696 Drobot, B., Steudtner, R., Raff, J., Geipel, G., Brendler, V., Tsushima, S., 2015. Combining
697 luminescence spectroscopy, parallel factor analysis and quantum chemistry to reveal metal
698 speciation – a case study of uranyl(VI) hydrolysis. *Chem. Sci*., 6, 964.
699

Flies, C.B., Jonkers, H.M., de Beer, D., Bosselmann, K., Bottcher, M.E., Schüler, D., 2005. Diversity and vertical distribution of magnetotactic bacteria along chemical gradients in freshwater microcosms. FEMS *Microbiol Ecol* 52: 185–195.

Frankel, R.B., Bazylinski, D.A., Johnson, M.S., Taylor, B.L., 1997. Magneto-Aerotaxis in Marine Coccoid Bacteria. *Biophys. J.* 73, 994.

Frankel, R.B., Papaefthymiou, G.C., Blakemore, R.P., O'Brien, W., 1983. Fe_3O_4 precipitation in magnetotactic bacteria. *Biochim. Biophys.Acta* 763, 147.

Gadd, G.M., 2008. Biosorption: critical review of scientific rationale, environmental importance and significance for pollution treatment. *J. Chem. Technol. Biot.*, 84, 13–28.

Gerber, U., Zirnstein, I., Krawczyk-Bärsch, E, Lünsdorf, H., Arnold, T., Merroun, M.L., 2016. Combined use of flow cytometry and microscopy to study the interactions between the gram-negative betaproteobacterium *Acidovorax facilis* and uranium(VI). *J. Hazard. Mat.* 317, 127–134.

Harms, H., Schlosser, D., Wick, L.Y., 2011. Untapped potential: exploiting fungi in bioremediation of hazardous chemicals. *Nature Reviews, Microbiology* 9, 177–192.

Hilpmann, S., Bader, M., Steudtner, R., Müller, K., Stumpf, T., Cherkouk, A., 2022. Microscopic and spectroscopic bioassociation study of uranium(VI) with an archaeal *Halobacterium* isolate. *PLoS ONE* 17(1): e0262275.

Hufton, J., Harding, J., Smith, T., Romero-González, M.E., 2021. The importance of the bacterial cell wall in uranium(VI) biosorption. *Phys.Chem.Chem.Phys*., 2021, 23, 1566.

Jacob, J.J., Suthindhiran, K., 2016. Magnetotactic bacteria and magnetosomes – Scope and challenges. *Mater. Sci. Eng.*, C, 68, 919–928.

730 Jayaraman, J., Sigamani, S., Ramamurthy, D., 2021. Metal biosorption by magnetotactic bacteria
731 isolated from fresh water sediments and characterization of extracted magnetosomes. *Arch.*
732 *Microbiol.* 203:5951–5962.
733
734 Jiang, W., Saxena, A., Song, B, Ward, B.B., Beveridge, T.J., Myneni, S.C.B., 2004. Elucidation of
735 Functional Groups on Gram-Positive and Gram-Negative Bacterial Surfaces Using Infrared
736 Spectroscopy. *Langmuir* 20, 11433–11442.
737
738 Jogler, C., Kube, M., Schübbe, S., Ullrich, S., Teeling, H., Bazylinski, D.A., Reinhardt, R., Schüler,
739 D., 2009. Comparative analysis of magnetosome gene clusters in magnetotactic bacteria provides
740 further evidence for horizontal gene transfer. *Environ Microbiol* 11: 1267–1277.
741
742 Kabary, H., Eida, M.F., Attia, M.M., Awad, N., Easa, S.M., 2017. Magnetotactic Characterization
743 and Environmental Application *P. aeruginosa* kb1 Isolate. *Annual Research & Review in Biology*
744 20(1): 1-10.
745
746 Kelly, S.D., Kemner, K.M., Fein, J.B., Fowle, D.A., Boyanov, M.I., Bunker, B.A., Yee, N., 2002.
747 X-ray absorption fine structure determination of pH-dependent U-bacterial cell wall interactions.
748 *Geochim. Cosmochim. Acta*, 66(22), 3855–3871.
749
750 Komeili, A., Vali, H., Beveridge, T.J., Newman, D.K., 2004. Magnetosome vesicles are present
751 before magnetite formation, and MamA is required for their activation. *PNAS* 101(11), 3839–3844.
752
753 Krawczyk-Bärsch, E., Gerber, U., Müller, K., Moll, H., Rossberg, A., Steudtner, R., Merroun, M.L,
754 2018. Multidisciplinary characterization of U(VI) sequestration by *Acidovorax facilis* for
755 bioremediation purposes. *J. Hazard. Mat.* 347, 233–241.
756
757 Kretzschmar, J., Tsushima, S., Lucks, C., Jäckel, E., Meyer, R., Steudtner, R., Müller, K.,
758 Rossberg, A., Schmeide, K., Brendler, V., 2021. Dimeric and Trimeric Uranyl(VI)−Citrate
759 Complexes in Aqueous Solution *Inorg. Chem.*, 60, 7998-8010.
760

Lefèvre, C.T. and Bazylinski, D.A., 2013. Ecology, Diversity, and Evolution of Magnetotactic Bacteria. *Microbiol. Mol. Biol. Rev.* 77(3), p. 497–526.

Lesmana, O., Febriana, N., Soetaredjo, F.E., Sunarso, J., Ismadji, S., 2009. Studies on potential applications of biomass for the separation of heavy metals from water and wastewater. *Biochem. Eng. J.* 44 (1), 19–41.

Li, B., Raff, J., Barkleit, A., Bernhard, G., Foerstendorf, H., 2010. Complexation of U(VI) with highly phosphorylated protein, phosvit in a vibrational spectroscopic approach. *J. Inorg. Biochem*. 104 (7), 718–725.

Li, J., Wang, X., Zhao, G., Chen, C., Chai, Z., Alsaedi, A., Hayat, T., Wang, X., 2018. Metal-organic framework-based materials: superior adsorbents for the capture of toxic and radioactive metal ions. *Chem. Soc. Rev*. 47, 2322–2356.

Li, X., Liu, Y., Zhang, C., Wen, T., Zhuang, L., Wang, X., Song, G., Chen, D., Ai, Y, Hayat, T., Wang, X., 2018. Porous Fe_2O_3 microcubes derived from metal organic frameworks for efficient elimination of organic pollutants and heavy metal ions. *Chem. Eng. J.* 336, 241–252.

Llorens, I., Untereiner, G., Jaillard, D., Gouget, B., Chapon, V., Carriere, M., 2012. Uranium interaction with two multi-resistant environmental bacteria: *Cupriavidus metallidurans* CH34 and *Rhodopseudomonas palustris*. *PLoS ONE* 7(12): e51783

Lloyd, J.R. and Macaskie, LE., 2002. Biochemical basis of microbe-radionuclide interactions. In: Interactions of Microorganisms with Radionuclides (ed. J. Miranda). Keith-Roach, M.J. and Livens, F.R., Elsevier Sciences, Oxford, UK, pp. 313–342.

Lütke, L., Moll, H., Bernhard, G,. 2012. Insights into the uranium(VI) speciation with *Pseudomonas fluorescens* on a molecular level. *Dalton Trans*., 41, 13370.

Macaskie, L.E., Bonthrone, K.M., Yong, P., Goddard, D.T., 2016. Enzymically mediated bioprecipitation of uranium by a Citrobacter sp.: a concerted role for exocellular lipopolysaccharide and associated phosphatase in biomineral formation. *Microbiology*-UK 146, 1855–1867.

Mann, S., Sparks, N.H.C., Frankel, R.B., Bazylinski, D.A., Jannasch, H.W., 1990. Biomineralization of ferromagnetic greigite (Fe_3S_4) and iron pyrite (FeS_2) in a magnetotactic bacterium. *Nature* 343, 258.

Merroun, M.L., Raff, J., Rossberg, A., Hennig, C., Reich, T., Selenska-Pobell, S., 2005. Complexation of uranium by cells and s-layer sheets of *Bacillus sphaericus* JG-A12. *Appl. Environ. Microbiol.* 71, 5532–5543.

Merroun, M.L. and Selenska-Pobell, S., 2008. Bacterial interactions with uranium: an environmental perspective. *J. Contam. Hydrol*. 102, 285–295.

Merroun, M.L., Nedelkova, M., Ojeda, J.J., Reitz, T., Fernandez, M-L., Arias, J.M., Romero-Gonzalez, M., Selenska-Pobell, S., 2011). Bio-precipitation of uranium by two bacterial isolates recovered from extreme environments as estimated by potentiometric titration, TEM and X–ray absorption spectroscopic analyses. *J. Hazard. Mater.* 197, 1–10.

Mokrani, N., Felfoul, O., Zarreh, F.A., Mohammadi, M., Aloyz, R., Batist, G., Martel, S., 2010. Magnetotactic bacteria penetration into multicellular tumor spheroids for targeted therapy. Engineering in Medicine and Biology Society (EMBC), 32nd Annual International Conference of the IEEE EMBS Buenos Aires, Argentina, p 4371–4374.

Monteil, C.L., Benzerara, K., Menguy, N., Bidaud, C.C., Michot-Achdjian, E., Bolzoni, R., Mathon, F.P., Coutaud, M., Alonso, B., Garau, C., Jézéquel, D., Viollier, E., Ginet, N., Floriani, M., Swaraj, S., Sachse, M., Busigny, V., Duprat, E., Guyot, F., Lefevre, C.T., 2021. Intracellular amorphous Ca-carbonate and magnetite biomineralization by a magnetotactic bacterium affiliated to the *Alphaproteobacteria*. *ISME J* 15: 1–18.

Moulin, C., Beaucaire, C., Decambox, P., Mauchien, P., 1990. Determination of uranium in solution at the ng L-1 level by time resolved laserinduced spectrofluorimetry - application to geological samples. *Anal. Chim. Acta* 238(2):291–296.

Moulin, C., Decambox, P., Moulin, V., Decaillon, J.G., 1995. Uranium speciation in solution by time-resolved laser-induced fluorescence. *Anal. Chem.* 67(2):348–353.

Müller, K., Brendler, V., Foerstendorf, H., 2008. Aqueous Uranium(VI) Hydrolysis Species Characterized by Attenuated Total Reflection Fourier-Transform Infrared Spectroscopy. *Inorg. Chem*., 47, 10127-10134.

Müller, K., Foerstendorf, H., Meusel, T., Brendler, V., Lefèvre, G., Comarmond, M.J., Payne, T.E., 2012. Sorption of U(VI) at the TiO2–water interface: an in-situ vibrational spectroscopic study. *Geochim. Cosmochim. Acta* 76, 191–205.

Müller, K., Foerstendorf, H., Brendler, V., Rossberg, A., Stolze, K., Gröschel, A., 2013. The surface reactions of U(VI) on gamma-Al_2O_3 – in-situ spectroscopic evaluation of the transition from sorption complexation to surface precipitation. *Chem. Geol.* 357, 75–84.

Ojeda, J.J., Romero-Gonzalez, M.E., Bachmann, R.T., Edyvean, R.G.J., Banwart, S.A., 2008. Characterization of the Cell Surface and Cell Wall Chemistry of Drinking Water Bacteria by Combining XPS, FTIR Spectroscopy, Modeling, and Potentiometric Titrations. *Langmuir* 24, 4032–4040.

Quilès, F. and Burneau, A., 2000. Infrared and Raman spectra of uranyl VI oxo-hydroxo complexes in acid aqueous solutions: a chemometric study. *Vib. Spectrosc*., 23, 231-241.

Schierz, A. and Zänker, H., 2009. Aqueous suspensions of carbon nanotubes: Surface oxidation, colloidal stability and uranium sorption. *Environ. Pollut.* 157,1088–1094.

Song, H.-P., Li, X.-G., Sun, J.-S., Xu, S.-M., Han, X., 2008. Application of a magnetotactic bacterium, *Stenotrophomonas* sp. to the removal of Au(III) from contaminated wastewater with a magnetic separator. *Chemosphere* 72, 616–621.

Spring, S., Amann, R., Ludwig, W., Schleifer, K.H., Vangemerden, H., Petersen, N., 1993. Dominating role of an unusual magnetotactic bacterium in the micro-aerobic zone of a fresh-water sediment. *Appl. Environ. Microbiol.* 59: 2397–2403.

Suzuki, Y. and Banfield, J.F., 2004. Resistance to and accumulation of, uranium by bacteria from a uranium-contaminated site. *Geomicrobiol. J.* 21, 113–121.

Tanaka, M., Knowles, W., Brown, R., Hondow, N., Arakaki, A., Baldwin, S., Staniland, S., Matsunaga, T., 2016. Biomagnetic Recovery and Bioaccumulation of Selenium Granules in Magnetotactic Bacteria. *Appl. Environ. Microbiol*. 82(13), 3886-3891.

Theodorakopoulos, N., Chapon, V., Coppin, F., Floriani, M., Vercouter, T., Sergeant, C., Camilleri, V., Berthomieu, C., Février, L., 2015. Use of combined microscopic and spectroscopic techniques to reveal interactions between uranium and *Microbacterium* sp. A9, a strain isolated from the Chernobyl exclusion zone. *J. Hazard. Mat.* 285, 285–293.

Vázquez-Campos, X., Kinsela, A.S., Collins, R.N., Neilan, B.A., Aoyagi, N., Waite, T.D., 2015. Uranium binding mechanisms of the acid-tolerant fungus *Coniochaeta fodinicola*. *Environ. Sci. Technol.* 49, 8487–8496.

Wang, Y., Gao, H., Sun, J., Li, J., Su, Y., Ji, Y., Gong, C., 2011. Selective reinforced competitive biosorption of Ag (I) and Cu (II) on *Magnetospirillum gryphiswaldense*. *Desalination* 270, 258–263.

Wei, L.L., Li, Y., Noguera, D.R., Zhao, N.B., Song, Y., Ding, J., Zhao, Q., Cui, F., 2017. Adsorption of Cu^{2+} and Zn^{2+} by extracellular polymeric substances (EPS) in different sludges: effect of EPS fractional polarity on binding mechanism. *J. Hazard. Mater.* 321, 473–483.

Wollenberg, A., Kretzschmar, J., Drobot, B., Hübner, R., Freitag, L., Lehmann, F., Günther, A., Stumpf, T., Raff, J., 2021. Uranium(VI) bioassociation by different fungi – a comparative study into molecular processes. *J. Hazard. Mat.* 411, 125068.

Wu, W.-M., Carley, J., Gentry, T., Ginder-Vogel, M.A., Fienen, M., Mehlhorn, T., Yan, H., Caroll, S., Pace, M.N., Nyman, J., Luo, J., Gentile, M., Fields, M.W., Hickey, R.F., Gu, B., Watson, D., Cirpka, O.A., Zhou, J., Fendorf, S., Kitanidis, P.K., Jardine, P.M., Criddle, C.S., 2006. Pilot-scale in situ bioremedation of uranium in a highlycontaminated aquifer. 2. Reduction of U(VI) and geochemical control of U(VI) bioavailability. *Environ. Sci. Technol*. 40, 3986–3995.

Zhang, Y.-J., Zhou, Z.-J., Lan, J.-H., Ge, C.-C., Chai, Z.-F., Zhang, P., Shi, W.-Q., 2017. Theoretical insights into the uranyl adsorption behavior on vanadium carbide MXene. *Appl. Surf. Sci*. 426, 572–578.

Zhong, J., Hu, X., Liu, X., Cui, X., Lv, Y., Tang, C., Zhang, M., Li, H., Qiu, L., Sun, W., 2021. Isolation and Identification of Uranium Tolerant Phosphate-Solubilizing *Bacillus* spp. and Their Synergistic Strategies to U(VI) Immobilization. *Front. Microbiol*. 12:676391.

Young, J., J.Y. Lee, Yang, J.-S., 2009. Biosorption of heavy metals and uranium by starfish and *Pseudomonas putida*. *J. Hazard. Mat*.161 (1), 157–162.

Yu, S., Tang, H., Zhang, D., Wang, S., Qiu, M., Song, G., Fu, D., Hu, B., Wang, X., 2022. MXenes as emerging nanomaterials in water purification and environmental remediation. *Sci. Total Environ.* 811, 152280.