

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

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

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19  
20  
21 **Abstract**

22  
23 The U(VI) bioassociation on *Magnetospirillum magneticum* AMB-1 cells was investigated using  
24 a multidisciplinary approach combining wet chemistry, microscopy, and spectroscopy methods to  
25 provide deeper insight into the interaction of U(VI) with bioligands of Gram-negative bacteria for  
26 a better molecular understanding. Our findings suggest that the cell wall plays a prominent role in  
27 the bioassociation of U(VI). In time-dependent bioassociation studies, up to 95 % of the initial  
28 U(VI) was removed from the suspension and probably bound on the cell wall within the first hours  
29 due to the high removal capacity of predominantly alive *Magnetospirillum magneticum* AMB-1  
30 cells. PARAFAC analysis of TRLFS data highlights that peptidoglycan is the most important  
31 ligand involved, showing a stable immobilization of U(VI) over a wide pH range with the formation  
32 of three characteristic species. In addition, *in-situ* ATR FT-IR reveals the predominant strong  
33 binding to carboxylic functionalities. At higher pH polynuclear species seem to play an important  
34 role. This comprehensive molecular study may initiate in future new remediation strategies on  
35 effective immobilization of U(VI). In combination with the magnetic properties of the bacteria, a  
36 simple technical water purification process could be realized not only for U(VI), but probably also  
37 for other heavy metals.

38

39

## 40 **1. Introduction**

41

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

65

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

71 magnetotactic bacteria has been determined by several researchers. [Monteil et al. \(2021\)](#) report  
72 about a large abundance up to  $5.8 \times 10^5$  cells mL<sup>-1</sup> of porewater in the sediments of Lake Pavin  
73 (France). In previous studies, the abundance was even found to be up to  $10^7$  cells mL<sup>-1</sup> in sediments  
74 of aquatic systems ([Spring et al., 1993](#); [Flies et al., 2005](#); [Jogler et al., 2009](#)), so that already [Spring](#)  
75 [et al., \(1993\)](#) assigned a dominating role to these bacteria in the microbial ecology in his studies of  
76 Lake Chiemsee sediments. Magnetotactic bacteria belong to the family of *Rhodospirillaceae* and  
77 are associated with the Alpha-, Gamma-, and Deltaproteobacteria classes of the Proteobacteria  
78 phylum and with the *Nitrospirae* phylum ([Amann et al., 2007](#)). They vary greatly in shape, existing  
79 in spiral, vibrio, rod, or even coccoid forms ([Lefèvre and Bazylinski, 2013](#)). The special feature of  
80 magnetotactic bacteria is that they synthesize intracellular magnetic mineral crystals, the so-called  
81 magnetosomes ([Balkwill et al., 1980](#)), which are protected by a lipid bilayer membrane about 3–4  
82 nm thickness. The magnetic crystals can be made of iron oxide magnetite (Fe<sub>3</sub>O<sub>4</sub>) ([Frankel et al.,](#)  
83 [1983](#)) or iron sulfide greigite (Fe<sub>3</sub>S<sub>4</sub>) ([Mann et al., 1990](#)). Some cells are able to form both types  
84 of mineral ([Bazylinski et al., 1993](#)). The magnetosomes are responsible that magnetotactic bacteria  
85 orient in the presence of external Earth's magnetic field lines ([Blakemore, 1975](#)) and actively swim  
86 by the means of their flagellar rotation toward a preferred oxygen concentration ([Frankel et al.,](#)  
87 [1997](#)). They are either microaerophiles, anaerobes, or both and are generally found at the oxic-  
88 anoxic interface and the anoxic regions of the habitat ([Bazylinski et al., 2013](#)).

89  
90 Due to the magnetic properties of the magnetosomes, potential applications in microbiology,  
91 biophysics, biochemistry, nanotechnology and also biomedicine are known, *e.g.*, immobilization  
92 of biomolecule, drug delivery, targeted and controlled delivery of anticancer agents for tumor  
93 therapy ([Jacob et al., 2016](#); [Afkhami et al., 2011](#); [Mokrani et al., 2010](#)). So far, a potential  
94 environmental application for magnetotactic bacteria in water purification processes was not yet  
95 implemented, although the use of the magnetic properties of these bacteria could play a major role  
96 in water treatment by removing heavy metal-loaded bacteria from water using simple technical  
97 means. Presumably it can be justified by the fact that so far only a few studies on the sorption of  
98 heavy metals by magnetotactic bacteria are known to date. In the past, the uptake of Pu by  
99 magnetotactic bacteria was shown by adding purified Pu, consisting of mixed  $\alpha$  (<sup>238</sup>Pu, <sup>239</sup>Pu, <sup>240</sup>Pu)  
100 and  $\beta$  (<sup>241</sup>Pu) emitters to a culture of magnetotactic bacteria ([Bahaj et al., 1998](#)). In a study from  
101 [Bahaj et al. \(1994\)](#) the effect of heavy metals (Al, Cd, Co, Cu, Fe, Mg, Mn, Pb, Zn) at different  
102 concentrations (1 ppm, 10 ppm, 100 ppm) were measured on the motility of magnetotactic

103 bacteria. In somewhat recent studies more detailed data are given by [Wang et al. \(2011\)](#). The  
104 removal efficiency of Ag(I) and Cu(II) by *Magnetospirillum gryphiswaldense* MSR-1 strain was  
105 mentioned to be 91 % and 62 %, respectively when using a wet weight biomass of 10 g/L and an  
106 initial concentration of 80 mg/L Ag(I) and Cu(II) at a pH of 4.0 and 5.0, respectively. In studies  
107 with the magnetotactic bacterial isolate *Stenotrophomonas* sp. it was shown, that Au(III) was  
108 removed from the contaminated water with a high biosorption capacity of 506, 369 and 308 mg  
109 Au(III) per g dry weight biomass at the initial pH values of 2.0, 7.0 and 12.0, respectively ([Song et  
110 al., 2008](#)). In recent studies, *Pseudomonas aeruginosa* Kb1, which was found to synthesis  
111 intracellular magnetosomes, was able to remove 99.4 % and 70 % of Cd and Pb at initial  
112 concentration of 4 ppm ([Kabary et al., 2017](#)). [Jayaraman et al. \(2021\)](#) showed that the strains of  
113 *Stenotrophomonas maltophilia*, *Pseudomonas aeruginosa* and *Achromobacter xylosoxidans* are  
114 able to tolerate higher concentration of Mn, Zn, Cu and Cr ranging from 20, 30, 50, 70, 90 and  
115 150 mg. The studies were performed on modified nutrient agar medium using MnCl<sub>2</sub>, ZnSO<sub>4</sub>,  
116 CuSO<sub>4</sub>·5H<sub>2</sub>O and K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub>. Although these studies already indicate, that magnetotactic bacteria  
117 could be a promising biosorbent for heavy metals, detailed information about binding properties,  
118 molecular identification of the bioassociation process, and statements on the formed species are  
119 missing. In the presented study, these issues are addressed using a multidisciplinary approach that  
120 includes wet chemistry, microscopy, and spectroscopy. The highly sensitive *cryo*-time resolved  
121 laser-induced fluorescence spectroscopy was used in combination with parallel factor analysis.  
122 This new type of evaluation offers the advantage over the previous spectroscopic method to obtain  
123 individual spectra of metal-ligand species from a sum spectrum. *In-situ* attenuated total reflection  
124 Fourier transform infrared spectroscopy can furthermore contribute to the molecular identification  
125 of the bioassociation process. These information are helpful for a better understanding of the  
126 mechanisms involved in the interaction of magnetotactic bacteria with heavy metals and could  
127 contribute to the development of remediation strategies of contaminated waters.

128  
129 Our studies focused on uranium (U) since, to our knowledge, there are no published studies to date  
130 showing the interaction of U with magnetotactic bacteria, although there should be great interest  
131 in it, since the existence of U in waters and sediments causes hazardousness to the environment  
132 and humans. The entry of U into the human body via the food chain, by inhalation of dusts, and  
133 through water can lead to serious illness or even death ([Lloyd and Macaskie, 2002](#)).

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

143

144

## 145 **2. Materials and methods**

146

### 147 *2.1. Cultivation*

148

149 The strain of *Magnetospirillum magneticum* AMB-1 was kindly provided by the Molecular and  
150 Environmental Microbiology Department of the Institute of Biosciences and Biotechnologies from  
151 CEA Cadarache in France. The cells were grown in 200 mL flasks in 1.5 mM MagMin medium  
152 containing Wolfe's mineral solution, Wolfe's vitamin elixir and iron-malate after Komeili et al.  
153 (2004), modified by CEA Cadarache with the addition of iron malate (1:2000) and vitamin elixir  
154 (1:2000). Before the cells were transferred to the flasks, the sealed flasks, filled with 98 mL of  
155 MagMin medium (pH 6.9), were gassed with N<sub>2</sub>/O<sub>2</sub> in a 98 % / 2 % ratio for 20 minutes to create  
156 a low oxygen medium with a low oxygen gas atmosphere. Subsequently, 50 µL vitamin solution  
157 and 50 µL iron malate solution were added to each flask. For inoculation of *Magnetospirillum*  
158 *magneticum* AMB-1 cells, 2 mL of an inoculum were added to the medium. During the incubation,  
159 the flasks were kept at 30 °C on a rotary shaker (Rotamax 120, Heidolph Instruments) at 80 rpm.  
160 The best growth conditions and the highest optical density at 600 nm of 0.4 was achieved using  
161 UV/Vis spectrometry (Specord 50 Plus, Analytik Jena, Germany) after an incubation of 24 h.  
162 Evidence that the bacteria synthesize magnetosomes was provided by placing a small magnet close  
163 to the flasks. After a few hours, an accumulation of bacteria formed (Supplementary Fig. 1).

164

165  
166 *2.2. U bioassociation experiments with varying biomass concentration*  
167  
168 For each experiment cells were grown in 200 mL flasks in MagMin medium for 24 h at 30 °C on  
169 a rotary shaker at 80 rpm. To reach a defined biomass concentration, a different number of flasks  
170 was used, concentrated and the optical density at 600 nm ( $OD_{600nm}$ ) was controlled. This resulted  
171 in a biomass concentration of 89.00, 77.04, 49.62 and 21.45 mg dry biomass (DBM) with an  
172  $OD_{600nm}$  of 2.32, 1.64, 1.02 and 0.45, respectively. The cells were centrifuged at  $7.870 \times g$  for 10  
173 minutes (Centrifuge Avanti J-20 CP, Beckman Coulter, Country?), suspended and washed twice  
174 in sterilized tap water at pH 6.5. The washed cells were suspended again for each experiment in  
175 100 mL sterilized tap water at pH 6.5. Subsequently, 100  $\mu$ L of a 0.1 M  $UO_2(NO_3)_2$  stock solution  
176 were added to each experiment to reach a final U(VI) concentration of 0.1 mM. During the  
177 incubation on a rotary shaker (Rotamax 120, Heidolph Instruments) at 30 °C, 1 mL of each cell  
178 suspension was collected three times at distinct time points (5, 15, 30 and 45 min, as well as after  
179 1, 2, 3, 4, 5, 6, 24 and 25 h). Each sample was centrifuged at  $13.225 \times g$  for 1 minute (Centrifuge  
180 5415R, Eppendorf AG, Hamburg, Germany). The supernatants were sampled and acidified with  
181  $HNO_3$  for Inductively Coupled Plasma Mass Spectrometry (ICP-MS) measurements using an  
182 ELAN 9000 type ICP-MS spectrometer (Perkin Elmer, Überlingen, Germany) to determine the  
183 residual U(VI) concentration within the supernatant.

184  
185  
186 *2.3. U bioassociation experiments with different pH*

187  
188 For the experiments a high biomass concentration was chosen (74.3–82.8 mg DBM) for each  
189 experiment, which was determined in prior by an  $OD_{600nm}$  in the range of 1.60–2.44. The cells were  
190 centrifuged, suspended and washed twice in sterilized tap water at defined pH related to each  
191 experiment (3.5, 4.5, 5.5, 6.5 and 7.5). The washed cells were suspended again for each experiment  
192 in 101 mL sterilized tap water at the defined pH and treated as described in the previous experiment.  
193 1 mL was taken for  $OD_{600nm}$  measurements. To reach a final U(VI) concentration of 0.1 mM,  
194 100  $\mu$ L of a 0.1 M  $UO_2(NO_3)_2$  stock solution was added to each experiment. 1 mL of each cell  
195 suspension was taken three times at distinct time points (5, 15, 30 and 45 min, 1, 2, 3, 4, 5, 6 and  
196 24 h) during the incubation, centrifuged, acidified and used for ICP-MS measurements to

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

202

203

#### 204 *2.4. Live-dead staining*

205

206 During the U(VI) interaction experiments, samples were taken at the beginning of the experiments  
207 and after 1, 3, 6 and 24 hours to check the viability of *Magnetospirillum magneticum* AMB-1 cells  
208 depending on the U(VI) incubation time, but also on the pH. The cells were harvested by  
209 centrifugation at 13.225 x g (Centrifuge 5415R, Eppendorf AG, Hamburg, Germany) for 1 min.,  
210 the supernatants discarded and the pellets resuspended in 200 µL of tap water. A volume of 0.3 µL  
211 of a Syto<sup>®</sup>9/propidium iodide mixture (vol/vol) (LIVE/DEAD<sup>™</sup> BacLight<sup>™</sup> Bacterial Viability  
212 Kit, Invitrogen<sup>™</sup>) was added to the cell suspensions and incubated in the dark for 15 min. The  
213 green fluorescent dye SYTO<sup>®</sup>9 can diffuse through intact cell walls into the cells. The red  
214 fluorescent dye propidium iodide can only enter dead cells through their defect cell walls. The cells  
215 were washed again to remove the unbounded stains. The washed and stained cells were diluted in  
216 330 µL 0.9% NaCl. The cell viability was observed using a confocal laser scanning microscope  
217 (Leica CLSM, Leica Microsystems, Wetzlar, Germany).

218

219

#### 220 *2.5. (Scanning) transmission electron microscopy ((S)TEM) and Energy-dispersive X-ray* 221 *spectroscopy (EDXS)*

222

223 For (S)TEM/EDXS studies, U(VI) interaction experiments were performed using cell suspensions  
224 with the same optical density at pH 6.5. An initial U(VI) concentration of 0.1 mM was adjusted  
225 and the sample was stored on a rotary shaker at 30°C. After 5 hours, the U(VI) incubation was  
226 stopped. At the end of the experiment, the cells were harvested by centrifugation for 10 minutes at  
227 7.870 x g (Centrifuge5804R, Eppendorf AG, Hamburg, Germany) at 30 °C. The supernatant was



228 removed and the cell pellet was washed twice with sterilized tap water at the defined pH.  
229 Subsequently, the cells were fixed with 2.5 % (vol/vol) glutardialdehyde from a 25 % (vol/vol)  
230 stock solution (Serva, Heidelberg, Germany) and stored at 4 °C. A reference sample without U was  
231 treated the same way. The fixed samples were prepared for transmission electron microscopy  
232 studies at the Center for Regenerative Therapies Dresden (CRTD) of the Technical University  
233 Dresden (Germany). In particular, ultrathin sections of < 100 nm were placed on carbon-coated Cu  
234 grids (200 mesh, Plano GmbH, Wetzlar, Germany). Bright-field transmission electron microscopy  
235 (TEM) images were recorded with an image-C<sub>s</sub>-corrected Titan 80-300 microscope (Field Electron  
236 and Ion Company (FEI), Eindhoven, The Netherlands) operated at an accelerating voltage of  
237 300 kV. Furthermore, high-angle annular dark-field scanning transmission electron microscopy  
238 (HAADF-STEM) imaging and spectrum imaging analysis based on energy-dispersive X-ray  
239 spectroscopy (EDXS) were performed with a Talos F200X (Thermo Fischer Scientific) microscope  
240 equipped with a high-brightness X-FEG electron source and a Super-X EDX detector system at an  
241 accelerating voltage of 200 kV (FEI). Prior to (S)TEM analysis, the specimen mounted in a high-  
242 visibility low-background holder was placed for 2 s into a Model 1020 Plasma Cleaner (Fischione,  
243 Export, PA, USA) to remove potential contamination.

244

245

## 246 2.6. *Cryo-time resolved laser-induced fluorescence spectroscopy (cryo-TRLFS)*

247

248 The *cryo*-TRLFS method was chosen due to the high sensitivity toward U(VI) complex formation  
249 in aqueous solutions (Moulin et al. 1990, 1995). For our experiments cultures of *Magnetospirillum*  
250 *magneticum* AMB-1 with an optical density of 1.6 at 600 nm were taken after 24 h of incubation  
251 and washed twice using sterilized tap water at defined pHs (3.5, 4.5, 5.5, 6.5, 7.5). Each washed  
252 cell suspension at the defined pH was resuspended in sterilized tap water and transferred into 4  
253 flasks. A 0.1 M stock solution of UO<sub>2</sub>(NO<sub>3</sub>)<sub>2</sub> was added to the flasks to adjust an initial U(VI)  
254 concentration of 0.1 mM in the cell suspension. The flasks were stored at 30 °C on a rotary shaker  
255 (Rotamax 120, Heidolph Instruments). After 0.5, 2, 5 and 24 h samples were taken and centrifuged  
256 for 5 min at 7870 x g (Centrifuge5804R, Eppendorf AG, Hamburg, Germany). A volume of 1 mL  
257 of each supernatant was used for determination of the U concentration by means of ICP-MS. For  
258 *cryo*-TRLFS measurements each pellet was washed twice in sterilized tap water at the defined pH,  
259 and transferred into a UV cuvette, shock frozen by N<sub>2</sub> and stored at -80 °C. In preparation of the

260 cryo-TRLFS measurements, the pellets were transferred just before into a copper holder. At 153 K  
261 the U(VI) luminescence was measured after excitation with laser pulses at 266 nm (Minilite high-  
262 energy solid-state laser; Continuum) and average pulse energy of 300  $\mu$ J. The emission of the  
263 samples was recorded using an iHR550 spectrograph (HORIBA Jobin Yvon) and an ICCD camera  
264 (HORIBA Jobin Yvon) in the 370.0–670.0 nm wavelength ranges by averaging 100 laser pulses  
265 and using a gate time of 2000  $\mu$ s. The measurements were performed from 0.1  $\mu$ s to 12.503  $\mu$ s to  
266 ensure that the lifetimes of all species present were detected. The specific separation of the sum  
267 spectra obtained from the measurements into individual spectra was performed using parallel factor  
268 analysis (PARAFAC). For more information see [Drobot et al. \(2015\)](#). Important ligands, *e.g.*,  
269 peptidoglycan (PGN) from *Bacillus subtilis* (Sigma Aldrich), lipopolysaccharide (LPS) from  
270 *Pseudomonas aeruginosa* (Sigma Aldrich), L-rhamnose (Sigma Aldrich), D-(+) galactose (Sigma  
271 Aldrich), D-(+) mannose (Sigma Aldrich) were used as reference ligands and measured for  
272 comparison and interpretation of possible binding sites of U(VI) to the cell wall. For this, each  
273 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  
274 excess of 1:20. A defined volume of a 0.1 M  $\text{UO}_2(\text{NO}_3)_2$  stock solution was added to reach a final  
275 U concentration of 0.1 mM. The samples were transferred into a UV cuvette, shock frozen by  $\text{N}_2$ ,  
276 stored at  $-80^\circ\text{C}$  and used for *cryo*-TRLFS measurements.

277

278

### 279 2.7. *In-situ attenuated total reflection Fourier-transform infrared spectroscopy (ATR FT-IR)*

280

281 For molecular identification of the U(VI) bioassociation process of *Magnetospirillum magneticum*  
282 AMB-1 cells, *in-situ* ATR FT-IR spectroscopy with a sub-minute time resolution was performed.  
283 On a Bruker Vertex 80/v vacuum spectrometer equipped with a Mercury Cadmium Telluride  
284 detector infrared spectra were measured from 4000 to 400  $\text{cm}^{-1}$ . Spectral resolution was 4  $\text{cm}^{-1}$ ,  
285 and spectra were averaged over 256 scans. A horizontal diamond crystal with nine internal  
286 reflections (DURA SamplIR II, Smiths Inc.) was used. Further details on the experimental ATR  
287 FT-IR spectroscopic setup are compiled in [Müller et al. \(2012\)](#). In our experiments a suspension  
288 of *Magnetospirillum magneticum* AMB-1 cells after 24 hours of incubation was centrifuged at  
289 7870 x g for 10 minutes, washed twice with autoclaved tap water (at pH 4.5 and 6.5) and  
290 resuspended in 100  $\mu$ L tap water at the same pH. A thin cell film was prepared directly on the

291 surface of the ATR diamond crystal as stationary phase by pipetting twice 1  $\mu\text{L}$  of the sample on  
292 the ATR crystal and subsequent drying with a gentle stream of  $\text{N}_2$ . The cell film was flushed using  
293 a flow cell with a blank solution (sterilized tap water, pH 4.5 and 6.5) with a flow rate of  
294 0.1  $\text{mL min}^{-1}$  for 60 min for conditioning. Subsequently, a 0.01 mM uranylchloride solution (in  
295 sterilized tap water, pH 4.5 and 6.5) was rinsed over the cell film. Difference spectra are calculated  
296 at several times during the U(VI) bioassociation process. After 120 min of bioassociation,  
297 equilibrium was reached and no further U(VI) accumulation or change of the cell film was observed  
298 within the spectral data. Then, the U(VI)-loaded cell film was flushed again with sterilized tap  
299 water for 60 min for controlling the possible reversibility of the sorbed species. Further details on  
300 the calculation of difference spectra are given in Müller et al. (2013).

301

302

### 303 **3. Results**

304

#### 305 *3.1. U(VI) bioassociation of Magnetospirillum magneticum AMB-1*

306

307 The U(VI) bioassociation of *Magnetospirillum magneticum* AMB-1 cells was first determined by  
308 time-dependent experiments, using different amounts of biomass (89.00, 77.04, 49.62 and  
309 21.45 mg DBM) and an initial U(VI) concentration of 0.1 mM. The experiments were performed  
310 at pH 6.5, each with an incubation time of 25 h. The results showed a fast increase of the U(VI)  
311 bioassociation during the first hour for all biomass concentrations (Fig. 1). Equilibrium conditions  
312 were already reached in the experiment with the highest biomass concentration after 1 h. After 24 h  
313 at the latest, a steady state has been established in all other experiments. The removal efficiency of  
314 U(VI) by *Magnetospirillum magneticum* AMB-1 cells appears to be very effective, independent of  
315 the biomass concentration. As shown in Fig. 1, between 85.7 and 95.5 % of the initial U(VI) was  
316 removed from the suspensions, except the experiment with the lowest biomass. Here, only 55.8 %  
317 of the U(VI) was bound.

318

319 In further U(VI) bioassociation experiments a defined biomass concentration, each in a range from  
320 74.3–82.8 mg DBM was chosen at different pH (3.5, 4.5, 5.5, 6.5, 7.5). These experiments provide

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

331

332

### 333 3.2. Cell viability by live-dead staining

334

335 During the U(VI) removal experiments, which were performed with an initial U(VI) concentration  
336 of 0.1 mM at pH 3.5–7.5 the cells were tested for their cell viability by live-dead staining (Syto®9  
337 and propidium iodide) using CLSM. The results show that high numbers of viable or living cells  
338 were detected after 1 h incubation, with the exception of pH 3.5, where individual agglomerates of  
339 cells were visible (Supplementary Fig. 2). Compared to samples after 3 h of incubation, the AMB-  
340 1 cells showed nearly the same number of viable cells. After 24 h the majority of cells were viable  
341 with the exception of the cells, which were handled in tap water at a pH of 3.5 and also at a pH of  
342 7.5. At acidic and basic pH the cells have only a very limited viability after 24 h. Agglomeration  
343 of dead cells are already determined at the beginning of the experiment at pH 3.5.

344

345

### 346 3.3. Localization of U by (S)TEM/EDXS analysis

347

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

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

354

355

#### 356 3.4. Determination of U species from cryo-TRLFS using PARAFAC analysis

357

358 After the incubation of *Magnetospirillum magneticum* AMB-1 cells with 0.1 mM U(VI) at different  
359 pH (3.5–7.5) and different contact times (0.5, 2, 5 and 24 h), the cells were washed, centrifuged  
360 and used for cryo-TRLFS measurements. A subsequent analysis of all emission spectra from the  
361 TRLFS data set by PARAFAC extracted the single component spectra of five U(VI) species, which  
362 were probably formed during the U(VI) bioassociation of *Magnetospirillum magneticum* AMB-1  
363 cells (Fig. 4b, c, d, f, g). The single component spectra are characterized by different emission  
364 bands. For the identification and assignment of the extracted spectra to possible binding sites of U  
365 on the cells, reference spectra of potential ligands loaded with U(VI) were measured. Since EDXS  
366 elemental distribution analysis clearly indicate that U(VI) is predominantly bound in the cell wall,  
367 important ligands, e.g., peptidoglycan (PGN), lipopolysaccharide (LPS), L-rhamnose, D-(+)  
368 galactose and D-(+) mannose were considered as possible complexants for U(VI) on  
369 *Magnetospirillum magneticum* AMB-1 cell walls. The luminescence properties have shown that  
370 there is no correspondence with the extracted single component spectra of U species formed on  
371 *Magnetospirillum magneticum* AMB-1 cells with LPS, L-rhamnose, D-(+) galactose and D-(+)  
372 mannose (Supplementary Fig. 3). However, the situation is completely different concerning PGN.  
373 In Fig. 4b, c, d three extracted single component spectra (species 1–3) are shown together with the  
374 appropriate reference spectra of U loaded PGN from *Bacillus subtilis*. Peak positions and ratios of  
375 the U associated species indicate a very good match with the three reference spectra of U-PGN  
376 (Tab. 1). Since the studies were performed in the pH range from 3.5 to 7.5, statements on the pH  
377 dependent formation of the U species with PGN are significant. As shown in the relative  
378 luminescence intensity distribution of the PARAFAC extracted species (1–5) versus pH and time  
379 (Fig. 4e), species (1) is mainly significant in the acidic pH range and is rather negligible in the  
380 neutral and basic pH range. Species (2) dominates the bioassociation of U(VI) in the mentioned  
381 broad pH range, with a dominance at pH 5.5 being emphasized. Species (3), on the other hand,  
382 only gains significance in the basic pH range. The detected properties of the species are also proved  
383 by studies performed on the U-PGN references of the relative luminescence intensity distribution

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

387  
388 In addition to the PGN species two more species should be mentioned. Species (4) shows four  
389 characteristic maxima of the luminescence emission peaks (Fig. 4f) and a dominance in the pH  
390 range from 3.5–4.5 (Fig. 4e). The emission peaks are comparable to literature data of reference  
391 spectra. These are spectra of molecules containing carboxylic acids. A study on the interaction of  
392  $\text{UO}_2^{2+}$  with malonate by using TRIFS and a metal concentration relevant for environmental aquatic  
393 systems in a 0.1M perchloric media at pH 4 shows comparable data (Brachmann et al., 2002). The  
394 position of the emission bands of  $\text{UO}_2\text{C}_3\text{H}_2\text{O}_4^0_{(\text{aq})}$  are in good agreement with those of species (4).  
395 Thus, we conclude the formation of a malonate-like species at acidic pH during the bioassociation  
396 of U(VI) on *Magnetospirillum magneticum* AMB-1 cells.

397  
398 The PARAFAC extracted single component spectra of species (5) is characterized by a broad  
399 emission band. Similar spectra are known from the literature as an unidentified species (Bader et  
400 al., 2018). In *Magnetospirillum magneticum* AMB-1 cells this species exists mainly at  $\text{pH} \geq 6.5$ .  
401 In this pH range it may be the dominant U species bound on *Magnetospirillum magneticum* AMB-  
402 1 cells and thus have to be considered as an important species in the bioassociation process at  
403 higher pH.

404  
405

### 406 3.5. Analysis of U(VI) coordination by in-situ ATR FT-IR

407  
408 *In-situ* ATR FT-IR spectroscopy allows to discriminate which functional groups are responsible  
409 for U(VI) complexation at microorganism interfaces, e.g., carboxylate, phosphoryl, amino or even  
410 hydroxyl groups (Barkleit et al., 2008; Barkleit et al., 2011; Comarmond et al., 2016; Jiang et al.,  
411 2004; Li et al., 2010; Theodorakopoulos et al., 2015; Krawczyk-Bärsch et al., 2018; Hilpmann et  
412 al., 2022). The experimental procedure involves the preparation of a cell film as stationary phase  
413 directly on the ATR crystal, which is then rinsed with aqueous background and U(VI) solutions at  
414 identical pH and ionic strength. In the meantime the cell film is continuously monitored by

415 recording single beam spectra in the sub-minute range. The calculation of difference spectra  
416 extracts only changes of the cell film that are associated with U(VI) interaction, constant spectral  
417 parts are not given. The time-resolved ATR FT-IR spectra at pH 4.5 and 6.5 are comparatively  
418 presented in Fig. 6. First, the cell film is equilibrated to aqueous sample conditions, *e.g.*, pH 4.5  
419 and 6.5 by flushing a blank solution. The red traces in Fig. 5 show only weak absorption bands  
420 during last 30 minutes of conditioning that can be attributed to cell interactions in contact with  
421 water. This is a measure of quality for the stability of the cell film and hence, prerequisite for the  
422 *in-situ* ATR FT-IR experiment. The black spectra are difference spectra calculated at different  
423 times of U(VI) accumulation, ranging from 5 to 120 minutes. At both pH conditions significant  
424 spectral changes are observed. The increasing intensities of the bands upon U(VI) contact time  
425 suggests metal accumulation at the cell surface. The bands below at  $940\text{ cm}^{-1}$  are attributed to the  
426 asymmetric stretching vibrational mode of the uranyl(VI) moiety,  $\nu_3(\text{UO}_2)$ . Generally, the  
427 frequency of this mode is observed at  $961\text{ cm}^{-1}$  for the fully hydrated ion in strong acidic aqueous  
428 solution (Quilès and Burneau, 2000). Upon complexation in solution and at biogeochemical  
429 interface it is shifted to lower frequencies (Müller et al., 2008; Kretzschmar et al., 2021; Hilpmann  
430 et al., 2022).

431  
432 At pH 4.5,  $\nu_3(\text{UO}_2)$  is observed at  $925\text{ cm}^{-1}$ , characteristic for complexation to carboxylate groups  
433 (Fig. 5a). This is in accordance with the spectral signature, we observe at higher wavenumbers.  
434 The bands at  $1453\text{ cm}^{-1}$  and  $1535\text{ cm}^{-1}$  can be assigned to the symmetrical  $\nu_s(\text{COO}^-)$  and  
435 asymmetrical  $\nu_{as}(\text{COO}^-)$  stretching vibrations of the carboxylate group (Barkleit et al., 2008;  
436 Theodorakopoulos et al., 2015; Krawczyk-Bärsch et al., 2018). The spectral region, where the  
437 asymmetric  $\nu_{as}(\text{PO})$  stretching modes are observed, shows also intense peaks at 1197 and  
438  $1106\text{ cm}^{-1}$ , suggesting that also phosphoryl functional groups are involved in U(VI) binding.  
439 However, since the symmetry of the  $\nu_3(\text{UO}_2)$  band hints strongly to the predominance of only one  
440 surface species and phosphoryl binding is characterized by a stronger shift of  $\nu_3(\text{UO}_2)$  to  $905\text{ cm}^{-1}$ ,  
441 this binding motif can be ruled out. With regard to the time-resolved data, the  $\nu_3(\text{UO}_2)$  band shows  
442 a fast bioassociation of U during the first minutes. After 60 min sorption still takes place, but to a  
443 less extent, possibly due to mostly occupied binding sites. These findings agree very well with the  
444 kinetic batch results (Fig. 1, 2).

445

446 At pH 6.5, the spectra offer some significant differences (Fig. 5b). First, it must be noted that the  
447 overall intensity is considerably reduced in comparison to lower pH. Second, the spectral signature  
448 has changed, the  $\nu_3(\text{UO}_2)$  mode is now characterized as an asymmetric-shaped broad band. Here,  
449 a multiple species set can be derived from 2<sup>nd</sup> derivative analysis of the time-resolved data. At  
450 15 min of U(VI) bioassociation, three local peak maxima at 923, 911 und 896  $\text{cm}^{-1}$  are extracted  
451 (data not shown). With ongoing bioaccumulation, the  $\nu_3(\text{UO}_2)$  mode is distinctly shifted to  
452 wavenumbers below 900  $\text{cm}^{-1}$  and contributions from 923 and 911  $\text{cm}^{-1}$  become less important.  
453 One may interpret this spectral data set as a combination of U(VI) bioassociated with carboxyl as  
454 well as phosphoryl functionalities, as this was observed for certain microorganisms (Bader et al.,  
455 2017; Krawczyk-Bärsch et al., 2018). The vibrational modes of the ligands  $\nu_{\text{as s}}(\text{COO}^-)$  and  $\nu_{\text{as}}(\text{PO})$   
456 would support this. However, the appearance of  $\nu_3(\text{UO}_2)$  mode is different in comparison to these  
457 previous works, where we clearly observed only two contributions, (1) the coordination to  
458 carboxylic groups with local band maximum at 925  $\text{cm}^{-1}$  and (2) to the phosphorylic groups at  
459 905  $\text{cm}^{-1}$ . Local band maxima below 900  $\text{cm}^{-1}$  were not observed so far. Unfortunately, the  
460 information given by (S)TEM/EDXS elemental distribution analysis, where phosphorous  
461 compounds together with U are detected in the cell wall, do not bring further insight on the binding  
462 motif. Therefore, an alternative interpretation of the IR data set becomes necessary. In a previous  
463 study of the complexation of U(VI) with citrate, a hydroxy-tricarboxylic acid, we observed in the  
464 experimental IR data a similar shifting of the  $\nu_3(\text{UO}_2)$  mode with increasing complex nuclearity  
465 and changing speciation from binary U(VI)-CA to ternary complexes involving also hydroxides  
466 (Kretzschmar et al., 2021). Spectral shifts were observed from 961 down to 879  $\text{cm}^{-1}$  with changing  
467 absorption properties of the different complexes.

468  
469 As a last step of the *in-situ* ATR FT-IR experiment, the U(VI) loaded cell film is again flushed  
470 with the blank solution at the respective pH (blue traces in Fig. 5). Thus, the reversibility of the  
471 bioassociation process is given by the difference spectra calculated at distinct times after changing  
472 aqueous solutions. Please note the differences in absolute intensities upon comparing the sorption  
473 (black) and flushing IR spectra (blue). At pH 4.5 the spectral features at both stages are very similar  
474 and the reversibility seems to be very low. That means, the coordination of U(VI) to carboxylic  
475 groups at pH 4.5 is very strong and only few quantities are removed from the cell walls by simply  
476 flushing the blank solution. Again, the situation is different at pH 6.5. From the set of coordinating  
477 species only those exhibiting the  $\nu_3(\text{UO}_2)$  mode mostly red shifted to 899  $\text{cm}^{-1}$  is reversible. From



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

480

481

#### 482 **4. Discussion**

483

484 The interaction of U(VI) with *Magnetospirillum magneticum* AMB-1 cells is characterized by a  
485 fast pH dependent bioassociation that takes place in the first hours of incubation due to the removal  
486 capacity of predominantly alive cells. Up to 95 % of the initial U(VI) concentration was bound to  
487 the cells, which tolerate a U(VI) concentration of 0.1 mM, but are not able to survive permanently  
488 at acidic and basic pH. Similar results, which were received under similar experimental conditions,  
489 were reported in [Gerber et al. \(2016\)](#). Batch sorption and flow cytometry studies using the live-  
490 dead staining approach confirmed that metabolically active cells of a Gram-negative *Acidovorax*  
491 *facilis* strain were able to remove nearly 100 % of the initial U(VI) concentration from the solution  
492 in the first hours of incubation with 0.05 mM and 0.1 mM U(VI) at pH 5. Also [Hufton et al. \(2021\)](#)  
493 mentioned the highest U(VI) binding to cell wall components in Gram-negative bacteria at pH 5.5.  
494 A high sorption capacity was also determined by the Gram-positive bacterium *Brachybacterium*  
495 sp. G1 ([Hilpmann et al., 2022](#)), which was explained by the high number of carboxylate groups  
496 within the PGN layer of the cell wall ([Bäuerlein, 2003](#)). In the previously performed infrared  
497 spectroscopic studies on *Brachybacterium* sp. G1 by [Bader et al. \(2018\)](#) it has already been shown  
498 that carboxylate groups of the cell wall were dominantly involved in the U(VI) bioassociation. It  
499 seems that high sorption capacities of U(VI) could be found both in both Gram-positive and in  
500 Gram-negative bacteria, although the structure of the cell walls differs. In Gram-positive bacteria  
501 the cell wall components are mainly PGN ([Hufton et al., 2021](#)), while only a thin, 4 nm thick, PGN  
502 layer is found in the cell walls of Gram-negative bacteria ([Bäuerlein, 2003](#)). In addition to the PGN  
503 layer the Gram-negative cell wall also contains an outer membrane, which consists of  
504 phospholipids and lipopolysaccharides, representing potential binding sites for the predominant  
505 positively charged  $\text{UO}_2^{2+}$  species. Involvement of phospholipids and lipopolysaccharides in the  
506 bioassociation would therefore be expected and has been demonstrated in previous studies on  
507 Gram-negative bacteria ([Kelly et al., 2002](#); [Merroun et al., 2005](#); [Llorens et al., 2012](#); [Luetke et al.,](#)  
508 [2012](#); [Krawczyk-Bärsch et al., 2018](#)). In contrast, in the presented work, it is clearly shown that  
509 phosphoryl sites could be excluded as major binding site for U(VI). The data obtained by ATR FT-

510 IR spectroscopy provide evidence for the formation of strong complexation of U(VI) with  
511 carboxylic functionalities at pH 4.5 during the first minutes of incubation. The spectroscopic proof  
512 of a fast U(VI) bioassociation agrees very well with the kinetic batch results. TRLFS studies in  
513 combination with PARAFAC confirm the complexation of U(VI) to carboxylate groups as  
514 described by ATR FT-IR. The comparison of three PARAFAC extracted single component spectra  
515 with the appropriate reference spectra of U(VI) loaded PGN from *Bacillus subtilis* showed an  
516 excellent match and emphasizes that PGN is the most important ligand in the bioassociation of  
517 U(VI) to *Magnetospirillum magneticum* AMB-1 cells. A pH dependent study indicates furthermore  
518 that the complexation of U(VI) with PGN even takes place in the investigated pH range from 3.5–  
519 7.5. In previous potentiometric titration and TRLFS studies of [Barkleit et al. \(2009\)](#) on the  
520 interaction of  $\text{UO}_2^{2+}$  with PGN reference samples, three different U-PGN complexes were  
521 identified as well. However, we must take into account that the previous studies were not conducted  
522 under the same conditions as our studies. All solutions in the previous studies were prepared with  
523 carbonate-free deionized water with an ionic strength of 0.1 M by adding stock solutions from  
524  $\text{NaClO}_4 \cdot \text{H}_2\text{O}$ . Also,  $\text{UO}_2^{2+}$  was added as  $\text{UO}_2(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$  and all samples were measured at room  
525 temperature. To obtain comparable data from the reference studies to our experiments with  
526 *Magnetospirillum magneticum* AMB-1 cells, tap water was used. High ionic strength was thus  
527 omitted in order to provide the bacteria with a natural environment in which they can survive. For  
528 the same reason, care was also taken to use the added  $\text{UO}_2^{2+}$  in form of  $\text{UO}_2(\text{NO}_3)_2$  stock solution.  
529 The most important difference, however, is that in the studies of [Barkleit et al. \(2009\)](#) mixed spectra  
530 were presented, whereas in our investigations with the help of PARAFAC single spectra of U-PGN  
531 could be shown. Nevertheless, the results of [Barkleit et al. \(2009\)](#) are indicating possible  
532 complexations due to the observed luminescence properties. Following these studies, species U-  
533 PGN 1 was determined in our studies at acidic pH. According to the previous studies, it could be  
534 assigned to the 1:1 uranyl carboxyl PGN complex  $\text{R-COO-UO}_2^+$  (glutamic acid or diaminopimelic  
535 acid), which appears between pH 3 and 5. U-PGN 2 could be assigned to the 1:2 uranyl carboxyl  
536 chelate complex  $(\text{R-COO})_2\text{-UO}_2$ , which dominates around pH 5.6 ([Barkleit et al. \(2009\)](#)). In the  
537 neutral pH range the U-PGN 3 species is dominating. This U-PGN species might be the uranyl  
538 chelate complex with the mixed coordination  $(\text{R-COO-UO}_2^{(+)}\text{-Ai-R})$  (Ai =  $\text{NH}_2$  or  $\text{O}^-$ ).

539  
540 The interpretation of time-resolved ATR FT-IR spectroscopy data obtained at pH 6.5 indicates  
541 differences. Considerable shifts, when increasing U(VI) loading at the cell surface, hint to multiple

542 surface species. Initially, possibly mononuclear U(VI) species are predominating, whereas at pH  
543 6.5, polynuclear species become more prominent. The interpretation of the TRLFS/PARAFAC  
544 data also reflect the transition from mononuclear to polynuclear U(VI) carboxylic coordination. In  
545 Bader et al. (2018) similar spectra were described as a typical loss of fine structure of polynuclear  
546 species, which they assigned to the 3:5 U(VI) hydroxo complex  $((\text{UO}_2)_3(\text{OH})_5^+)$ . Wollenberg et al.  
547 (2021) summarizes it more generally and assume that this polynuclear species could consist of  
548 ligands in which amino, hydroxyl or sulphonyl groups could be important. In *Magnetospirillum*  
549 *magneticum* AMB-1 cells they may become the dominant U(VI) species, which mainly exists at  
550  $\text{pH} \geq 6.5$ . In contrast to the data obtained at pH 4.5, ATR FT-IR spectroscopy data indicate that the  
551 polynuclear species seems to be not as strongly coordinated and presents a higher reversibility  
552 under the given experimental conditions. When using magnetotactic bacteria for remediation  
553 purposes, this higher reversibility at  $\text{pH} \geq 6.5$  will not be a disadvantage. In open systems there  
554 will always be an inflow and thus a continuous flow of a high amount of bacteria.

555  
556 Even though magnetotactic bacteria show similar strong sorption capacities of heavy metals and  
557 radionuclides as other Gram-negative or Gram-positive bacteria, there are some advantages that  
558 make magnetotactic bacteria interesting for the use in bioremediation of contaminated waters:  
559 i) Magnetotactic bacteria prefer to live planktonically (Lefèvre et al., 2013) and thereby provide an  
560 ideally large surface area for interactions with metals. ii) They do not form biofilms, as is known  
561 from many other bacteria such as *Bacillus* spp. (Zhong et al., 2021) or *Shewanella* sp. (Cao et al.,  
562 2011). Biofilm consists of multilayered cell clusters embedded in a matrix of exopolymeric  
563 substance (EPS), which facilitate the adherence of microorganism. As shown in Zhong et al.  
564 (2021), the EPS of these biofilms might also play a key role in U(VI) sequestration where U(VI)  
565 could be bound through cellular phosphate, hydroxyl, carboxyl, and amide groups through  
566 enzymatic bioprecipitation (Wei et al., 2017). However, biofilms are less suitable for flow-through  
567 systems than planktonic bacteria due to the risk of clogging and sedimentation, and the need for a  
568 carrier matrix which results in larger amounts of waste during disposal. The removal of heavy  
569 metal-loaded biofilms thus seems difficult and cost intensive. Similar problems could also arise  
570 with filament networks, e.g., fungal systems, although fungi are also known as very good  
571 biosorbents for heavy metals and radionuclides due to the immobilization in the mycosphere, the  
572 sorption to cell walls or the uptake into fungal cells (Baeza and Guillén, 2006; Harms et al., 2011;  
573 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) bioassociation 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

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611

612

### 613 **Appendix A. Supporting information**

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

615

616

### 617 **References**

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