Uranium adsorption by *Shewanella oneidensis* MR-1 as a function of dissolved inorganic carbon concentration

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Although the existence of uranyl-carbonate surface complexes on bacterial cell walls has been documented, the identities and thermodynamic stabilities of those complexes are poorly constrained by previous studies. We measured the adsorption of aqueous uranium [U(VI)] onto *Shewanella oneidensis* MR-1 with different initial concentrations of NaHCO₃. Experiments were conducted in 0.1 M NaClO₄ to buffer ionic strength, and pH was varied from 3 to 9. The observed extent of U(VI) adsorption onto *S. oneidensis* was independent of dissolved inorganic carbon (DIC) concentration below pH 5. Above pH 5, the extent of adsorbed U(VI) decreased with increasing DIC concentration. A thermodynamic surface complexation modeling approach was used to interpret the adsorption data. A series of uranyl-, uranyl-hydroxide-, and uranyl-hydroxide-carbonate-bacterial surface complexes are required in order to account for the observed adsorption behavior, and we used the adsorption measurements to constrain values for the stability constants of these complexes. The modeling results suggest that uranyl-hydroxide-carbonate-bacterial surface complexes form and that competition between these complexes and uranyl-carbonate aqueous complexes controls the U(VI) adsorption behavior under high pH conditions in systems with high DIC concentrations. Hence, our results may be used to predict the extent of U(VI) adsorption onto bacteria in a range of natural and engineered DIC-bearing systems.

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1. Introduction

Metal adsorption onto bacterial surfaces can affect the mobility and transport of metals in geologic systems (e.g., Beveridge and Murray, 1976; Harvey and Leckie, 1985; Konhauser et al., 1993; Fein, 2000). Several studies have examined U(VI) adsorption onto bacterial cells. U(VI) adsorbs strongly onto bacteria, exhibiting pH-dependent adsorption behavior that is likely caused by a range of uranyl surface complexes on the cell wall (Fowle et al., 2000; Haas et al., 2001; Sar and D’Souza, 2001; Gorman-Lewis et al., 2005; Sheng et al., 2011). In addition to the direct effect of adsorption on the partitioning of mass in geologic systems, metal adsorption onto bacteria may control the bioavailability of metal ions by bacteria (e.g., Pawlik et al., 1993; Franklin et al., 2000; Borrok et al., 2005a; Sheng et al., 2011). For example, the extent and speciation of U(VI) adsorbed onto bacterial cell walls may control the initial rate of U(VI) enzymatic reduction by bacteria under anaerobic conditions (Sheng et al., 2011). Hence, a better understanding of uranyl-bacterial surface complexation reactions is needed not only to better constrain uranium partitioning in bacteria-bearing aqueous systems, but also to develop quantitative models of the bacterial bioavailability of uranium.

U(VI) adsorption onto bacteria increases with increasing pH under the low pH conditions (less than approximately pH 5) where the uranyl cation dominates the aqueous U(VI) speciation. In systems containing dissolved inorganic carbon (DIC), as pH increases above 5, a series of aqueous uranyl-hydroxide, uranyl-hydroxide-carbonate, and uranyl-carbonate complexes become important. The presence of these aqueous complexes competes to some extent with bacterial cell wall binding sites for uranyl, causing a decrease in the extent of U(VI) that adsorbs onto bacteria (Haas et al., 2001; Gorman-Lewis et al., 2005; Sheng et al., 2011). Surface complexation modeling represents a flexible approach for quantifying the extent of metal adsorption onto bacterial cell walls (e.g., Plett et al., 1996; Fein et al., 1997; Ngwenya et al., 2003; Guiné et al., 2006), and hence can be used to predict the fate and transport of metals in water–rock systems (Fein, 2000). The increase in adsorption with increasing pH up to pH 5 can be modeled as uranyl cation adsorption onto deprotonated cell wall functional groups according to the following reaction (Fowle et al., 2000; Haas et al., 2001; Gorman-Lewis et al., 2005):

$$\text{UO}_2^{2+} + \text{A}^- \Leftrightarrow \text{A}^- \cdot \text{UO}_2$$

where >A⁻ represents a deprotonated functional group bound to the bacterial cell wall. Because the uranyl cation activity becomes negligible with increasing pH above pH 5, models based on Reaction (1), including those that involve UO₂⁺ adsorption onto multiple sites, predict dramatically decreasing U(VI) adsorption with increasing pH above pH 5 (Haas...
et al., 2001; Gorman-Lewis et al., 2005). However, between approximately pH 5 and 8, observed extents of U(VI) adsorption onto bacteria are extensive both in DIC-free systems and systems open to atmospheric CO\(_2\), and adsorption only decreases slightly with increasing pH in this pH range (Gorman-Lewis et al., 2005). The elevated extent of U(VI) adsorption that is observed relative to predicted extents of adsorption based only on Reaction (1) represents strong evidence that other aqueous uranyl species adsorb to a significant extent onto bacterial cell walls.

Although the available data are unequivocal regarding the existence of these additional uranyl-bacterial surface complexes, there is considerable uncertainty regarding the identities and thermodynamic stabilities of these complexes, because only a limited number of conditions have been probed. X-ray absorption spectroscopy (XAS) studies indicate that adsorption of U(VI) onto bacterial cell walls under low pH conditions (pH < 5) is controlled by uranyl complexation with phosphoryl and carboxyl functional groups within the bacterial cell wall (Hennig et al., 2001; Kelly et al., 2002; Merroun et al., 2005). However, XAS studies of uranyl adsorption onto bacteria above pH 5 are limited (e.g., Ravel et al., 2007), and the identities of the uranyl-bacterial surface complexes that control U(VI) adsorption onto bacteria above pH 5 in the presence of DIC are not well constrained.

It is imperative to place rigorous constrains on the identities and stability constants of the important uranyl-carbonate-bacterial surface species under conditions with pH greater than 5 and high concentrations of DIC in order to model uranium remediation and mining strategies that involve carbonate leaching (IAEA, 1993; Mason et al., 1997; Francis et al., 1999). Haas et al. (2001) modeled U(VI) adsorption onto Shewanella putrefaciens up to pH 10, invoking binding of UO\(_2^+(\text{OH})\)\(^2+\) onto cell wall functional groups above pH 5, and Gorman-Lewis et al. (2005) modeled the enhanced U(VI) adsorption onto Bacillus subtilis above pH 5 in the presence of DIC concentrations in equilibrium with the atmospheric CO\(_2\) with reactions involving the adsorption of UO\(_2^-(\text{CO}_3)^2-\), (UO\(_2^+\)(\text{CO}_3)(\text{OH})\)\(^2+\), and UO\(_2^-(\text{CO}_3)^3-\). Each of these previous studies involved experiments with solutions open to atmospheric CO\(_2\), so only one DIC concentration was investigated at each pH condition and hence the measurements do not provide rigorous constraints on the stoichiometries of the important adsorbed uranyl complexes.

In this study, we measured the adsorption of aqueous U(VI) onto Shewanella oneidensis strain MR-1 as a function of DIC concentration in solution. The molar ratio of DIC/U(VI) in this study ranged from 0 to 120:1. A thermodynamic surface complexation modeling approach was used to interpret the experimental adsorption data in order to determine the dominant adsorption reactions and the stability constants for the important uranyl-bacterial surface complexes. The wide range of DIC concentrations that we studied is needed in order to rigorously constrain the identities and stability constants of the important uranyl-carbonate-bacterial surface species. It is particularly important to determine these parameters in order to model the effects of bacterial adsorption of uranium and the bioavailability of uranium in a range of natural and engineered DIC-bearing aqueous systems.  

2. Experimental methods  

2.1. Bacteria preparation  

U(VI) adsorption was measured onto S. oneidensis strain MR-1, a facultative Gram-negative bacterial species. All of the growth media and solutions used in this study were made with ultrapure 18 MΩ water. Cells of S. oneidensis were cultured aerobically and prepared following similar procedures to those described in Sheng et al. (2011). The bacterial cells were first transferred from an agar plate to a test tube containing 7 mL of autoclaved trypticase soy broth (TSB) with 0.5% yeast extract, and incubated at 32 °C for 24 h. The cell suspension in the tube was then transferred to 2 L of autoclaved TSB with 0.5% yeast extract, and incubated at 32 °C for another 24 h. Cells were collected by centrifugation at 10970 g for 5 min after the 48 h growth. Collected cells were then rinsed five times in 0.1 M NaClO\(_4\). The cells were recollected by centrifugation at 8100 g for 5 min after each rinse. After the fifth rinse, the supernatant was discarded, and the cells were then centrifuged twice at 8100 g for 30 min without any further addition of 0.1 M NaClO\(_4\). The wet mass is approximately 8 times the dry mass of the biomass (Borrok et al., 2005b). A known wet mass of cells was added to a weighed volume of 0.1 M NaClO\(_4\) electrolyte solution to form a concentrated parent cell suspension, and this parent suspension was diluted gravimetrically for the U(VI) adsorption experiments.

2.2. U(VI) adsorption experiments  

An aqueous filter-sterilized (using a nylon membrane with pore size of 0.2 μm) uranyl acetate stock solution was prepared according to procedures described previously (Sheng et al., 2011). The concentration of uranium in the uranyl acetate stock solution was approximately 160 mM, which was determined by inductively coupled plasma optical emission spectroscopy (ICP-OES). A weighed amount of solid NaHCO\(_3\) was dissolved in 0.1 M NaClO\(_4\) electrolyte solution to achieve initial NaHCO\(_3\) concentrations of 0.0, 0.2, 2.4, 11.9 or 30.0 mM. Experiments with 0.0 mM NaHCO\(_3\) were conducted inside a glovebox chamber with a gas composition of 95% N\(_2\)/5% H\(_2\). The NaClO\(_4\) solution was bubbled with 95% N\(_2\)/5% H\(_2\) for ~30 min before being transferred into the glovebox. All the other experiments with dissolved NaHCO\(_3\) were conducted outside the glovebox open to the atmosphere. For each concentration of NaHCO\(_3\), an aliquot of the uranyl acetate solution was added to attain an initial dissolved U(VI) concentration of 0.25 mM in each experiment. Before the addition of bacterial cells to the experiments, samples were taken of the experimental solution in order to determine the initial total dissolved uranium concentration by ICP-OES. A weighed volume of the parent cell suspension as prepared above was added to the 0.25 mM U(VI)-electrolyte solution to form a parent bacteria-U(VI)-electrolyte suspension with 1.0 g/L (wet mass) of S. oneidensis. The pH of the parent suspension was then adjusted to be between 6 and 8 using small aliquots of concentrated HNO\(_3\) and/or NaOH. The parent suspension was then separated into Teflon tubes with approximately 8 mL in each. The pH of the suspension in each Teflon tube was adjusted to cover the pH range of approximately 3 to 9 using small aliquots of concentrated HNO\(_3\) and/or NaOH. After the pH adjustment, all of the Teflon tubes were placed on a rotating rack (~30 rpm) for 3 h. After the 3 h equilibration period, the final pH of the suspension was measured, and then each suspension was centrifuged at 6600 g for 2 min and finally filtered through a 0.2 μm Millipore Millex GF/P filter. The filtered solution was then acidified with concentrated HNO\(_3\) in preparation for ICP-OES analysis of the final concentration of dissolved uranium. The concentration of adsorbed U(VI) was determined by difference between the measured initial and final aqueous uranium concentrations. Cell-free control experiments for each concentration of NaHCO\(_3\) were also conducted, and did not indicate any significant or consistent uranium loss (~5% maximal loss) due to adsorption onto the experimental apparatus in the pH range studied (data not shown).

A Perkin-Elmer Optima 2000DV ICP-OES system was used to analyze the concentration of total dissolved uranium in solution. Matrix-matched blanks and standards covering the probable range of uranium concentrations in solution were prepared. The standards and the blanks were re-analyzed after running every 30–40 samples in order to check machine drift. Analytical uncertainty was approximately ±2%, as determined by repeat analyses of an aqueous uranium standard, and the operational detection limit for uranium on the ICP-OES was determined to be approximately 60 ppb.

Control experiments were conducted to test if U(VI) reduction occurs under the conditions of the 0.0 mM NaHCO\(_3\) adsorption experiments. The preparation of bacteria and the experimental conditions were identical to those for the 0.0 mM NaHCO\(_3\) adsorption experiments. Samples were taken for initial U(VI) concentration measurements.
before adding the bacteria. After a 3 h exposure period, each system was acidified to pH ~ 1.5 with aliquots of 12.1 M HCl for 40 min in order to desorb U(VI) from the bacterial cells for final U(VI) concentration measurements. The initial and final U(VI) concentrations were measured by spectrofluorometry following the analytical procedures described in Sheng et al. (2011). The results of these control experiments did not indicate any loss of U(VI) due to U(VI) reduction under the conditions of 12%, and 1% in the 2.4, 11.9, and 30.0 mM NaHCO3 experiments, respectively. The extent of adsorption that we observed varies significantly as a function of pH to determine the pH dependence of the total DIC concentration in solution. At pH 6.7, the measured extent of U(VI) adsorption varies significantly as a function of DIC concentration, with the maximum adsorption in the concentration in solution. At pH 6.7, the measured extent of U(VI) adsorption varies significantly as a function of DIC concentration, with the maximum adsorption in the range of 3–9. Each bottle was rotated (~30 rpm) for 3 h, and the final pH of the solution in each bottle was measured and samples were collected for final DIC measurements. All the samples were acidified with 12.1 N HCl and sealed immediately, and then analyzed using a Shimadzu TOC-V analyzer. For all the NaHCO3 concentrations studied, the final DIC concentrations were relatively constant from pH 3 to approximately 5.5, which increased with increasing pH from pH 5.5 to pH 7–8, and the concentrations remained constant at the initial NaHCO3 concentration above pH 7–8 (Fig. S2 in Supplementary data). We used a polynomial fit to the DIC concentration data as a function of pH to determine the pH dependence of the total DIC concentrations for each set of experiments with a given amount of added NaHCO3.

3. Results and discussion

3.1. U(VI) adsorption experiments

The observed extent of U(VI) adsorption onto S. oneidensis is independent of DIC concentration below pH 5 (Fig. 1). The extent of adsorption in the 0.0 mM and 0.2 mM NaHCO3 experiments is similar to each other across the pH range of these experiments, with the concentration of adsorbed U(VI) continuing to increase above pH 5 to approximately 90% at pH 8.7 and 79% at pH 8.4 for the 0.0 and 0.2 mM NaHCO3 experiments, respectively. However, above pH 5, the extent of U(VI) adsorption that we observed varies significantly as a function of DIC concentration, with U(VI) adsorption decreasing with increasing DIC concentration in solution. At pH 6.7, the measured extent of U(VI) adsorption in the experiments with an initial NaHCO3 concentration of 0.2 mM is approximately 63%, and decreases to approximately 46%, 12%, and 1% in the 2.4, 11.9, and 30.0 mM NaHCO3 experiments, respectively (Fig. 1). Because of the effect of dissolved DIC on the adsorption behavior under the higher pH conditions studied, the pH at which the observed maximum extent of U(VI) adsorption occurs also changes as a function of DIC concentration, with the maximum adsorption in the presence of 0.1 M NaClO4 and varying initial concentrations of NaHCO3 as a function of pH. Total concentration of uranium was 0.25 mM in each experiment; initial concentrations of NaHCO3 were 0.0, 0.2, 2.4, 11.9 and 30.0 mM.

% U Adsorbed

Fig. 1. The percentage of U(VI) adsorbed onto 1 g (wet mass)/L of S. oneidensis MR-1 in the presence of 0.1 M NaClO4 and varying initial concentrations of NaHCO3 as a function of pH. Total concentration of uranium was 0.25 mM in each experiment; initial concentrations of NaHCO3 were 0.0, 0.2, 2.4, 11.9 and 30.0 mM.

2.4, 11.9 and 30.0 mM NaHCO3 experiments occurring at approximately pH 5.9, 5.9 and 5.0, respectively.

3.2. Thermodynamic modeling

We use a thermodynamic surface complexation approach to model the adsorption of U(VI) onto the cell surface of S. oneidensis. In this approach, U(VI) adsorption onto bacteria is modeled as interactions between a range of adsorbing aqueous uranyl species and specific sites on the bacterial cell wall. The adsorption reactions are modeled to involve discrete, negatively-charged deprotonated sites, with the deprotonation reactions of the bacterial cell wall functional groups expressed as:

\[
R - L_n - H^+ \leftrightarrow R - L_n + H^+ \quad (2)
\]

where \(R\) is the bacterial cell wall macromolecule to which the functional groups are attached, and \(L_n\) represents a particular functional group type that is present on the bacterial cell wall. We use a non-electrostatic model (Fein et al., 2005), so the mass action equation for Reaction (2) is expressed as:

\[
K_n = \frac{[R - L_n - H^+]}{[R - L_n]} \quad (3)
\]

where \([R - L_n - H^+]\) and \([R - L_n]\) represent the concentration of deprotonated and protonated functional groups of each bacterial cell wall species in moles per liter of solution, respectively, and \(a_{H^+}\) represents the activity of H+ in solution. We use the 4-site model of Mishra et al. (2010) to characterize the proton-active binding sites on S. oneidensis, with Sites 1–4 exhibiting \(pK_a\) values of 3.3 ± 0.2, 4.8 ± 0.2, 6.7 ± 0.4, and 9.4 ± 0.5, respectively. The corresponding site densities for Sites 1–4 are \(8.9(±2.6) \times 10^{-5}\), \(1.3(±0.2) \times 10^{-4}\), \(5.9(±3.3) \times 10^{-5}\) and \(1.1(±0.6) \times 10^{-4}\) mol per gram of wet mass, respectively.

The adsorption of an aqueous metal cation (\(M^{n+}\)) onto bacteria has been represented as the following reaction (e.g., Fein et al., 1997):

\[
R - L_n + M^{n+} \leftrightarrow R - L_n - M^{(n-1)+} \quad (4)
\]

with a mass action equation for this reaction expressed as:

\[
K_{ads} = \frac{[R - L_n - M^{(n-1)+}]}{[R - L_n] [a_{M^{n+}}]} \quad (5)
\]
where $K_{ads}$ represents the equilibrium constant for Reaction (4). This approach is suitable for conditions at which the dissolved metal is present exclusively as the aqueous cation. Below approximately pH 5, dissolved U(VI) is present in solution dominantly as the aqueous uranyl cation, $\text{UO}_2^{2+}$, and we use this approach to account for the observed adsorption behavior under conditions with pH < 5. However, with increasing pH above pH 5, the aqueous speciation of U(VI) becomes much more complex, with a range of aqueous uranyl-hydroxide, uranyl-carbonate, and mixed uranyl-hydroxide-carbonate complexes forming. In general, the speciation of U(VI) in solution can be represented by the following generic aqueous complexation reaction:

$$x\text{UO}_2^{2+} + y\text{CO}_3^{2−} + z\text{OH}^{−} \leftrightarrow (\text{UO}_2)_{x}y(\text{CO}_3)_{y}(\text{OH})_{z}^{2x−2y−z−z}.$$  (6)

Because both the speciation of aqueous U(VI) and the speciation of the bacterial cell wall change as a function of pH, it is impossible to unequivocally determine both the adsorbing U(VI) species and the binding sites responsible for the observed adsorption behavior based on bulk adsorption measurements only. However, we can place constraints on these parameters, and we do so by modeling U(VI) adsorption with the following generic reaction:

$$R - L^{−}_n + x\text{UO}_2^{2+} + y\text{CO}_3^{2−} + z\text{OH}^{−} \leftrightarrow R - L^{−}_n - (UO_2)_{x}y(\text{CO}_3)_{y}(\text{OH})_{z}^{2x−2y−z−z}.$$  (7)

The goal of this study is to construct a model that could quantitatively account for the observed U(VI) adsorption behavior as a function of both pH and DIC concentration. Without spectroscopic constraints on the binding sites involved in adsorption, we take the approach of modeling the adsorption starting with the low pH data, collected under conditions at which the aqueous U(VI) speciation is most simple and where only Site 1 sites are deprotonated to a significant extent. With increasing pH, we ascribe the observed adsorption to the dominant aqueous U(VI) species present at each experimental pH (Fig. S3 in the Supplementary data, calculated from the reactions given in Table S1 in the Supplementary data), binding onto the dominant deprotonated surface site type at the pH of interest. The calculated uranyl aqueous speciation for each initial NaHCO$_3$ concentration in Fig. S3 (except for the 0.0 mM NaHCO$_3$ experiments which had no DIC) accounted for the measured pH dependence of the total DIC concentration, which was determined by the control experiments described above (Fig. S2). The general modeling approach is similar to the approach described by Gorman-Lewis et al. (2005) and Sheng et al. (2011).

Because the speciation of the aqueous U(VI) is most simple under low pH conditions and in the DIC-free 0.0 mM NaHCO$_3$ experiments, and because only Site 1 is deprotonated under these conditions, we begin the construction of our adsorption model by modeling only the 0.0 mM NaHCO$_3$ data below pH 5. Increasing pH causes more sites to deprotonate and also complicates the aqueous U(VI) speciation, so starting with the low pH data provides a firm foundation for the modeling. Using the pH < 5 data from the 0.0 mM NaHCO$_3$ experiments, we attributed the observed adsorption to the formation of $R - L^{−}_1 - \text{UO}_2^{2+}$, using $x = 1, y = z = 0$ in Reaction (7). Using FITEQ (Herbelin and Westall, 1994), the data were used, in conjunction with the aqueous complexation reactions listed in Table S1 including reactions 1–10 and 18, in which carbonate is not involved in the aqueous speciation, to calculate the stability constant for $R - L^{−}_1 - \text{UO}_2^{2+}$. If we use this calculated stability constant to predict the extent of U(VI) adsorption as a function of pH across the entire pH range studied, the predictions (dashed curve labeled $R - L^{−}_1 - \text{UO}_2^{2+}$ in Fig. 2) fit the data well below pH 4.5, but the model predicts a decrease in adsorption above pH 4.5 to negligible adsorption at approximately pH 6 due to the dramatic decrease in the concentration of aqueous $\text{UO}_2^{2+}$ above pH 4.5. Clearly, our data, which show a continued increase in adsorption above pH 4.5, are strong evidence that additional adsorption reactions become important with increasing pH.

In the next step, we modeled the enhanced adsorption relative to that predicted by accounting for $\text{UO}_2^{2+}$ adsorption only progressively with increasing pH, choosing the aqueous uranyl species that predominates over a particular pH range as the adsorbing species for that pH range. Furthermore, we ascribe adsorption of that species to the deprotonated site that predominates over that same pH range. As described previously, the aqueous complexation reactions listed in Table S1, including reactions 1–10 and 18, were used to calculate the aqueous speciation of U(VI) from pH 3 to 9 under the 0.0 mM NaHCO$_3$ experimental conditions (Fig. S3–S5). Based on these calculations, we ascribe the enhanced adsorption between pH 4.5 and 6.5 to an adsorption reaction involving the dominant aqueous U(VI) species in this pH range, $\text{UO}_2^{2+}(\text{OH})_{3}^{−}$. We modeled all of the pH < 6.5 data simultaneously by invoking the formation of both $R - L^{−}_1 - \text{UO}_2^{2+}$ and $R - L^{−}_2 - \text{UO}_2^{2+}$ complexes.

![Fig. 2. The open diamonds represent experimental adsorption data for the 0.0 mM NaHCO$_3$ experiments. The dashed and solid curves represent predicted extents of U(VI) adsorption (see text).](image-url)

**Table 1**

Calculated Log K values* for uranyl surface complexes formed on the cell wall of *S. oneidensis* MR-1.

<table>
<thead>
<tr>
<th>[NaHCO$_3$] (mM)</th>
<th>Uranyl surface complexes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R - L^{−}_1 - \text{UO}_2^{2+}$</td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------</td>
</tr>
<tr>
<td>0.0</td>
<td>4.4</td>
</tr>
<tr>
<td>0.2</td>
<td>4.6</td>
</tr>
<tr>
<td>2.4</td>
<td>5.0</td>
</tr>
<tr>
<td>11.9</td>
<td>4.8</td>
</tr>
<tr>
<td>30.0</td>
<td>4.4</td>
</tr>
<tr>
<td>Avg.</td>
<td>4.6</td>
</tr>
<tr>
<td>2σ</td>
<td>0.5</td>
</tr>
</tbody>
</table>

*Calculation of Log K value was not conducted for this dataset (see text).

* Log K values are reported in the form of following equation: $R - L^{−}_n + (\text{uranyl species})^{x} \leftrightarrow R - L^{−}_n - (\text{uranyl species})^{x−1}$.

* $R - L^{−}_n$ represents *S. oneidensis* MR-1 functional groups, Sites 1–4, with pH, values of $3.3 \pm 0.2, 4.8 \pm 0.2, 6.7 \pm 0.4$, and $9.4 \pm 0.5$, respectively (Mishra et al., 2010).
Since Site 2 becomes deprotonated and available for U adsorption above pH 4.8, we ascribed adsorption of (UO$_2$)$_2$(OH)$_2^{2-}$ onto Site 2. R - L$_1$ - UO$_2^2$ and R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$ can account for the data up to pH 6 well, but cannot account for the increase in adsorption with increasing pH above pH 6 (dashed curve labeled R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$ in Fig. 2). Because the increase in concentration of (UO$_2$)$_4$(OH)$_7^{3-}$ at the expense of (UO$_2$)$_3$(OH)$_5^{2-}$ from pH 6.0 to 8.0 matches the increase in the extent of adsorption over this pH region, we invoked R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$ to account for these data. We do not ascribe the enhanced adsorption in this pH region to (UO$_2$)$_4$(OH)$_7^{3-}$ adsorbing onto Site 3 because at pH 6.0, where the discrepancy begins, Site 2 is the dominant deprotonated site. Furthermore, the concentration of Site 3 is not high enough to account for both the adsorption observed in this pH region as well as the adsorption that occurs above pH 8, and it is more likely that Site 3 is involved in the higher pH adsorption reaction. Again, a model involving these three bacterial surface complexes accounts for the data below approximately pH 7.2 very well, at higher pH values, the model predicts a decrease in adsorption due to the decrease in importance of (UO$_2$)$_4$(OH)$_7^{3-}$ (dashed curve labeled R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$ in Fig. 2). Above pH 8.0 (UO$_2$)$_3$(OH)$_5^{2-}$ dominates the aqueous U(VI) speciation, and Site 3 is the dominant deprotonated site, so we invoke R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$ to account for the observed extent of adsorption under these highest pH values studied. The entire 0.0 M NaHCO$_3$ dataset can be modeled by invoking formation of the 4 uranyl-bacterial surface complexes described above: R - L$_1$ - UO$_2^2$, R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$, R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$ and R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$. The calculated stability constants for these complexes are tabulated in Table 1, and the model fit to the data is shown in Fig. 2 labeled R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$. The three dashed curves in Fig. 2 represent predicted extents of adsorption based on models that include R - L$_1$ - UO$_2^2$ only, R - L$_1$ - UO$_2^2$ and R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$ only, and R - L$_1$ - UO$_2^2$, R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$ and R - L$_2$ - (UO$_2$)$_2$(OH)$_2^{2-}$ only, and these curves are shown as evidence for the need for additional uranyl-bacterial complexes in order to account for the observed adsorption behavior across the entire pH range studied. The final model involving all 4 uranyl-bacterial surface complexes with all data modeled simultaneously yields an excellent fit to the experimental data over the entire pH range studied.

In the next stage of modeling, we used the stability constants that we calculated from the 0.0 mM NaHCO$_3$ dataset to predict the extent of U(VI) adsorption that we would expect in the 0.2 mM NaHCO$_3$ experiments (dashed curve in Fig. 3). The prediction model included all the reactions listed in Table S1, including those which involve aqueous uranyl-carbonate complexation reactions (Reactions 11–17), but did not include any adsorption reactions that form uranyl-carbonate-bacterial surface complexes. The total DIC concentration in the prediction model at each pH was determined from the DIC concentration data of the 0.2 mM NaHCO$_3$ controls which were conducted as a function of pH (Fig. S2–S5). The prediction curve fits the data reasonably well up to approximately pH 6. Moreover, this model prediction dramatically underestimates the extent of adsorbed U(VI) above pH 6, where the aqueous U(VI) speciation becomes dominated by uranyl-carbonate species (Fig. S3–S5). The observed enhanced extent of U(VI) adsorption relative to that predicted from the model derived from the 0.0 mM NaHCO$_3$ data represents strong evidence that at least one aqueous uranyl-carbonate species must adsorb onto the bacteria above pH 6.
causing the adsorption that we observed under those conditions. The dominant aqueous U(VI) species above pH 6 in the 0.2 mM NaHCO₃ system is (UO₂)₂CO₃(OH)₃⁻, so to account for the observed enhanced adsorption, we added the adsorption reaction that creates $R - L_2 - (UO_2)_2CO_3(OH)_3$ to the reactions that we used to model the 0.0 mM NaHCO₃ data and solved for the stability constant of this new uranyl-bacterial surface complex. Invoking adsorption onto Site 2 was required because the extent of adsorption in the 0.2 mM NaHCO₃ experiments above pH 6 exceeds the capacity of Site 3 or Site 1. The stability constants for the surface complexes that control the U(VI) adsorption in the 0.2 mM NaHCO₃ system (listed in Table 1) were calculated based on the 0.2 mM NaHCO₃ data only, independent of the 0.0 mM NaHCO₃ data. However, we fixed the stability constants for $R - L_2 - (UO_2)_4(OH)_7$ and $R - L_3 - (UO_2)_3(OH)_7$ at the values that were calculated from the 0.0 mM NaHCO₃ data, because (UO₂)₄(OH)₇⁺ and (UO₂)₃(OH)₇⁻ are minor species in the 0.2 mM NaHCO₃ system, and an independent calculation of those stability constants with the 0.2 mM NaHCO₃ data is not possible. In these calculations, the total DIC concentration at the pH of each data point was set to the value determined from the DIC concentration data (Fig. S2-Sa). The model curve calculated using the set of K values that were determined from the 0.2 mM NaHCO₃ data fits the data very well across the studied pH range (Fig. 3).

For the three highest NaHCO₃ concentrations studied, we used a similar modeling approach as we did for the 0.0 and 0.2 mM NaHCO₃ datasets. First, we used the averaged stability constants that were calculated from the 0.0 and 0.2 mM NaHCO₃ data to predict the extent of adsorption that we would expect under those DIC concentrations (dashed curves in Fig. 4a, b, and c). For each of these conditions, the prediction curve fits the observed extent of U(VI) adsorption reasonably well in the pH range studied. There are no pH regions in which the predicted curves significantly and consistently underestimate the observed extents of adsorption, indicating that while the data may yield slightly different K values, the same bacterial surface complexes are likely responsible for the observed adsorption behavior at each of these DIC conditions. We used each set of experiments to independently calculate stability constants for each of the important bacterial surface complexes in the 2.4, 11.9, and 30.0 mM NaHCO₃ datasets. In these cases, because aqueous uranyl-hydroxide complexes are not major species under any of the studied conditions, we could not use the 2.4, 11.9, or 30.0 mM NaHCO₃ data to independently solve for the stability constants for the uranyl-hydroxide-bacterial surface complexes, and we fixed these values at the averaged values shown in Table 1, calculated from the 0.0 and 0.2 mM NaHCO₃ data. For all of these calculations, the total DIC concentration at each pH studied in each system was set to the value determined from the DIC concentration data (Fig. S2-Sb, S2-Sc).

Fig. 5. The open diamonds represent experimental adsorption data for the (a) 0.0 mM (b) 0.2 mM (c) 2.4 mM (d) 11.9 mM and (e) 30.0 mM NaHCO₃ experiments. The solid curves represent predicted extents of U(VI) adsorption using the set of average K values listed in Table 1.
The stability constants for $R - L_1 - UO_2$ and $R - L_2 - (UO_2)CO_3(OH)\_2$ were calculated for each of the three highest NaHCO\_3 concentration datasets separately, and the resulting set of calculated stability constants for each condition (tabulated in Table 1) yields an excellent fit to each dataset across the pH range studied, as shown by the solid curves in Fig. 4.

In summary, our U(VI) adsorption data and modeling results indicate the formation of a series of uranyl-, uranyl-hydroxide-, and uranyl-hydroxide-carbonate-bacterial surface complexes. Contrary to the results of Gorman-Lewis et al. (2005), our data do not require the adsorption of $UO_2CO_3$ or $UO_2CO_3$ onto the bacterial cell wall. The average values of the calculated stability constants are listed in Table 1. The uncertainty associated with each stability constant value was calculated directly from the range of K values calculated for each complex, or, for stability constants that were calculated from fewer than three sets of data (e.g., for $R - L_2 - (UO_2)CO_3(OH)^-\_0$, $R - L_2 - (UO_2)CO_3(OH)^-\_2$) and $R - L_1 - (UO_2)CO_3(OH)^-\_2$, we determined the uncertainty by varying the K value while fixing all others in order to determine the K value range needed to encompass 95% of the experimental data under the pH range where the associated complex dominates the adsorption. In general, the calculated K values from the different datasets in this study are consistent with each other within their calculated uncertainties, especially the K values for $R - L_1 - UO_2$ and $R - L_2 - (UO_2)CO_3(OH)^-\_2$. However, the K values for $R - L_2 - (UO_2)CO_3(OH)^-\_0$ and $R - L_1 - (UO_2)CO_3(OH)^-\_2$ are not as well constrained by our data as are the K values for the other surface complexes because only the 0.0 mM NaHCO\_3 dataset could be used to solve for it independently. In order to test the accuracy of the overall set of calculated K values to account for the range of adsorption behaviors that we observed as functions of pH and NaHCO\_3 concentration, we used the average stability constant values to predict the extent of adsorption expected under each experimental condition studied. These predicted adsorption curves are shown in Fig. 5. The set of average stability constants from this study provides excellent fits to all of the datasets in general, accounting for the effects of both pH and DIC concentration on the adsorption behavior of U(VI) by S. oneidensis.

4. Conclusions

The extent of U(VI) adsorption onto S. oneidensis below pH 5 is independent of DIC concentration in solution, likely because the adsorption is caused by complexation of the uranyl cation with cell wall functional groups and uranyl-carbonate complexes are not involved in the binding. Above pH 5, increasing DIC concentration causes the extent of U(VI) adsorption to decrease, but the observed extent of U(VI) adsorption is greater than that predicted by accounting for aqueous uranyl-carbonate complexation only and neglecting adsorption of uranyl-carbonate species onto the bacteria. Therefore, our adsorption data provide strong evidence for the formation of a series of uranyl-, uranyl-hydroxide-, and uranyl-hydroxide-carbonate-bacterial surface complexes. Contrary to the results of Gorman-Lewis et al. (2005), our data do not require the adsorption of $UO_2CO_3$ and $UO_2CO_3$ onto the bacterial cell wall. However, adsorption reactions involving binding of $(UO_2)CO_3(OH)_2$ onto bacterial cell wall functional groups control the U(VI) adsorption onto bacteria under conditions with elevated DIC concentrations. The thermodynamic model that we construct in order to account for the observed U(VI) adsorption behavior is not unique. We have assumed that the dominant aqueous uranyl species at each pH studied is the adsorbing species, and we have ascribed binding of these species onto the dominant deprotonated site on the bacterial cell wall in a given pH range. Clearly, detailed spectroscopic data are required as a function of pH and DIC concentration in order to place better constraints on the identities and compositions of the important uranyl-bacterial surface complexes. However, the model that we have developed is a reasonable one and provides an excellent overall fit to the data, capturing the pH and DIC dependencies well. Our results demonstrate that bacteria can compete with DIC to control the distribution of U(VI) in aqueous systems, and the calculated stability constants for the uranyl-bacterial surface complexes from this study provide a framework for estimating the adsorption and speciation of U(VI) on bacterial cell walls in complex environments. These modeling results may further improve our ability to understand bacterial effects on U(VI) speciation, bioavailability, and remediation in geologic systems.

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Appendix A. Supplementary data

Aqueous speciation reactions used in the thermodynamic modeling (Martell and Smith, 2001; Guillaumont et al., 2003); experimental control results to demonstrate that U(VI) reduction did not occur under the conditions of the 0.0 mM NaHCO\_3 adsorption experiments; final DIC concentration measurements as a function of pH for each initial NaHCO\_3 concentration studied in the adsorption experiments; aqueous U(VI) speciation diagrams for each system studied. Supplementary data associated with this article can be found, in the online version, at doi:

References

Herbelin, A., Westall, J.C., 1994. PTEG 3.1, a computer program for determination of chemical equilibrium constants from experimental data. Report 94-01. Department of Chemistry, Oregon State University, Cornvallis, OR, USA.


