UO₂²⁺ SPECIATION DETERMINES URANIUM TOXICITY AND BIOACCUMULATION IN AN ENVIRONMENTAL *PSEUDOMONAS* SP. ISOLATEMICHAEL R. VANENGELN,^{†‡} ERIN K. FIELD,^{‡§} ROBIN GERLACH,^{†‡} BRADY D. LEE,^{||} WILLIAM A. APEL,^{||} and BRENT M. PEYTON^{*†‡}[†]Department of Chemical and Biological Engineering, Montana State University, Bozeman, Montana 59717-3920, USA[‡]Center for Biofilm Engineering, Montana State University, Bozeman, Montana 59717-3980, USA[§]Department of Microbiology, Montana State University, Bozeman, Montana 59717, USA^{||}Idaho National Laboratory, Biological Systems Department Idaho Falls, Idaho 83415, USA

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Abstract—In the present study, experiments were performed to investigate how representative cellulosic breakdown products, when serving as growth substrates under aerobic conditions, affect hexavalent uranyl cation (UO₂²⁺) toxicity and bioaccumulation within a *Pseudomonas* sp. isolate (designated isolate A). Isolate A taken from the Cold Test Pit South (CTPS) region of the Idaho National Laboratory (INL), Idaho Falls, ID, USA. The INL houses low-level uranium-contaminated cellulosic material and understanding how this material, and specifically its breakdown products, affect U-bacterial interactions is important for understanding UO₂²⁺ fate and mobility. Toxicity was modeled using a generalized Monod expression. Butyrate, dextrose, ethanol, and lactate served as growth substrates. The potential contribution of bicarbonate species present in high concentrations was also investigated and compared with toxicity and bioaccumulation patterns seen in low-bicarbonate conditions. Isolate A was significantly more sensitive to UO₂²⁺ and accumulated significantly more UO₂²⁺ in low-bicarbonate concentrations. In addition, UO₂²⁺ growth inhibition and bioaccumulation varied depending on the growth substrate. In the presence of high bicarbonate concentrations, sensitivity to UO₂²⁺ inhibition was greatly mitigated, and did not vary between the four substrates tested. The extent of UO₂²⁺ accumulation was also diminished. The observed patterns were related to UO₂²⁺ aqueous complexation, as predicted by MINTEQA2 (ver. 2.5.2) (Easton, PA, USA). In the low-bicarbonate medium, the presence of positively charged and unstable UO₂²⁺-hydroxide complexes explained both the greater sensitivity of isolate A to UO₂²⁺, and the ability of isolate A to accumulate significant amounts of UO₂²⁺. The exclusive presence of negatively charged and stable UO₂²⁺-carbonate complexes in the high bi-carbonate medium explained the diminished sensitivity of isolate A to UO₂²⁺ toxicity, and limited ability of isolate A to accumulate UO₂²⁺. Environ. Toxicol. Chem. 2010;29:763–769. © 2010 SETAC

Keywords—Uranium Low-level waste Speciation Toxicity Bioaccumulation

INTRODUCTION

Uranium (U) is a widely distributed subsurface contaminant at several Department of Energy (DOE) sites [1], and across the globe [2]. At sites where molecular oxygen is present, most U in the aqueous phase will be in the form of the hexavalent uranyl cation (UO₂²⁺). Low-level waste (LLW) sites, including the Idaho National Laboratory (Idaho Falls, ID, USA) [3] and the Drigg site (Cumbria, NW England, UK) [4], are among U-contaminated sites housing buried contaminated cellulosic material, such as paper towels, cardboard, and lab coats. These materials, and specifically their breakdown products, can potentially contribute to both UO₂²⁺ immobilization and mobilization. For example, these breakdown products include short-chain fatty acids that can potentially enhance UO₂²⁺ mobility through chelation [5–7]. Mechanisms of UO₂²⁺ immobilization include UO₂²⁺ accumulation within biomass metabolizing the cellulose and cellulosic breakdown products [8–10]. In its reduced valence state (U(IV)), uranium can readily precipitate as UO₂, which exhibits limited mobility and toxicity

[11,12]. The ability of iron- and sulfate-reducing bacteria to couple oxidation of organic substrates to U(VI) reduction under anaerobic conditions plays a prominent role in many uranium bioremediation schemes [13]. However, many LLW sites are aerobic, including the INL and Drigg sites [3,4], and, therefore, UO₂²⁺ reduction, an anaerobic process, is not expected to play a large role in decreasing uranium mobility in these systems.

Bacterial bioaccumulation, which could potentially contribute to UO₂²⁺ immobilization, has been studied extensively in laboratory settings as a means of immobilizing toxic metals, including Cd, Pb, Cu, Zn, and UO₂²⁺ [14]. A considerable amount of research has investigated this phenomenon as a potential component of bioremediation schemes in aerobic systems [15–17]. Among the factors that have been shown to influence bioaccumulation are the conditions under which the bacteria are grown, including choice of buffer and carbon source [18,19].

Understanding the impact such growth conditions have on UO₂²⁺ bacterial bioaccumulation processes in LLW sites is important, given the range of carbon and energy sources made available during cellulosic breakdown [20]. In addition, understanding how these conditions affect UO₂²⁺ toxicity is equally important because bioaccumulation will affect UO₂²⁺ mobility

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significantly only if native bacteria are able to reach appreciable numbers in the U-contaminated system. While specific mechanisms of UO_2^{2+} toxicity to bacteria are yet to be described, UO_2^{2+} is known to inhibit bacterial growth generally through oxidative stress [21]. The purpose of this study was to investigate how model cellulose breakdown products, including butyrate, dextrose, ethanol, and lactate, when used as primary carbon and energy sources, affect UO_2^{2+} bioaccumulation and toxicity in a *Pseudomonas* sp. isolate (hereafter referred to as isolate A) cultured from the Cold Test Pit South (CTPS) at the INL. Previous studies have concluded that UO_2^{2+} toxicity and bioaccumulation potential is strongly influenced by complexation and speciation of the UO_2^{2+} [22,23], and, therefore, when possible, differences in toxicity and bioaccumulation patterns were associated with changes in UO_2^{2+} speciation.

MATERIALS AND METHODS

Media composition and growth conditions

Isolate A, the only *Pseudomonas* sp. collected, was isolated from the CTPS at the INL by repeated re-streaking of an aerobic liquid enrichment on agar methylcellulose (0.1%) plates. Cells were grown from frozen (-80°C) glycerol stocks (20% v/v) at $20^\circ \pm 0.5^\circ\text{C}$ in chemically defined liquid media (pH = 7.0) consisting of simulated INL groundwater [24] containing the following (per L): 1.0 mg KCl, 12.7 mg Na_2SO_4 , 3.5 mg CaO, 1.1 mg MgSO_4 , 0.825 g NH_4Cl , 0.261 g K_2HPO_4 , amended with 5 ml of Wolfe's vitamin solution [25], and 1 ml SL-4 trace elements solution [25]. Unamended medium was sterilized by autoclaving at 121°C for 25 min, and the vitamin and trace-metal solutions were filter sterilized ($0.2\ \mu\text{m}$). Because cellulose breakdown can release a variety of organic molecules which can potentially serve as carbon and electron sources for native bacteria [20], representative carbon substrates were chosen from four general categories: butyrate as a model carboxylate, ethanol as an alcohol, dextrose as a sugar, and lactate, a fatty acid commonly used in studies involving UO_2^{2+} -microbe interactions. Carbon sources were added to a concentration of 15 mM carbon. Carbon dioxide (CO_2) is the ultimate cellulosic breakdown product, which in contaminated systems would exist in equilibrium with several bicarbonate species in the aqueous phase. While the presence of bicarbonates is expected regardless of metabolic activity, due to equilibrium between the aqueous phase and atmospheric CO_2 , the CO_2 generated by the activity of the native bacteria could potentially lead to an excess of bicarbonate species in the system. The influence of excess bicarbonate was therefore investigated by repeating each growth experiment with 10 mM NaHCO_3 added to the media, making eight possible growth conditions. Media with added NaHCO_3 is referred to as high-bicarbonate media, and media in equilibrium with atmospheric CO_2 as low-bicarbonate media. Media pH maintained at pH = 7.0 and was buffered with 10 mM piperazine- N , N' -bis(2-ethanesulfonic acid) (PIPES).

U bioaccumulation experiments

The ability of isolate A to accumulate UO_2^{2+} was measured in each of the medium combinations described above. Cells were grown, harvested by centrifugation (6,000 g for 20 min), washed three times in fresh media, then added to Teflon

screw-cap vials to give a concentration of $80 \pm 5\ \text{mg} \cdot \text{protein/L}$ (measured using the Bradford assay [26]). The cells were allowed to incubate for 1 h (allowing the cells to resume growth, as measured by optical density at 600 nm). $50\ \mu\text{M}$ of depleted UO_2^{2+} (as UO_2Cl_2 , International Bio-Analytical Industries) was then added to the cell cultures. Preliminary experimentation carried out in our lab demonstrated the UO_2^{2+} bioaccumulation reaches equilibrium after 10 min, at which point UO_2^{2+} concentrations in filtered samples ($0.2\ \mu\text{m}$) were measured with a kinetic phosphorescence analyzer (KPA, ChemChek Instruments). Cells were then centrifuged (6,000 g for 5 min) and washed five times with a $100\ \mu\text{M}$ EDTA (pH = 5.5) solution to remove loosely bound UO_2^{2+} [27]. Cells were then digested in 50% HNO_3 and UO_2^{2+} concentrations were measured with a KPA. Cell-free, heat-killed cells (exposed to 80°C for 15 min), UO_2^{2+} -free, and carbon-free controls served as comparisons.

U toxicity experiments and modeling

The sensitivity of isolate A to UO_2^{2+} under each of eight growth conditions was investigated. Cells were grown in the same medium to be tested and allowed to reach late exponential growth phase. Cells were washed (three cycles of centrifugation at 6,000 g for 20 min) and suspended in 50 ml of fresh medium. Growth in high-bicarbonate media took place in 125-ml serum bottles sealed with butyl stoppers and crimped with aluminum seals. Growth in low-bicarbonate media took place in 250-ml baffled shaker flasks. UO_2^{2+} concentrations (as uranyl chloride, UO_2Cl_2 , International Bio-Analytical Industries) ranged from 0 to $250\ \mu\text{M}$. Cultures were shaken at 100 rpm at $20^\circ \pm 0.5^\circ\text{C}$. Liquid samples were periodically removed to measure protein concentrations using the Bradford assay [26]. UO_2^{2+} inhibition of growth was modeled using the following generalized Monod expression demonstrated in Levenspiel Equation 1 [28]:

$$\frac{\mu_i}{\mu_0} = \left(1 - \frac{[\text{UO}_2^{2+}]}{[\text{UO}_2^{2+}]_{\text{crit}}} \right)^n \quad (1)$$

where μ_i is the first order growth rate in the presence of UO_2^{2+} , μ_0 is the growth rate in UO_2^{2+} free medium, and $[\text{UO}_2^{2+}]$ is the concentration (in μM) in the medium. The $[\text{UO}_2^{2+}]_{\text{crit}}$ value corresponds to the theoretical minimum $[\text{UO}_2^{2+}]$ that completely inhibits growth. The exponent, n , is referred to as the toxic power. The quotient on the left side of Equation 1, hereafter referred to as the relative inhibition, was plotted against the UO_2^{2+} concentration to generate toxicity curves. Cell-free and carbon-free controls were performed in parallel. The $[\text{UO}_2^{2+}]_{\text{crit}}$ and n values were calculated according to Levenspiel [28]. The calculated $[\text{UO}_2^{2+}]_{\text{crit}}$ values were confirmed empirically (data not shown). Experiments were performed in triplicate. Cell-free and carbon-free controls were performed in parallel.

U speciation modeling

Uranium speciation in substrate-free media was determined using Visual MINTEQ, ver. 2.52. The partial pressure of atmospheric CO_2 was incorporated into the model and was assumed to stay constant at 38.5 Pa. Due to a lack of published thermodynamic data, PIPES buffer was not included in the modeling. Incorporation of the four substrates was also

excluded, again due to a lack of published thermodynamic data for all substrates. Thermodynamic data were available for butyrate and lactate, but these substrates were not predicted to alter UO₂²⁺ speciation significantly (data not shown).

Statistical analysis

All reported values represent the mean of triplicate experiments, and error bars correspond to 95% confidence intervals. Single factor ANOVA was performed on the calculated parameters. Results were determined to be significantly different if $p < 0.05$.

RESULTS AND DISCUSSION

UO₂²⁺ toxicity and modeling results

Figure 1 shows the growth curves of isolate A on each of the four substrates over a range of [UO₂²⁺] (0–150 μM) in low-bicarbonate media. With the exception of ethanol as a substrate, cells tolerated [UO₂²⁺] up to 50 μM without significant inhibition, and when grown on either butyrate or dextrose, [UO₂²⁺] up to 100 μM showed only moderate inhibition both in terms of growth rate and cell yield. Cultures grown on lactate were significantly inhibited by 100 μM UO₂²⁺. Remarkably, cells grown on ethanol were sensitive to [UO₂²⁺] as low as 0.1 μM, a concentration which falls below the United States Environmental Protection Agency (U.S. EPA) prescribed drinking water limit of 0.126 μM. To our knowledge, this is the first reported example of submicromolar UO₂²⁺ concentrations inhibiting microbial growth.

Equation 1 was used to model the results obtained for each substrate, the results of which are shown graphically in Figure 2. Important parameters are summarized in Table 1. The toxicity of UO₂²⁺ to isolate A was dependent on the carbon source tested, as indicated by the significantly different n terms. However, the three [UO₂²⁺]_{crit} values associated with growth on butyrate, dextrose, and lactate were not found to be statistically different ($p < 0.05$) and averaged 154 μM. Growth on ethanol produced a dramatically different [UO₂²⁺]_{crit} value of 1.0 ± 0.22 μM. The impact of the toxic power can be seen when the IC₅₀ values are compared between the four substrates (Table 1). As n approaches zero, the inhibition curve becomes more sharply concave downward and the IC₅₀ approaches UO₂²⁺_{crit}. As n approaches 1, the inhibition curve becomes more linear, and IC₅₀ approaches $\frac{1}{2}$ UO₂²⁺_{crit}. For $n > 1$, which is the case for the ethanol system, the corresponding inhibition curve becomes concave upward as IC₅₀ approaches zero. For this reason, the IC₅₀ values did vary significantly between all four substrates. Cells grown on butyrate had the highest IC₅₀ (138 ± 8 μM) followed by dextrose (103 ± 3 μM), lactate (91 ± 6 μM) and ethanol (0.19 ± 0.04 μM) (Table 1). Butyrate,

Fig. 1. (a–c): Growth curves of isolate A in low carbonate media measured in hours on butyrate (a), dextrose (b), and lactate (c) over a range of UO₂²⁺ concentrations (◇ = UO₂²⁺ free; □ = 50 μM UO₂²⁺; △ = 100 μM UO₂²⁺; ○ = 125 μM UO₂²⁺; + = 150 μM UO₂²⁺). Part (d) shows growth curves of isolate A in low carbonate media on ethanol (◇ = UO₂²⁺ free; □ = 0.10 μM UO₂²⁺; △ = 0.15 μM UO₂²⁺; ○ = 0.25 μM UO₂²⁺; + = 0.50 μM UO₂²⁺; × = 1.0 μM UO₂²⁺). Carbon source had a significant effect on UO₂²⁺ toxicity, particularly when ethanol served as carbon source, which led to growth inhibition at UO₂²⁺ concentrations as low as 0.10 μM UO₂²⁺. Error bars represent 95% confidence intervals of triplicate measurements.

Fig. 2. UO_2^{2+} toxicity modeling results: (a) = butyrate, dextrose and lactate results, (b) = ethanol results. Data points represent the relative inhibition induced by the corresponding UO_2^{2+} concentration. Error bars represent 95% confidence intervals of experiments performed in triplicate. Lines represent the predicted relative inhibition using Equation 1 and the kinetic parameters summarized in Table 1. UO_2^{2+} was found to be so toxic to isolate A when grown on ethanol that these modeling results had to be graphed separately. The dashed vertical line in graph (b) corresponds to the U.S. EPA drinking water limit for U.

which yielded the highest IC_{50} value, also yielded the lowest n value of 0.38 ± 0.10 . This trend continued with dextrose ($n = 0.62 \pm 0.09$), lactate ($n = 0.75 \pm 0.07$), and ethanol ($n = 3.3 \pm 0.20$).

In high-bicarbonate media, isolate A was equally sensitive to UO_2^{2+} regardless of substrate, including ethanol (Fig. 3). A single n and UO_2^{2+} crit value was sufficient to characterize all the data collected under these conditions with 95% confidence (Table 1). The UO_2^{2+} crit value was $217 \pm 20 \mu\text{M}$, and the n value was calculated to be 0.57 ± 0.16 . Isolate A was more than two orders of magnitude more tolerant to UO_2^{2+} when grown on ethanol due to the addition of 10 mM bicarbonate.

UO_2^{2+} speciation modeling results

The significant impact of bicarbonate on UO_2^{2+} toxicity to isolate A can be partially explained using MINTEQA2 speciation modeling (ver. 2.52) (Table 2). These results are similar to previous studies [23,29–31], where UO_2^{2+} toxicity was mitigated by the presence of tightly binding UO_2^{2+} ligands, including bicarbonate species [23,30], and dissolved organic carbon (DOC) [28], both of which can form stable UO_2^{2+} complexes [29]. Stability constants for UO_2^{2+} -bicarbonate complexes range from 8.0 to 20.0, compared with the stability constants of UO_2^{2+} -hydroxide complexes which range from -3.0 to -5.0 [32]. The bioavailability of UO_2^{2+} , and thus toxicity, appears to correlate with stability of the UO_2^{2+} complexes present in solution. It is expected that UO_2^{2+} toxicity will depend heavily on the abundance of weakly bound UO_2^{2+} -hydroxide complexes which appear to interact more readily with important cellular functions.

In high-bicarbonate media, MINTEQA2 predicted that UO_2^{2+} would be mostly present as $\text{UO}_2(\text{CO}_3)_3^{4-}$ (43% of total component concentration), UO_2CO_3 (35% of total component concentration), and $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ (18% of total component concentration). The balance of the UO_2^{2+} (4% of total component concentration) was present as either phosphate complexes (UO_2HPO_4 , and UO_2PO_4^-) or minor bicarbonate complexes ($(\text{UO}_2)_2\text{CO}_3(\text{OH})_3$, $\text{UO}_2(\text{CO}_3)_2^{2-}$, and $\text{CaUO}_2(\text{CO}_3)_3^{2-}$) (Table 2). In low-bicarbonate media, MINTEQA2 predicted that UO_2^{2+} aqueous equilibrium speciation would be dominated by $(\text{UO}_2)\text{CO}_3(\text{OH})_3^-$, accounting for 84% of the total component concentration, followed by UO_2PO_4^- (10% of total component concentration), and UO_2HPO_4 (2.5% of total component concentration). However, 2.5% of the total component concentration was predicted to be present as uranyl-hydroxide complexes, which were not present in the high-bicarbonate system. Consistent with previous studies, isolate A appears less sensitive to aqueous UO_2^{2+} tightly bound to bicarbonate and phosphate complexes than to UO_2^{2+} present as less stable hydroxide complexes. Even though a relatively small amount of the UO_2^{2+} present was weakly bound by hydroxides, the difference in associated bioavailability appears to cause the overall UO_2^{2+} toxicity to be significantly enhanced, particularly to the cells growing on ethanol.

UO_2^{2+} bioaccumulation results

Parallel experiments were performed to investigate the influence carbon source and added bicarbonate has on UO_2^{2+} bioaccumulation (Fig. 4). Previous studies have demonstrated the ability of *Pseudomonas* spp. to rapidly accumulate

Table 1. Summary of the kinetic parameters describing UO_2^{2+} toxicity toward isolate A in each medium combination^a

Bicarbonate	Carbon source	Toxic power (n)	$[\text{UO}_2^{2+}]_{\text{crit}}$ (μM)	Calculated IC_{50} (μM)
Low	Butyrate	0.38 ± 0.10	158 ± 11	138 ± 8
	Dextrose	0.62 ± 0.09	153 ± 5	103 ± 3
	Lactate	0.75 ± 0.07	151 ± 9	91 ± 6
	Ethanol	3.3 ± 0.20	1.0 ± 0.22	0.19 ± 0.04
High	All c-sources	0.57 ± 0.16	217 ± 20	152 ± 14

^a The $[\text{UO}_2^{2+}]_{\text{crit}}$ values correspond to the theoretical minimum $[\text{UO}_2^{2+}]$ that completely inhibits growth. The exponent, n , is referred to as the toxic power. Analysis of variance was carried out on all calculated n values for both the low and high carbonate systems. Between the different substrates, the n values associated with the limited carbonate systems were found to be significantly different ($\alpha < 0.05$). Using the same α value, all n values associated with the high carbonate systems were not significantly different, and thus the average of all n values are reported. IC_{50} = half maximal inhibitory concentration.

Fig. 3. UO₂²⁺ toxicity and toxicity modeling results in the high-carbonate systems. Data points represent the relative inhibition induced by UO₂²⁺. Error bars represent 95% confidence intervals of experiments performed in triplicate. The line represents the predicted relative inhibition using Equation 1 and the kinetic parameters summarized in Table 1. Because the kinetic parameters did not differ significantly in the carbonate buffered systems ($p < 0.05$) only one model fit was needed to summarize the results.

significant amounts of UO₂²⁺ [18,19]. The results of the present study showed that between the filtered samples and the digested cells, virtually all the UO₂²⁺ originally added could be recovered. This suggested that a minimal amount of UO₂²⁺ was being washed off the cell surface, and that virtually all UO₂²⁺ was accumulated within the cell. Figure 4 shows the concentration of UO₂²⁺ accumulated (μmol UO₂²⁺ per mg-protein basis) where, consistent with previous results [33], the results showed the type of carbon source had a significant impact on UO₂²⁺ accumulation. In low- bicarbonate media, actively metabolizing cells of isolate A accumulated between 56 and 88% of the UO₂²⁺ present. Cells metabolizing dextrose accumulated the most UO₂²⁺ (0.56 μmol/mg · protein),

Fig. 4. Micromoles UO₂²⁺ accumulated by isolate A (added to a concentration of 80 ± 5 mg-protein/L) metabolizing each of the four substrates, including carbon-free and heat-killed cell controls. Error bars represent 95% confidence intervals of experiments performed in triplicate.

while the lactate system accumulated the least (0.35 μmol/mg · protein). Cells metabolizing butyrate and ethanol accumulated 0.44 μmol UO₂²⁺/mg · protein and 0.40 μmol UO₂²⁺/mg · protein, respectively. Carbon-free and heat-killed controls accumulated the least amount of UO₂²⁺ (0.17 and 0.16 μmol UO₂²⁺/mg · protein respectively). This suggests that actively metabolizing cells accumulate significantly more UO₂²⁺, which is consistent with a previous study which used granular biomass for UO₂²⁺ removal from aqueous systems [9].

Bioaccumulation of UO₂²⁺ by isolate A in the high-bicarbonate system was significantly affected by carbon source as well (Fig. 4). Among the metabolizing cells, those in the dextrose medium accumulated the most UO₂²⁺ (0.12 μmol UO₂²⁺/mg · protein), while cells metabolizing lactate accumulated the least (0.066 μmol UO₂²⁺/mg · protein). However, on average, metabolizing cells only accumulated 20% as much UO₂²⁺ compared with cells in low-bicarbonate media. The carbon-free and heat-killed controls accumulated the least amount of UO₂²⁺ (0.054 and 0.062 μmol UO₂²⁺/mg · protein, respectively).

The limited ability of isolate A to accumulate UO₂²⁺ in high-bicarbonate media can be explained using speciation modeling results obtained by MINTEQ (ver. 2.52) (Table 2). In both systems, MINTEQ predicted that UO₂²⁺ aqueous equilibrium speciation would be dominated by neutrally or negatively charged bicarbonate and phosphate complexes. As described earlier, in low-bicarbonate media, MINTEQ predicted that UO₂²⁺ aqueous equilibrium speciation would include uranyl-hydroxide complexes, absent in high-bicarbonate media. These complexes included neutral species (UO₂(OH)₂), negatively charged species (UO₂(OH)₃⁻, and, further unique to this system, positively charged species, including UO₂OH⁺, (UO₂)₃(OH)₅⁺, and (UO₂)₄(OH)₇⁺ (Table 2). While only predicted to be a small percentage of the total UO₂²⁺ in the system at equilibrium (2.25%), the presence of these positively charged species could explain the increased potential for bioaccumulation when the negative zeta potential of *Pseudomonas* spp. isolates is taken into consideration [34,35]. Positively charged UO₂²⁺ complexes will be electrostatically attracted to the surface of the cells. This is in contrast to the high-bicarbonate media, in which none of the UO₂²⁺ is predicted to be present as a positive complex and therefore not electrostatically attracted to the cells.

Table 2. MINTEQ modeling results showing the expected U(VI) species present in the carbonate limited and excess carbonate systems^a

Species	Low bicarbonate	High bicarbonate
	% of total component concentration	% of total component concentration
UO ₂ OH ⁺	0.20	NP
(UO ₂) ₃ (OH) ₅ ⁺	1.4	NP
(UO ₂) ₄ (OH) ₇ ⁺	0.65	NP
UO ₂ (OH) ₃ ⁻	0.02	NP
UO ₂ (OH) ₂ (aq)	0.23	NP
UO ₂ HPO ₄ (aq)	2.4	0.03
UO ₂ PO ₄ ⁻	10.2	0.12
(UO ₂) ₂ CO ₃ (OH) ₃ ⁻	84	1.2
UO ₂ CO ₃ (aq)	0.73	35
UO ₂ (CO ₃) ₂ ⁻	0.11	1.3
UO ₂ (CO ₃) ₃ ⁻	NP	43
CaUO ₂ (CO ₃) ₃ ⁻	NP	0.96
Ca ₂ UO ₂ (CO ₃) ₃ (aq)	NP	18

^a NP denotes species not present. In the presence of excess carbonate, UO₂²⁺ is mostly present as tightly bound carbonate or phosphate complexes, which might explain the apparent lower bioavailability of UO₂²⁺. By contrast, the carbonate limited media is predicted to contain a number of loosely bound UO₂²⁺-hydroxide complexes, which might account for the relatively higher bioavailability of UO₂²⁺ in these systems.

In addition, electrostatic considerations can explain the relatively minimal accumulation observed for heat-killed and carbon-limited cells. Recent experiments with *Pseudomonas aeruginosa* (ATCC 10145) showed that starved and dead cells had more negative zeta potentials compared with metabolically active cells [34]. This difference may partially explain why starved and killed cells would accumulate less positively charged species. Similar reasoning explains why starved and heat-killed cells accumulated relatively more UO_2^{2+} in the presence of high bicarbonate compared UO_2^{2+} accumulated in the absence of added bicarbonate (Fig. 4). On average, heat-killed and carbon-limited cells accumulated only $74 \pm 17\%$ and $65 \pm 26\%$ of the amount of UO_2^{2+} accumulated by metabolizing cells, respectively, in high-bicarbonate media. In the low-bicarbonate media, heat-killed and carbon-limited cells accumulated only $37 \pm 2\%$ and $40 \pm 2\%$ of the amount of UO_2^{2+} accumulated by metabolizing cells, respectively. Positively charged UO_2^{2+} species will be less electrostatically attracted to killed and starved cells, as will negatively charged species be less electrostatically repulsed from killed and starved cells.

Electrostatic considerations do not explain the influence of carbon source on UO_2^{2+} accumulation, because in both high- and low-bicarbonate media cells, metabolizing dextrose accumulated the most UO_2^{2+} . Substrate binding to UO_2^{2+} also does not appear to explain the observed accumulation patterns, as there is no correlation between the extent of accumulation in cells metabolizing a particular substrate, and the ability of that substrate to form stable complexes with UO_2^{2+} . For example, of the four substrates only lactate and butyrate form complexes with UO_2^{2+} , in low-carbonate media cells metabolizing these substrates accumulated the least and second most UO_2^{2+} . However, metabolism does appear to impact UO_2^{2+} bioaccumulation in isolate A.

The UO_2^{2+} speciation modeling results explained many of the toxicity and bioaccumulation patterns observed between high- and low-bicarbonate systems. Our results show between the two systems an 80% decrease in UO_2^{2+} bioaccumulation potential corresponded with an average 40% increase in UO_2^{2+} tolerance. However, this trend was not conserved across substrates within either the high- or low-bicarbonate systems. If a strong correlation between accumulation and toxicity existed, it would be expected that conditions that lead to more UO_2^{2+} accumulation would also lead to proportionately greater UO_2^{2+} toxicity. However, in low-bicarbonate media, cells metabolizing ethanol accumulated only 24% less UO_2^{2+} than cells metabolizing dextrose, but the IC_{50} of cells growing on ethanol was only 0.2% that of cells growing on dextrose. In high-bicarbonate media, bioaccumulation depended on the carbon sources tested, but toxicity did not, indicating that UO_2^{2+} accumulation in the presence of 10 mM bicarbonate has a negligible effect on toxicity. While adding bicarbonate reduced both bioaccumulation and toxicity, it did not do so proportionately (the fivefold decrease in accumulation led to a less than twofold reduction in UO_2^{2+} toxicity). To our knowledge, no studies in which correlations between UO_2^{2+} toxicity and accumulation in bacterial cells have been published. The results of the present study are in contradiction to a study of UO_2^{2+} and a *Chlorella* sp. which found that a twofold increase in intracellular UO_2^{2+} resulted in a roughly twofold increase in UO_2^{2+} toxicity [31].

Ongoing research is aimed at understanding the relationship between UO_2^{2+} toxicity and bioaccumulation.

CONCLUSIONS

The effect of cellulose breakdown products on UO_2^{2+} toxicity and bioaccumulation within an environmental *Pseudomonas* sp. isolate (isolate A) under aerobic conditions was investigated. Among the breakdown products considered, the presence of high bicarbonate concentrations was found to have the most significant impact on both UO_2^{2+} toxicity and bioaccumulation. UO_2^{2+} was found to be more toxic in systems with low bicarbonate species concentrations, a result attributed to the predicted presence of unstable UO_2^{2+} -hydroxide complexes. This was especially true when cells were grown on ethanol. Under these conditions, cells were inhibited by UO_2^{2+} concentrations which fell below the U.S. EPA drinking water limits. In media with high bicarbonate concentrations, UO_2^{2+} was predicted to be present mostly as stable UO_2^{2+} -bicarbonate complexes, which were found to be less toxic due to their limited bioavailability. Cells in low-bicarbonate media were found to yield more UO_2^{2+} bioaccumulation. This was largely attributed to electrostatic effects predicted based on UO_2^{2+} speciation data. Given the potential impact of UO_2^{2+} speciation on bacterial – UO_2^{2+} interactions, efforts aimed at controlling UO_2^{2+} speciation might prove to be worthwhile when developing UO_2^{2+} bioremediation strategies. While UO_2^{2+} toxicity and bioaccumulation patterns could largely be explained through MINTEQ speciation modeling results, the final relationship between UO_2^{2+} toxicity and bioaccumulation remains unclear and is the subject of ongoing research.

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