Effects of *Bacillus subtilis* on the reduction of U(VI) by nano-Fe$^0$

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Received 2 November 2014; accepted in revised form 19 May 2015; available online 1 June 2015

Abstract

The effects of *Bacillus subtilis* (*B. subtilis*, a typical model bacterium) on the reduction of U(VI) by nanoscale zero-valent iron (nano-Fe$^0$) were investigated using batch techniques. The reaction products were analysed using spectroscopic techniques, and a kinetics model was developed to elucidate the mechanisms of U(VI) reduction by nano-Fe$^0$. The presence of *B. subtilis* enhanced the U(VI) sorption rate at pH 3.5–9.5 but inhibited the reduction rate of U(VI) to U(IV) at pH > 4.5. According to the FTIR and XRD analysis, the reduction of U(VI) to U(IV) was inhibited due to the formation of inner-sphere surface complexes between the oxygen-containing functional groups of *B. subtilis* or extracellular polymeric substances with the Fe(II)/Fe(III) generated by nano-Fe$^0$, which blocked electron transport from the Fe$^0$ core to U(VI). Based on the EXAFS analysis, a fitting of U–Fe shell at $\sim$3.44 Å revealed inner-sphere bidentate complexes between uranyl and the oxide film of nano-Fe$^0$. For the nano-Fe$^0$ + *B. subtilis* system, the U–Fe shell (at $\sim$3.44 Å) and the U–C/P shell (at $\sim$2.90 Å) further indicated the formation of inner-sphere surface complexes. The kinetics model supported that U(VI) reduction was triggered by U(VI) sorption on the oxide shell of nano-Fe$^0$. The XPS and XANES analyses showed that reductive precipitation was the main mechanism of U(VI) removal by nano-Fe$^0$, whereas the sorption process dominated the removal of U(VI) in the presence of *B. subtilis*, which was further demonstrated by TEM images.

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1. INTRODUCTION

Uranium is a common radionuclide contaminant in soils and aquifers that results from nuclear fuel production, weapons manufacturing and research activities (Stewart et al., 2010; Sun et al., 2013, 2014b). The environmental risk of uranium is mainly dependent on the degree of its mobility, which is greatly affected by its valence state (Bi et al., 2013). Hexavalent uranium (U(VI)O$_2^{2+}$) is soluble and easily forms U(VI) complexes with carbonates in groundwater (Gui et al., 2009), which increases the solubility of uranium by several orders of magnitude (Dong and Brooks, 2006; Stewart et al., 2010). However, tetravalent uranium U(IV) is often immobilised as solid U(IV) (such as uraninite (UO$_2$)), which inhibits uranium transport in contaminated soils and groundwater (Langmuir, 1978). Therefore, the reduction of mobile U(VI) to U(IV)O$_{2\delta}$ by reducing agents has been extensively investigated for the remediation and prediction of U(VI) in groundwater and soils (Riba et al., 2008; Bernier-Latmani et al., 2010; Veeramani et al., 2011; Troyer et al., 2014). Recent studies have revealed that reduced U species include the U(IV) uraninite (UO$_2$) and non-uraninite species (monomeric U(IV)) species lacking the 3.85 Å U–U associations in the corresponding

http://dx.doi.org/10.1016/j.gca.2015.05.036
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has widely been employed as in the standard between 3.0 and octahedra and carbonate (or bicarbonate) ligands in. Charlet et al. (1998) determined that is tetrahedra of sepiolite through a bidentate configuration. However, to the corrosion products and U(VI) (Gu et al., 1998; Fein et al., 2005; Leone et al., 2007). Gorman-Lewis et al. (2005) found that uranyl species could form stable surface complexes on the cell walls of B. subtilis via electrostatic interactions and covalent bonding. In addition, Fowle et al. (2000) determined that the neutral phosphate and deprotonated carboxyl functional groups of B. subtilis played important roles in the formation of uranyl complexes. However, bacteria can adhere to Fe-oxide minerals through the formation of inner-sphere Fe-phosphate/phosphonate complexes (Parikh and Chorover, 2006; Ojeda et al., 2008). Parikh and Chorover (2006) reported that inner-sphere Fe-phosphate complexes were formed between Fe(II)/Fe(III) on Fe-oxides and the terminal phosphate/phosphonate and phosphodiester groups of bacteria. In addition, it is determined that B. subtilis can secrete bacterial extracellular polymeric substances (EPS), which are a complex mixture of macromolecule such as polysaccharides, proteins, and nucleic acids. EPS can also adsorb to the iron oxide-film, which would decrease the reduction activity of nano-FeO (Cao et al., 2011; Hong et al., 2013). Therefore, it is essential to investigate the effect of non-metal reducing bacteria on the reduction of U(VI) by nano-FeO. However, to the author’s knowledge, investigations relevant to the simultaneous biosorption and reduction of U(VI) in ternary system have not been well documented.

Extended X-ray absorption fine structure (EXAFS) spectroscopy has widely been used to explore the interaction mechanisms of U(VI) at water–mineral interfaces. Waite et al. (1994) identified the surface complexes of U(VI) on ferrihydrite (a bidentate complex formed by polyhedral edge sharing, (Fe-O2)UO2(2H2O)3) as having a U–Fe distance of approximately 3.5 Å based on EXAFS analysis. In addition, a similar distance was observed by Reich et al. (1998). Bargh et al. (2000) observed that U(VI) was simultaneously coordinated with FeO6 octahedra and carbonate (or bicarbonate) ligands in a bidentate configuration (hematite-U(VI)–carbonate). Sun et al. (2014b) proposed the coordination of UO22+ with the SiO4 tetrahedra of sepiolite through a bidentate configuration based on EXAFS analysis (U–Si at ~3.16 Å). The U–P distances in phosphate precipitates (~3.58 Å) indicate that uranyl formed a monodentate complex with phosphate (Beazley et al., 2007). In addition, it was demonstrated that the doublet feature on a UO2(s)25 doublet feature on a UO2(s)

On the other hand, the enhanced biosorption of U(VI) by non-metallic reducing bacteria have been extensively investigated due to a variety of oxygen-containing functional groups of cell surface (Gadd, 2007). Bacillus subtilis (B. subtilis, Gram-positive bacterium) is a non-metallic reducing bacterium, which was selected as a model Gram-positive bacterium for this study because it is a common soil microorganism and its cell wall properties have been well-characterized and described in previous reports (Beveridge and Murray, 1980; Fowle et al., 2000; Fein et al., 2005; Leone et al., 2007). The kinetics model
could describe evolution of the thermodynamic quantities of a chemical reaction occurring on water-adsorbent interfaces with time. Wang et al. (2005) quantified the differences in the \(^{243}\)Am(III) desorption behaviour based on a pseudo-first-order kinetics model. In addition, Yan et al. (2010) used a kinetics model to investigate the kinetics of U(VI) removal and reduction on nano-Fe\(^0\). A kinetics model that is consistent with our experimental results was developed to elucidate the mechanisms of U(VI) reduction by nano-Fe\(^0\) in the presence and absence of \(B.\) subtilis.

The objectives of this study were (1) to investigate the effects of \(B.\) subtilis on the immobilisation of U(VI) by nano-Fe\(^0\) under different environmental conditions, such as reaction time, carbonate concentration, pH, initial U(VI) concentration and temperature; and (2) to distinguish the reduction mechanisms of aqueous U(VI) via sorption to nano-Fe\(^0\) using X-photoelectron spectroscopy (XPS), X-ray diffraction (XRD), Fourier transformed infrared (FTIR) spectroscopy, X-ray absorption near-edge spectroscopy (XANES), EXAFS spectroscopy, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and a kinetics model. This paper highlights the reduction of U(VI) by nano-Fe\(^0\) in the presence of non-metallic reducing bacteria and EPS in the PRB, and the findings play a vital role in predicting the fate and transformation of U(VI) in the geochemical environment.

2. MATERIALS AND METHODS

2.1. Materials

All chemicals used in this study were analytical grade, and all solutions were prepared using Milli-Q water. The U(VI) stock solution (1.0 mmol/L) was prepared from UO\(_2\)(NO\(_3\))\(_2\)-6H\(_2\)O in a 0.01 M HNO\(_3\) solution. The Na\(_2\)CO\(_3\) stock solution (0.1 mol/L) was used as a background electrolyte because CO\(_3^{2-}\) is common in groundwater (Gui et al., 2009) and can greatly influence uranium sorption on mineral surface. These stock solutions were prepared with pre-boiled, N\(_2\)(g)-purged distilled water and placed in an anaerobic chamber prior to use. Nano-Fe\(^0\) was purchased from the Aladdin Industrial Corporation Company (Shanghai, China). The \(B.\) subtilis strain was provided by the College of Life Science at Sichuan University. \(B.\) subtilis cells were cultured in a beef extract-peptone medium at 30 °C. Next, the cells were harvested by centrifugation (4000 x g, 5 min) during the stationary phase and were washed three times using 0.05 mol/L NaCl solutions.

2.2. Experimental methods

2.2.1. Kinetics experiments

The kinetics experiments were conducted in an anaerobic chamber that was purged with N\(_2\)(g) at a flow rate of 0.1 L/min throughout the reaction period with 1.0, 5.0 and 10.0 mmol/L of Na\(_2\)CO\(_3\) as the background electrolyte. Briefly, 2, 10 and 20 mL of 0.1 mol/L Na\(_2\)CO\(_3\) stock solution and 168, 160 and 150 mL of distilled water, respectively, were added to polypropylene tubes containing 30 mL of U(VI) stock solution (1.0 mmol/L). Then, 0.4 g of nano-Fe\(^0\) was added to each tube at a solid/solution ratio of 2 g/L. For nano-Fe\(^0\)+ \(B.\) subtilis systems, 2, 10 and 20 mL of 0.1 mol/L Na\(_2\)CO\(_3\) and 148, 140 and 130 mL of distilled water, respectively, were added to 20 mL of the \(B.\) subtilis suspension (~3.0 × 10\(^6\) cells/mL). Then, 30 mL of the 1.0 mmol/L U(VI) stock solution and 0.4 g of nano-Fe\(^0\) were added to the suspension in a glovebox that was purged with N\(_2\) gas. The initial pH of suspensions was adjusted to 3.5, 5.0, 7.0 or 9.0 by the drop-wise addition (<0.1 mL) of a 1.0 mol/L HNO\(_3\)/NaOH solution. Aliquots of the suspension were periodically withdrawn from the tubes by using a 6 mL polypropylene syringe. Subsamples containing 3 mL of the suspensions (solid plus aqueous phase) were used to extract surface-adsorbed U(VI) by using a 0.2 mol/L Na\(_2\)CO\(_3\) solution. The remaining samples (3 mL) were centrifuged and filtered through a 0.1 μm syringe filter. The filtrate was used to determine the U(VI) concentration in the aqueous phase and the collected particles were freeze-dried for analysis of the solid phase.

2.2.2. Reaction edge experiments

Reaction edge experiments were prepared in an anaerobic chamber using a 1.0 mmol/L Na\(_2\)CO\(_3\) background electrolyte for nano-Fe\(^0\), nano-Fe\(^0\)+ \(B.\) subtilis and \(B.\) subtilis, respectively. For nano-Fe\(^0\), 0.2 mL of a 0.1 mol/L Na\(_2\)CO\(_3\) stock solution and 16.8 mL of distilled water were added to 3.0 mL of the U(VI) stock solution. Next, 0.04 g of nano-Fe\(^0\) was added to the suspension. For \(B.\) subtilis, the suspensions included 0.2 mL of the 0.1 mol/L Na\(_2\)CO\(_3\) stock solution, 14.8 mL of distilled water, 3.0 mL of the U(VI) stock solution, and 2 mL of the \(B.\) subtilis suspension (~3.0 × 10\(^6\) cells/mL). For nano-Fe\(^0\)+ \(B.\) subtilis, 0.04 g of nano-Fe\(^0\) was added to the suspension. The initial pH of the resulting suspension was adjusted to 3.5–9.5 by the drop-wise addition of negligible amounts of HNO\(_3\)/NaOH. Next, the suspensions were purged and sealed under N\(_2\)(g) and continuously stirred at 25 °C for 24 h. The final pH values of the experimental suspensions were measured before centrifugation and filtration.

2.2.3. Effects of \(B.\) subtilis, EPS and the U(VI) concentrations

The removal of U(VI) in the nano-Fe\(^0\)+ \(B.\) subtilis treatments with different \(B.\) subtilis concentrations (~1.5–9.0 × 10\(^6\) cells/mL) was determined at 298 K in an anaerobic chamber. The removal and reduction of U(VI) by nano-Fe\(^0\) in the presence of EPS-free \(B.\) subtilis (3.0 × 10\(^8\) cells/mL) and different EPS concentration (0–0.5 g/L) were measured at pH 3.5 and pH 7.0, respectively, at 25 °C. Obtainment of EPS and EPS-free \(B.\) subtilis was adapted to the protocol of Omoike and Chorover (2004). The detailed extraction process was provided in Supporting Information (S1). The removed capacities of U(VI) by nano-Fe\(^0\) and nano-Fe\(^0\)+ \(B.\) subtilis were determined at U(VI) concentrations of 150–450 μmol/L at 5, 15 and 25 °C. The initial pH was adjusted to 5.0 to avoid the formation of schoepite precipitates (as shown in Fig. 3). All of the experimental data were averages of triplicate data (the resulting error bars (within ±5%) are provided).
2.3. Analytical techniques

The U(VI) concentrations were measured using a Fluorolog-3 fluorescence spectrometer (Jobin-Yvon-SPEX instruments, New Jersey). However, this method cannot detect the U(IV) species because it does not emit fluorescence (Kaminski et al., 1981; Brina and Miller, 1992). Briefly, 0.1 mL of the U(VI) solution was added to 3 mL of deoxygenated phosphoric acid (10%) to enhance its phosphorescence. Then, the mixed solutions were sent from the anaerobic chamber to the Fluorolog-3 fluorescence spectrometer in serum bottles sealed with butyl rubber stoppers. The detection limit of this method is 0.1 mg/L. A 450-W Xenon arc lamp was used as the excitation source, and the emission spectra were recorded from 482 to 555 nm with an excitation wavelength of 280 nm. The U(VI) concentration in solution was calculated at the peak emission of 515.4 nm.

The percentages and capacities of U(VI) removal ([U]_{\text{removal}} (%) and [U]_{\text{removal}} (mg/g), respectively) were determined according to Eqs. (1) and (2), respectively.

$$[U]_{\text{removal}}(\%) = \left(\frac{[U(VI)]_{0} - [U(VI)]_{\text{aq}}}{[U(VI)]_{0}}\right) \times 100\% \quad (1)$$

$$[U]_{\text{removal}}(\text{mg/g}) = V \times \left(\frac{[U(VI)]_{0} - [U(VI)]_{\text{aq}}}{m}\right) \quad (2)$$

where [U(VI)]_{0} (mg/L) and [U(VI)]_{aq} (mg/L) are the initial U(VI) concentrations and the aqueous U(VI) concentration after the reaction, respectively, and m (g) and V (mL) represent the mass of nano-Fe^{0} and the volume of the suspension, respectively.

The adsorbed U(VI) ([U(VI)]_{\text{adsorbed}}) can be extracted by following the methods of Gu et al. (1998). Briefly, equal volumes of a 0.2 mol/L Na_{2}CO_{3} solution were added to the suspensions (solid plus aqueous phase) after reaction. Next, the suspensions were reacted for 30 min with vigorously stirring. The U(VI) concentrations in the ([U(VI)]_{aq}) extract were regarded as the sum of [U(VI)]_{aq} and [U(VI)]_{adsorbed} (i.e., [U(VI)]_{aq} = [U(VI)]_{aq} + [U(VI)]_{adsorbed}) due to the absence of fluorescence by the U(IV) species. Therefore, the reduced U(VI) concentration ([U(VI)]_{\text{reduced}}) is the difference between [U(VI)]_{0} and [U(VI)]_{aq} concentrations (Yan et al., 2010), and the reduction percentage of U(VI) ([U]_{\text{reduction}} (%)) was expressed as shown in Eq. (3).

$$[U]_{\text{reduction}}(\%) = \left(\frac{[U(VI)]_{0} - [U(VI)]_{\text{aq}}}{[U(VI)]_{0}}\right) \times 100\% \quad (3)$$

For this method, the recovery percentage of U(IV) to U(VI) can be obtained >95% (Table S1, SI).

2.4. Characterisation

The morphological analyses of nano-Fe^{0}, nano-Fe^{0} + B. subtilis and B. subtilis were performed using SEM (FEI-JSM 6320F) and TEM (JEM-2010, Japan). Samples were prepared in an anaerobic chamber as described by Zhang et al. (2007). Generally, samples were fixed in a 2.5% glutaraldehyde solution for 4 h and dehydrated them sequentially in 10, 30, 70, 90 and 100% ethanol. The fixed dehydrated samples were mounted onto a SEM stub for SEM analysis, and placed on copper grids for TEM and selected area electron diffraction (SAED) analyses. The zeta potentials of the nano-Fe^{0} and B. subtilis were determined using an acoustic spectrometer (Dukhin et al., 2001). The corrosion products of the nano-Fe^{0} were identified by XRD (RigakuMiniFlex 600, Japan) by using a Cu-Kα radiation source (λ = 1.5406 Å) of 10 to 70° with a step size of 0.02° and a count time of 8 s. The FTIR spectra were collected from a KBr pellet by using a JASCO FT1R 410 spectrophotometer. Briefly, 2 mg of sample and 200 mg of KBr (sealed in a desiccator) were ground before pressing into a disc. The spectrum of each sample was collected from 400 to 4000 cm^{-1}. Surface analysis of the solid phase was performed using a Thermos Escalab 250 XPS with Al Kα radiation at 150 W. The samples were attached to a sample supporting plate by using tape before placing in a chamber and evacuating to ~10 Torr. The energies were corrected using the C 1 s peak at 284.6 eV as a reference. The XPS data were processed using the XPSPEAK software (version 4.1).

2.5. XANES and EXAFS analyses

A uranyl nitrate solution (1 mmol/L UO_{2}(NO_{3})_{2}) and solid U^{IV}O_{3}(s) were used as U(VI) and U(IV) standards for the XANES and EXAFS analysis. Four samples were prepared, nano-Fe^{0} at pH 5.0 and pH 7.0, nano-Fe^{0} + B. subtilis at pH 7.0, and B. subtilis at pH 7.0. The experimental details were identical to those described for the reaction edge experiments, except for the background electrolyte. To explore the effects of B. subtilis on U(VI) reduction by nano-Fe^{0}, Na_{2}CO_{3} background solutions were replaced by NaClO_{4} solutions to prevent interference by C atoms. Thus, the EXAFS analysis here just considered the interaction between free UO_{2}^{2+}, nano-Fe^{0} and B. subtilis. On this basis, the influence of CO_{3}^{2−} on the EXAFS spectra of the ternary system will be investigated in further study. After equilibration, each experimental system was centrifuged at 10,000 × g for 10 min. The wet and homogeneous samples were placed in an anaerobic chamber under N_{2}(g) conditions for the XANES and EXAFS measurements.

U L_{III}-edge XANES and EXAFS spectra were collected at the BL14W1 beamline at the Shanghai Synchrotron Radiation Facility (SSRF, Shanghai, China) to investigate the local structure of uranium on the solid phases. The samples were measured in fluorescence mode using a high-throughput 30-element solid-state germanium array detector due to the low uranium concentration, whereas the U^{IV}O_{3}(s) reference sample was measured in transmission mode. A Silicon (1 1 1) double-crystal monochromator was used to tune the desired energies of the incident X-ray beam, and a Zr-foil was used for energy calibration. The spectral data were analysed using Athena and Artemis, which are included in the IFFEFIT 7.0 software package (Newville, 2001). The accuracies of the bond distances and coordination numbers (CNs) were estimated to be ±0.03 Å and ±30%, respectively, when compared to the crystal structures. In addition, the multiple scattering (MS) paths from the axial oxygen atoms were determined (i.e., O_{ax} = U = O_{ax}', O_{ax}' = U = O_{eq}).
3. RESULTS AND DISCUSSION

3.1. Characterisation

As shown by the SEM images, the nano-Fe\(^0\) particles were strongly aggregated (Fig. 1A). In the presence of *B. subtilis*, some nano-Fe\(^0\) particles adhered to the surface of this bacterium (Fig. 1B). As shown in Fig. 1C, the size distribution of nano-Fe\(^0\) particle was 20–100 nm. The surface area of nano-Fe\(^0\) was 15.0 m\(^2\)/g according to the BET analysis (Table 1). Nano-Fe\(^0\) particles exhibited a core–shell structure, which can be clearly seen in high resolution TEM images (Fig. 1D). The EDX spectra demonstrated that an Fe(0) core and an outer shell of iron oxide resulted from surface oxidation, which was consistent with previous studies (Riba et al., 2008; Crane et al., 2011). The thickness of outer shell was approximately 3–4 nm, based on the high resolution TEM and XPS analysis. The surface oxidation of nano-Fe\(^0\) was further confirmed by XRD and XPS analyses. As shown in Fig. 1E, the FTIR spectrum of the nano-Fe\(^0\) did not show any obvious peaks, whereas significant differences were observed for *B. subtilis* and nano-Fe\(^0\) + *B. subtilis* (at pH 3.5 and 7.0). The two bands at 1582 and 1407 cm\(^{-1}\) for *B. subtilis* were corresponded to the asymmetrical and symmetrical C=O stretching bands of the carboxylate groups (COO–) of the terminal amino acid (Yu et al., 2007). However, these bands were not observed for nano-Fe\(^0\) + *B. subtilis*. The enhanced
intensities of the peaks at 1664, 1535 and 1384 cm\(^{-1}\) for nano-Fe\(^0\) + \textit{B. subtilis} potentially resulted from shifts in the asymmetrical and symmetrical C=O stretching, which generally occurred at carboxylate anions coordinated with iron cations (Kazy et al., 2009). A decreased intensity and red shift of the P=O stretching peaks of C–PO\(_3\)\(^2\) was observed for nano-Fe\(^0\) + \textit{B. subtilis} (at \(\sim1187\) cm\(^{-1}\) and at \(\sim1189\) for pH 3.5 and 7.0, respectively) compared with \textit{B. subtilis} (at \(\sim1230\) cm\(^{-1}\)). This evidence indicated that the iron was easily bound with the phosphate groups (Kazy et al., 2009). The FTIR analysis suggested that the oxygen-containing functional groups of \textit{B. subtilis} or EPS (i.e., carboxylate and phosphate groups) were easily coordinated with Fe(II) or Fe(III) on nano-Fe\(^0\). According to the zeta potentials over the studied pH range (Fig. 1F), the isoelectric point (pH\(_{\text{pzc}}\)) values for the nano-Fe\(^0\) and \textit{B. subtilis} were \(\sim7.9\) and \(\sim3.5\), respectively. \textit{B. subtilis} exhibited the negatively charged at pH > 3.5, whereas nano-Fe\(^0\) particles was positive charge at pH < 7.9. Thus, \textit{B. subtilis} can adhere to nano-Fe\(^0\) surface by electrostatic attraction.

### 3.2. Batch macroscopic experiments

#### 3.2.1. Removal rates

The effects of reaction time on the removal of U(VI) by nano-Fe\(^0\), \textit{B. subtilis}, and nano-Fe\(^0\) + \textit{B. subtilis} as a function of pH and carbonate concentration were shown in Fig. 2. At 1.0 mmol/L Na\(_2\)CO\(_3\), the removal rates of U(VI) on nano-Fe\(^0\) decreased as follows: pH 7.0 > pH 5.0 > pH 9.0 > pH 3.5. Approximately 98% (pH 7.0), 80%
of the U(VI) was removed after 5 h (Fig. 2A). However, approximately 95%, 85%, 55% and 30% of the U(VI) was removed in the nano-Fe0 + B. subtilis treatments at pH 7.0, pH 5.0, pH 9.0 and pH 3.5, respectively, following only 1.5 h (Fig. 2B). By contrast, the removal percentage of U(VI) by B. subtilis was lower than those of nano-Fe0 and nano-Fe0 + B. subtilis, whereas the rapid removal rate (removal equilibrium reached at approximately 30 min) was observed (Fig. 2C). Approximately 70%, 45%, 40% and 10% of U(VI) was removed by B. subtilis at pH 7.0, pH 5.0, pH 9.0 and pH 3.5, respectively. Compared to nano-Fe0, the more rapid removal rate of nano-Fe0 + B. subtilis was also observed. Therefore, the fast removal rate of B. subtilis and nano-Fe0 + B. subtilis for U(VI) could be attributed to the abundant oxygen-containing functional groups of the cell surface. When the finite sorption sites on B. subtilis were occupied, the removal rate of nano-Fe0 + B. subtilis was predominated by nano-Fe0.

Consistent with previous reports (Gu et al., 1998; Yan et al., 2010), the kinetics of U(VI) removal by nano-Fe0 were described by pseudo-first-order model ($R^2 > 0.98$), which indicates that the removal of U(VI) by nano-Fe0 was controlled by the transition of free aqueous U(VI) to adsorbed state (Rudzinski and Plazinski, 2006). The fitting parameters were shown in Table 2. However, U(VI) removal by nano-Fe0 + B. subtilis and B. subtilis cannot be satisfactorily fit by the pseudo-first-order model, which suggested that B. subtilis could influence the interaction mechanisms between U(VI) and nano-Fe0.

The removal rate of nano-Fe0, B. subtilis, and nano-Fe0 + B. subtilis decreased with increasing Na2CO3 concentration. At 5.0 mmol/L Na2CO3, 48, 15 and 2 h were required to remove 80%, 90% and 70% of the U(VI), respectively, in the nano-Fe0, nano-Fe0 + B. subtilis and B. subtilis treatments at pH 7.0 (Fig. 2D-F). At 10 mmol/L Na2CO3, 80%, 90% and 70% of the U(VI) was removed following 96, 48 and 12 h in the nano-Fe0, nano-Fe0 + B.

Table 2
Fitting parameters of U(VI) removal or reduction on nano-Fe0 in the absence or presence of B. subtilis based on the pseudo-first-order rate law.

<table>
<thead>
<tr>
<th>Removal or reduction</th>
<th>Na2CO3 (mM)</th>
<th>pH</th>
<th>Rate constants (h⁻¹)</th>
<th>Half-lives (h)</th>
<th>$R^2$</th>
</tr>
</thead>
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<tr>
<td>U(VI) immobilisation (nano-Fe0)</td>
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<td>3.5</td>
<td>0.020</td>
<td>34.66</td>
<td>0.97</td>
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<td></td>
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<td>7.0</td>
<td>0.781</td>
<td>0.89</td>
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<td>9.0</td>
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<td>0.008</td>
<td>86.64</td>
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<td>0.012</td>
<td>57.76</td>
<td>0.89</td>
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<td>4.47</td>
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subtilis} and {B. subtilis} treatments at pH 7.0, respectively (Fig. 2G–I). However, the removal of U(VI) on nano-Fe<sup>0</sup> and nano-Fe<sup>0</sup> + {B. subtilis} at pH = 3.5 was slightly influenced by Na<sub>2</sub>CO<sub>3</sub> solution. The strong inhibition of U(VI) removal could result from the formation of a U(VI)–carbonate species, which were more stable in aqueous solutions and were not favourable for sorption on nano-Fe<sup>0</sup> (Yan et al., 2010). Fig. 3 showed the distribution of U(VI) species calculated by MINEQL + 3.0 varied with pH and the Na<sub>2</sub>CO<sub>3</sub> and U(VI) concentrations. When the concentration of U(VI) was 150 μmol/L (the experimental concentration for kinetics), the concentration of the U(VI)–hydroxyl species was significantly decreased as the increase of Na<sub>2</sub>CO<sub>3</sub> concentration, whereas more U(VI)–carbonate species (e.g., UO<sub>2</sub>CO<sub>3</sub>(aq), UO<sub>2</sub>(CO<sub>3</sub>)<sub>3</sub><sup>4-</sup> and UO<sub>2</sub>(CO<sub>3</sub>)<sub>2</sub><sup>2-</sup>) species were observed at pH > 4.0 (Fig. 3A–C). Thus, the decreased removal rates with increasing concentration of Na<sub>2</sub>CO<sub>3</sub> can be attributed to the electrostatic repulsion between negatively charged U(VI)–carbonate species and negative charged surface of nano-Fe<sup>0</sup>. However, the subtle impacts of Na<sub>2</sub>CO<sub>3</sub> on the U(VI) species were observed at pH < 4.0 over the investigated Na<sub>2</sub>CO<sub>3</sub> concentrations, which could explain the weak effects of the Na<sub>2</sub>CO<sub>3</sub> concentrations on U(VI) removal at pH 3.5 for nano-Fe<sup>0</sup>, nano-Fe<sup>0</sup> + {B. subtilis} and {B. subtilis}.

### 3.2.2. Reduction rates

The amounts of adsorbed U(VI) can be determined by extracting the uranium-containing solid samples with 0.1 mol/L Na<sub>2</sub>CO<sub>3</sub> solution (Gu et al., 1998). Therefore, the amount of U(VI) reduced to U(IV) can be determined as the difference between the initial removal and extraction concentrations. The U(VI) reduction rates, as indicated by the time evolution of the residual U(VI) solutions ([U(VI)]<sub>res</sub>), were shown in Fig. 4 for nano-Fe<sup>0</sup> and nano-Fe<sup>0</sup> + {B. subtilis}. The reduction amounts for nano-Fe<sup>0</sup> + {B. subtilis} at pH 5.0, pH 7.0 and pH 9.0 (~42%, 45%, and 24%) were lower than those of the nano-Fe<sup>0</sup> (~65%, 90% and 35%) at 1.0 mmol/L Na<sub>2</sub>CO<sub>3</sub> at 8 h, suggesting that {B. subtilis} inhibited the reduction of U(VI). However, this inhibition was not observed at pH 3.5 (i.e., both ~30% and ~29% reduced U(VI) for nano-Fe<sup>0</sup> and nano-Fe<sup>0</sup> + {B. subtilis}, respectively). As shown in Fig. S1 of SI, the reduction capacity of {B. subtilis} for U(VI) (9%) can be negligible as compared with nano-Fe<sup>0</sup> (90%). The decrease in the reduction amounts for nano-Fe<sup>0</sup> + {B. subtilis} may result from the interactions between {B. subtilis} and nano-Fe<sup>0</sup> at high pH. Approximately 65% of {B. subtilis} cells were attached with nano-Fe<sup>0</sup> at 10 min (Fig. S2, SI). In addition, EPS secreted by {B. subtilis} can also bind with nano-Fe<sup>0</sup>. As discussed in the FTIR analysis, these functional groups from {B. subtilis} might occupy some active sites (i.e., structural Fe(II)) (White and Peterson, 1996; Charlet et al., 1998) on the oxide shell of nano-Fe<sup>0</sup>, which would decrease the reduction capacity of the nano-Fe<sup>0</sup>. Structural Fe(II) in the oxide shell of the nano-Fe<sup>0</sup> was more effective for reducing U(VI) (Liger et al., 1999; Boland et al., 2014). The reduction rates for nano-Fe<sup>0</sup> and nano-Fe<sup>0</sup> + {B. subtilis} decreased as the Na<sub>2</sub>CO<sub>3</sub> concentration increased at pH > 3.5. However, the reduction rate was only slightly influenced by Na<sub>2</sub>CO<sub>3</sub> at pH 3.5. At pH 7.0, approximately 5, 60 and 160 h were.
required for the nano-Fe\(^0\) to reduce 80% of the U(VI) at Na\(_2\)CO\(_3\) concentrations of 1.0, 5.0 and 10.0 mmol/L, respectively (Fig. 4A, C and E). The decrease in U(VI) reduction could result from the inhibition of U(VI) sorption due to Na\(_2\)CO\(_3\).

3.2.3. pH effect

The effects of pH on the removal and reduction of U(VI) by nano-Fe\(^0\), B. subtilis and nano-Fe\(^0\) + B. subtilis at 24 h were shown in Fig. 5. The removal of U(VI) by nano-Fe\(^0\), B. subtilis and nano-Fe\(^0\) + B. subtilis increased with increasing pH from 3.5 to 7.0, whereas decreased removal was observed at pH > 7.0 (Fig. 5A). Fig. 5B showed the corresponding reducing amounts of U(VI) to U(IV). The increased reduction of U(VI) on nano-Fe\(^0\) and nano-Fe\(^0\) + B. subtilis was observed at pH 3.5–7.0, whereas the reducing capacity of B. subtilis was limited (~10% reduced U(VI) at pH 7.0). Mangaiyarkarasi et al. (2011) demonstrated that the reduction of Chromate by B. subtilis was attributed to the membrane bound enzymes. The reduction of U(VI) on B. subtilis might be mediated by U(VI) reductase on the cell membrane under circumneutral conditions, whereas extreme acidic and basic conditions can make the enzyme inactivation. As a control, heat killed B. subtilis cells did not present the reduction of U(VI) over a wide range of pH from 3 to 10 (Fig. S3, SI). The relatively high reducing amounts of U(VI) on nano-Fe\(^0\) (~70–90%) from pH 5.0 to 7.0 potentially resulted from the high sorption of U(VI). Li and Zhang (2007) determined that the oxide shell of nano-Fe\(^0\) offered surface coordination and sorption sites for heavy metal ions. The density of the negative charge on the oxide shell increases as the pH increases and the solubility of U(VI) decreases (Kanel et al., 2006; Scott et al., 2011), which favoured U(VI) sorption and/or co-precipitation on the oxide shell at high pH (Shao et al., 2014). The decreased sorption of U(VI) at pH > 7.0 may be attributed to the electrostatic repulsion between the negative oxide shell and the negative U(VI) species (i.e., UO\(_2\)(CO\(_3\))\(_6\)\(^{4-}\) and UO\(_2\)(CO\(_3\))\(_2\)(OH)\(_2\)\(^{2-}\)) (Fig. 3A) (Song et al., 2014). In addition, the U(VI) reduction was triggered by the sorption of U(VI) on the nano-Fe\(^0\) surface (Yan et al., 2010). Riba et al. (2008) determined that U(VI) was

![Fig. 4](image)

**Fig. 4.** The kinetics of U(VI) reduction on nano-Fe\(^0\) at 1.0 (A), 5.0 (C) and 10.0 (E) mmol/L Na\(_2\)CO\(_3\); and on nano-Fe\(^0\) + B. subtilis at 1.0 (B), 5.0 (D) and 10.0 (F) mmol/L Na\(_2\)CO\(_3\), [U(VI)]\(_0\) = 150 μmol/L, T = 25 °C, and \(m/V = 2\) g/L.
adsorbed on the oxide shell, which then mediated the transfer of electrons from the Fe\textsuperscript{0} core to U(VI). The formation of surface complexes provided a favourable environment for electron transfer from iron to uranium (Liger et al., 1999).

The presence of *B. subtilis* was noted to increase the U(VI) removal on the nano-Fe\textsuperscript{0}, but inhibited the U(VI) reduction at pH > 4.5 (Fig. 5). At pH 5.0, 70% of U(VI) was reduced by nano-Fe\textsuperscript{0} compared to nano-Fe\textsuperscript{0} + *B. subtilis* (~50%) and *B. subtilis* (10%). The enhanced removal of U(VI) on nano-Fe\textsuperscript{0} + *B. subtilis* can be attributed to the increased sorption sites from the functional groups of *B. subtilis*, as indicated by Table 1. The increased sorption of U(VI) on *B. subtilis* with increasing pH was due to the deprotonation of functional groups of cell wall, resulting in the increase of massive surface sites. Likewise, the chemical bonding between structural Fe(II) or Fe(III) of nano-Fe\textsuperscript{0} and oxygen-containing groups of the surface or EPS secreted by *B. subtilis* can be attributed to the increased sorption sites from the functional groups of *B. subtilis*, as indicated by Table 1. The increased sorption of U(VI) on *B. subtilis* with increasing pH was due to the deprotonation of functional groups of cell wall, resulting in the increase of massive surface sites. Likewise, the chemical bonding between structural Fe(II) or Fe(III) of nano-Fe\textsuperscript{0} and oxygen-containing groups of the surface or EPS secreted by *B. subtilis* can be attributed to the increased sorption sites from the functional groups of *B. subtilis*, as indicated by Table 1. The increased sorption of U(VI) on *B. subtilis* with increasing pH was due to the deprotonation of functional groups of cell wall, resulting in the increase of massive surface sites.

The FTIR results also showed that the phosphate groups. For nano-Fe\textsuperscript{0} and eps were inclined to coordinate FeOH groups of nano-Fe\textsuperscript{0}. Parikh and Chorover (2006) demonstrated that the interaction of *B. subtilis* and Fe-oxides was mediated by the formation of inner-sphere Fe-phosphate/phosphonate complexes and/or terminal Fe-phosphate/phosphonate and phosphodiester groups. The complexation of methylphosphonic acid/phospholipid vesicle with iron oxide mineral was also evidenced.

3.2.4. Effect of *B. subtilis* and EPS

The effects of the *B. subtilis* concentrations on the removal and reduction of U(VI) by nano-Fe\textsuperscript{0} + *B. subtilis* at 24 h were shown in Fig. 6A and B. At pH 3.5 (Fig. 6A), the removal and reduction percentages of U(VI) on nano-Fe\textsuperscript{0} were ~30 and 24%, respectively, which were higher than those of *B. subtilis* (~25% for removal and ~5% for reduction). However, approximately 90% of U(VI) was removed by nano-Fe\textsuperscript{0} + *B. subtilis* at pH 3.5 at 9.0 × 10\textsuperscript{6} cells/mL. It was also observed that the reducing amount of U(VI) on nano-Fe\textsuperscript{0} + *B. subtilis* (~27%) was slightly lower than nano-Fe\textsuperscript{0} at 9.0 × 10\textsuperscript{6} cells/mL. Therefore, the increased removal of U(VI) on nano-Fe\textsuperscript{0} + *B. subtilis* potentially resulted from the increased sorption site density of the functional groups on *B. subtilis*. As shown in Fig. 6B, the removal of U(VI) nano-Fe\textsuperscript{0} + *B. subtilis* at pH 7.0 (~80% at 0.6 × 10\textsuperscript{6} cells/mL) was still lower than the nano-Fe\textsuperscript{0} (~100%). This inhibition effect potentially resulted from the adhesion between the nano-Fe\textsuperscript{0} and *B. subtilis*, which occupied the sorption sites. As the *B. subtilis* concentration increased, a slight inhibition effect was observed due to the greater number of sorption sites on *B. subtilis*. Thus, the removal of U(VI) to U(IV) was significantly inhibited by the nano-Fe\textsuperscript{0} + *B. subtilis* at concentrations of 6.0, 7.0 and 9.0 × 10\textsuperscript{6} cells/mL. As indicated in previous discussion, a favourable coordination environment for the formation of structural Fe(II) or Fe(III) surface complexes with oxygen-containing groups on *B. subtilis* was provided at high pH, which decreased the reducing capacity of nano-Fe\textsuperscript{0}. The greater *B. subtilis* concentration provided more functional groups for binding with structural Fe(II) or Fe(III), which further inhibited the reduction of U(VI) to U(IV). Sorption of U(VI) was the main removal mechanism for *B. subtilis* at pH 7.0 because only ~10% of the U(VI) was reduced. The charged property of *B. subtilis* was derived from a variety of oxygen- and phosphoryl-containing functional groups. As a Gram-positive bacterium, the cell wall of *B. subtilis* was characterized by the components of peptidoglycan (i.e., N-acetylglucosamine and N-acetylmuramic acid), crosslinks of short peptides and polymers such as teichoic acid, teichuronic acid and lipoteichoic acid (Yee and Fein, 2001). The surface reaction sites derived from these polymers and the EPS secreted by *B. subtilis* into the external aqueous solution can coordinate with U(VI) ions. Leone et al. (2007) based on surface-specific molecular-scale measurements identified three surface reaction sites, including \( \equiv\text{COOH}, \equiv\text{NH}^+ \) and \( \equiv\text{PO}^- \) groups. For nano-Fe\textsuperscript{0}, the only surface site (amphoteric \( \equiv\text{FeOH} \) group, mainly resulted from its oxide-shell in contact with water molecules). The FTIR results also showed that the phosphate and carboxylate groups of *B. subtilis* or EPS were inclined to coordinate \( \equiv\text{FeOH} \) groups of nano-Fe\textsuperscript{0}. Parikh and Chorover (2006) demonstrated that the interaction of *B. subtilis* and Fe-oxides was mediated by the formation of inner-sphere Fe-phosphate/phosphonate complexes and/or terminal Fe-phosphate/phosphonate and phosphodiester groups. The complexation of methylphosphonic acid/phospholipid vesicle with iron oxide mineral was also evidenced.
by previous studies (Barja et al., 1999; Cagnasso et al., 2010). Hence, an increase in *B. subtilis* can enhance the sorption of U(VI) on *B. subtilis* and can inhibit the reduction of U(VI) by nano-Fe$_0$, especially at high pH.

The inhibition effect of *B. subtilis* on nano-Fe$_0$ may result from EPS. Thus, the influence of EPS-free *B. subtilis* with variable EPS concentration on U(VI) removal or reduction by nano-Fe$_0$ was investigated by batch techniques (Fig. 6C and D). As shown in Fig. 6C, the removal of U(VI) on nano-Fe$_0$ + EPS-free *B. subtilis* (37%) at pH 3.5 was larger than that of nano-Fe$_0$ (30%) in Fig. 6A. It was also observed that the removal of U(VI) increased with increasing EPS concentration, suggesting that both EPS and EPS-free *B. subtilis* provided sorption sites for U(VI). A slight decrease was observed for the reduction of U(VI) by nano-Fe$_0$ with increasing EPS concentration. As shown in Fig. 6D, the reduction amount of U(VI) decreased from 56% to 5% with increasing EPS concentration from 0.05 to 0.5 mg/L, indicating that EPS greatly inhibited the reduction activity of nano-Fe$_0$. The similar trend was also observed for *B. subtilis* (Fig. 6B). The reduction amount of U(VI) (60%) by nano-Fe$_0$ + *B. subtilis* (EPS-free) was lower than nano-Fe$_0$ (74%), suggesting that the oxygen-containing functional groups of *B. subtilis* surface also inhibited the reduction of U(VI) on nano-Fe$_0$.

Omoike and Chorover (2004) also demonstrated EPS secreted from *B. subtilis* were capable of binding iron oxide film. These results indicated that the inhibited reduction of U(VI) can be caused by the complexation of nano-Fe$_0$ with EPS or oxygen-containing groups of *B. subtilis* surface.

### 3.2.5. Effects of temperature and U(VI) concentrations

The removal capacities of U(VI) varied from 150 to 450 μmol/L on nano-Fe$_0$ and nano-Fe$_0$ + *B. subtilis* at pH 5.0 and 24 h and were shown in Fig. 7. From Fig. 3, the initial concentrations of U(VI), even at 450 μmol/L, were below the precipitation concentration required for schoepite at pH 5.0. The removal of U(VI) by nano-Fe$_0$ and nano-Fe$_0$ + *B. subtilis* significantly increased as the initial U(VI) concentration increased (Fig. 7). The maximum removal capacities of nano-Fe$_0$ and nano-Fe$_0$ + *B. subtilis* at pH 5.0 and $T=25^\circ C$ were 215.17 and 95.64 mg/g, respectively, which demonstrated that *B. subtilis* inhibited the removal of U(VI) by nano-Fe$_0$ at pH 5.0. The effects of temperature on the removal of U(VI) by nano-Fe$_0$ and nano-Fe$_0$ + *B. subtilis* were shown in Fig. 7. It was observed that the removal of U(VI) by nano-Fe$_0$ and nano-Fe$_0$ + *B. subtilis* were the highest at 25 $^\circ C$ and the lowest at 5 $^\circ C$, indicating that U(VI) removal by nano-Fe$_0$ and nano-Fe$_0$ + *B. subtilis* was promoted at
higher temperatures. The improved performance of nano-Fe$^0$ for treating heavy metal (Rangsivek and Jekel, 2005), trichloroethylene (Trux et al., 2011) and nitrite (Liang et al., 2008) was observed with an increase in temperature. Figure S4 in SI showed the U(VI) sorption isotherms at pH 5.0 and 25 °C for nano-Fe$^0$ and B. subtilis, respectively. The maximum sorption capacities of nano-Fe$^0$ and B. subtilis were calculated from Langmuir model to be 12.75 mg/g and 335.25 mg/g, respectively. Therefore, surface site density of nano-Fe$^0$ and B. subtilis availability for sorption processes was calculated to be 0.053 and 6.06 sites/nm$^2$, respectively (Dixit and Hering, 2003). The surface density of B. subtilis was comparable to previous studies reported by Leone et al. (2007) who calculated the surface site density (8.52 site/nm$^2$) based on the total amounts of \( \equiv \text{COOH}, \equiv \text{NH}_2 \) and \( \equiv \text{PO}^- \) by the potentiometric titration. Similarly, the surface site density of nano-Fe$^0$ and B. subtilis availability for reduction processes was calculated 62.14 and 0.14 sites/nm$^2$, respectively. It was observed that the site density of B. subtilis for U(VI) sorption was two orders of magnitude higher than that of nano-Fe$^0$, suggesting the great sorption ability of B. subtilis than nano-Fe$^0$. This results may explain why the sorption of U(VI) was the main removal mechanism for nano-Fe$^0$ + B. subtilis. However, the surface site density of nano-Fe$^0$ for U(VI) reduction (62.14 sites/nm$^2$) was great larger than that of B. subtilis (0.14 sites/nm$^2$), which indicated that more reducing ability of nano-Fe$^0$ was observed. As shown in Fig. 7, approximately 100% of U(VI) was removed by nano-Fe$^0$ at 25 °C even at high U(VI) loading conditions due to its high reducing ability. Gu et al. (1998) determined that no maximum U(VI) removal capacity could be defined as long as sufficient amounts of Fe$^0$ were present in the system to maintain an electron flow and a favourable reducing environment. Thus, the Fe(0) core could be considered as an electron bank for the oxide shell (i.e., structural Fe(II) or Fe(III)), which in turn reduces U(VI) to U(IV). By contrast, the presence of B. subtilis may block the electron flow due to the complexion of oxygen-containing functional groups with structural Fe(II) or Fe(III)). Consequently, the active sites of nano-Fe$^0$ cannot be regenerated, which results in the inhibition of reduction reaction.

3.3. Spectroscopic Analysis

3.3.1. XRD Analysis

The XRD patterns of the nano-Fe$^0$ and nano-Fe$^0$ + B. subtilis before and after reaction were shown in Fig. 8A. The corrosion products of nano-Fe$^0$ included predominant Fe, magnetite/maghemite and lepidocrocite, which were consistent with the results obtained from the TEM images. For the U(VI)-reacted nano-Fe$^0$, relatively higher intensities of magnetite and lepidocrocite were observed at pH 3.5, which suggested that the corrosion of nano-Fe$^0$ is accelerated at low pH (Matheson and Tratnyek, 1994; Song and Carraway, 2005). In addition, the intensities of magnetite/maghemite peaks from the U(VI)-reacted nano-Fe$^0$ + B. subtilis (pH 3.5 and 7.0) were lower than those from the nano-Fe$^0$, indicating that the presence of B. subtilis inhibited the corrosion of nano-Fe$^0$, potentially due to the complexation of Fe(II) or Fe(III) with the oxygen-containing functional groups of B. subtilis. According to Fig. 3A, schoepite (UO$_3$2H$_2$O) was the thermodynamically prevalent precipitate that resulted from oversaturated U(VI) species at pH > 5.0. Riba et al. (2008) proved that the gradual precipitation of UO$_3$2H$_2$O became significant after 7 days. However, UO$_3$2H$_2$O was not detected in the XRD patterns of the samples. Instead, the low intensity of peak at \( 2\theta = 28.1^\circ \) for U(VI)-reacted nano-Fe$^0$ at pH 7.0 could be assigned to UO$_{280}$ (Frazier et al., 2005), which suggested that the removal of U(VI) on nano-Fe$^0$ at pH 7.0 could be attributed to the formation of reductive UO$_{280}$ precipitates. The formation of precipitates (U(IV)O$_2$) was further corroborated by the EXAFS analysis. Therefore, the aqueous U(VI) was adsorbed on the nano-Fe$^0$ and B. subtilis surfaces first.

The XRD results indicated that corrosion of nano-Fe$^0$ was accelerated, whereas the high level removal of U(VI) on nano-Fe$^0$ was observed (Fig. 7B). This contradictory phenomenon indicated that the Fe(0) was unlikely to reduce U(VI) directly. Moura et al. (2005) demonstrated that active structural Fe(II) was formed by the electron transfer of Fe(0) to Fe(III) of iron oxide:

\[
\text{Fe}(0) + \text{Fe(III)}(\text{oxide}) \rightarrow \text{Fe(II)} + \text{structural Fe(II)}(\text{oxide})
\]
Therefore, it should be the Fe(0) core that provide electron via indirect corrosion to regenerate structural Fe(II), enabling continued reactivity of nano-Fe\(^0\). The increased intensity of magnetite after reaction may result from the indirect corrosion of bulk Fe(0) core. Crane et al. (2011) also determined the great removal of U(VI) on nano-Fe\(^0\) due to the supply of electron form Fe(0) core to near-stoichiometric magnetite surface. It was reported that the interface between Fe(0) core and magnetite (oxide shell) can mediate electron transfer from Fe(0) to the oxide shell (Moura et al., 2005). Magnetite can be an n or p semiconductor, and the ohmic junction with very low resistance was formed on the interface of metal-magnetite (White, 1990; Sutton and Balluffi, 1995). The production of structural Fe(II) during the electron transfer from Fe\(^0\) to iron oxide has already been demonstrated as a precursor mechanism in Cr(VI) reduction (dos Santos Coelho et al., 2008) and in the Fenton reaction (Moura et al., 2005) by metallic iron. However, the coordination of B. subtilis with structural Fe(II) or Fe(III) blocked the electron flow and prevented further corrosion of nano-Fe\(^0\). Therefore, it was assumed that the structural Fe(II) of the outer layer of nano-Fe\(^0\) was donated electrons to U(VI).

### 3.3.2. TEM analysis

The TEM-SAED images of nano-Fe\(^0\) and nano-Fe\(^0\) + B. subtilis after reaction with U(VI) were shown in Fig. 8B and C. As shown in Fig. 8B, the aggregated nano-Fe\(^0\) nanoparticles were observed accompanied with some precipitate. The inset selected area electron diffraction (SAED) pattern from these precipitate displayed diffuse powder rings. The d-spacing and intensities of ring electron diffraction pattern were matched well with (hkl) indices of UO\(_2\)(s) such as (111), (200), (220) and (113) planes (Lee et al., 2010; Alessi et al., 2012). The ring-pattern structures revealed the formation of poor crystallinity or nanoparticles (Lee et al., 2010), which was consistent with our XRD results. In contrast to nano-Fe\(^0\), few precipitates were observed on the surface of B. subtilis for the system of nano-Fe\(^0\) + B. subtilis (Fig. 8C). The inset SAED pattern of B. subtilis displayed a non-crystalline solid phase, indicating that U(VI) was predominantly as non-uraninite U(IV) species on B. subtilis. The SAED pattern from precipitates in nano-Fe\(^0\) + B. subtilis exhibited a broad and diffuse ring relative to that of pure nano-Fe\(^0\), suggesting the relatively low bulk abundance of uraninite. Bargar et al. (2013) also determined that U(VI) was reduced to monomeric U(IV) species (complexes or nanoscale precipitates) likely associated with biomass and to an ordered species resembling uraninite. The presence of multiple products suggested multiple redox transition pathways. B. subtilis and/or these EPS could inhibit the reducing reactivity of nano-Fe\(^0\). The result indicated that the biosorption of U(VI) on the oxygen-containing functional groups of B. subtilis represented a large contribution for the removal of U(VI) by nano-Fe\(^0\) + B. subtilis. As shown in Fig. S5 of SI, energy dispersive X-ray spectrum (EDS) mapping of B. subtilis showed that oxygenated- and phosphorous-containing groups were main coordination sites for U(VI) surface complexes, which was further demonstrated by FTIR analysis (Fig. S6 of SI). Fowle et al. (2000) also evidenced that the sorption of U(VI) on B. subtilis was coordinated with neutral phosphate and deprotonated carboxyl functional groups of B. subtilis. These observations showed that reductive precipitation was a major contributor to U(VI) removal by nano-Fe\(^0\), whereas the sorption process dominated the removal of U(VI) in the presence of B. subtilis.

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**Fig. 8.** (A) XRD patterns of the control (un-reacted nano-Fe\(^0\)) and U(VI)-reacted samples at pH 3.5 and 7.0, M (magnetite/\(\gamma\)-maghemite) and L (\(\gamma\)-lepidocrocite); TEM images of U(VI)-reacted samples with corresponding SAED pattern (inset), (B) nano-Fe\(^0\), (C) nano-Fe\(^0\) + B. subtilis.
3.3.3. XPS Analysis

The XPS analyses of nano-Fe\(^0\) and nano-Fe\(^0\) + \(B.\) subtilis before and after their reaction with U(VI) at pH 3.5 and 7.0 were shown in Fig. 9A. For all of the samples after the reaction, the curve fitting results of the U 4f \(^{7/2}\) and U 4f \(^{5/2}\) peaks clearly displayed the non-stoichiometric U(IV) centred at 380.4 ± 0.3 and 391.3 ± 0.3 eV, and the U(VI) located at 382.2 ± 0.3 and 393.1 ± 0.3 eV (Allen et al., 1974, 1981; Riba et al., 2008) for all of the samples. The evidence showed that U(IV) phase was also presented by U 5f line (centred at 1.5 eV) at surfaces of these samples. Hexavalent U with a U 5f \(^0\) electron configuration didn’t produce a U 5f peak, whereas tetravalent U with a U 5f \(^2\) configuration was observed (Neal et al., 2004). As shown in Fig. S7, the presence of U 5f line at the spectra of U(VI)-reacted samples compared with U(VI)-free samples revealed the reduction of U(VI). Therefore, U 4f peaks at 380.4 ± 0.3 and 391.3 ± 0.3 eV were contributed to U(IV) phase. These results revealed that both sorption and reductive precipitation of U(VI) contributed to the removal of aqueous U(VI) by nano-Fe\(^0\) in the absence and presence of \(B.\) subtilis. Consistent with the kinetics data of the reduction, the ratios of U(IV)/U(VI) for the nano-Fe\(^0\) at pH 7.0 were larger than those at pH 3.5, suggesting that the reductive reaction occurred easily at neutral pH (Riba et al., 2008). Previous reports demonstrated that the affinity of the U(VI)-hydroxyl species (i.e., \(\text{UO}_2\text{O}_2\text{OH}^{2+}\), \(\text{UO}_2\text{OH}^{3+}\), \(\text{UO}_2\text{O}_2\text{OH}^{4+}\), and \(\text{UO}_2\text{O}_2\text{OH}^{5+}\) ) to adsorbent at high pH was stronger than that of the UO\(^2+\) species at low pH (Ding et al., 2014; Sun et al., 2014b). Therefore, the U(VI)-hydroxyl species was more rapidly adsorbed on the oxide film of nano-Fe\(^0\) than UO\(^2+\), which contributed to the subsequent reductive reaction. Additionally, the surface complexes \(\text{=[Fe}^{\text{III}}\text{O}^{\text{VI}}\text{U(OH)}\text{O}_2\text{OH]}\) between the U(VI)-hydroxyl species and nano-Fe\(^0\) may be more kinetically efficient for the occurrence of reductive reactions due to the donation of electron density from OH\(^-\) groups (Liger et al., 1999). Liger et al. (1999) determined that the surface complexes of \(\text{=[Fe}^{\text{III}}\text{O}^{\text{III}}\text{Fe}^{\text{III}}\text{OH]}\) were more efficient reductants than \(\text{=[Fe}^{\text{III}}\text{O}^{\text{III}}\text{Fe}^{\text{III}}\text{OH]}\) because the reactivity of the structural Fe(II) was enhanced by the donation of electron density from OH\(^-\) groups. Compared with nano-Fe\(^0\), the higher proportion of U(VI) on nano-Fe\(^0\) + \(B.\) subtilis at pH 3.5 and 7.0 indicated that U(VI) sorption was the main mechanism for the removal of U(VI) by nano-Fe\(^0\) in the presence of \(B.\) subtilis, which was ascribed to the greater number of sorption sites provided by \(B.\) subtilis as discussed above. For the nano-Fe\(^0\) + \(B.\) subtilis at pH 7.0, the amounts of U(VI) greatly decreased compared with nano-Fe\(^0\) at pH 7.0, whereas the decrease of U(IV) was not obvious at pH 3.8, demonstrating the inhibition effect of \(B.\) subtilis on U(VI) reduction by nano-Fe\(^0\) at high pH.

A detailed Fe 2p XPS analysis of the unreacted nano-Fe\(^0\) and U(VI)-treated nano-Fe\(^0\) in the absence or presence of \(B.\) subtilis at pH 7.0 was shown in Fig. 9B. The Fe 2p peaks displayed broad Fe 2p \(^{3/2}\) and Fe 2p \(^{1/2}\) lines located at 710.8 ± 0.2 and 724.4 ± 0.2 eV, respectively, that were assigned to Fe(II)/Fe(III)-bearing oxides, as reported in previous works (Missana et al., 2003; Scott et al., 2005). Concurrently, an Fe 2p \(^{3/2}\) peak of metallic iron (Fe(0)) was observed at 706.9 ± 0.2 (Li and Zhang, 2007). The peak at 719.7 ± 0.2 eV may result from the overlap of oxidised iron (Fe 2p \(^{3/2}\)) and zerovalent iron (Fe 2p \(^{1/2}\)) (Egert and Panzner, 1982). The Fe(0) peak was also examined at the surface of the U(VI)-treated nano-Fe\(^0\) indicated that the nanoparticles can still provide electrons if favourable conditions were supplied. It had been reported that oxide shells can protect or preserve the Fe(0) core, prolonging the performance of nano-Fe\(^0\) for U(VI) aqueous remediation (Li and Zhang, 2007). The binding energy of nano-Fe\(^0\) + \(B.\) subtilis at pH 7.0 (~709.68 eV) was lower than that of

![Fig. 9. (A) Curve fitting of the U 4f XPS peaks for nano-Fe\(^0\) and nano-Fe\(^0\) + \(B.\) subtilis; (B) curve fit of the Fe 2p peaks for the samples at pH = 7.0 and with 150 µmol/L and \(m/V\) = 2 g/L.](image-url)
nano-Fe\textsuperscript{0} at pH 7.0 (~710.88 eV), indicating the complexation of structural Fe(II) or Fe(III) with oxygenated functional groups on \textit{B. subtilis}. Compared with the U(VI)-treated nano-Fe\textsuperscript{0}, a higher proportion of Fe(II) was observed on nano-Fe\textsuperscript{0} + \textit{B. subtilis}, which suggested more Fe(II) on the nano-Fe\textsuperscript{0} complexes with the oxygen-containing functional groups of \textit{B. subtilis}. The Fe(II) complexes inhibit the reduction of U(VI) to U(IV) and the oxidation of Fe(II) to Fe(III). Therefore, the inhibition reduction of U(VI) by \textit{B. subtilis} may be attributed to the complexation between structural Fe(II) or Fe(III) and \textit{B. subtilis}, which blocked the electron transport chain from the Fe\textsuperscript{0} core to U(VI).

### 3.3.4. XANES and EXAFS Analysis

The normalized U L\textsubscript{III}-edge XANES spectra for the samples reacted with the U(VI) solutions (nano-Fe\textsuperscript{0} at pH 5.0 (a) and 7.0 (b), nano-Fe\textsuperscript{0} + \textit{B. subtilis} at pH 7.0 (c), \textit{B. subtilis} at pH 7.0 (d)) and the standard references (i.e., U\textsuperscript{VI}O\textsubscript{2}\textsuperscript{2+} and U\textsuperscript{IV}O\textsubscript{2}) were shown in Fig. 10A. As shown in Fig. 10A, the absorption edge of U\textsuperscript{IV}O\textsubscript{2}(s) (17,176 eV) shifted to a lower energy than that of U\textsuperscript{VI}O\textsubscript{2}\textsuperscript{2+} (17,179 eV), which was consistent with the results reported by O’Loughlin et al. (2010). The edge positions for the nano-Fe\textsuperscript{0} at pH 7.0 and 5.0 were close to the edge position of U\textsuperscript{IV}O\textsubscript{2}(s), suggesting the predominance of U(IV) in both samples. However, the edge position of \textit{B. subtilis} at pH 7.0 (d) was near the edge of U\textsuperscript{VI}O\textsubscript{2}\textsuperscript{2+} due to the low weight percentage of U(IV) on \textit{B. subtilis} (<5%), indicating that U(VI) was dominant. The edge position of nano-Fe\textsuperscript{0} + \textit{B. subtilis} at pH 7.0 was between the edge positions of the nano-Fe\textsuperscript{0} and \textit{B. subtilis}, which indicated that \textit{B. subtilis} significantly inhibited the reduction of U(VI) on nano-Fe\textsuperscript{0} at pH 7.0.

The Fourier transformed (FT, uncorrected phase shift) EXAFS spectra of these samples were shown in Fig. 10B and the corresponding parameters were tabulated in Table 3. The EXAFS spectra of nano-Fe\textsuperscript{0} at pH 5.0 and 7.0 displayed similar features, whereas slight differences in the features of nano-Fe\textsuperscript{0} + \textit{B. subtilis} (pH 7.0) and \textit{B. subtilis} (pH 7.0) were observed. As shown in Fig. 10B, the FT features of U\textsuperscript{VI}O\textsubscript{2}\textsuperscript{2+} at ~1.4 and 1.9 Å can be satisfactorily fit by ~2 axial oxygen atom (O\textsubscript{ax}) at ~1.79 Å and ~6 equatorial oxygen atom (O\textsubscript{eq}) at ~2.30 Å, respectively, which agreed with the distances (1.70–1.81 Å and 2.41–2.43 Å, respectively) reported for other uranyl aqueous species (Allen et al., 1997; Antonio et al., 2001; Sun et al., 2015). The FT features for U\textsuperscript{IV}O\textsubscript{2}(s) can be fit by ~8.0 O at 2.35 Å and ~10.9 U at 3.87 Å, respectively, which was consistent with the structure of UO\textsubscript{2} reported by O’Loughlin et al. (2003). For the nano-Fe\textsuperscript{0} at pH 5.0 and 7.0, the FT feature at ~1.2 Å can be fit by ca. 2 O\textsubscript{ax} at 1.79 Å, whereas the coordination numbers of U–O\textsubscript{eq} were 7.51 and 7.63 for nano-Fe\textsuperscript{0} at pH 5.0 and 7.0, respectively. This evidence revealed the contributions of U(IV) and U(VI) (i.e., 25% and 18% U(VI) with 6 equatorial oxygen atoms, respectively) (O’Loughlin et al., 2010). The predominance of U(IV) species on nano-Fe\textsuperscript{0} can also be evidenced by the presence of backscattering from U neighbours at ~3.7 Å, which indicated the presence of U=O=U interaction and was consistent with the presence of uraninite (Latta et al., 2011, 2012). The presence of the U–U pair correlation in the EXAFS spectra of nano-Fe\textsuperscript{0} (pH 5.0 and 7.0) can be attributed to the presence of UO\textsubscript{2}(S) (Bargar et al., 2013), which agreed with the results of TEM-SAED. The smaller amplitudes of the U–U pair for nano-Fe\textsuperscript{0} (pH 5.0 and 7.0) may be caused by the small and partially disordered clusters.
and the presence of other U(VI) species without a U–U pair and hence diluted signal of uraninite-like species (Stoliker et al., 2013). The FT feature at /C24 3.2 Å for nano-Fe0 (pH 5.0 and 7.0) can be fit by the U–Fe neighbours at 3.44 Å (O’Loughlin et al., 2010), which indicated the formation of inner-sphere U(VI) complexes on the oxide shells of the nano-Fe0 particles. The fitted U–Fe distance for nano-Fe0 was consistent with that (near 3.5 Å) of the (>Fe–O2−)UO2(H2O)n complexes reported by Waite et al. (1994), who indicated that a bidentate complex was formed by polyhedral edge-sharing between the hydrated uranyl ion, (UO2)(H2O)n2+, and a single FeO6 surface site. In addition, a similar distance was observed by Reich et al. (1998). The fitting results of EXAFS support the sorption of U(VI) on the oxide film of nano-Fe0, and the following reduction of U(VI) by structural Fe(II).

For B. subtilis, the fitting CNs of the O ax and O eq shells (/C24 2.04 and /C24 6.05, respectively) were consistent with the structure of the U(VI) standard, indicating the predominant U(VI) species on B. subtilis. The FT feature at /C24 2.2 Å did not dip as much as the U(VI) standard, which corresponded to the contribution of a carbon atom (Dunham-Cheatham et al., 2011). The FT feature for B. subtilis at /C24 2.6 Å was fitted by the C shell at 2.9 Å (Fletcher et al., 2010) because of the poor match of the multiple scattering of O=U=O paths between 2.5 and 3.0 Å (Bargar et al., 1999). Within experimental uncertainty, the EXAFS results for the U–O eq bond length (~2.45 Å) in the B. subtilis sample were comparable to the bond length (~2.6 Å) (Kelly et al., 2002) of the aqueous uranyl acetate standard, in which all of the equatorial oxygen atoms result from the carboxyl groups. The fitted U–O eq bond lengths were also within the range of bond lengths (2.41–2.47 Å) reported for several types of solid U–O eq–C compounds (Allen et al., 1995; Bargar et al., 2000), indicating the formation of inner-sphere U(VI) with the carbon-containing functional groups, especially with the carboxyl groups on B. subtilis. Since the fitting quality obtained when fits were performed using P was similar to that obtained with C, it was impossible to distinguish between C and P coordination on the basis of EXAFS alone (Bargar et al., 2013; Stoliker et al., 2013). Overall, the fitting results suggested U(VI) coordination to C/P-containing groups in B. subtilis. This result was consistent with the findings of Kelly et al. (2002) who demonstrated that U(VI) adsorption to B. subtilis depended on pH. At low pH, the inner-sphere uranyl-phosphoryl complexes were dominant. However, adsorption was ascribed to the inner-sphere complex with two oxygen atoms shared between the uranyl and the carboxyl ligands with increasing pH.

For the nano-Fe0 + B. subtilis spectrum, the fitting results suggested a binding environment with /C24 2.0 axial atoms at /C24 1.79 Å, /C24 6.1 equatorial oxygen atoms at /C24 2.36 Å, /C24 1.1 carbon/phosphorous atoms at 2.88 Å, and approximately 1.23 iron atoms at 3.84 Å (Table 3). As with nano-Fe0 (pH 5.0 and 7.0), the lower amplitude of the U–U pair for nano-Fe0 + B. subtilis suggested that B. subtilis

<table>
<thead>
<tr>
<th>Sample</th>
<th>Shell</th>
<th>CNa</th>
<th>Rd (Å)</th>
<th>σd (Å²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>U(IV)O2 (s)</td>
<td>U–O</td>
<td>8.05 (3)</td>
<td>2.35 (4)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>U–U</td>
<td>10.89 (4)</td>
<td>3.87 (6)</td>
<td>0.009</td>
</tr>
<tr>
<td>U(IV)O22+</td>
<td>U–O ax</td>
<td>1.74 (8)</td>
<td>1.79 (7)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>U–O eq</td>
<td>5.87 (9)</td>
<td>2.25 (5)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>U–U</td>
<td>0.23 (3)</td>
<td>3.85 (3)</td>
<td>0.016</td>
</tr>
<tr>
<td>Nano-Fe0 (pH 5.0)</td>
<td>U–O ax</td>
<td>2.02 (5)</td>
<td>1.77 (4)</td>
<td>0.004</td>
</tr>
<tr>
<td></td>
<td>U–O eq</td>
<td>7.51 (3)</td>
<td>2.30 (3)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>U–Fe</td>
<td>1.50 (9)</td>
<td>3.42 (6)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>U–U</td>
<td>1.70 (5)</td>
<td>3.86 (7)</td>
<td>0.005</td>
</tr>
<tr>
<td>Nano-Fe0 (pH 7.0)</td>
<td>U–O ax</td>
<td>2.04 (5)</td>
<td>1.79 (7)</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>U–O eq</td>
<td>7.63 (3)</td>
<td>2.30 (1)</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>U–Fe</td>
<td>1.84 (7)</td>
<td>3.42 (2)</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td>U–U</td>
<td>2.26 (5)</td>
<td>3.90 (7)</td>
<td>0.007</td>
</tr>
<tr>
<td>Nano-Fe0 + B. subtilis (pH 7.0)</td>
<td>U–O ax</td>
<td>2.01 (2)</td>
<td>1.79 (2)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td>U–O eq</td>
<td>6.12 (3)</td>
<td>2.36 (4)</td>
<td>0.040</td>
</tr>
<tr>
<td></td>
<td>U–C/P</td>
<td>1.10 (5)</td>
<td>2.88 (6)</td>
<td>0.045</td>
</tr>
<tr>
<td></td>
<td>U–Fe</td>
<td>1.23 (8)</td>
<td>3.48 (9)</td>
<td>0.052</td>
</tr>
<tr>
<td></td>
<td>U–U</td>
<td>0.05 (5)</td>
<td>3.84 (6)</td>
<td>0.026</td>
</tr>
<tr>
<td>B. subtilis (pH 7.0)</td>
<td>U–O ax</td>
<td>2.04 (2)</td>
<td>1.90 (9)</td>
<td>0.025</td>
</tr>
<tr>
<td></td>
<td>U–O eq</td>
<td>6.05 (7)</td>
<td>2.12 (2)</td>
<td>0.009</td>
</tr>
<tr>
<td></td>
<td>U–C/P</td>
<td>1.35 (4)</td>
<td>2.90 (7)</td>
<td>0.022</td>
</tr>
</tbody>
</table>

a CN, coordination numbers of neighbours.
b Rd, the bond distance.
c σd, the Debye-Waller factor.
inhibited the reduction of U(VI) by nano-Fe\(^0\). As discussed above, the functional groups on \textit{B. subtilis} blocked the active sites in the outer layer of nano-Fe\(^0\), hence hindering the regeneration of structural Fe(II). In addition, U(VI)-phosphate complexes formed on \textit{B. subtilis} may be not favourable for the reduction of U(VI) by nano-Fe\(^0\). The slow bioreduction of phosphate-precipitated U(VI) was observed by Rui et al. (2013). The EXAFS results

![Figure 11](image)

**Table 4**
The sorption \((k_1)\), desorption \((k_{-1})\) and reduction rate constants \((k_2)\) calculated from the kinetics model fitting for nano-Fe\(^0\) and nano-Fe\(^0\) + \textit{B. subtilis} at pH 7.0 and the variable Na\(_2\)CO\(_3\) concentration.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Na(_2)CO(_3) (mM)</th>
<th>(k_1) ((h^{-1}))</th>
<th>(k_{-1}) ((h^{-1}))</th>
<th>(k_2) ((h^{-1}))</th>
<th>(^{a})SSE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano-Fe(^0)</td>
<td>1.0</td>
<td>2.650 (3)</td>
<td>0.900 (5)</td>
<td>0.800 (2)</td>
<td>0.055</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.071 (2)</td>
<td>0.280 (2)</td>
<td>0.212 (2)</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0.051 (5)</td>
<td>1.498 (3)</td>
<td>0.202 (6)</td>
<td>0.041</td>
</tr>
<tr>
<td>Nano-Fe(^0) + \textit{B. subtilis}</td>
<td>1.0</td>
<td>3.500 (6)</td>
<td>0.400 (7)</td>
<td>0.080 (7)</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.950 (3)</td>
<td>0.200 (5)</td>
<td>0.018 (4)</td>
<td>0.051</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>0.850 (3)</td>
<td>0.380 (4)</td>
<td>0.012 (5)</td>
<td>0.098</td>
</tr>
</tbody>
</table>

\(^{a}\) SSE, sum of squares error.
demonstrated that \textit{B. subtilis} enhances the sorption of U(VI) due to its variety of functional groups while inhibiting the reduction of U(VI) by nano-Fe$^{0}$. 

3.4. Modelling the reduction of U(VI) by nano-Fe$^{0}$ and nano-Fe$^{0}+\textit{B. subtilis}$

According to the kinetics results and the product analysis described above, a reaction mechanism was proposed in which the U(VI) was first adsorbed on the oxide of shell of nano-Fe$^{0}$ and then subsequently reduced by structural Fe(II). The sorption of U(VI) to the nano-Fe$^{0}$oxide film favoured subsequent reduction by the structural Fe(II) (Liger et al., 1999; Riba et al., 2008). Therefore, a kinetics model was used to simulate the removal of U(VI) on nano-Fe$^{0}$ according to the method reported by Yan et al. (2010). In addition, it was hypothesised that the removal of U(VI) by nano-Fe$^{0}$ undergoes a two-step reaction process, which was conceptualised by Laidler (1996) for surface reactions:

(i) sorption reaction : \[ \text{SOH} + \text{U}^{\text{VI}}\text{O}_{2}^{2+} \times k_1 \Rightarrow \text{SOH} \text{U}^{\text{VI}}\text{O}_{2}^{2+} \] (5)

where \( \text{SOH} \) designated the species associated with the surfaces of nano-Fe$^{0}$ and nano-Fe$^{0}+\textit{B. subtilis}$. The forward and backward (sorption and desorption) rate constants are indicated by \( k_1 \) and \( k_{-1} \), respectively.

(ii) reduction reaction : \[ \text{SOH} \text{U}^{\text{VI}}\text{O}_{2}^{2+} \times k_2 \Rightarrow \text{SU}^{\text{IV}}\text{O}_2(s) + \text{iron (hydr)oxides} \] (6)

Here, \( k_2 \) is the reduction rate constant.

The surface concentration of \( \text{SOH} \) was considered as a constant in the model due to the large specific surface area of nano-Fe$^{0}$ (Yan et al., 2010). Then, the time evolution of aqueous U(VI) depletion, U(VI) sorption and U(IV) production in the system can be described as following by Eqs. (7–9):

\[
\frac{d[U^{\text{VI}}\text{O}_{2}^{2+}]}{dt} = -k_1 [U^{\text{VI}}\text{O}_{2}^{2+}] + k_{-1} \text{SOH} U^{\text{VI}}\text{O}_{2}^{2+}
\] (7)

\[
\frac{d[\text{SOH} U^{\text{VI}}\text{O}_{2}^{2+}]}{dt} = k_1 [U^{\text{VI}}\text{O}_{2}^{2+}] - (k_{-1} + k_2) \text{SOH} U^{\text{VI}}\text{O}_{2}^{2+}
\] (8)

\[
\frac{d[U^{\text{IV}}]}{dt} = k_2 [\text{SOH} U^{\text{VI}}\text{O}_{2}^{2+}]
\] (9)

The kinetics experimental data for nano-Fe$^{0}$ and nano-Fe$^{0}+\textit{B. subtilis}$ at pH 7.0 were simulated by these kinetics equations using the MATLAB R 2009a software. The experimental data were simulated by optimizing \( k_1 \), \( k_{-1} \) and \( k_2 \) values constrained from Eqs. (7–9). The fitting results were shown in Fig. 11 and the values of \( k_1 \), \( k_{-1} \) and \( k_2 \) obtained from the curve fitting results were shown in Table 4. As summarised in Table 4, the \( k_1 \) values for nano-Fe$^{0}$ (2.65) and nano-Fe$^{0}+\textit{B. subtilis}$ (3.50) were much larger than the \( k_{-1} \) values for nano-Fe$^{0}$ (0.90) and nano-Fe$^{0}+\textit{B. subtilis}$ (0.40) at 1.0 mmol/L Na$_2$CO$_3$, which suggested that the removal of U(VI) on nano-Fe$^{0}$ and nano-Fe$^{0}+\textit{B. subtilis}$ was favourable for the sorption process. Furthermore, the \( k_1 \) values of nano-Fe$^{0}$ (2.65) and nano-Fe$^{0}+\textit{B. subtilis}$ (3.50) were larger than the values of \( k_2 \) nano-Fe$^{0}$ (0.80) and nano-Fe$^{0}+\textit{B. subtilis}$ (0.08), indicating that the reduction of U(VI) on nano-Fe$^{0}$ and nano-Fe$^{0}+\textit{B. subtilis}$ was the rate-controlling step for U(VI) removal. The \( k_1 \) value of nano-Fe$^{0}+\textit{B. subtilis}$ (3.50) was larger than that of nano-Fe$^{0}$ (2.65), whereas the \( k_2 \) value of nano-Fe$^{0}+\textit{B. subtilis}$ (0.08) was smaller.

![Fig. 12. (A) Schematic of the reaction between nano-Fe$^{0}$, U(VI) and \textit{B. subtilis}; (B) tetravalent uranium species; (C) bidentate binding of uranyl to a carboxyl group on \textit{B. subtilis}; (D) bidentate binding of uranyl to the surface of nano-Fe$^{0}$ (FeO$_4$).](image-url)
than that of nano-Fe\(^0\) (0.80), which suggested that \(B.\ subtilis\) enhanced U(VI) sorption but inhibited the reduction of U(VI) by nano-Fe\(^0\). As the carbonate concentration increased, the \(k_1\) and \(k_2\) values decreased for the nano-Fe\(^0\) and nano-Fe\(^0\)+\(B.\ subtilis\) and the \(k_v\) value increased. The results indicated that carbonate inhibited the sorption and reduction of U(VI) but increased the desorption of U(VI) from nano-Fe\(^0\) and nano-Fe\(^0\)+\(B.\ subtilis\). This result was potentially ascribed to the formation of U(VI)-carbonate species, as indicated by Fig. 3. The negatively charged U(VI)-carbonate species decreased the electrostatic repulsion, which in turn inhibited U(VI) reduction. The rate-limiting step of U(VI) removal on nano-Fe\(^0\) shifted from reduction to sorption as the carbonate concentration increases. However, this transformation was not observed for the nano-Fe\(^0\)+\(B.\ subtilis\). For example, at 10.0 mmol/L carbonate, the \(k_1\), \(k_\perp\) and \(k_2\) values of the nano-Fe\(^0\) were 0.051, 1.498 and 0.202, respectively, while the \(k_1\), \(k_\perp\) and \(k_2\) values of the nano-Fe\(^0\)+\(B.\ subtilis\) were 0.850, 0.380 and 0.012, respectively. The reduction reaction was the rate-determining step for nano-Fe\(^0\)+\(B.\ subtilis\) over a range of carbonate concentrations (1.0–10.0 mmol/L), which further indicated the inhibition effect of \(B.\ subtilis\) for U(VI) reduction by nano-Fe\(^0\).

The schematics of the reaction mechanisms from the combined macroscopic experimental, spectroscopic and model fitting data were depicted in Fig. 12. It was assumed that U(VI) was preferentially adsorbed to the nano-Fe\(^0\) oxide shell before the adsorbed U(VI) was directly reduced by the structural Fe(II) to produce Fe(III). Subsequently, the Fe(III) was reduced by an electron from the Fe(0) core. The structural Fe(II) on the oxide shell of the nano-Fe\(^0\) serves as an effective electron shuttle from the core of Fe(0) to the adsorbed U(VI). The presence of \(B.\ subtilis\) was coordinated with the structural Fe(II) and Fe(III), which decreased the reductive capacity of the nano-Fe\(^0\). Therefore, U(VI) was predominantly present as monomeric U(IV) and/or as bidentate U(VI)-Fe surface complexes on nano-Fe\(^0\), whereas bidentate U(VI)-C/P and U(VI)-Fe surface complexes were the major species in the presence of \(B.\ subtilis\).

4. CONCLUSIONS

In conclusion, the effect of \(B.\ subtilis\) on the removal of U(VI) by nano-Fe\(^0\) was investigated in batch techniques. Overall, \(B.\ subtilis\) can significantly enhance the sorption rates of U(VI) and inhibit the reduction rates of U(VI) by nano-Fe\(^0\), especially at high pH. The observed inhibition effect could result from the complexation of structural Fe(II) or Fe(III) with the oxygen-containing functional groups (e.g., carboxyl and phosphate groups) of both \(B.\ subtilis\) and EPS, which would inhibit electron transport from Fe(0) to the Fe(II)/Fe(III) oxide shell. The combined macroscopic, spectroscopic and model fitting data revealed that the reduction of U(VI) by nano-Fe\(^0\) was triggered by the sorption of U(VI) on the oxide shell of nano-Fe\(^0\). For nano-Fe\(^0\)+\(B.\ subtilis\), the rate-determining step was predominant for U(VI) removal at 1.0–10.0 mmol/L Na\(_2\)CO\(_3\). However, the rate-determining step shifts from a reduction reaction (at 1.0 mmol/L Na\(_2\)CO\(_3\)) to adsorption reaction (at 5.0 and 10.0 mmol/L Na\(_2\)CO\(_3\)) for nano-Fe\(^0\). Therefore, the removal of U(VI) on nano-Fe\(^0\) was greatly influenced by \(B.\ subtilis\) over short-term periods. The findings in this study may be generally applied to other bacteria and these EPS with massive carboxyl and phosphoryl groups. Accordingly, the reduction of U(VI) on nano-Fe\(^0\) was retarded due to the coordination of these organisms with oxide film of nano-Fe\(^0\). The interaction mechanism obtained by spectroscopic techniques at molecular-scale can provide significant information to estimate the fate and transportation of U(VI) in aqueous environments in ternary system such as water-Gram-positive bacteria – nano-Fe\(^0\) interface. Further investigations regarding the roles of roles of other microorganisms, especially metal reducing bacteria, biofouling by EPS and environmentally relevant organic matters on the reduction of U(VI) by nano-Fe\(^0\) should be taken into account to evaluate the fate and transportation of U(VI) in more complex environments.

ACKNOWLEDGMENTS

Financial support from National Natural Science Foundation of China (21207135, 91126020; 21225730; 91326202; 41273134), 973 project from Ministry of Science and Technology of China (2011CB933700), Anhui Provincial Natural Science Foundation (1408085MB28), the Jiangsu Provincial Key Laboratory of Radiation Medicine and Protection, the Priority Academic Program Development of Jiangsu Higher Education Institutions, Hefei Center for Physical Science and Technology (2012FXZYY05) and MCTL Visiting Fellowship Program from Key Laboratory of Marine Chemistry Theory and Technology (Ocean University of China), Ministry of Education are acknowledged.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.gca.2015.05.036.

REFERENCES


Associate editor: Annie B Kersting