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Zhang, C. and Francis, A. J.

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Brookhaven National Laboratory

P.O. Box 5000
Upton, NY 11973-5000
www.bnl.gov

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Interactions of Ionic Liquids with Uranium and its Bioreduction

Chengdong Zhang^{1,3}, Arokiasamy J Francis^{1,2}

¹Environmental Sciences Department, Brookhaven National Laboratory, Upton, NY, USA; ²Division of Advanced Nuclear Engineering, Pohang University of Science and Technology, Pohang, Korea

³College of Environmental Science and Engineering, Nankai University, Tianjin, China

Abstract. We investigated the influence of ionic liquids (ILs) 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM]⁺[PF₆]⁻, N-ethylpyridinium trifluoroacetate [EtPy]⁺[CF₃COO]⁻ and N-ethylpyridinium tetrafluoroborate [EtPy]⁺[BF₄]⁻ on uranium reduction by *Clostridium* sp. under anaerobic conditions. Potentiometric titration, UV-vis spectrophotometry, LC-MS and EXAFS analyses showed monodentate complexation between uranyl and BF₄⁻ PF₆⁻; and bidentate complexation with CF₃COO⁻. Ionic liquids affected the growth of *Clostridium* sp. as evidenced by decrease in optical density, changes in pH, gas production, and the extent of U(VI) reduction and precipitation of U(IV) from solution. Reduction of U(VI) to U(IV) was observed in the presence of [EtPy][BF₄] and [BMIM][PF₆] but not with [EtPy][CF₃COO].

Introduction

Ionic liquids (ILs) are ion-pair compounds containing an organic cation and an organic or inorganic anion. They are versatile since their chemical properties such as hydrophobicity, melting point and viscosity can be adjusted to obtain the desired reaction. They are used as green solvents in organic synthesis and in materials chemistry (Anastas and Warner, 1998). Visser et al. (2003) determined the molecular association of UO₂²⁺ with hydrophobic ILs [C₄mim][PF₆] and [C₈mim][N(SO₂CF₃)₂] in the presence of CMPO and TBP using EXAFS. The uranium formed a UO₂(NO₃)⁻ (CMPO) complex in the IL phase while IL-associated anion complexation with uranium was not observed. Gaillard et al. (2005) used time-resolved emission spectroscopy and EXAFS analysis to determine the complexation ability of fluorinated anions with uranyl ion in aqueous solution at room temperature. Both [BF₄]⁻ and [PF₆]⁻ formed inner-sphere monodentate complexes with uranyl ion while [Tf₂N]⁻ did not complex with uranium.

Microbial reduction of uranium is of considerable interest because of its potential application in the bioremediation of contaminated sites and in the pretreatment of uranium containing wastes. In this study, we investigated the interaction of uranium (VI) with N-ethylpyridinium tetrafluoroborate [EtPy][BF₄], 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM][PF₆] and N-ethylpyridinium trifluoroacetate [EtPy][CF₃COO], (Fig. 1) in aqueous solution and examined their effects on uranium reduction by anaerobic bacteria.

Fig 1. Molecular structures of ILs N-ethylpyridinium tetrafluoroborate [EtPy][BF₄], 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM][PF₆] and N-ethylpyridinium trifluoroacetate [EtPy][CF₃COO].

Characterization of Uranium associated with ILs.

Potentiometric titration. Potentiometric titration of uranyl nitrate showed the inflection point at 2 mM OH⁻/mM U at pH 6.4 (Fig. 2). The release of 2 protons is due to the association of uranium ion with 2 OH⁻ in water. The titration curves for the U:[BMIM][PF₆] and the U:[EtPy][BF₄] with uranyl nitrate alone indicated no complex formation between the metal and the ILs. Addition of one-fold excess IL had no effect on the complexation. However, titration of the U in the presence of [EtPy][CF₃COO] showed a release of one more proton into the medium as indicated by the inflection point at 3 mM OH⁻/mM U. This phenomenon shows that there is an interaction of the uranium with the [EtPy][CF₃COO]. The interaction increases with addition of one-fold excess [EtPy][CF₃COO] (more acid released).

Fig 2. Potentiometric titration curve of U and ILs mixture: (A) [BMIM][PF₆] (B) [EtPy][BF₄]; (C) [EtPy][CF₃COO].

UV-vis absorption spectroscopy. The UV-vis absorption spectrum for the pH unadjusted uranyl nitrate in the range of 300 nm to 500 nm showed fine structure in the 380 nm to 440 nm range characteristic of the presence of uranyl ion species. The maximum absorbance was at 413 nm and two shoulder peaks were noted at 402 nm and 425 nm. The UV-vis absorbance spectra for the 1:1 U:[EtPy][BF₄] and 1:2 U:[EtPy][BF₄] mixtures showed the absorption maxima for both spectra are higher by 25 to 30% compared to uranyl nitrate alone and this enhanced absorption indicates there is interaction of the IL with uranium. However, the 1:1 and 1:2 of U:[BMIM][PF₆] mixtures showed absorbance maxima similar to the uranyl nitrate. The [BMIM][PF₆] compound is the least soluble of the three ILs in this study and therefore it was difficult to determine the presence of a U-IL complex using this method. In the presence of uranyl ion and [EtPy][CF₃COO] there was a shift in absorbance maximum to 408 nm from 402 and to 419 nm from 425 nm compared to uranyl nitrate alone. The observed shift to lower energy is most probably due to the electron withdrawing ability of the fluoride ions, confirming the complexation of uranyl ion with the IL.

Fig 3. UV-vis spectrophotometry of uranium nitrate and U-IL mixtures: uranium-nitrate (A); [EtPy][BF₄] (B); [BMIM][PF₆] (C); [EtPy][CF₃COO] (D).

Electrospray Ionization Mass Spectrometry (ESI-MS) analysis. In figure 4 uranium nitrate in water showed peaks at 270 (m/z), 287 (m/z) and 305 (m/z) due to the uranium ion and its association with hydroxyl ions in water. In the MS of 1:1 U: [EtPy][BF₄] (Fig 5), 108(m/z) is the molecular ion peak of N-ethyl pyridi-

mium cation. The small peak at 418(m/z) may come from the anion exchange between uranium nitrate and [EtPy][BF₄].

Figure 4. Mass spectroscopy of uranium nitrate in water under positive mode.

Fig 5. Mass spectroscopy of 1:1 U: [EtPy][BF₄] mixture under positive mode.

In Figure 6, the ESI-MS spectrum under negative mode for uranyl nitrate shows peaks at 365 (m/z) [UO₂(NO₃)(OH)₂]⁻ and 455 (m/z) [UO₂(NO₃)₃]⁻. They are due to uranyl ion complexation with nitrate and hydroxyl ions. Figure 7 shows the ESI-MS spectrum under negative mode for U:[EtPy][CF₃COO]. In addition to the peaks observed in Figure 6 for uranyl nitrate, there is a molecular ion peak at 413 (m/z). We believe the appearance of this peak is due to formation of [UO₂(NO₃)(OH)(H₂O)(COO)]⁻ species with loss of the CF₃ moiety.

Fig 6 Electrospray ionization (ESI-MS) spectra under negative mode showing the molecular ions produced by uranyl nitrate solution.

Fig 7. Electrospray ionization (ESI-MS) spectra under negative mode showing the molecular ions produced by U:[EtPy][CF₃COO] solution.

X-ray Absorption Spectroscopy. X-ray Absorption Near-edge Spectroscopy (XANES) analysis of the absorption edge energies for U(VI)-nitrate and mixtures of U(VI)-nitrate and various ILs revealed that they have the same absorption edge energy (17175 eV). This confirms that the U(VI) added to the IL's solution was present as U(VI) species and not reduced to U(IV). Extended X-ray absorption fine structure (EXAFS) spectroscopy analysis of the U ILs samples showed the following complexes formed between uranyl and [EtPy][BF₄], [EtPy][CF₃COO], and [BMIM][PF₆]: monodentate complex between uranyl and BF₄⁻, and PF₆⁻, bi-

dentate complex between uranyl and CF_3COO . Based on the ESI-MS and EXAFS results we propose the following structure (Fig 8).

Fig 8. Proposed structures of U-Ionic Liquid complexes.

Effect of ILs on growth of *Clostridium* sp. Addition of ILs to bacterial growth medium affected the growth of *Clostridium* sp. as evidenced by decrease in optical density (Fig. 9).

Fig. 9. Effects of IL on the optical density of *Clostridium* sp.

The optical density decreased with increase in concentration of $[\text{BMIM}][\text{PF}_6]$. Similar effects were observed with $[\text{EtPy}][\text{BF}_4]$ and $[\text{EtPy}][\text{CF}_3\text{COO}]$ (Fig. 9B and C). In comparison with $[\text{EtPy}][\text{BF}_4]$, the two ILs, $[\text{BMIM}][\text{PF}_6]$ and $[\text{EtPy}][\text{CF}_3\text{COO}]$, showed higher inhibition on the growth of *Clostridium* sp. Addition of 0.1% of $[\text{BMIM}][\text{PF}_6]$ or $[\text{EtPy}][\text{CF}_3\text{COO}]$, the optical density decreased to 0.3 and 0.25 respectively. As the concentration of these two ionic liquids increased $> 0.5\%$ (V/V), no growth of bacteria was observed.

After 48 hours incubation the pH of the medium changed from 5.5 to 3 due to organic acids produced from glucose metabolism. However, in presence of ILs, the growth was inhibited, and less glucose was consumed, thus less acids produced and the pH changed slowly. The drop in pH with the high concentrations of ILs may be due to the hydrolysis of ILs. Commensuration with growth, the amount of total gas, CO_2 , H_2 production was less in ILs containing samples. We found that the growth of *Clostridium* sp and *Pseudomonas fluorescens* was affected by one more of these ILs (Wang et al. 2011; Zhang et al 2011; Nancharaih and Francis, 2011).

Effects of Ionic Liquids on Reduction of U(VI) by *Clostridium* sp. Addition of uranyl nitrate to growing culture of *Clostridium* sp. resulted in the rapid reduction of U(VI) to U(IV) which precipitated from solution (Fig 10A). In the presence of [EtPy][BF₄] reduction of U(VI) proceeded similar to uranyl nitrate but the concentration of reduced(IV) in solution was 3-fold higher and it precipitated much more slowly (Fig 10B). In presence of [BMIM][PF₆] U(VI) reduction followed the same pattern as uranyl nitrate (Fig.10C). Uranium which formed a bidentate complex with EtPy[CF₃COO] was not reduced (Fig 10D).

Fig. 10. Reduction of U(VI) by *Clostridium* sp in presence of various ILs: (A) uranyl nitrate; (B) [EtPy][BF₄]; (C) [BMIMPF₆]; (D) [EtPy][CF₃COO].

Analysis of Uranium (VI) and U(IV) by UV-vis Spectroscopy. Uranium in the culture sample was extracted with citric acid under anaerobic conditions and the absorption spectra of U(VI) and U(IV) were determined using a Hewlett Pack-

ard 8453 diode array scanning UV-vis spectrophotometer. Before bacterial reduction, the maximum absorbance was at 435nm which is the characteristic absorbance of U(VI). After bioreduction, the absorbance shifted to 555 and 665nm, which indicated the presence of U(IV) (Fig.11). XANES analysis showed energy shift from 17175 eV to 17169 eV indicating the reduction of U(VI) to U(IV).

Fig. 11. UV-vis spectroscopy of precipitates extracted by citric acid (A) before bioreduction; (B) after bioreduction.

These results show that the ILs affected the growth of *Clostridium* sp. as evidenced by decrease in optical density, changes in the pH of the medium, total gas production and rate and extent of reductive precipitation of uranium.

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