Biodegradation of PuEDTA and Impacts on Pu Mobility

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Summary

The contamination of many DOE sites by Pu presents a long-term problem because of its long half-life (240,000 yrs) and the low drinking water standard ($<10^{-12}$ M). EDTA was co-disposed with radionuclides (e.g., Pu, ^{60}Co), formed strong complexes, and enhanced radionuclide transport at several DOE sites. Biodegradation of EDTA should decrease Pu mobility. One objective of this project was to determine the biodegradation of EDTA in the presence of PuEDTA complexes. The aqueous system investigated at pH 7 (10^{-4} M EDTA and 10^{-6} M Pu) contained predominantly $Pu(OH)_2EDTA^{2-}$. The EDTA was degraded at a faster rate in the presence of Pu. As the total concentration of both EDTA and PuEDTA decreased (i.e., 10^{-5} M EDTA and 10^{-7} M PuEDTA), the presence of Pu decreased the biodegradation rate of the EDTA. It is currently unclear why the concentration of Pu directly affects the increase/decrease in rate of EDTA biodegradation. The soluble Pu concentration decreased, in agreement with thermodynamic predictions, as the EDTA was biodegraded, indicating that biodegradation of EDTA will decrease Pu mobility when the Pu is initially present as Pu(IV)EDTA. A second objective was to investigate how the presence of competing metals, commonly encountered in geologic media, will influence the speciation and biodegradation of Pu(IV)-EDTA. Studies on the solubilities of $Fe(OH)_3(s)$ and of $Fe(OH)_2(s)$ plus $PuO_2(am)$ in the presence of EDTA and as a function of pH showed that Fe(III) out competes the Pu(IV) for the EDTA complex, thereby showing that Pu(IV) will not form stable complexes with EDTA for enhanced transport of Pu in Fe(III) dominated subsurface systems. A third objective is to investigate the genes and enzymes involved in EDTA biodegradation. BNC1 can use EDTA and another synthetic chelating agent nitrilotriacetate (NTA) as sole carbon and nitrogen sources. The same catabolic enzymes are responsible for both EDTA and NTA degradation except that additional enzymes are required for EDTA degradation. When the catabolic genes were cloned and sequenced, the gene cluster also contained genes encoding a hypothetical ABC-type transporter. RT-PCR analysis showed that the transporter genes and EDTA monooxygenase gene (emoA) are co-transcribed. EppA is one of the transporter genes, and it codes for a periplasmic binding protein responsible for binding to the substrate before transport across the membrane can occur. EppA was cloned, expressed, and purified in *Escherichia coli* and found to bind, MgEDTA, CaEDTA, Fe(III)EDTA, MgNTA, CaNTA, and Fe(III)NTA. Our data also suggest that BNC1 uses the same ABC-type transporter for both EDTA and NTA uptake. Results from these studies can provide mechanistic understanding and approaches to assist in the bioremediate PuEDTA and other radionuclide-EDTA complexes at DOE sites .

Introduction

EDTA (Figure 1) can form strong water-soluble complexes with radionuclides and metals and has been used to decontaminate and process nuclear materials. EDTA was co-disposed with radionuclides (e.g., ^{60}Co, Pu) and has enhanced their transport in the subsurface (Bolton et al. 2000, Cleveland and Rees 1981, Means et al. 1978, Riley and Zachara 1992). An understanding of EDTA biodegradation is essential to help mitigate enhanced radionuclide transport by EDTA. Three research areas are discussed in this poster. First, the biodegradation of EDTA in the presence of Pu. Second, the speciation of Pu(IV)-EDTA as a function of pH (Rai et al. 2001) and the effects of Fe(III) and Ca on Pu(IV)-EDTA speciation (Rai et al. 2004). Third, the genetics of EDTA biodegradation by BNC1 and the periplasmic binding protein, which may be responsible for EDTA transport into the cell (Bohuslavek et al. 2001, Liu et al. 2001). Our first hypothesis was that EDTA would be degraded in the presence of Pu and that the biodegradation of EDTA would result in the formation of insoluble Pu(IV) species. Our second hypothesis was that the Fe(III) would be able to out compete the Pu(IV) for the EDTA complex. Our third hypothesis was that the periplasmic binding protein and ABC-type transported cotranscribed with the genes for EDTA degradation codes for EDTA transport into the cell.

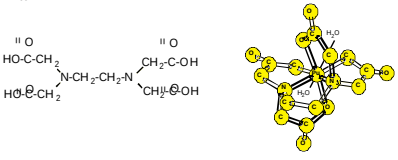

Figure 1. Structure of EDTA and the PuEDTA complex.

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Research Area 1

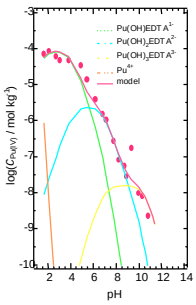

Figure 2. Pu(IV)EDTA species present as a function of pH in equilibrium with PuO_2(am) and 0.1 mM EDTA. The $Pu(OH)_xEDTA$ (x =z 1, 2, or 3) species are the dominant Pu(IV)EDTA species from pH 2 to 11. Based on the solubility of PuO_2 as a function of pH and EDTA concentrations the following thermodynamic data were developed to reliably predict Pu(IV)EDTA species.

$Pu^{4+} + OH^- + EDTA^{4-} = PuOHEDTA^-$ (log K=36.39)

$Pu^{4+} + 2OH^- + EDTA^{4-} = Pu(OH)_2EDTA^{2-}$ (log K=44.74)

$Pu^{4+} + 3OH^- + EDTA^{4-} = Pu(OH)_3EDTA^{3-}$ (log K=49.64)

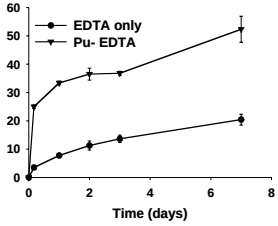

Figure 3. Mineralization of 100 micromolar EDTA by BNC1 with and without Pu(IV) at pH 7. The Pu(IV)EDTA solution was equilibrated as discussed in Figure 6. The same concentration of cells was added to both treatments. The EDTA was degraded by BNC1 in the presence of Pu more rapidly than when Pu was not present. Thus the presence of low concentrations of PuEDTA did not inhibit EDTA deoradation.

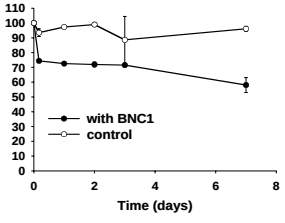

Figure 4. Aqueous Pu(IV) concentrations at various stages of EDTA mineralization, which are shown in Figure 7. A control was included without BNC1 added. The soluble concentrations of Pu decreased significantly when the EDTA was biodegraded.

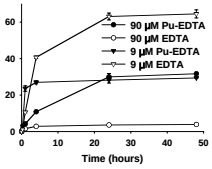

Figure 5. Mineralization of 90 and 9 micromolar EDTA by BNC1 with and without Pu(IV) at pH 7. The Pu(IV)EDTA solution was equilibrated as discussed in Figure 6. The same concentration of cells was added to both treatments. The present of high concentrations of PuEDTA (90 _M total EDTA) enhanced EDTA degradation, but this enhancement was not present as total concentration of EDTA decreased to 9 _M total EDTA.

Research Area 2

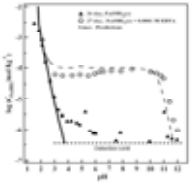

Figure 6. Solubility of $Fe(OH)_3(s)$ (2-line ferrihydrite) in the absence and presence of 0.0001 M EDTA. Lines represent predicted concentrations (Rai et al. 2004). The data provides validated thermodynamic constants for use in mixed systems and shows that EDTA binds strongly to Fe(III) and thus increases the solubility of $Fe(OH)_3(s)$ and the aqueous activity of Fe(III) such that Fe(III) would be expected to compete strongly with Pu(IV) for complexation with EDTA in a large range of pH values.

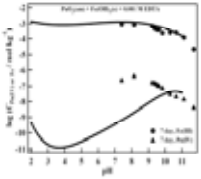

Figure 7. The solubility of PuO_2(am) plus $Fe(OH)_3(s)$ (2-line ferrihydrite) in the presence of 0.001 M EDTA and as a function of pH (Rai et al. 2004). Solid lines represent predicted concentrations. The data shows that Fe(III) is strong competitor for Pu(IV) and that in the environmental range of interest of pH values >99.9% of the added EDTA is complexed with Fe(III).

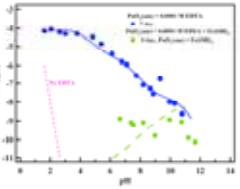

Figure 8. The solubility of PuO_2(am) in the presence of 10^{-4} M EDTA and $Fe(OH)_2$ decreased up to three orders of magnitude compared with the solubility of PuO_2(am) in the presence of 10^{-4} M EDTA. Thus the Fe(III) was able to out-compete the Pu(IV) for the EDTA. Thus Pu(IV)EDTA complexes are not stable in the presence of Fe(III). Contrary to implications in the literature, EDTA is NOT expected to significantly mobilize Pu(IV) in Fe(III) containing aerobic subsurface systems.

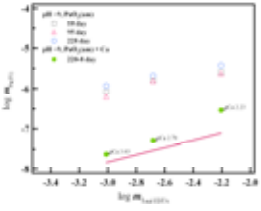

Figure 9. The observed solubility of PuO_2(am) in the presence of different concentrations of Ca^{2+} and a s a function of EDTA concentrations was lower than in the absence of Ca^{2+}, but was similar to that predicted from the available thermodynamic data indicating that the available data are reliable. Thus Ca2+ can also out-compete the Pu(IV) for the EDTA. Implications are that aerobic Ca2+ rich groundwaters should not have significant mobilization of Pu(IV) by EDTA.

Research Area 3

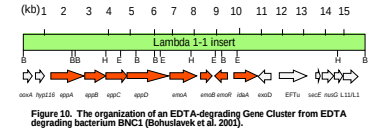

Figure 10. The organization of an EDTA-degrading Gene Cluster from EDTA degrading bacterium BNC1 (Bohuslavek et al. 2001).

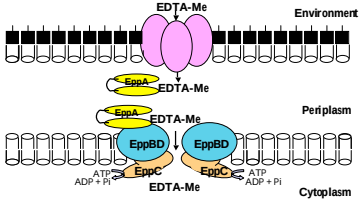

Fig. 11. EppABCD code for Encoding an EDTA-uptake ABC-type transporter, of which EppA is a periplasmic binding protein (Herman et al. 2002). EppA binds only CaEDTA and MgEDTA but not CuEDTA for cellular uptake, explaining why most stable metal-EDTA complexes are not degraded by BNC1.

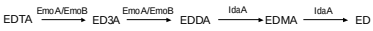

Fig. 12. EDTA degrading pathway. EDTA monooxygenase (encoded by *emoA*), an FMNH2-utilizing monooxygenase, and NADH:FMN oxidoreductase (*emoB*) working together to catalyze sequential oxidation of EDTA to EDA (ethylenediaminetriacetate) and then to EDDA (ethylenediaminediacetate). EDDA oxidase (*idaA*) oxidizes EDDA to EDMA (ethylenediaminemonoacetate) and finally to ED (ethylenediamine) (Liu et al. 2001). ED is structurally similar to putrescine and can be degraded by *Escherichia coli*.

Why is EDTA recalcitrant in the environment?

EDTA is a simple organic compound and should be easily degraded by microorganisms, but it is resistant to biodegradation. After we have characterized the EDTA uptake and its metabolic pathway, it becomes clear that the recalcitrance is due to lack of cellular uptake for stable metal-EDTA complexes. Only CaEDTA and MgEDTA are transported into the cells by the transport system. Stable metal-EDTA complexes, e.g. CuEDTA, are not transported by the BNC1 uptake system. It is interesting that metals in organic complexes affect their degradation by microorganisms. Stable metal-EDTA only slowly change ligands with Ca^{2+} or Mg^{2+} with the thermodynamic equilibrium favoring to the stable metal-EDTA complexes. Thus, stable metal-EDTA complexes are either degraded directly by other microorganisms (unknown to date) or form small amount CaEDTA or MgEDTA, which are degraded by BNC1 and related microorganisms.

Conclusions and Implications

Research Area 1

- Pu(IV) forms strong Pu(OH)xEDTA complexes with thermodynamic data developed to model speciation and PuEDTA biodegradation. Pu(IV) solubility and mobility increased with decreasing pH and increasing EDTA concentration.
- The concentration of Pu influenced the rates of EDTA degradation.
- The soluble concentrations of Pu decreased significantly when the EDTA was biodegraded. Mobile PuEDTA concentrations will decrease as the EDTA is degraded, resulting in the formation of insoluble Pu(IV) species

Research Area 2

- Fe(III) and Ca2+ strongly compete with the Pu(IV) for the EDTA.
- Pu(IV)EDTA complexes are not stable in the presence of Fe(III). EDTA is NOT expected to significantly mobilize Pu(IV) under aerobic conditions.
- If EDTA is responsible for mobilizing Pu, it is more likely as Pu(III)EDTA under anaerobic conditions.

Research Area 3

- EDTA degrading genes and transporter genes are organized in a operon.
- The periplasmic binding protein, EppA, selectively binds several metal-EDTA and metal-NTA complexes, suggesting only selected metal-EDTA and metal-NTA species are transported into the cell.
- The metabolic enzymes for EDTA and NTA degradation are cytoplasmic.
- Stable metal-EDTA complexes are resistant to biodegradation because of lack of cellular uptake.

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