Anaerobic Biotransformation and Mobility of Pu and Pu-EDTA

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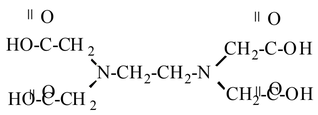
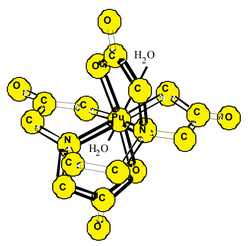
Summary

The complexation of radionuclides (e.g., plutonium (Pu) and ^{60}Co) by co-disposed ethylenediaminetetraacetate (EDTA) has enhanced their transport in sediments at DOE sites. Our previous NABIR research investigated the aerobic biodegradation and biogeochemistry of Pu(IV)-EDTA. Plutonium(IV) forms stable complexes with EDTA under aerobic conditions and an aerobic EDTA degrading bacterium can degrade EDTA in the presence of Pu and decrease Pu mobility. However, our recent studies indicate that while Pu(IV)-EDTA is stable in simple aqueous systems, it is not stable in the presence of relatively soluble Fe(III) compounds (i.e., $Fe(OH)_3$(s) - 2-line ferrihydrite). Since most DOE sites have Fe(III) containing sediments, Pu(IV) in likely not the mobile form of Pu-EDTA in groundwater. The only other Pu-EDTA complex stable in groundwater relevant to DOE sites would be Pu(III)-EDTA, which only forms under anaerobic conditions. Research is therefore needed in this brand new project to investigate the biotransformation of Pu and Pu-EDTA under anaerobic conditions. The biotransformation of Pu and Pu-EDTA under various anaerobic regimes is poorly understood including the reduction kinetics of Pu(IV) to Pu(III) from soluble (Pu(IV)-EDTA) and insoluble Pu(IV) as PuO2(am) by metal reducing bacteria, the redox conditions required for this reduction, the strength of the Pu(III)-EDTA complex, how the Pu(III)-EDTA complex competes with other dominant anoxic soluble metals (e.g., Fe(II)), and the oxidation kinetics of Pu(III)-EDTA. Finally, the formation of a stable soluble Pu(III)-EDTA complex under anaerobic conditions would require degradation of the EDTA complex to limit Pu(III) transport in geologic environments. Anaerobic EDTA degrading microorganisms have not been isolated. These knowledge gaps preclude the development of a mechanistic understanding of how anaerobic conditions will influence Pu and Pu-EDTA fate and transport to assess, model, and design approaches to stop Pu transport in groundwater at DOE sites.

Introduction

EDTA (Figure below) 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. 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 speciation of Pu(IV)EDTA complexes, how Fe(III) and Ca compete with Pu(IV) for EDTA, and speciation of mixed systems. Second, planned studies of reduction of PuO_2(am) and Pu(IV)EDTA by dissimilatory metal reducing bacteria. Third, initial studies on anaerobic EDTA degradation.





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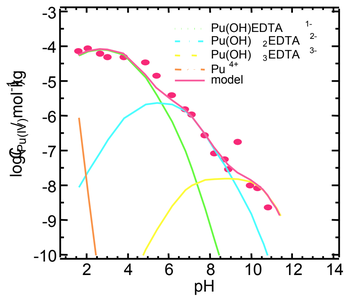
Pu(IV) System



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.

Reaction	log K^0	Reference
$PuO_2(am) + L^{4-} + 3H^+ = PuOHL^- + H_2O$	21.58	Rai et al. 2001
$PuO_2(am) + L^{4-} + 2H^+ = Pu(OH)_2L^{2-}$	15.93	Rai et al. 2001
$PuO_2(am) + L^{4-} + H_2O + H^+ = Pj(OH)_3L^{3-}$	6.94	Rai et al. 2001

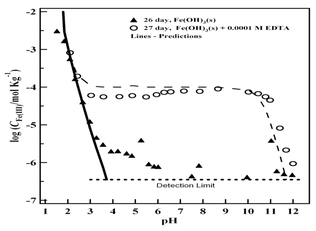
Thermodynamic data developed from PuO_2(am) solubility as a function of pH and $EDTA^{4-}$(L^{4-}) concentrations (Rai et al. 2001).

Microbial Reduction of Pu(IV)

Objectives are to determine the interactions between dissimilatory metal reducing bacteria and PuO_2(am) and Pu(IV)EDTA and answer the following questions:

- Can DMRB reduce Pu(IV) to Pu(III)? What are the comparative rates? Will this reduction enhance Pu solubility and mobility?
- Will EDTA enhance the rate and extent of reduction for PuO2(AM) and the subsequent solubility of Pu(III)?
- Where is the Pu located (e.g., extracellularly sorbed, intracellularly bioaccumulated, or in solution) and is the Pu soluble during and after reduction?

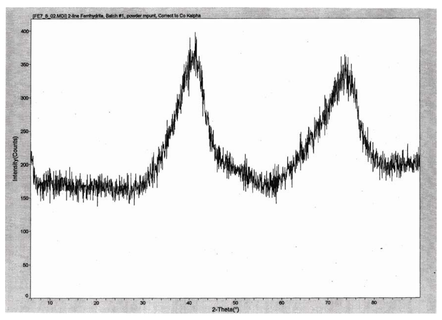
Fe(III) Systems



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. 2005). 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.

Reaction	log K^0	Reference
$Fe^{3+} + OH^- = FeOH^{2+}$	11.81 ± 3	Smith et al. 2001
$Fe^{3+} + 2OH^- = Fe(OH)_2^+$	23.4 ± 1	Smith et al. 2001
$Fe^{3+} + 4OH^- = Fe(OH)_4^-$	34.4	Smith et al. 2001
$2Fe^{3+} + 2OH^- = Fe_2(OH)_2^{4+}$	25.14 ± 2	Smith et al. 2001
$3Fe^{3+} + 4OH^- = Fe_3(OH)_4^{5+}$	49.7	Smith et al. 2001
$Fe_3(OH)_3(s) = Fe^{3+} + 3OH^-$	-38.8 ± 2 -40.36	Smith et al. 2001 This study

Thermodynamic data developed/verified from solubility of 2-line ferrihydrite as a function of pH



Solubility X-ray diffraction pattern of 2-line ferrihydrite used in studies

Reaction	Log K^0
$Fe^{3+} + L^{4-} = FeL^-$	27.66
$FeL^- + H^+ = FeHL^0$	1.51± 1
$FeOHL^{2-} + H^+ = FeL^- + H_2O$	7.818± 3
$2FeOHL^{2-} = Fe_2(OH)_2\ L_2^{4-}$	1.94± 2

Verification of thermodynamic data based on Smith et al. 2001 for Fe(III) – EDTA complexes

Anaerobic Degradation of EDTA

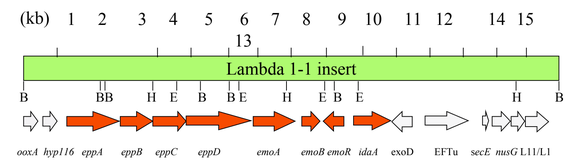
Several aerobic bacteria have been isolated for their abilities to degrade either EDTA or NTA. Interestingly, none of the isolated NTA-degraders can degrade EDTA; whereas, all the bacteria that was initially enriched to degrade EDTA can also use NTA as a the sole carbon source. We have worked out the metabolic pathways for EDTA and NTA degradation by an aerobic bacterium BNC1. Same enzymes are used for both EDTA and NTA degradation (below). Our speculation is that NTA degrading enzymes have been evolved to degrade EDTA and retained the ability to degrade NTA.

EDTA —EmoA/EmoB→ ED3A —EmoA/EmoB→ EDDA —IdaA→ EDMA —IdaA→ ED

EDTA degrading pathway. EDTA monooxygenase (encoded by *emoA*), an $FMNH_2$-utilizing monooxygenase, and NADH:FMN oxidoreductase (*emoB*) working together to catalyze sequential oxidation of EDTA to ED3A (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*.

NTA —EmoA/EmoB→ IDA —IdaA→ Glycine

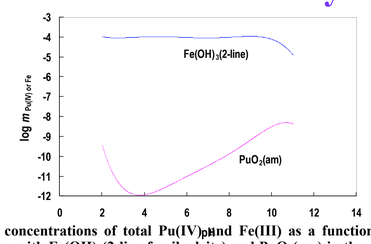
NTA degrading pathway. EmoA/B also oxidize NTA to iminodiacetic acid (IDA). IdaA also converts IDA to glycine (Liu et al. 2001).



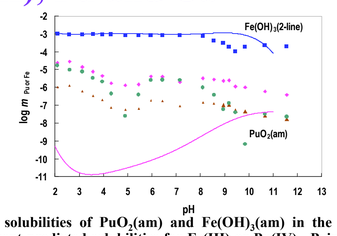
The organization of an EDTA-degrading Gene Cluster from EDTA degrading bacterium BNC1 (Bohuslavek et al. 2001). EppABCD constitute the EDTA uptake system, EmoAB are the two-components EDTA and NTA monooxygenase, and IdaA is EDDA, EDMA, and IDA oxygenase.

To date, no anaerobic EDTA-degrading microorganisms have been isolated. However, several anaerobic NTA-degrading bacteria have been reported. On the basis of our work on aerobic degradation of EDTA and NTA, we hypothesize that some bacteria may have evolved their ability for NTA degradation into EDTA degradation under anaerobic conditions. Since some NTA oxidizing and nitrate reducers have been described, we have set up similar enrichment culture for EDTA degraders. The purpose is to isolate EDTA degrading nitrate reducers. Efforts are also under way to isolate EDTA degrading metal reducers.

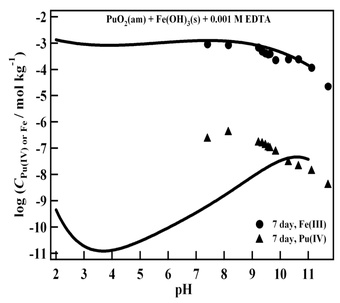
Mixed Systems: Pu and Fe(III), Pu and Ca



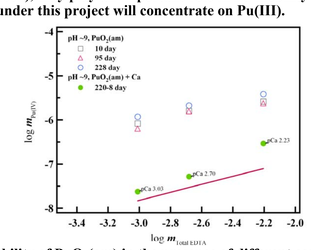
Predicted concentrations of total Pu(IV) and Fe(III) as a function of pH in equilibrium with $Fe(OH)_3$(2-line ferrihydrite) and PuO_2(am) in the presence of 0.0001 M EDTA. The data shows that Pu(IV)-EDTA complexes are not expected to be important in the presence of Fe(III) species and contrary to the implications in the literature these species could not possibly have been the transported species in geologic environments.



Observed and predicted solubilities of PuO_2(am) and $Fe(OH)_3$(am) in the presence of 0.001 M EDTA. Solid lines represent predicted solubilities for Fe(III) or Pu(IV). Points represent observed solubilities. Concentrations of total Pu and different species [Total Pu (diamonds), Pu(III) (circles), and Pu(IV) (triangles)] are also represented. Smaller triangles are used to indicate that the measured Pu(IV) concentrations in these samples are uncertain. The data shows that Fe(III) effectively out competes Pu for complexation with EDTA and that in the entire pH region over 99% of the added EDTA forms complexes with Fe(III). The dominant Pu oxidation state at pH values less about 9 is Pu(III), surmised resulting from minor Fe(II) impurities in $Fe(OH)_3$(am). The dominant oxidation state at pH values >9 is Pu(V)/Pu(VI) (data not shown). These data in general are consistent with the predictions from simple systems that Pu(IV) in oxygenated waters is not likely the mobile species and that Pu(III), previously ignored but consistent with our recent data in Fe(II) systems (Rai et al. 2002), may play an important role in Pu mobility in geologic environments. Future chemistry studies under this project will concentrate on Pu(III).



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. 2005). 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).



The observed solubility of PuO_2(am) in the presence of different concentrations of Ca^{2+} and as 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 Ca^{2+} can also out-compete the Pu(IV) for the EDTA. Implications are that aerobic Ca^{2+} rich groundwaters should not have significant mobilization of Pu(IV) by EDTA.

CONCLUSIONS

- Fe(III) and Ca^{2+} strongly compete with Pu(IV) for EDTA
- Pu(IV) 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
- Work has started on Pu(III) and Pu(III)EDTA
- Work will begin on microbial reduction of PuO_2(am) and Pu(IV)EDTA
- Enrichments for an anaerobic EDTA degrader have been started