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Subsurface Interactions of Actinide Species and Microorganisms: Implications for the Bioremediation of Actinide-Organic Mixtures

by J. E. Banaszak, B. E. Rittmann, and D. T. Reed

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SUBSURFACE INTERACTIONS OF ACTINIDE SPECIES AND MICROORGANISMS: IMPLICATIONS FOR THE BIOREMEDIATION OF ACTINIDE-ORGANIC MIXTURES‡

by

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Table of Contents

List of Figures

List of Figures (Contd.)

List of Tables

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Abstract

By reviewing how microorganisms interact with actinides in subsurface environments, we assess how bioremediation controls the fate of actinides. Actinides often are co-contaminants with strong organic chelators, chlorinated solvents, and fuel hydrocarbons. Bioremediation can immobilize the actinides, biodegrade the co-contaminants, or both. Actinides at the IV oxidation state are the least soluble, and microorganisms accelerate precipitation by altering the actinide's oxidation state or its speciation. We describe how microorganisms directly oxidize or reduce actinides and how microbiological reactions that biodegrade strong organic chelators, alter the pH, and consume or produce precipitating anions strongly affect actinide speciation and, therefore, mobility. We explain why inhibition caused by chemical or radiolytic toxicities uniquely affects microbial reactions. Due to the complex interactions of the microbiological and chemical phenomena, mathematical modeling is an essential tool for research on and application of bioremediation involving co-contamination with actinides. We describe the development of mathematical models that link microbiological and geochemical reactions. Throughout, we identify the key research needs.

1. Introduction

Microbiological processes will have an important role in defining the speciation and mobility of actinides in the subsurface. Although this important fact has been largely ignored, microbiological activity plays a major role in a wide variety of subsurface processes. As we show later, microorganisms can reduce/oxidize multivalent actinides, can biodegrade the solvents and complexants that coexist with actinide contaminants, can cause the precipitation of insoluble inorganic actinide phases, and can become strongly associated with actinides.

For this review, our goal is to identify what is now known about key interactions between microbes and actinide-organic mixtures under conditions prevalent in the subsurface. Our focus is on subsurface interactions that relate to the chemistry and physical dynamics of actinide migration and bioremediation. Understanding these interactions is critical to existing efforts and future approaches to use bioremediation as part of a remediation strategy to cleanup existing organic-radionuclide contaminants. Although few published studies directly relate to actinide-microbiological interactions, we are able to extrapolate from what is known in a general sense about metal-microbiological interactions and biodegradation processes to systems of relevance to actinides.

The U.S. Department of Energy (DOE) is responsible for management of existing and future radionuclide-organic subsurface contaminants. A scientifically sound understanding of the key interactions between actinides and microbes is essential if DOE is to define and guide risk-based analysis of the potential for radionuclides and organic contaminants to become available in the biosphere. Needs include assessments of existing contaminant plumes, as well as development of future remediation, containment, and waste management strategies. Examples of future strategies include controlling and/or manipulating the subsurface microbiology via engineered bioremediation, relying on intrinsic bioremediation to prevent migration, and using *ex situ* bioprocesses in the treatment and separation of radionuclides from organic-metal waste mixtures.

What we need to know about the key interactions between actinides and actinide-organic mixtures with microbes is largely defined by the expected range of contaminated sites and the likely success, based on existing non-radionuclide applications, of bioremediation. Therefore, the remainder of Section 1 provides overviews of (1) the issues faced by DOE in its nuclear waste management; (2) successful applications of bioremediation; (3) bioprocessing of nuclear waste; and (4) key aspects of metal-microbiological interactions.

1.1 Issues in managing nuclear waste problems

The many current problems regarding nuclear waste within the DOE complex have been extensively described in DOE site publications and reports [1-11]. Research related to these problems has been reported in annual symposia proceedings on the Scientific Basis for Nuclear Waste Management and biannual proceedings of the Chemistry and Migration Behavior of Actinides and Fission Products in the Geosphere. Significant radionuclide migration and waste problems occur at all the weapons laboratories. Additionally, radionuclide migration is the key concern for the nuclear waste repositories being developed to dispose of this waste.

Nuclear waste problems are extraordinarily varied and wide ranging. For example, radionuclides have migrated into the groundwater at the Hanford site from several sources:

Leaking high-level nuclear waste tanks, leaching of near-surface contaminants, and escape of low-level/transuranic (TRU) nuclear waste from surface disposal sites. The groundwater environment at Hanford is alkaline, anoxic, high in carbonate, and strongly reducing, with significant levels of sulfide, methane, and Fe^{2+}. Problems associated with the high-level nuclear waste tanks, near-surface TRU storage facilities, and past contaminants also exist at the Savannah River site. Here, radionuclide transport is in organic-rich, near-surface groundwaters. Defense-related activities at Oak Ridge National Laboratory also have led to well-documented radionuclide plumes in anoxic groundwaters of low-to-high ionic strength. Weapons production operations have caused extensive soil contamination at the Rocky Flats site. Radionuclide contaminants exist at the Los Alamos and Idaho National Engineering Laboratory sites and, to a lesser extent, at the Lawrence Livermore site. Lastly, two repositories are being developed for the permanent disposal of radioactive waste. The Waste Isolation Pilot Plant (WIPP) repository, currently in the licensing phase, is proposed for the final disposal of transuranic waste (< 100 nCi/g) in an unsaturated, but anoxic, salt basin. The Yucca Mountain Project is evaluating the Yucca Mountain site in Nevada for the final disposal of commercial spent fuel and glass from the reprocessing of defense high-level nuclear waste. This proposed site is well above the water table, but the path to the biosphere is through release into the groundwater, which is mildly alkaline, is low in ionic strength, and has limited oxygen availability.

Despite the diversity in nuclear-waste problems and contaminated sites, there are some key attributes in common:

- The radionuclides of most interest in TRU waste, high-level waste, low-level wastes, and existing groundwater contaminants are the long-lived isotopes of Pu, Am, Np, U, and Tc. Of these, the radionuclide of greatest concern is, without doubt, plutonium.

- The radionuclides present in the vast majority of nuclear waste coexist with other contaminants [11] that potentially have dominant roles in determining the fate and transport of radionuclides in the subsurface. The most important of these contaminants are the organic solvents (e.g., halogenated hydrocarbons) and complexants (e.g., organic acids, phosphate-organic derivatives, and other specialized extractants) used to process nuclear spent fuel.

- Lastly, the release of radionuclides to the accessible environment, even at contaminated sites where the groundwater table is very near the surface, involves an important transition from oxic, often unsaturated zones, to oxygen-depleted groundwaters that are more reducing. From this perspective, aerobic and anaerobic processes, as well as the transition at the oxic/anoxic interface, are important in defining the mobile radionuclide species.

1.2 *In-situ remediation of existing contaminated sites*

Among the most successful and widely used "innovative technologies" for cleanup of sites contaminated with hazardous chemicals is *in situ* bioremediation [12, 13]. It is used routinely to restore sites contaminated with petroleum hydrocarbons; is beginning to have applications for the decontamination of chlorinated solvents, pesticides, explosives, polynuclear aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs); and is being studied for

cleanup of sites contaminated with metals [13]. Its advantages include reduced costs over most other alternatives and, in some cases, reduced exposure risks [12, 14].

Bioremediation exploits the metabolic reactions of microorganisms to destroy contaminants or to transform them into species whose mobility is controlled [12, 14]. In many cases, these beneficial effects are caused directly by oxidation or reduction reactions catalyzed by enzymes that the microorganisms use to gain energy. In some cases of mobility control, the effects are indirect, because the oxidation/reduction reactions also produce or consume chemical species that react with the target contaminant. In either case, the driving force behind bioremediation is catalysis of oxidation/reduction reactions.

In situ bioremediation takes place in sites whose soils remain undisturbed. Because most of the contaminants of concern at hazardous-waste sites--including actinides and other contaminants of concern to DOE--are "trapped" in the soil matrix by adsorption, formation of a nonaqueous liquid phase, or precipitation, they cannot be "flushed out" by the pump-and-treat approach [12, 15]. *In situ* bioremediation overcomes this limitation by letting high densities of microorganisms locate themselves very close to the nonaqueous source of the contaminants. If the biodegradation activity is strong and close to the nonaqueous source, transport of the contaminants is completely controlled.

The key to complete control is ensuring that sufficient microorganisms are located near enough to the source. To accomplish this goal, bioremediation technologists must make sure that growth-supporting substrates and nutrients are supplied to the zone of bioremediation at rates high enough to grow and sustain the microorganisms and their metabolic reactions [16].

In situ bioremediation can be divided into two major classes that are distinguished by how fast the substrates and nutrients are supplied. The first class is engineered *in situ* bioremediation, in which engineering technologies are used to greatly increase the rate of substrate and nutrient supply to biologically active zones [12, 14, 16]. By far the most common applications for engineered bioremediation have been petroleum hydrocarbons, such as leaked gasoline. Oxygen and macronutrient (N and P) supplies are the foci for bioremediating petroleum hydrocarbons, and a wide range of engineered supply techniques are used successfully [12, 13]. Engineered bioremediation is best applied when success requires a short cleanup time.

The second class is intrinsic *in situ* bioremediation, which relies on the intrinsic (or naturally occurring) supply rates of substrates and nutrients. Instead of achieving the fastest possible cleanup, the prime goal of intrinsic bioremediation is to prevent the migration of contaminants from the source [16]. Thus, it is a form of biological containment. When sources are small or otherwise give slow contaminant-dissolution rates, the intrinsic supply rates can be high enough to allow full biodegradation (or immobilization) before the contaminants are transported a significant distance. Intrinsic bioremediation also has been called "natural attenuation."

Critical to the success of *in situ* bioremediation are site characterization before the project begins and comprehensive monitoring during the life of the bioremediation. Because contamination normally is trapped in the soil matrix, heterogeneously distributed, and difficult to sample, characterization and monitoring are major challenges. Nevertheless, they are absolutely essential for any successful project. General guidelines for characterization and monitoring are available [e.g., 14, 16, 17], and more detailed protocols are being developed and tested. Since bioremediation is driven by microbially catalyzed oxidation/reduction reactions, characterization

and monitoring must focus on the supply rates and transformation rates of biologically available electron donors and acceptors.

1.3 Bioprocessing of nuclear waste

Biological processes have been used to treat domestic wastewaters for nearly a century. Today, biological treatment is routinely used to remove many organic wastes, toxic compounds, and biological nutrients, such as nitrogen and phosphorus, from water and wastewater. Biological treatment is attractive because, when applicable, it is usually more cost-effective than traditional physical-chemical treatment alternatives: The amount of catalyst, or biomass, grows as the target compounds are stabilized and/or detoxified, eliminating the need for expensive chemical additions to achieve the desired treatment goals. Capitalizing on the progress we have made in the development of biological treatment options for organic wastes and contaminants, the application of microbiological processes to the treatment of inorganic industrial wastes and remediation of metal and radionuclide contaminated waste streams and groundwaters is currently a rapidly evolving technology that is the subject of much active research [18-26].

In contrast to the biological treatment of organic wastes and contaminants, which are often completely mineralized to harmless products during treatment, the treatment of metals and radionuclides by any method cannot affect the elemental composition of the contaminant or waste. Thus, the treatment goals for nuclear wastes are different from those for organic wastes and could include, for example, the selective accumulation of radionuclides for possible recovery or ultimate disposal, the immobilization of radionuclides in a subsurface aquifer to prevent migration into water supplies, or the selective leaching of radionuclides from contaminated soil [27]. Also, any biological treatment alternative for nuclear waste streams cannot be a stand-alone system, but must be integrated into a more complex overall treatment strategy.

Numerous examples of the biological manipulation of metals are found in the mining industry. Biological processes are used in every facet of metal recovery, from the bioleaching of precious metals from low grade ores to the selective recovery of metals from aqueous streams by biosorption or sulfide precipitation [28]. Similar processes have been attempted or envisioned for the treatment of actinide-containing waste streams and contaminated sites. Some examples of proposed processes for treating actinide-bearing wastes are:

- Macaskie and co-workers [20, 21, 29-32] demonstrated the removal of U and other actinides from pilot-scale aqueous streams by bioprecipitation of phosphate solids, and they proposed that their system may be suitable as the final polishing step of treatment for nuclear-waste streams.

- Francis and Dodge [25, 33] suggested that U and other metals may be recovered from contaminated soils by chelation with citric acid to solubilize the metals sorbed onto soil particles, followed by microbial and/or photodegradation of the citric acid to release the chelated metals for recovery.

- Phillips et al. [34] proposed the removal of U from soils by complexation with bicarbonate and demonstrated that U was recovered from solution by precipitation following reduction by metal-reducing bacteria.

- Uranium removal from aqueous waste streams by biosorption onto non-viable biomass has been demonstrated by several researchers [35, and references therein].

All of these proposed treatment processes share several attributes. First, each of them includes a chemical and a biological step as part of the combined treatment system; thus, detailed understanding of the coupling between equilibrium aquatic chemistry and the kinetics of microbiological degradation reactions is required to accurately predict process performance. Second, all four processes are examples of *ex situ* treatment systems, i.e., the wastes are treated in an engineered vessel or reactor. Out of the four processes, only nonspecific biosorption does not depend on the direct metabolic activity of microorganisms. The other three processes highlight the importance of coupled biological and chemical processes in the treatment of actinide-contaminated waste streams. It is this coupling that is the focus of Section 2.

1.4 Microbe-metal interactions

Metal cations are essential to all living organisms. Metals function as key components of enzymes and cofactors required for biochemical pathways [36, 37]. For example, iron is found in many cellular enzymes associated with respiration; magnesium is an essential element in chlorophyll; cobalt is required for the synthesis of vitamin B_{12}; nickel is required by methanogenic bacteria for synthesis of key enzymes that catalyze methane fermentation; and calcium and magnesium act as bridging ions between the lipid by-layers that make up many cellular membranes.

Although metal ions are essential micronutrients in all biological systems, microorganisms are unique in that they can derive energy for growth by catalyzing the oxidation or reduction of metals [38-40]. Reduced metal species act as electron-donor substrates, supplying microorganisms with the electrons needed to generate energy and reduce nutrients for biosynthesis of new cellular material. Oxidized metals are used as electron-acceptor substrates in anaerobic respiration. Thus, the biogeochemical cycling of many abundant metals is influenced by the metabolic activity of microorganisms.

Among the metals, Fe, Co, Zn, Mo, Cu, Mn, Ni, W, and Se are required in trace amounts for aerobic microorganisms [37]. Anaerobic microorganisms often are more sensitive to nutrient deficiencies because their specialized biochemical pathways require relatively high amounts of trace metals [41]. Also, nutrient deficiencies may limit the growth of microorganisms if trace metals are not bioavailable to the organism [37, 41]. Nonessential metals, including the actinides and metals such as Hg and Pb, have no known beneficial function in biological processes and are considered toxic when present in elevated quantities.

Even though certain metals are required in trace amounts by all living organisms, when essential metals are present in high enough concentrations they can inhibit the activity of living organisms. The nonessential metals normally are toxic at elevated concentrations. The mechanisms of metal toxicity toward microorganisms are varied and complex [42], and many organisms have genetically encoded mechanisms to combat the harmful effects of toxic metals [36, 43]. Essential metals sometimes stimulate the growth of bacteria until the metal concentration reaches a level at which it becomes inhibitory to the organism [41]. Toxic metals often inhibit the growth of microorganisms by substituting for essential metals in key cofactors, rendering them inoperable, or by saturating membrane transport proteins in place of essential metal micronutrients.

Although much literature is available detailing the interactions between metals and microorganisms [44], relatively little is known about the interactions between actinides and microorganisms. Much of our discussion in this review extrapolates from what is known about metal-microbe interactions to actinide-microbe systems. In Section 2, we discuss the impact of microbial activity on the speciation and mobility of actinides in the subsurface. In Section 3, we examine the effects of actinides on microbial processes. In Section 4, we describe modeling techniques that can be used to predict the important interactions between microorganisms and actinides and identify areas where modeling improvements are needed. Finally, in Section 5, we discuss the potential application of bioremediation strategies to subsurface sites contaminated with mixtures of organic compounds and actinides, and we identify key research challenges that need to be overcome to immobilize subsurface actinides during the bioremediation of organic contaminants.

2. Impact of Microorganisms on Actinide Speciation and Mobility

The mobility of actinide species in groundwater environments depends on many competing mechanisms. Redox conditions, pH, temperature, colloids, and complexing ligand concentrations play roles in determining the chemical speciation and migration behavior of actinide elements [45]. For example, complexation with organic chelating agents has been implicated in mobilizing radionuclides in several groundwater systems [8, 46-48]. However, other mechanisms, such as colloid association [49-52], biosorption onto mobile bacteria [53], and complexation with natural organic matter (NOM) [54-57] also increase actinide migration. Microorganisms and their metabolic activities can be central in enhancing or retarding most of these mobilizing effects. Many details of specific interactions between microorganisms and metal contaminants/radionuclides have been reviewed [26, 38-40, 58], as has the environmental chemistry of the actinides [59-61]. The activity of subsurface microorganisms has been recognized as being potentially significant in radioactive waste disposal [23, 62, 63].

Microorganisms are ubiquitous in nature, even in deep subsurface environments [64-66], and some species survive in the most extreme conditions: pH ranging from less than 1 to greater than 10, temperatures from near 0 to over 100°C, pressures of up to 1100 atm, and salinities up to 5 M [37, 67, 68]. Biological activity has been implicated in the formation of diverse geologic phenomena [58, 69], including uraniferous hydrocarbon formations in southwest Scotland [70], placer gold deposits in Alaska, and magnetite accumulation in the Banded Iron Formations [39]. Thus, interactions between microorganisms and actinides should not be constrained by general environmental conditions in the subsurface.

In general, we can place the interactions between microorganisms and chemical compounds in two broad categories: indirect or direct. Bacteria can *indirectly* affect actinide speciation by changing or controlling overall chemical parameters in the system through their normal metabolic cycles, such as pH and redox potential. Prediction of biological impacts on actinide mobility in the indirect case involves linking microbial growth and metabolism to chemical reactions, such as complexation, oxidation/reduction, sorption, or precipitation/dissolution. As we examine later, a change in the chemical speciation of an actinide element may dramatically affect its potential toxicity to microorganisms. Additionally, the biodegradability of an organic compound is in many cases controlled by its chemical properties.

So, we can envision coupled cycles in which biological activity affects actinide and organic speciation, which in turn alters biological activity.

Microorganisms *directly* interact with compounds by utilizing substances in energy-generating or biosynthesis reactions as electron donors, electron acceptors, or nutrients, or by mitigating potential detrimental effects of toxic substances. We examine the basics of microbial metabolism in more detail later, but at this point we define a direct interaction as the involvement of an actinide in an enzymatically catalyzed biological reaction. In contrast to the indirect case, the properties of actinides in the system are directly related to biological activity, as opposed to chemical parameters in the environment. To understand actinide fate when direct interactions are important, we must know what enzymatic mechanisms are involved.

Direct and indirect interactions between microorganisms and actinides are by no means exclusive processes, nor are they the totally dominant forces responsible for actinide speciation in the environment. In this section we detail the known, suspected, or potential interactions related to actinide series elements in contact with biological processes. We draw on results of experimental work with actinides and bacteria, as well as infer possible interactions from results of work with other metals.

2.1 Bioutilization of actinides as electron donor or acceptor substrates

Microorganisms harness energy for growth by catalyzing oxidation/reduction reactions. In addition to an available source of trace metal micronutrients and macronutrients like N, P, and S, bacteria require a source of cellular carbon and usable electron-donor and -acceptor substrates. Oxidation of electron-donor substrates provides bacteria with a source of electrons to use in energy generating and biosynthesis reactions. The carbon source, which is often, but not always the same compound as the electron-donor substrate, provides a source of cellular carbon. Finally, reduction of the electron-acceptor substrate provides a terminal endpoint for the electrons used in energy-generating reactions by bacteria.

The maximum amount of energy available to bacteria from any given combination of electron-donor and -acceptor substrates is simply the free energy derived from combining appropriate electron-donor and -acceptor half reactions. Like humans and animals, microorganisms obtain the maximum amount of energy from aerobic respiration, in which O_2 is the ultimate electron acceptor. However, many species of bacteria either can or must grow by anaerobic respiration, the reduction of an electron acceptor other than oxygen. Organisms that can switch between aerobic or anaerobic respiration are termed "facultative," whereas bacteria that can only grow in the complete absence of oxygen are known as "obligate anaerobes."

In the environment, common alternative electron acceptors include nitrate, nitrite, and sulfate. These electron acceptors are also common constituents of nuclear wastes. In many subsurface environments, oxygen, nitrate, and nitrite are rapidly depleted. Solid metal oxides like FeOOH and MnO_2 then become energetically attractive alternative terminal electron acceptors, since more free energy is available from Fe(III) and Mn(IV) reduction than sulfate reduction [71]. Many species of bacteria are able to couple oxidation of organic and inorganic electron donors to reduction of metal ions [40, 72, 73]. Reduction of Fe(III) and Mn(IV) oxides can result in solubilization of free reduced Fe(II) and Mn(II) ions. Dissimilatory metal reduction,

the enzymatic transfer of electrons to metal ions for energy conservation, is a critical part of the geochemical cycling of trace metals in the environment [38-40, 58, 69].

Although the detailed biochemistry of electron transport systems in microorganisms is beyond the scope of this review, the general pattern for electron flow in bacteria is of the highest importance. (For a full description of electron transport systems in bacteria, consult a general microbiology textbook such as [37]). During respiratory oxidation of an organic electron-donor substrate, electrons are transferred to intracellular electron carriers, usually nicotinamide-adenine dinucleotide (NAD), creating its reduced form (NADH):

$$NAD^+ + 2e^- + 2H^+ \rightarrow NADH + H^+ \qquad E_0' = -0.32 \text{ volt}$$

For example, consider the oxidation of acetate, a common electron-donor substrate in microbiological reactions. Acetate releases 8 electrons, forming 4 NADH molecules:

$$CH_3COOH + 4NAD^+ + 4H_2O \rightarrow 2H_2CO_3 + 4NADH + 4H^+$$

For purposes of clarity, all stoichiometric reactions presented in this section neglect biomass synthesis; the role of biomass synthesis is considered in Section 4 on mathematical modeling of microbiological systems. The reduced form of the carrier, NADH, carries 2-electron "packets" to the cell membrane, where they are transferred to membrane-bound redox-active coenzymes, such as flavoprotein (a derivative of riboflavin), regenerating NAD^+ and reducing the membrane-bound coenzyme. The electrons are successively transported along the membrane through a redox "chain" of coenzymes, called cytochromes. Energy harvested from the redox-potential difference between each pair of cytochromes is used to produce an electrochemical gradient across the cell membrane, which drives energy generation and membrane transport processes in the organism. Finally, the electrons are transferred to the terminal electron acceptor by a specialized enzyme, a *reductase*, after the maximum amount of useful energy has been harvested.

In the case of aerobic respiration, the overall electron-acceptor reaction across the membrane-bound cytochromes is:

$$O_2 + 2NADH + 2H^+ \rightarrow 2H_2O + 2NAD^+$$

Even though NAD is not involved in the transfer of electrons to the terminal acceptor, it is the net product of the overall chain of reactions, and the internal cycling of intracellular electron carriers need not be explicitly expressed. Combination of the electron-donor and electron-acceptor reactions yields:

$$CH_3COOH + 2O_2 \rightarrow 2H_2CO_3$$

Up to this point, we have shown an example using an organic electron donor. However, microorganisms are not limited to organic electron donor substrates. Autotrophic bacteria, organisms capable of utilizing inorganic carbon as their carbon source for biosynthesis of cellular components, couple metal oxidation and oxidation of other inorganic electron donors, such as ammonium, sulfide, elemental S, and H_2, to respiratory processes for energy generation [37].

Although great variety in electron acceptors and their electron transport systems is being documented among different species of microorganisms, the laws of thermodynamics are never violated: NAD^+ only functions as the internal electron carrier when the reduction potential of the electron donor is higher (more electronegative) than the NAD/NADH couple. Similarly, the reduction potential of the last cytochrome in the electron transport chain must be more electronegative than the redox potential of the terminal electron acceptor. In that light, Fig. 2.1 superimposes the standard reduction potentials (at pH=7) of common internal electron carriers with potentials for key electron acceptors, electron donors, and actinide redox couples. Inspection of Fig. 2.1 shows that some organisms, such as sulfate-reducing bacteria (SRB) and methanogens, are able to capture energy from very small potential differences between electron-donor and -acceptor substrates; thus, electron-transport chains for 1, 2, and 3 in Fig. 2.1 are very short and generate little energy per electron pair transferred from the donor to the acceptor. Other organisms, such as aerobic or denitrifying heterotrophs, have long chains, which provide much more energy per electron pair extracted from the donor (chains 4-6).

For a redox couple to be useful in an electron-transfer reaction, the relative positions of the redox couples must define a thermodynamically possible pairing, the organism must possess the necessary enzymes to effectively interact with the substrate, and the substrate must be in a form that can be readily utilized by the organism, or bioavailable [40].

We can infer some possible electron donor/acceptor interactions involving actinides by correlating the redox couples shown in Fig. 2.1 to some of the actual actinide species observed in the subsurface. As an example, we focus on the Pu system, which is shown in the +3, +4, +5, and +6 oxidation states in Fig. 2.1. Although high pH can shift Pu speciation toward the +5 and +6 oxidation states, in general, Pu(V) and (VI) are found in oxidizing waters [51, 59, 60, 74-76]. Because aerobic microorganisms rapidly utilize oxygen as an electron-acceptor substrate, oxic subsurface waters often correlate to environments with low biodegradable organic substrate loading rates and/or high oxygen recharge capacity. In an isolated aquifer with high concentrations of organic electron-donor substrates, oxygen and nitrate, the two most energetically favorable electron acceptors, are rapidly depleted, and metal reduction becomes the dominant energy-generating mechanism in the presence of capable organisms. By comparison of their potentials to the O_2/H_2O couple, we would expect that Pu(VI) could be reduced to Pu(V) concurrently with oxygen depletion, since the potential for the PuO_2^{2+}/PuO^{2+} couple is more positive that that of O_2/H_2O. On the other hand, Pu(V) probably would be reduced to Pu(IV) after oxygen is consumed. The reduction of Pu(IV) to Pu(III) (chain 8) is thermodynamically less favorable, yielding much less energy per electron than reduction of CO_2 or SO_4^{2-}. Nonetheless, Fig. 2.1 shows that reduction of Pu(IV) to Pu(III) should be energetically favorable for microorganisms that oxidize organic compounds or H_2. In summary, we would expect that Pu(IV) (in the form of a complex or PuO_2 solid) is the dominant oxidation state in most biologically active anoxic environments. In fact, measurements in anaerobic groundwaters have shown that Pu speciation is dominated by PuO_2(s) [75]. Similarly, U(VI) (in the form of UO_2^{2+}) and Np(V) are potential electron acceptors, and Fig. 2.1 shows that they have energy-generating potentials superior to Fe(III) and SO_4^{2-}, but substantially less than NO_3^- and O_2.

Electron transfer from an electron donor substrate, through a membrane-bound electron transport chain and terminal reductase, and to a metallic electron acceptor defines a possible direct interaction that is energy generating for microorganisms. In many cases, one organism can

produce alternative terminal reductase enzymes, depending on the abundance of available electron acceptors [40, 77].

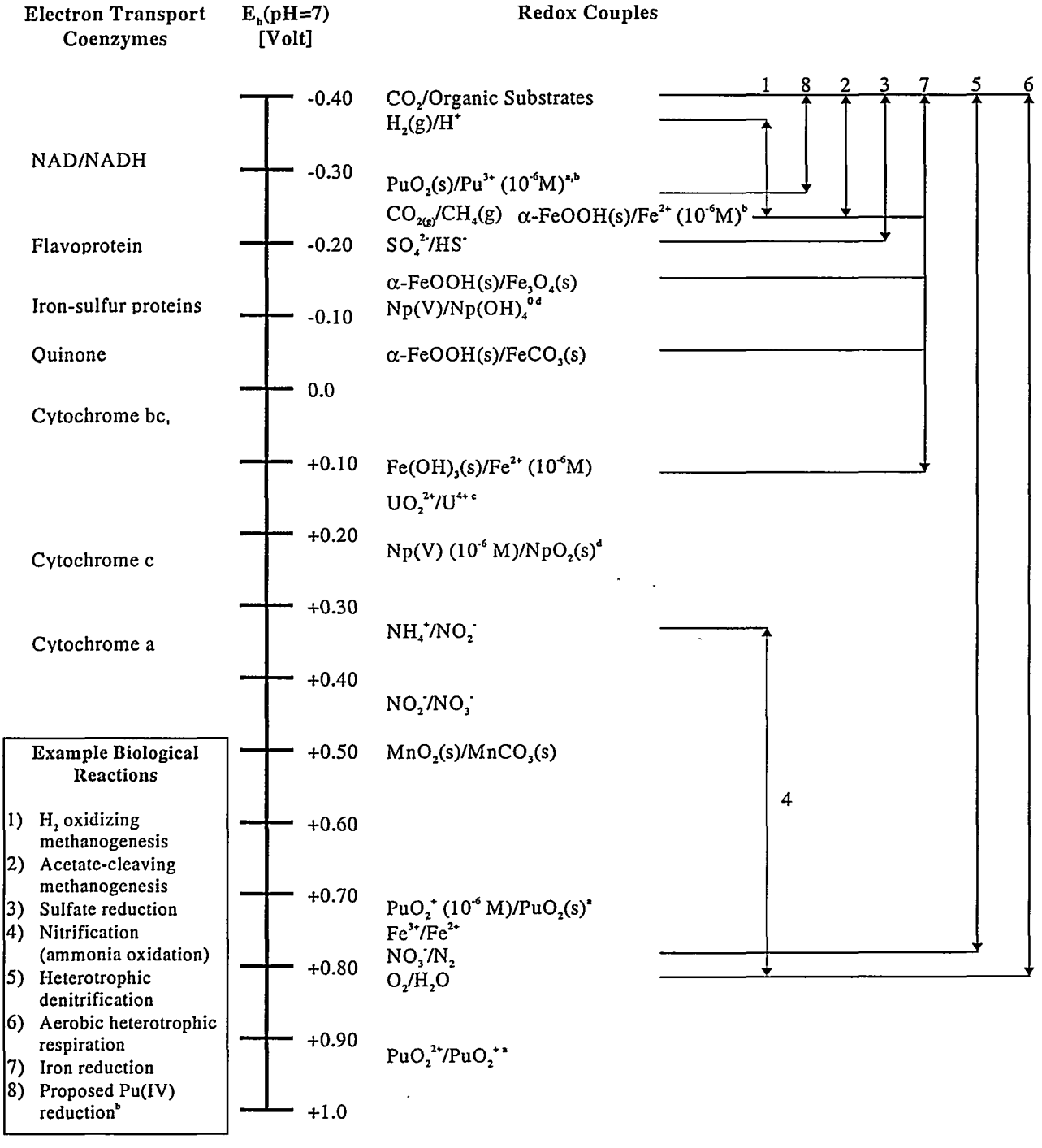


Fig. 2.1 - Conceptual electron transport tower, compared to important biological electron acceptor and actinide redox couples. Adapted from Madigan et al. *[37]* and Stumm and Morgan *[90]*. Letter superscripts defined as follows: a*[117]*, b*[79]*, c*[60]*, d(calculated from Fahey *[291]* and Yamaguchi et al. *[275]*).

With one exception, published evidence describing use of actinides as terminal electron acceptors in biological energy conservation reactions is limited to the uranium system. Enzymatic reduction of soluble U(VI) species to insoluble U(IV) solids has been documented for several metal-reducing bacteria [39, 40]. *Geobacter metallireducens* (formerly strain GS-15) can grow using acetate as a sole carbon and energy source coupled to uranium reduction by a c-type cytochrome:

$$CH_3COOH + 4UO_2^{2+} + 4H_2O \rightarrow 4UO_2(s) + 2H_2CO_3 + 8H^+$$

Other organisms are capable of reducing UO_2^{2+}. *Shewanella putrefaciens* and several *Desulfovibrio* species couple U(VI) reduction to H_2 oxidation [39, 40]:

$$H_2 + UO_2^{2+} \rightarrow 2H^+ + UO_2(s)$$

S. putrefaciens is an excellent example of the remarkable adaptability of some microorganisms. This organism can grow aerobically when O_2 is available and anaerobically using either NO_3^-, NO_2^-, $S_2O_3^{2-}$, S^0, Fe^{3+}, Mn^{4+}, or fumarate as electron acceptors. In other studies, Francis et al. [78] showed uranium reduction by a *Clostridium* sp. growing on glucose.

Among the other actinides, use of Pu as an electron acceptor has been hypothesized by Rusin et al. [79], who suggested that reduction of Pu^{4+} to Pu^{3+} by iron-reducing bacteria may be responsible for increased solubilization rates of Pu, although they were not able to directly observe Pu^{3+} formation. Their proposed mechanism, shown schematically in Fig. 2.2, includes reduction and solubilization of $PuO_2(s)$ by the organism, followed by the rapid abiotic reoxidation of the Pu^{3+} to Pu^{4+} to form the stable Pu(IV)-nitrilotriacetic acid (NTA) complex [80, 81]. The electrochemical potential for the reduction of Pu(IV) to Pu(III) (chain 8 in Fig. 2.1) is similar to the reduction potentials for other metals used as known electron acceptors in biological reactions, such as the reduction of FeOOH to Fe^{2+} (other iron species, such as Fe_2O_3 or $FeCO_3$, are produced by biological iron reduction, see chain 7 in Fig. 2.1). Two iron-reducing *Bacillus* species, *B. polymyxa* and *B. circulans*, solubilized up to 90% of PuO_2 in the presence of NTA, while *E. coli* and NTA control samples showed insignificant dissolution of the solid. The results of this work have important implications for the mobility of Pu in the subsurface, and more controlled studies are needed to determine the exact dissolution mechanism. For example, we need evidence concerning whether or not the reduction of Pu(IV) to Pu(III) results in energy capture by the cells.

Metal-reducing capabilities are not restricted to the organisms described above, as the heterotrophic bacteria *Pseudomonas*, *Bacillus*, *Geovibrio*, and *Desulfuromonas* species, among others, mediate metal reduction [39, 40, 79, 82, 83]. Our current knowledge of metal-reducing organisms is limited by our isolation and identification capabilities, and new discoveries are likely to occur regularly.

In order to take into account substrate bioavailability, details of reaction stoichiometry must be considered. For example, we presented the uranium product and reactant as soluble U(VI) (UO_2^{2+}) being reduced to insoluble U(IV) [$UO_2(s)$], which correlates with much of the experimental evidence described for dissimilatory uranium reduction [39, 40, 78, 84, 85] and serves as an example of one mechanism of *bioprecipitation*. However, uranium has many

possible oxidized and reduced forms, and metal reduction is usually represented in the nonspecific form U(VI) → U(IV) to avoid these speciation complications [39]. Avoiding complications has its price, because the overall free energy derived from each possible reaction and the reaction kinetics depend strongly on the exact initial and final species.

The highly dependent nature of biological electron transfer reactions on the forms of metal electron acceptors is exemplified by the Fe(III)/Fe(II) system. Chain 7 in Fig. 2.1 compares the redox potentials of several Fe(III)/Fe(II) couples and illustrates the effect of chemical speciation on the energy available from iron reduction. The potential of the $Fe(OH)_3(s)/Fe^{2+}$ couple is about 0.30V more positive than the $FeOOH(s)/Fe^{2+}$ couple. Thus, more energy is available to organisms catalyzing the reduction of $Fe(OH)_3(s)$ to Fe^{2+} than to organisms reducing FeOOH(s) to the same reduced iron species.

Dissimilatory iron reduction can result in mobilization of solid oxidized mineral Fe(III) to reduced Fe(II) species, since Fe(II) is more soluble than Fe(III). In these cases, bacteria must have direct physical contact between themselves and the solid Fe(III) substrates. Bioavailability of Fe(III) as an electron acceptor is related to mineralogical form [40]. Highly crystalline oxide surfaces are difficult, if not impossible to reduce, while microorganisms can more rapidly utilize amorphous minerals. Iron reduction is also accelerated by the presence of strong complexing agents that form soluble Fe(III) complexes [86], some of which can be secreted by the bacteria themselves. Additionally, the rate of biological iron reduction in some environments is dependent on mineral surface area, especially if iron reduction is limited by the soil's ability to remove soluble Fe(II) species by sorption [87].

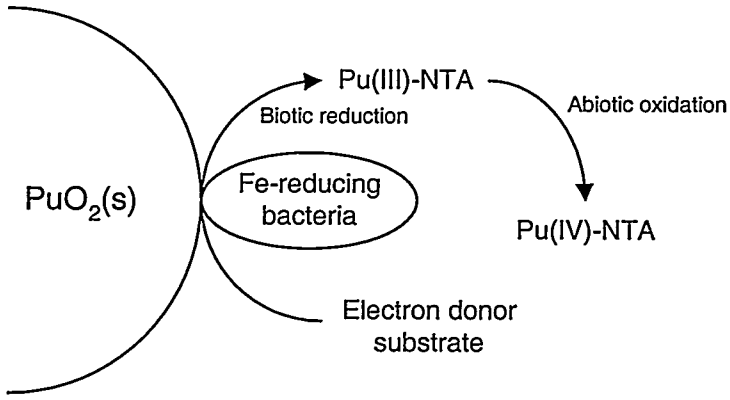


Fig. 2.2 - Schematic representation of the plutonium solubilization mechanism proposed by Rusin et al. *[79]* based on the results of experiments conducted with several species of metal-reducing bacteria. Reduction of Pu(IV) in the solid oxide results in dissolution of Pu(III), formation of the Pu(III)-NTA complex, and subsequent abiotic oxidation of Pu to form the stable Pu(IV)-NTA complex.

Dissimilatory reductive solubilization of common mineral oxides by microorganisms has implications on actinide mobility, as well. Actinides are known to co-precipitate with common mineral compounds [88]. Solubilization of Fe(III) and Mn(IV) mineral oxides also releases other metals sorbed on the oxide surfaces or coprecipitated in the mineral matrix [63, 89, 90]. Also, it is often impossible to differentiate direct electron transfers from indirect or abiotic processes [39]. Matters are further complicated by the fact that other direct biological electron transfer processes are possible. For example, many, if not most, microorganisms *assimilate* and reduce metals for biosynthesis of their necessary cofactors [40]. Additionally, microorganisms may fortuitously shuttle electrons to alternative electron acceptors when electron donors are abundant, making growth limited by the availability of primary electron acceptors [91]. In the next section, we discuss some of the possible indirect biologically mediated redox interactions between microorganisms and actinides.

So far, we have considered actinides as electron acceptors. However, reduced forms of actinides might serve as inorganic electron donors, as is documented for Fe(II) and Mn(II) [37, 92]. In such cases, bioavailability becomes a key factor. The most well known example of metal electron donor utilization is the oxidation of Fe(II) to Fe(III) in mine wastewaters by *Thiobacillus* sp [37, 93]. The low pH typical of many iron mines stabilizes iron as Fe(II). *Thiobacillus*, an autotrophic organism, oxidizes the iron by using oxygen as the terminal electron acceptor, resulting in precipitation of Fe(III) oxide. Microbial metal oxidation may be a contributing factor in the microbially induced corrosion of steels. Linhardt [94] proposed that the formation of manganese oxide deposits by Mn-oxidizing bacteria may initiate the corrosion of stainless steel.

Biotic iron oxidation by *Thiobacillus* is used in the mining industry for the recovery of metals from sulfide deposits. In a process called "bioleaching," the ferric ions directly produced by biotic iron oxidation abiotically oxidize the metal sulfide precipitates, resulting in solubilization of the metal into the leachate [95, 96]. Bioleaching is used for the production of uranium from ore. As the insoluble U(IV) in the ore is oxidized, it is released as soluble U(VI) and collected in the leachate [63, 93].

Once again, the laws of thermodynamics govern the biological processes we observe in nature. Referring to Fig. 2.1, we can see from their relative positions on the potential diagram that Fe(II), Mn(II), and U(IV) are energetically capable of serving as electron donors during aerobic respiration [37-40, 84]. While Pu(III) and Np(IV) are candidates to be electron donors, no evidence is yet available that they are. Detailed investigations are needed to determine if any actinides other than uranium can interact directly in biological electron transfer reactions.

2.2 Change in subsurface redox and/or acid base chemistry

Microbiological processes have a pronounced effect on, and, in many cases, control the pH and redox environment in the subsurface. This effect has very significant implications on the speciation and mobility of actinides in the subsurface, since the actinides of most concern are multivalent species with solubilities that are dependent on pH and the oxidation state.

In the absence of significant concentrations of complexing ligands, the solubility and mobility of the actinides are highly dependent on their oxidation state and the pH of the aqueous medium. In general, the solubility of the metals decreases with increasing ionic charge [60]. For example, in pH 7 water exposed to atmospheric CO_2, the relative calculated solubilities of the actinide (An) oxidation states are $AnO_2^+ > AnO_2^{2+} > An^{3+} >> An^{4+}$; the solubility of the +4

oxidation state is about five orders of magnitude less than that of the +5 oxidation state [60]. The tendency toward formation of polymeric colloidal species follows the opposite trend as that for solubility: species with greater ionic charge show increased propensity toward colloid formation [59, 60, 97]. Colloid formation can increase or decrease actinide mobility, depending on whether the particles remain suspended in solution, sorb onto natural mobile colloidal particles, or sorb onto the soil matrix [50, 56, 57, 59-61, 97-99]. Sorption of dissolved actinide species onto soil matrices, usually as oxy-hydroxide complexes, again follows the same trend of increasing affinity for sorption with increasing ionic charge. Thus, oxidation state has a dramatic effect on the migration of dissolved actinide ions. For example, Lieser and Mühlenweg [100] found that the adsorption ratio of Np increased nearly four orders of magnitude following the reduction of Np(V) to Np(IV).

In this section, we discuss the indirect chemical influences that microbiological activity can have on actinide speciation and mobility via normal metabolic processes. Obviously, we cannot always conclusively label all biologically mediated processes as *direct* or *indirect*; we use the terminology here to provide a clear distinction between the two possible interactions.

2.2.1 Effects of microbiological activity on redox conditions

The most important indirect effect on actinide speciation is the influence of biological activity on subsurface redox conditions. In the most general case, microorganisms live under either aerobic (the presence of oxygen) or anaerobic (the absence of oxygen) conditions. Because of the high energy availability when O_2 is the electron acceptor, aerobic microorganisms tend to grow most rapidly when they have sufficient electron-donor substrates and essential nutrients. Thus, in subsurface environments with low groundwater recharge rates, oxygen depletion eventually occurs, causing the aquifer to become anaerobic. After the onset of anoxia, degradation of organic material is controlled by the availability of terminal electron acceptors, the presence of microbial species capable of utilizing the available substrates, and the cooperative or competitive interactions among various microbial populations. In the presence of capable microorganisms, oxidation of organic compounds is coupled to reduction of the thermodynamically favored terminal electron acceptor. As shown in Fig. 2.1, reductions of nitrate, Mn(IV), Fe(III), sulfate, and carbon dioxide provide successively less free energy to support growth. When sufficient degradable organic matter is present and multiple electron acceptors are available in subsurface environments, the mass transport and hydrodynamics imposed by groundwater movement may cause general or localized depletion of the more favorable electron acceptors [101, 102]. Thus, microbial colonization may occur in large zones following complete depletion of a particular electron acceptor or in small microcosms due to localized acceptor depletion.

If nitrate or oxidized metals are absent, if the electron donor substrate is a complex organic polymer, or if bacteria with metal-reducing capabilities are not present, fermenting microorganisms can conserve energy for growth by utilizing organic compounds as both primary substrates, i.e., electron donor and acceptor. In fermentation reactions, a portion of the electron donor is oxidized to CO_2, and the remainder is reduced to intermediate products such as long-chain fatty acids, simple sugars, organic acids, and alcohols. The amount of free energy that can be derived from fermentation reactions is directly dependent on the fraction of electrons the organism can shuttle to proton reduction and hydrogen gas (or formate) production. If the H_2 (or formate) produced from the fermentation is utilized through a syntrophic relationship with

hydrogen-consuming bacteria, the fermenting organism can gain the maximum energy by transferring most of the electrons derived from the electron donor to proton reduction. However, if the H_2 (or formate) produced from the fermentation is not removed from the system, the organism transfers a portion of its electrons to organic intermediate production. It appears that in many anaerobic settings, the coexistence or competition between SRB and methanogens--both H_2- (or formate-) consuming organisms--governs the delicate balance among the whole anaerobic community by mediating the H_2 partial pressure (or formate concentration) in the system [66, 103, 104].

The connection between microbial activity and actinide redox speciation (e.g., oxidation state) is clear. As organic-substrate degradation becomes dependent on each successive terminal electron acceptor, the overall redox potential of a portion of the aquifer changes from oxidizing to more reducing conditions. In this case, actinide speciation may be indirectly influenced by the microbial community utilizing their "normal" electron-acceptor substrates, as opposed to a direct electron transfer reaction between the microorganism and the metal. For example, microbial activity may provide a kinetic pathway to achieving thermodynamic equilibrium or generate reducing agents that are reactive toward actinides.

The effect of redox potential on actinide environmental chemistry has been discussed by numerous authors [19, 59-61, 75, 89, 105-110]. Although oxidation/reduction reactions in the environment are often dependent on kinetics, we can make some important generalizations by studying the predicted equilibrium redox behavior of the actinides. Figure 2.3 shows the expected dominant oxidation state in water for the actinides U, Pu, Np, and Am at pH 7 and in equilibrium with atmospheric carbon dioxide. Superimposed on Fig. 2.3 are the predicted redox potentials associated with the major biological redox couples. From Fig. 2.3, we predict that Pu(V/VI), Np(V), and U(VI) are the dominant oxidation states in oxygenated waters (+3 is the stable oxidation state for Am under most environmental conditions). Under most anaerobic conditions, the thermodynamically stable oxidation state for U, Pu, and Np is +4. We already discussed the biotic reduction of U in Section 2.1. In cases where the redox potential of the system can be reliably determined, the predicted redox speciation of Np, U, and Pu agrees well with experimental data. Cleveland and Mullin [75] found that +3 and +4 were the dominant Pu oxidation states in anaerobic groundwaters; Ahonen et al. [111] showed that groundwater E_h measurements could be used to predict the dissolved U(VI)/U(IV) ratio; and Hakanen and Lindberg [112] found that Np(IV) was the major oxidation state in pH 9 groundwater with a measured E_h of -290 mV.

It is important to note that the effects of microbial processes on subsurface redox processes are not completely understood. However, it is clear that in localized microcosms or in systems with large, active microbial populations, the redox potential can be correlated to the biological electron-acceptor couple. It remains to be seen to what extent these processes can affect actinide oxidation state distribution.

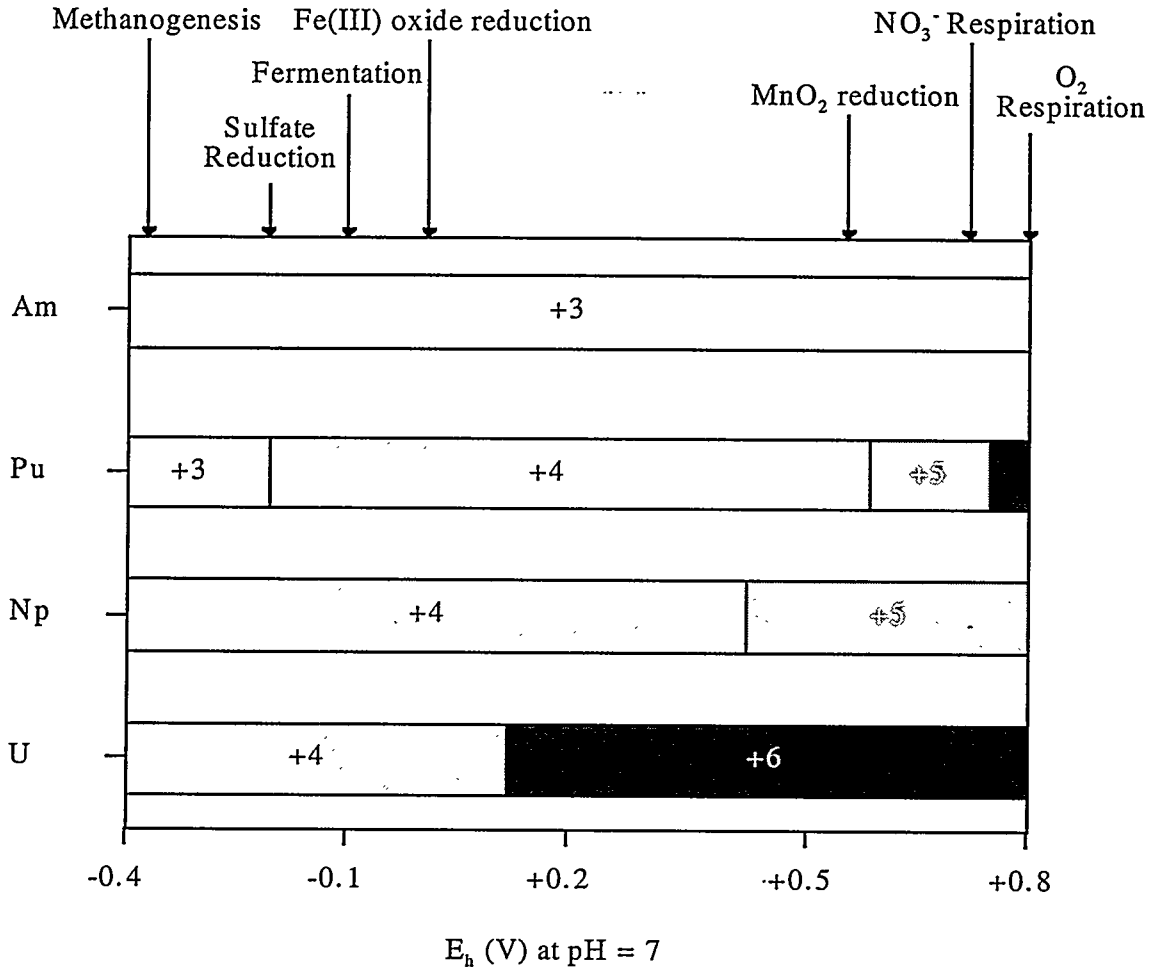


Fig. 2.3 - Expected dominant oxidation states of the actinides as a function of standard reduction potential in pH 7 water at equilibrium with atmospheric CO_2. Arrows at the top of the figure show the expected redox potentials associated with common microbial electron-acceptor couples. Adapted from Silva and Nitsche *[60]* and Stumm and Morgan *[90]*.

2.2.2 Effects of microbiological activity on pH

In addition to affecting the redox conditions in the subsurface, normal metabolic microbial processes can influence subsurface chemistry by exerting control over the pH of the system. As we have briefly discussed already, microorganisms routinely produce or consume a variety of organic compounds, many with acid-base and metal complexing properties. Likewise, many biologically mediated reactions include the consumption or production of acidic hydrogen, and nearly all microbial reactions evolve or consume $CO_2(g)$, which reacts with water to form carbonic acid. In subsurface environments, the response to microbial activity may or may not be manifested as a pH change, as the buffering capacity of many groundwaters can be relatively high [90, 113]. However, in laboratory systems pH changes due to microbial activity can be quite significant.

The simplest way to describe the potential effects of microbial metabolism on pH is to consider a few example degradation pathways. Recall that in Section 2.1 we combined an electron-donor reaction, acetate oxidation, with an electron-acceptor reaction, oxygen reduction, to develop the overall stoichiometry for acetate mineralization to carbon dioxide. If we now account for the production of biomass during acetate utilization (the methodology used to develop the stoichiometry of biological reactions is discussed in Section 4.1.1), the acetate degradation reaction becomes:

$$CH_3COOH + 0.234\,NH_4^+ + 0.828\,O_2 \rightarrow$$
$$0.234\,C_5H_7O_2N + 0.828\,H_2CO_3^* + 0.704\,H_2O + 0.234\,H^+$$

where $C_5H_7O_2N$ represents the average chemical composition of microbial biomass [114] and $H_2CO_3^*$ is the sum of carbonic acid (H_2CO_3) and dissolved carbon dioxide ($CO_2(aq)$). All species having an acid/base character are shown in their most protonated form, which creates a consistent reference level. By inspection of this reaction stoichiometry, we can see that one mole of acetate degradation results in:

1. consumption of one mole of acetic acid ($pK_a = 4.7$)
2. consumption of 0.234 moles of a much weaker acid, NH_4^+ ($pK_a = 9.3$)
3. production of 0.828 moles of a weaker acid, $H_2CO_3^*$ ($pK_{a,1} = 6.3$)
4. direct formation of 0.234 acid equivalents, H^+

The carbonic acid produced may or may not stay in solution, because $H_2CO_3^*$ can exchange with $CO_2(g)$ if a gas phase exists.

To quantify the potential pH effect, we consider the microbial degradation of one-half of an initial 10^{-3} M acetate in pH 7.0 water containing a total ammonia concentration of 10^{-3} M. We compute the effects for open, closed, and externally buffered systems. In an open system, carbonate evolves as $CO_2(g)$ and has no effect on pH. In this case, degradation of 5×10^{-4} M acetate consumes 1.17×10^{-4} M ammonia and produces 1.17×10^{-4} M acid equivalents, resulting in a pH increase to 7.90. In the case of a closed system, the degradation of the same amount of acetate produces 4.14×10^{-4} M carbonic acid, which is retained in the system, increasing the number of acid equivalents added to the water and resulting in a final pH of 7.25. In the case of an externally buffered system, the pH is no longer dominated by carbonate equilibria. For example, if our hypothetical reaction is carried out in a 10^{-2} M phosphate buffer, the pH only increases to about 7.07 and 7.01 in open and closed systems, respectively. This type of buffering is more characteristic of the subsurface.

Utilization of different electron-donor and -acceptor substrates can yield dramatically different pH effects. An example is the partial oxidation of pyruvate to acetate by sulfate-reducing bacteria:

$$CH_3COCOOH + 0.858\,H_2O + 0.0472\,NH_4^+ + 0.132\,SO_4^{2-} + 0.216\,H^+ \rightarrow$$
$$0.0472\,C_5H_7O_2N + CH_3COOH + 0.764\,H_2CO_3^* + 0.132\,H_2S$$

Investigation of this reaction in a manner parallel to our acetate example shows that degradation of one mole of pyruvate results in:

1. consumption of one mole of pyruvic acid ($pK_a = 2.5$)
2. consumption of 0.0472 moles of ammonia ($pK_a = 9.3$)
3. production of one mole of acetate ($pK_a = 4.7$)
4. production of 0.764 moles of carbonic acid ($pK_{a,1} = 6.3$)
5. production of 0.132 moles of hydrogen sulfide ($pK_a = 7.1$)
6. consumption of 0.216 acid equivalents, H^+

During the oxidation of 5×10^{-4} M of pyruvate in our pH 7 water initially containing 10^{-3} M ammonia and no hydrogen sulfide, 2.37×10^{-5} M ammonia and 1.08×10^{-4} M acid equivalents are consumed, and 3.82×10^{-4} M carbonic acid, 6.60×10^{-5} M hydrogen sulfide, and 5×10^{-4} M acetate are produced. If no hydrogen sulfide leaves the system, pyruvate degradation results in a change in pH to 7.34 and 6.12 in our unbuffered open and closed systems, respectively. Addition of 10^{-2} M phosphate buffers the pH change to 7.01 and 6.96, respectively. Thus, partial oxidation of pyruvate by SRB can cause either an increase or decrease in pH, depending on the fate of the carbonic acid produced by the reaction, while aerobic oxidation of an equal molar amount of acetate causes a pH rise in all cases.

The control of pH is a very important component in performing actinide-microbiological studies in the laboratory. Observed pH changes during microbial substrate utilization in weakly buffered systems can be significant. For example, Joshi-Topé and Francis [115] observed a pH increase from 6.1 to 7.9 during the aerobic degradation of 0.52 mM citrate by a *Pseudomonas fluorescens* sp. Microbially mediated pH effects of this magnitude can substantially alter metal solubility and speciation. Joshi-Topé and Francis [115] also found that the pH increase associated with citrate degradation caused the precipitation of Fe, Zn, and Ni from solution. Banaszak et al. [116] showed that a pH increase from 7.0 to 7.9, caused by the degradation of nitrilotriacetate (NTA) by *Chelatobacter heintzii,* resulted in the formation of a colloidal/precipitate Np-phosphate phase and a decrease in Np solubility.

Biologically mediated changes in pH have several possible effects on actinide speciation. The pH dependence of actinide redox couples is well known: Acidic conditions shift the equilibrium toward the +4 and +3 oxidation states, while neutral to basic pHs tend to stabilize the +5 and +6 species [105, 107, 117, 118]. However, the role of microorganisms as catalysts of redox reactions expands the range of compounds that can serve as electron donors or acceptors. Thus, the pH dependence of actinide oxidation or reduction in biological systems cannot be discerned without including the electron-acceptor or -donor half reaction. For example, if we consider actinide reductions using H_2 as the electron donor and ignore biomass formation, the full redox reactions are written as:

$$\frac{1}{2}H_2 + AnO_2^{2+} \Leftrightarrow H^+ + AnO_2^+ \qquad An(VI/V)$$

$$H_2 + 2H^+ + AnO_2^+ \Leftrightarrow An^{4+} + 2H_2O \qquad An(V/IV)$$

$$\frac{1}{2}H_2 + An^{4+} \Leftrightarrow H^+ + An^{3+} \qquad An(IV/III)$$

Thus, the reduction of the An +6 and +4 oxidation states using hydrogen as an electron donor generates acid, while the reduction of the +5 oxidation state consumes acid.

The aqueous speciation of actinides in a given oxidation state is also affected by changes in pH. Fig. 2.4 shows the calculated speciation of Np(V) as a function of pH in water open to the atmosphere. In general, the speciation behavior of Np follows that seen for other metals in waters without strong organic complexants. At low pH, most of the actinide is present as the free aquo ion, NpO_2^+ in this case. As the pH increases, the metal ion forms complexes with the hydroxide, bicarbonate, and/or carbonate anions. In the case of Np(V), the aquo ion is totally dominant up to pH 7, carbonate complexation dominates Np speciation at pHs above 8.5, and hydroxide complexation is relatively unimportant. In the general case, the relative stability of the free metal actinide species in water decreases as the charge-to-ionic radius ratio increases due to shifting of the onset of hydroxide complexation to lower pHs, as is shown in Fig. 2.4 [60]. Thus, while hydroxide complexation is almost negligible in the case of the An(V) oxidation state, it dominates An(VI) speciation in the pH range 5-7, An(III) speciation in the pH range 6-9, and An(IV) speciation at all pHs greater than 2-4, depending on the total actinide concentration [60].

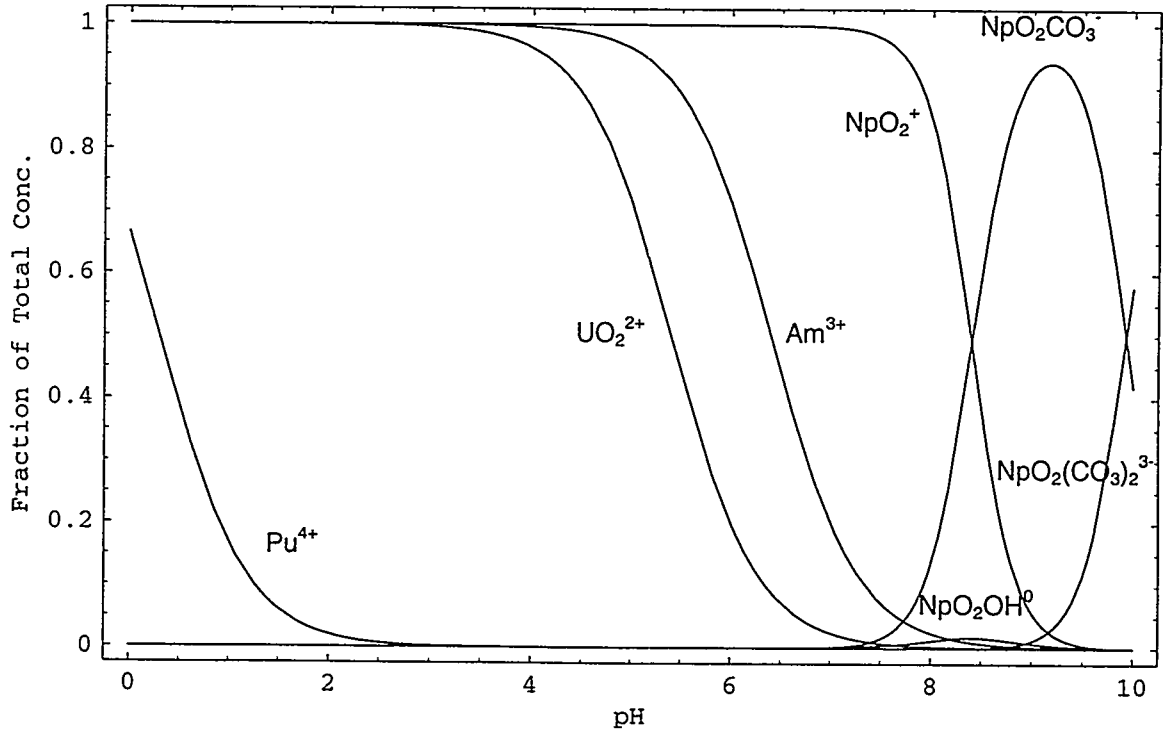


Fig. 2.4 - Calculated relative speciation of 10^{-5} M Np(V) in water at equilibrium with atmospheric CO_2. The free metal ion (NpO_2^+) dominates neptunium speciation up to pH 8.4. At higher pH values, carbonate complexes dominate Np speciation. Shown for comparison are the calculated free metal ion concentrations for Am(III), Pu(IV), and U(VI) under the same conditions. As the charge-to-ionic radius on the actinide increases, the onset of hydroxide complexation with the metal shifts to lower pH values, reducing the pH range of stability of the aquo metal species.

Changes in subsurface pH also can have a major impact on the sorption of actinides to soil surfaces, colloidal particles, and bacterial cell membranes. The mechanisms of adsorption of radionuclides onto mineral surfaces and particles have been discussed in by numerous investigators [19, 35, 59, 119-130]. The surfaces of organic matter and biological structures and compounds are characteristically composed of functional groups that, depending on pH, can ionize; this ionization results in a localized surface charge and the attraction or repulsion of ionic actinide species [90]. For example, we consider a model surface site composed of one carboxyl and one amine functional group. Depending on the pH, which controls the equilibrium of the functional groups with the surrounding solution, the net charge of the surface site is positive, neutral, or negative:

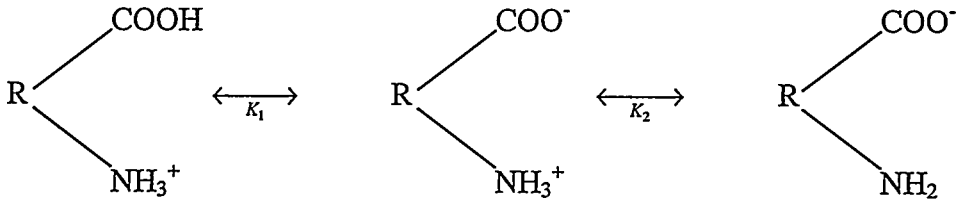


Figure 2.5 illustrates the transition from +1 net charge to -1 net charge as the pH increases from 4 to 10. Although organic surfaces in the environment contain a wide diversity of functional groups, the surface charge on organic material is negative for pHs greater than about 5 [90].

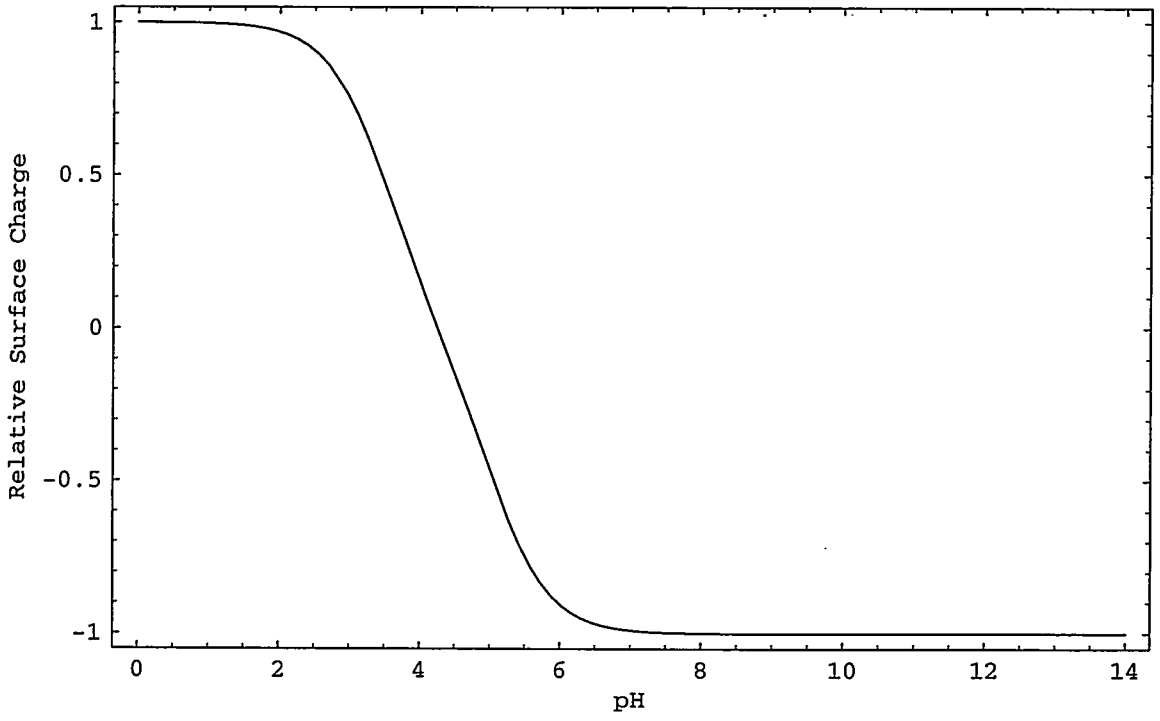


Fig. 2.5 - The relative surface charge associated with the hypothetical functional groups described in the text. The overall charge on surfaces can be positive or negative, depending on pH. For this example, $pK_1 = 3.5$ and $pK_2 = 5$.

In a manner similar to aqueous coordination chemistry, radionuclides (and metal-ligand complexes) undergo pH-dependent complexation reactions with surface functional groups and sorbed ligands, resulting in the partitioning of the metal between the solid and solution [59, 121, 124, 125, 127, 128, 131]. Since sorption is one of the primary mechanisms affecting radionuclide migration in the subsurface, it is imperative that we correctly account for changes in the acid/base chemistry brought about by microbial reactions [19].

In some cases, biological activity affects the surfaces themselves. Of course, the microorganisms are organic surfaces having adsorptive properties. Microbial activity also affects the amount and characteristics of mineral surfaces by controlling rates of mineral precipitation and/or dissolution in response to microbially catalyzed formation of acids or bases and production or consumption of ions that form the minerals themselves (e.g., CO_3^{2-}, OH^-, S^{2-}, and Fe^{3+}) [63, 89, 113, 132, 133]. Thus, the biological activity, equilibrium chemistry, and geology of a particular subsurface site are intimately linked. Because of the highly complex interactions possible in the subsurface, it is difficult to predict *a priori* what effect biological activity will have on the fate of actinides in the system. In Section 4.1, we discuss methods for coupling biological reactions to chemical speciation.

2.3 Biodegradation of organic compounds and chelating agents

Organic compounds, naturally occurring and anthropogenic, are present in most subsurface environments. Biodegradation of these organics, even those that do not strongly complex actinides, can have a pronounced effect on actinide speciation and migration. The fate of actinides in a biologically active subsurface environment is an important issue for existing contaminated subsurface sites, as well as for proposed low-level, high-level, and TRU nuclear waste repositories.

In many cases, organic chelating agents [e.g., NTA, ethylenediaminetetraacetic acid (EDTA), citric acid, oxalic acid, and organic phosphates] used in the processing of nuclear fuel usually were disposed of along with the actinide contaminants. Chlorinated hydrocarbons and fuel hydrocarbons also are present in some cases [11] and are targets for *in situ* bioremediation efforts. A strategy designed to remediate these hazardous organic species must also account for the effects of bioremediation on the mobility of the actinide species present. For these reasons, we summarize biodegradation possibilities for some important organic compounds to highlight their possible impact on actinide fate [we refer the reader to several comprehensive discussions of organic biodegradation mechanisms for more detailed information [14, 16, 17, 134-138]].

Table 2.1 shows biodegradation routes and degradation by-products for several of the organic contaminants commonly found co-disposed with actinides on DOE lands [11]. We illustrate the range of possible biodegradation mechanisms for representative organic compounds in each class.

The chlorinated solvent, trichloroethylene (TCE), is a key example. Under aerobic conditions, TCE is co-metabolized to CO_2, H_2O, and HCl by bacteria utilizing a small range of primary electron-donor substrates that require monooxygenation as a first step. Stimulation of aerobic TCE biodegradation requires a supply of O_2 and a suitable electron donor, such as methane, as well as microorganisms carrying out monooxygenation of that donor. On the other hand, the anaerobic biodegradation of TCE proceeds via reductive dechlorination of the halogenated hydrocarbon catalyzed by a variety of anaerobic organisms. As opposed to the

complete mineralization of TCE under aerobic conditions, the anaerobic biotransformation of TCE produces dichloroethylene (DCE) and vinyl chloride (VC) as intermediate compounds and ethylene as the final reduced product. During anaerobic degradation of TCE, the relative abundance of the intermediate compounds DCE and VC, as compared to the amount of contaminant fully mineralized to harmless products, may be governed by the interactions among several organisms in anaerobic microbial communities and the availability of specific electron donors [139, 140], and it is not completely understood. For example, although the rate of reductive dehalogenation of TCE to DCE can be catalyzed by pure cultures of bacteria, complete mineralization of TCE requires a consortia of microbial species [14, 16, 17, 134-138]].

Bioremediation scenarios using one or the other of the two bioremediation alternatives for TCE, co-metabolic oxidation vs. reductive dechlorination, will have dramatically different effects on the actinide species co-disposed with the solvent. Co-metabolic degradation involves a strongly oxic environment and aerobic microorganisms carrying out specialized mono-oxygenation reactions. Reductive dechlorination involves strongly reducing conditions and consortia of anaerobic microorganisms that utilize simple electron donors, such as H_2, formate, or organic substrates fermented to them.

Carbon tetrachloride is a second chlorinated solvent. It represents a chlorinated aliphatic that is reductively dechlorinated by a range of microorganisms carrying out anaerobic respiration [91, 102, 135, 136, 141, 142]. Carbon tetrachloride is not susceptible to aerobic biotransformation.

Another class of organic compounds listed in Table 2.1, the fuel hydrocarbons, is represented by the routes of benzene and toluene biodegradation. As we mentioned in Section 1.2, the *in situ* bioremediation of petroleum hydrocarbons is a relatively mature technology and usually results in mineralization of the hydrocarbons in the presence of a capable consortium of microorganisms. However, petroleum-hydrocarbon bioremediation has never been attempted in the presence of actinides, warranting studies into the possible effects of fuel bioremediation on actinide fate. Biodegradation of petroleum hydrocarbons is usually accomplished with aerobic conditions in which O_2 transfer is critical; however, anaerobic biodegradation of petroleum hydrocarbon is being documented [86, 143, 144].

The final group of organic compounds listed in Table 2.1 is the organic acids, including the organic chelating agents. As opposed to the halogenated and petroleum hydrocarbons, which probably do not directly interact with actinides in the subsurface, many of the organic acids and chelates form soluble complexes with actinides, potentially enhancing their mobility. Also, many biologically produced organic compounds have similar properties to anthropogenic chelating agents. For these reasons, much research has focused on the interactions of organic chelates and actinides in biologically active systems. We detail much of this work here by describing the biodegradation of one natural organic acid, citrate, and two anthropogenic chelates, NTA and EDTA.

Table 2.1 - Biodegradation routes for naturally occurring and anthropogenic organic compounds found co-disposed with actinides on DOE lands.

Organic Class and Representative Compounds	Biodegradation Routes	Products	Example References
Chlorinated Solvents			
Trichloroethylene (TCE)	Aerobically by bacteria carrying out monooxygenation.	CO_2, HCl	[287, 288]
	Anaerobically under sulfate-reducing and methanogenic conditions.	Dichloroethylene (DC), vinyl chloride (VC), ethylene, and HCl	[240, 289]
Carbon tetrachloride (CT)	Anaerobically under denitrifying, metal-reducing, sulfate reducing, and methanogenic conditions.	Chloroform, dichloromethane, methane	[91, 102, 135, 136, 141, 142, 290]
Fuel Hydrocarbons			
Benzene and toluene	Aerobically via mono- or di-oxygenation.	CO_2	[16]
	Anaerobically by iron-reducing bacteria.	CO_2	[86, 143, 144]
Organic Acids			
Citrate	Aerobically by a variety of organisms via the tri-carboxylic acid (TCA) cycle.	CO_2	[37]
	Fermented by a variety of organisms. Anaerobically by nitrifying and metal-reducing bacteria.	CO_2, various organic acids and alcohols	[37, 158]
NTA	Aerobically by organisms with the NTA-monooxygenase enzyme.	CO_2, NH_4^+	[150, 159-164]
	Anaerobically by denitrifying bacteria with the NTA-dehydrogenase enzyme.	CO_2, NH_4^+	[168]
EDTA	Aerobically by BNC1 and during wastewater treatment.	CO_2, NH_4^+, and unidentified organic products	[147, 149, 151, 172]

2.3.1 Properties of organic chelates

Chelates are natural or anthropogenic multi-dentate ligands that form stable soluble complexes with metals, including actinides. Because of their strong affinity for complexation with radionuclides, chelating agents were frequently used as actinide extractants and for decontaminating nuclear reactors at weapons production facilities [8, 11, 47], although they can occur as the result of natural processes, as in the case of citrate [145, 146]. The most common chelates found on U.S. DOE lands include NTA, EDTA, citric and oxalic acids, and other organic acids and chelating agents [11]. Although the focus of current research is on the relationship between these organic ligands and radionuclides, the applicability of these studies is far-reaching, since many of these same compounds are among the most common contaminants found at other sites where toxic metals are also present. For example, NTA and EDTA were originally added to detergents as a phosphate substitute and bleach stabilization element, respectively, and EDTA is a common contaminant in pharmaceutical and photo-processing wastewaters [147-152].

In many cases, the presence of a chelate in the subsurface defines the chemical speciation of the resident metals and radionuclides. Free metal ions, hydroxides, and oxides often sorb onto soil particles, effectively becoming immobilized. However, complexation by a strong ligand solubilizes the metal species, dramatically increasing its mobility in the groundwater [8, 45, 48]. Thus, mobile radionuclides now are viewed as a potentially major threat to populations near contaminated lands [8, 11, 55].

Consistent with the other chemical properties of the actinides, the strength of the metal-chelate complex--thus, the degree to which the chelate dominates actinide speciation--depends on the oxidation state of the actinide. In general, the magnitude of the formation constant for a given ligand with a particular oxidation state decreases in the order $An^{4+} > An^{3+} \cong AnO_2^{2+} > AnO_2^{+}$ [60, 105]. In natural systems, the speciation of an actinide is determined by competition between these organic chelates, natural organic matter, and the inorganic ligands present. At high pHs, the carbonate and hydroxide ligands often out compete the strongest organic chelates for actinide complexation [60, 105, 153].

Figure 2.6 presents the calculated speciation of 10^{-6} M Pu(IV) and 10^{-6} M Pu(V) in pH 6 water in contact with atmospheric CO_2 as the total NTA concentration is increased. This figure illustrates two key aspects of actinide-chelate formation constants. By comparison of their formation constants alone, log β = 12.82 for Pu(IV)-NTA and = 6.75 for Pu(V)-NTA [81], it would appear that NTA should complex more with Pu(IV) than with Pu(V). The opposite is true. Whereas PuO_2^{+} is the dominant form of uncomplexed Pu in the +5 oxidation state at moderate pH, Pu(IV) occurs as the oxyhydroxy polymer and as hydroxide, carbonate, and mixed hydoxy-carbonate complexes. As NTA is added to the system, it complexes with the most available metal. When the ligand-to-metal ratio is 1:1, only about 5% of the Pu(IV) is bound as the NTA complex, while about 75% of the Pu(V) is bound to NTA. In this case, NTA is out competed by other ligands (OH^-, HCO_3^-/CO_2^{2-}) for the Pu(IV) ion, in particular by $Pu(OH)_4^0$. Even when the ligand-to-metal ratio is 50:1, NTA complexes with only about 45% of the available Pu(IV), although essentially all of the Pu(V) is complexed.

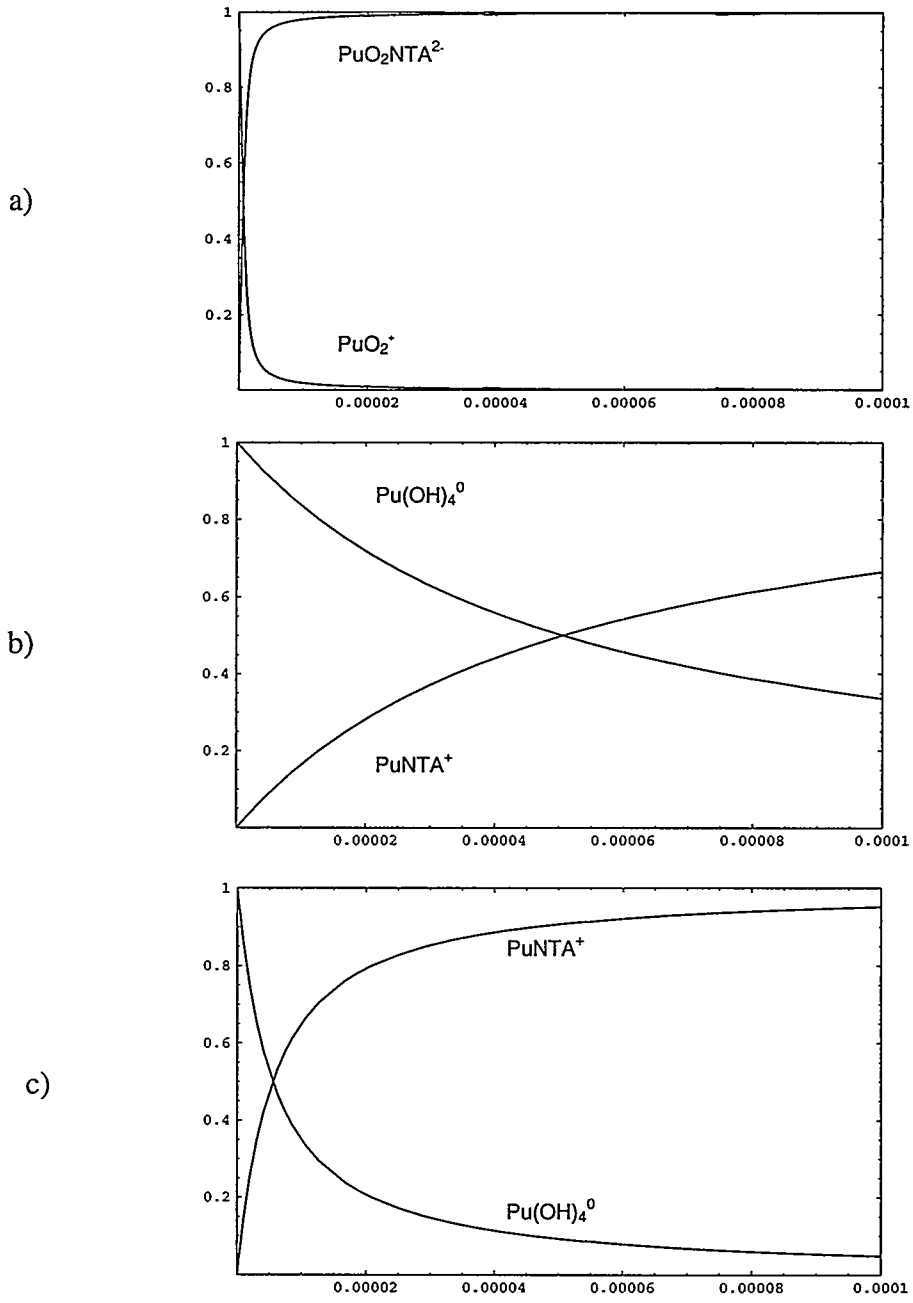


Fig. 2.6 - Aqueous speciation of 10^{-6} M Pu(V) (a) and Pu(IV) (b) as a function of total NTA concentration, in pH 6 water at equilibrium with atmospheric CO_2, based on calculations made using published stability constants (refer to Section 4.2.1 for a detailed discussion of the species considered in the creation of this figure) for NTA, carbonate, hydroxide, and carbonate-hydroxide complexes. For (c), the Pu(IV)-NTA complexation constant is one order of magnitude higher than reported. The relative strength of the Pu-NTA complex depends on the difference between the organic complex strength and the stability of inorganic Pu complexes.

The second aspect illustrated by Fig. 2.6 is that a relatively small error in the formation constant can translate into a large difference in calculated speciation. This factor is significant because complex-formation constants are not known for as many actinide-ligand species or with the same degree of confidence as constants determined for other metals. As the strength of the actinide/chelate complex increases relative to the affinity of the actinide ion for the common inorganic ligands found in natural waters, the speciation behavior of the system shifts toward the actinide-chelate. This is shown in Fig. 2.6c for Pu(IV) when the formation constant is increased from $10^{12.8}$ (Figs. 2.6a and b) to $10^{13.8}$; Pu(IV)NTA is a much more important species for all NTA concentrations, and $Pu(OH)_4^0$ becomes less important.

The presence of organic chelating agents can preferentially stabilize certain actinide oxidation states [154]. Because of the relative complex strength, chelation tends to stabilize the +4 oxidation state, expanding the range of E_h and pH values for which the An(IV)-chelate complex predominates. Al Mahamid et al. [81] showed that Pu complexed with NTA as either the +3, +5, or +6 oxidation state eventually was reduced (or, in the case of Pu^{3+}, oxidized) to the Pu(IV) complex. Additionally, recent evidence suggests that chelates function as "catalysts" for biotic and abiotic electron transfer reactions. Reed et al. [154] found that EDTA, citrate, and oxalate reduce An(VI) to An(V/IV) in WIPP brines. In biological systems, the presence of organic chelating agents accelerates the growth of organisms utilizing solid electron-acceptor substrates [155]. Much more research is needed before we fully understand the role chelates play in defining the actinide oxidation state.

2.3.2 Biodegradation of organic chelates

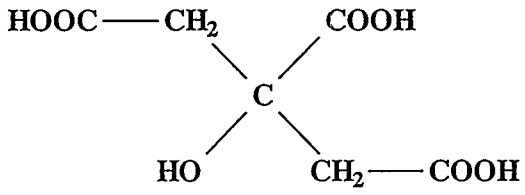
The importance of the interrelationships between organic complexants and microbial activity in determining actinide speciation and mobility in subsurface environments cannot be overstated. Biodegradation of chelates affects radionuclide speciation by three mechanisms: Chelate biodegradation (1) removes complexant from solution, liberating metals from the complex; (2) affects the system pH, since the chelates are conjugate bases of organic acids; and (3) alters redox speciation, since complexants tend to preferentially stabilize certain actinide oxidation states. Another interaction between chelates and microorganisms is that the presence of complexants can mitigate or promote radionuclide toxicity to microorganisms. Finally, since the properties of many natural organic compounds and microbially derived by-products found in the subsurface mimic the behavior of anthropogenic complexants, understanding the effect of chelate biodegradation on the fate of radionuclides in the subsurface has broad applicability to many *in situ* bioremediation efforts.

In addition to their effect on radionuclide chemistry, most chelating agents can serve as substrates for bacterial growth. The highest biodegradation rate among common chelates is associated with naturally occurring organic acids, like citric acid. Citric acid, shown schematically in Fig. 2.7a, is found as a naturally occurring compound, as it can be produced by microorganisms, especially fungi [37]. Citrate can form monodentate, bidentate, tridentate, and polynuclear complexes with a variety of metals. Since citrate is ubiquitous in nature and is a key intermediate in the tricarboxylic acid cycle, it can act as a sole carbon and energy substrate for a wide variety of bacteria [37]. The type of complex formed can significantly affect the rate of substrate utilization, which is discussed in detail in Section 4.1.3.

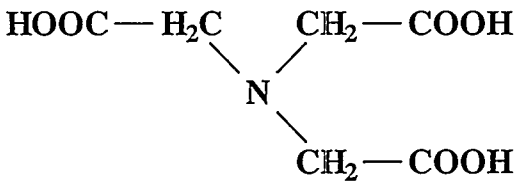
Microorganisms oxidize citrate in the presence of toxic or radioactive metals. For example, a *Pseudomonas* sp. was able to degrade citrate in the presence of a variety of toxic

metals [115, 146, 156], and a *Citrobacter* sp. immobilized uranium as a phosphate precipitate while metabolizing citrate [21, 157]. Citrate can also be fermented anaerobically by members of *Clostridia* sp., phototrophic bacteria, enteric bacteria, and lactic acid bacteria through an incomplete tricarboxylic acid cycle catalyzed by the citrate lyase ligase enzyme [37, 158].

a)



b)



c)

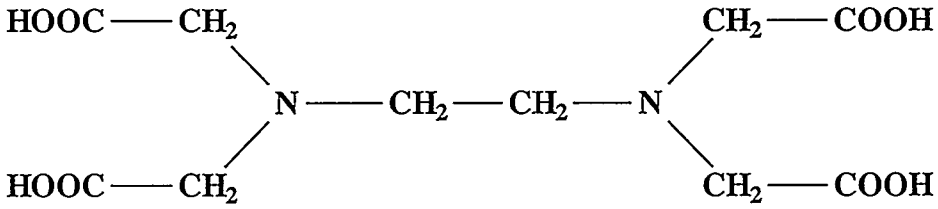


Fig. 2.7 - Structure of the organic chelating agents citrate (a), NTA (b), and EDTA (c).

The structure of NTA is similar to that of citrate, the major difference being the replacement of the central carbon-hydroxyl group in the citrate molecule with nitrogen (Fig. 2.7b). NTA is degradable in the presence of other carbon sources, and it is rapidly degraded in aerobic wastewater treatment systems, where daily loading rates can average 0.13 gm person^{-1} day^{-1} [147]. It is also anaerobically degradable by facultative denitrifying bacteria [159-161]. The ability to degrade NTA is widespread among aqueous and soil microorganisms [150, 159-164], and bacteria will degrade NTA in the presence of heavy metals [163] and the actinides Np, U, and Pu [116, 165].

NTA can be used as a sole C and N source by bacteria and is aerobically cleaved to glyoxalate and iminodiacetate (IDA), which is later oxidized to glyoxalate and glycine [162]. Much work has focused on NTA degradation by *Chelatobacter* spp. The first step in the aerobic degradation of NTA by these organisms is the activation of the molecule by the NTA-monooxygenase (NTA-MO) enzyme [159, 166], implying that the aerobic degradation of NTA by these organisms is strongly O_2 dependent. Various studies showed that the NTA-MO reaction required the presence of Mg^{2+}, Mn^{2+}, and Co^{2+} as co-substrates for the enzyme [159, 160, 162, 166, 167], and NTA utilization is much greater in systems with mixed carbon sources, which would be the predicted response for a monooxygenase-catalyzed reaction because of its requirement for an electron-donor co-substrate. In the presence of Mg^{2+}-scavenging compounds, such as high concentrations of EDTA, the activity of the NTA-MO enzyme decreases [167]. The anaerobic degradation of NTA proceeds via a NTA-dehydrogenase reaction that is coupled to the reduction of nitrate via the nitrate reductase enzyme [168].

The EDTA molecule consists of four carboxylic groups and two amine groups, all of which are capable of interacting in acid-base and complexation reactions (Fig. 2.7c). Because of the availability of multiple complexation sites, metal-EDTA complexes tend to be extremely stable and usually control the speciation of any metals in moderate pH systems when the ligand is present in concentrations in excess of the total metal concentration. The biological behavior of EDTA is quite different from that of citrate and NTA. Originally, EDTA was completely resistant to degradation by microorganisms in a variety of systems [169-171]. More recent studies show that EDTA is slowly degradable in soils, sediments, and wastewater by pure and mixed cultures, as well as during wastewater treatment under alkaline conditions [147, 149, 151, 172]. The data on the degradability of EDTA are by no means conclusive at this point, and current studies are focusing on the behavior of EDTA in biological systems contaminated with metals and radionuclides [151, 173, 174].

We stop our general summary of the biodegradation of organic complexants. Subsequent sections provide detailed discussions of specific interactions among actinides, microorganisms, and organic chelates. In Section 3.2, we discuss the importance of complexation for mitigating actinide toxicity; in Section 3.3, we evaluate the potential effects of actinides on organic substrate bioavailability; and in Section 4.1.3, we show how coupled processes, namely organic substrate biodegradation and chemical speciation, can be accurately modeled.

2.4 Bioprecipitation

Bioprecipitation of a metal is the biologically mediated precipitation of the metal in an insoluble form, sometimes referred to as *biomineralization*. Biologically induced precipitation of actinides is currently the focus of much research, because it is being considered as a strategy to reduce actinide migration in the subsurface. We already introduced one form of bioprecipitation in Section 2.1: the precipitation of a reduced metal species after a respiratory electron transfer from the organism to the metal. Although it may be argued that the actual precipitation reaction was an *indirect* chemical process caused by *direct* metal reduction, precipitation in some cases occurs spontaneously only in the presence of biological activity. Thus, although the precipitation could be reproduced using chemical manipulations, sometimes precipitation requires the presence of the bacteria themselves. Our discussion of bioprecipitation, then, focuses on the direct bioprecipitation of metals by microorganisms.

The possible mechanisms leading to bioprecipitation of metals and radionuclides have been reviewed in considerable detail [35, 175], and many of the discussions focused on the possible immobilization of radionuclides at contaminated sites and repositories [26, 30, 35, 109, 176, 177]. Recent evidence implicates biological processes as the drivers for diverse mineralogical deposits [e.g., see 38, 39, 69, 70, 178]. Our goal in this section is to describe the relationships between the thermodynamics and kinetics of metal/actinide precipitation and the possible biochemical influences of an active microbial population.

In a homogeneous system, solid precipitation is controlled by chemical equilibrium and kinetics. In the absence of nucleation sites, precipitation only occurs after the solution has become supersaturated to the point where the energy released during precipitation can overcome the energy required for initial nucleation of the solid [90, 113]. In the case of heterogeneous nucleation, however, the presence of nuclei lowers the free energy barrier to precipitation, decreasing the amount of supersaturation required to initiate precipitation. The degree to which the nucleation free-energy barrier is reduced is related to the structural similarity between the nuclei and the precipitate; the more similar the precipitate is to the nuclei, the lower the energy barrier to precipitation [90, 178].

As we discuss in detail in Section 2.5, the cell walls and membranes of microorganisms are not uniform structures. Rather, they consist of large areas of lipid bi-layers interspersed with various membrane-bound proteins, many of which can act as specific metal binding sites and can be viewed as ideal heterogeneous nucleation sites [175, 176]. Thus, oftentimes bacteria can accelerate precipitation reactions by merely acting as nucleation sites, reducing the free energy barrier to the point where precipitation becomes possible. Additionally, the external membranes of microorganisms usually have an overall negative charge that can attract positively charged metal species as counter ions. The resulting sorbed metal complex can serve as a nucleation site for solid precipitation [175].

Another way that microorganisms can accelerate precipitation reactions is by influencing the local chemical gradients in the system. Once precipitation is initiated, the rate of solid crystal growth is a function of the degree of solution supersaturation (i.e., the thermodynamic driving force between the solution chemistry and equilibrium), mass transfer of chemical species to the solid surface, and the interfacial area of the solid (i.e., area available for solid accumulation). The relationship can be described as [90, 113]:

$$\frac{dSp_{solid}}{dt} = k\,a\left(1-\frac{K_{sp}}{Q}\right)[Sp]$$

where the left-hand rate term represents the rate of precipitation (or dissolution if it has a negative sense) of the solid phase, Q equals the ion activity product of the species in solution, k represents a mass-transfer rate constant for precipitation or dissolution, a is the interfacial area of the solid surface, and [Sp] is the concentration of the rate-controlling species in solution. Since Q is proportional to [Sp], higher concentrations of the species of importance increase the rate of solid precipitation. As we discussed in previous sections, microbial metabolic reactions release various by-products, some of which can increase Q and/or [Sp].

A prime example is carbonic acid generation. As an organism metabolizes its substrates, H_2CO_3 is excreted from (or, in the case of autotrophic growth, transported into) the cell into the

surrounding environment. The carbonate concentration immediately adjacent to the cell is then governed by the rate of H_2CO_3 mass transfer away from the bacteria. When the rate of H_2CO_3 production exceeds the rate at which it is removed from the surroundings, the carbonate concentration adjacent to the active cells is elevated. If the carbonate concentration adjacent to the cells builds up enough to exceed the solubility of a metal carbonate species, metal-carbonate precipitation can take place on the surface of the organism.

Local concentration gradients of other species affected by metabolic reactions can cause bioprecipitation. In addition to carbonate, microbial activity can alter the concentration of precipitating anions such as S^{2-}, SO_3^{2-}, NO_3^{2-}, and PO_4^{3-}, and can affect the speciation of ligands and metal cations adjacent to the cell by direct production or consumption of H^+.

Precipitation due to local gradients in concentration or pH is likely important in systems with relatively high resistance to mass transport. Typical environments where conditions such as these predominate include groundwater aquifers, lake and ocean sediments, and thermo- and chemo-clines in stratified lakes. Each type of environment yielded bacteria encased with metal precipitates formed from metabolic by-products. For example, Schultz-Lam et al. [175] isolated SRBs from a mine tailings pond covered with iron sulfide precipitates; Sawicki et al. [179] found organisms surrounded by siderite ($FeCO_3$) in an anaerobic biofilm growing on the rock walls of an underground laboratory; and Lienemann et al. [180] discovered cobalt co-precipitated in the manganese oxide coating of bacteria inhabiting the oxy-cline in a stratified lake.

In systems with high by-product formation rates, metal bioaccumulation can occur in regions exposed to flowing aqueous streams, implying that bioprecipitation processes may be effective methods for cleaning contaminated waste streams. In a series of studies, Macaskie and co-workers [20, 21, 29-32, 157, 181-184] investigated the removal of actinide and lanthanide elements from dilute aqueous streams via bioprecipitation using a mutant *Citrobacter* species capable of overexpression of a membrane-bound phosphatase enzyme. The function of the phosphatase enzyme is to liberate inorganic phosphate from an organic phosphate substrate, in this case, glycerol phosphate. The continuous expression of the phosphatase enzyme resulted in the decoupling of enzyme activity from the growth of the organism. By immobilizing the mutant *Citrobacter* sp. in a biofilm column reactor, these researchers were able to relate the removal of U, Am, Pu, and Th by phosphate precipitation to the activity of the enzyme and the solution chemistry of the influent stream [30-32, 157, 183].

Table 2.2 summarizes the actinide removal rates found by Macaskie and co-workers using phosphate bioprecipitation, along with some of their observations regarding important system parameters. The results of their work highlight the importance of completely unraveling the relationships among the biological, hydrodynamic, and chemical mechanisms active in the system under study. It also serves as a preview of our discussion of the importance of actinide toxicity, substrate bioavailability, and modeling coupled processes in Sections 3 and 4. At first glance, the maximum removal rates shown in Table 2.2 appear to say that the solubilities of An(IV) phosphates are higher than those for the III and VI oxidation states. However, the opposite is true; the IV actinide oxidation state in general has the lowest solubility in phosphate solution [32, 60]. However, the An(IV) oxidation state also tends to complex with the hydroxide anion and polymerize at neutral pH, effectively lowering the free metal concentration and, hence, the thermodynamic driving force for phosphate precipitation [32].

Table 2.2 - Summary of actinide removal rates by phosphate bioprecipitation using a Citrobacter sp.

Actinide	Maximum Removal (%)	Removal *Increased* by: Low flow rates and phosphatase overproduction in every case[a,b,c,d,e]	Removal *Decreased* by: High flow rates and normal phosphatase activity in every case[a,b,c,d,e]
U(VI)	100[a]	Citrate addition to reduce metal toxicity[b] Addition of NH_4Acetate to form lower solubility precipitate[c]	High or low pH due to phosphate speciation[c]
Am(III)	100[d]	Column priming w/ $LaPO_4$ for precipitate nucleation[d]	
Pu(IV)	50[d]	Column priming w/ $LaPO_4$ for precipitate nucleation[d]	
Th(IV)	80[e]	Column priming w/ $LaPO_4$ for precipitate nucleation[d] Lower citrate concentration to increase free metal concentration[e] Addition of NH_4Acetate to form lower solubility precipitate[e]	Low pH (below 5) due to unavailability of HPO_4^{2-} species[e]

[a][31].
[b][182].
[c][183].
[d][30].
[e][32].

The same reasoning can be used to describe the sensitivity of An(IV) removal to the citrate concentration in these experiments. Although complexation with citrate increased the measurable solubility (total amount of actinide in solution), the free metal concentration was reduced, thereby decreasing the driving force for precipitation. On the other hand, citrate addition for U(VI) increased removal rates by reducing the chemical toxicity of the actinide [32]. Thus, the optimum citrate addition had to be tailored to the oxidation state of the target actinide.

Changing pH had several effects on actinide removal rates: low pH (below 5) increased the concentration of the free metal species but decreased the concentration of HPO_4^{2-}, the precipitating anion; the net effect was to reduce the removal efficiency in all cases [32, 183]. High pH increased the HPO_4^{2-} concentration but decreased the free metal concentration, negatively affecting the removal efficiency for U(VI) [32]. These researchers are continuing their investigation into the complex interactions in this system.

Bioprecipitation can be an internal process as well, and the internal sequestering of metal precipitates by microorganisms is often defined as *biouptake*. An example of internal metal sequestering by *P. syringae* was described by Cooksey [185]. In this case, the organism possessed a genetically encoded resistance to copper toxicity. As the bacteria were exposed to inhibitory concentrations of copper, a specific copper-binding protein was synthesized, causing the accumulation of blue Cu^{2+} in the periplasm and outer membrane of the organism. Genetically encoded resistance to metal toxicity is common, and the response mechanisms are

quite varied. Two *P. aeruginosa* strains accumulated and concentrated high amounts of cadmium intracellularly [186], a process that has been linked to the formation of insoluble sulfides and phosphates inside the cells of *Klebsiella aerogenes* strain NCTC 418 [187]. Studies on the organism *Alcaligenes eutrophus* (CH34) described plasmid-encoded resistance to Co^{2+}, Ni^{2+}, CrO_4^{2-}, Hg^{2+}, Tl^{+}, Cd^{2+}, Cu^{2+}, and Zn^{2+} ions. This process operates by an efflux mechanism that causes an increase in the pH outside of the cytoplasmic membrane. Sequestered metal carbonates are then bioprecipitated in the saturated zone around the cell membrane [188].

As in abiotic surface chemistry, the line where precipitation ends and surface complexation begins has not yet been clearly delineated in biological systems. However, we limited our discussion in this section to true solid formation reactions, which are often irreversible and dramatically retard the migration of radionuclides in the subsurface.

2.5 Biosorption/extracellular complexation

One of the most prevalent and well-studied interactions between metal ions and microorganisms is the sorption or complexation of metals by cellular materials. As organic "particles," microbial cell walls can specifically or non-specifically bind metal ions through physical or chemical adsorption mechanisms. Some microorganisms produce compounds as part of their normal metabolic processes or to facilitate their adhesion onto solid surfaces. Many of these compounds have metal-binding capabilities. Additionally, microorganisms are known to excrete metal complexing agents to uptake metals as nutrients or that reduce or eliminate the toxicity of specific metal ions. Except in cases where the cells are artificially permanently immobilized, all of the mechanisms described above can enhance the mobility of the sorbed or complexed metal.

Analogous to the world of macroscopic biology, the properties of the external cell membrane in microorganisms are as diverse as the number of microbial species and, in many instances, are related to the external environment to which an organism is exposed. It should come as no surprise, then, that the interactions between the "zone of influence" of a living cell and the trace elements in contact with that cell are also quite diverse and complex. A significant body of research details the numerous possible interactions among microorganisms, their cell membranes and extracellular excretions, and metal ions in the environment. A number of excellent reviews summarize much of this work [175, 189-191] and the possible implications of these processes on radionuclide migration [21, 23, 35, 109, 176, 177, 189, 192]. Our goal in this section is to focus attention on the possible effects of the various processes on actinide chemistry.

We start our discussion by considering the general similarities between the cell membrane and wall structures of single-celled microorganisms [consult Madigan et al. [37] for a detailed discussion of the properties and function of biological membranes]. The first "line of defense" between a cell and its surrounding environment is the cell membrane. Although cell membranes differ chemically among different species of microorganisms, structurally the membrane properties are remarkably consistent among all cells. The membrane is constructed of two phospholipid[1] layers, oriented such that the hydrophobic fatty acid "tails" are directed toward the inside of the membrane and the hydrophilic glycerol-phosphate groups make up the internal

[1] Lipid molecules containing a substituted phosphate group and two fatty acid chains on a glycerol backbone.

and external membrane surfaces exposed to the cell cytoplasm and outside environment, respectively. The lipid bilayer membrane is held together by hydrophobic forces and is stabilized by ionic bonding between Ca^{2+} and Mg^{2+} cations and the negatively charged phospholipids. The properties of the cell membrane make it a highly selective barrier to solute transport: Only nonpolar and fat-soluble molecules and water can penetrate the cell membrane (e.g., alcohol disinfection works by destroying the integrity of microbial cell membranes). The transport of ionic and polar nutrients into, and metabolic by-products out of, the cell is carried out through the action of specific proteins that span the thickness of the membrane.

Because of the semi-fluid nature of the cell membrane, its exposure to the forces normally present in the surrounding environment would cause a rapid loss in cell integrity if there were no other means of mechanical support. Bacteria maintain cell shape and rigidity through their cell walls, which fall into two distinct categories: Gram-positive and Gram-negative.[2] The cell walls of both types of bacteria are composed of peptidoglycan, a layer of cross-linked "chains" constructed from sugar derivatives and amino acids. The Gram-positive cell wall consists of a relatively thick (as compared to the membrane thickness) uniform layer of peptidoglycan. Attached to the outer surface of the peptidoglycan layer are acidic polysaccharides containing glycerophosphate or ribitol phosphate residues. On the other hand, Gram-negative cells have a thin layer of peptidoglycan surrounding the cell membrane to provide mechanical support for the cell structure, and the peptidoglycan layer is encased by an outer lipid bilayer, resulting in a three-component outer structure. The outer lipid bilayer found in Gram-negative bacteria is not identical in structure to the cell membrane. It consists of a lipid bilayer backbone that is attached to the peptidoglycan layer by proteins on the inside but, on the outside, serves as a support for a lipopolysaccharide layer constructed from branched sugar moieties attached to lipid molecules anchored in the outer cell wall. Because of the "branched" outer layer, the cell wall of Gram-negative bacteria appears quite "rough" as compared to the Gram-positive cell wall. The outer cell structure is not highly selective toward the type of compounds that are allowed to pass through it, as it contains trans-membrane proteins that are permeable to compounds with molecular weights of less than 5000. The difference in selectivity between the inner membrane and outer cell structure gives Gram-negative bacteria the ability to regulate *extracellular* processes, i.e., conduct biotic transformation process *outside* the cell, but *within* the limits of the cell structure.

The basic building block of all cell membranes and the outer cell wall of Gram-negative bacteria--the phospholipid--and the phosphate groups associated with the outer surface of Gram-positive bacteria are deprotonated at neutral to alkaline pHs. Cells have other surface functional groups that include carboxylates, amines, sulfhydryls, and hydroxls. Although the amine groups can be positively charged, in general the ligand groups exposed to the environment give a net negative charge on the outer cell surface [37, 90].

With this brief introduction to bacterial cell walls and membranes, we now relate some of the chemical properties of the outer cell surfaces to various *bioaccumulation* mechanisms. We begin with *bioprecipitation,* one type of bioaccumulation already considered in Section 2.4. The

[2] The names given to the two types of cell wall structures are derived from the results of the *Gram test*, a differential staining procedure used to identify and characterize bacteria. Gram-positive bacteria retain the dye used in the procedure due to the structural difference between their cell walls and those of Gram-negative bacteria.

negatively charged ligand groups exposed at the outer surface of the cell can act as selective nucleation sites for phosphate precipitates [20, 175, 176].

Once the outer cell surfaces have been synthesized by an organism, they retain many of their same chemical properties whether or not the organism remains viable. It is the chemical properties associated with living or dead biomass that can selectively bioaccumulate metals and actinides from aqueous solution by *biosorption.* Biosorption is analogous to other physicochemical sorption processes [35, 176, 192]. Metals are removed by ion exchange or surface complexation with functional groups associated with cell products. In engineered systems, the removal of toxic heavy metals by non-specific sorption by biomass can be quite significant. On the negative side, biosorption is a main reason that digested activated sludge derived from many municipal wastewater treatment plants in industrialized regions is considered a hazardous waste [193, 194]. On the positive side are the many biosorption processes used for treating contaminated waste streams [35, 176, 190]. In the subsurface environment, biosorption processes by living and dead cell matter surely are taking place. However, it appears that in natural systems, biosorption may in some cases act to facilitate contaminant migration, rather than retard it, because the cells onto which the metals are sorbed can be transported [19, 45, 120, 195, 196].

Biosorption may be enhanced in living cells by *extracellular complexation*, the external secretion of enzymes or other cofactors that selectively or fortuitously bind metal ions. Good examples are the substances bacteria secrete to help them adhere to each other in flocs or to attach themselves to solid surfaces in the form of a biofilm. These extracellular secretions, normally polymeric polysaccharides, contain a number of functional groups that show high affinity for binding cations and can increase the amount of "biomass" available to non-specifically adsorb metal species [176].

Often, extracellular secretions by bacteria are formed in direct response to the presence of toxic metals in solution [189, 197-199]. As we discuss in detail in Section 3.2, chemical toxicity due to metals is correlated with the aqueous concentration of the free metal ion. Secretion of extracellular complexing agents reduces the free ion concentration by isolating the toxic metal in a non-bioavailable form, either retained in solution as a mobile metal ligand complex or immobilized in a polymeric "slime" layer outside the cell wall [189, 197, 198]. Direct evidence for extracellular actinide complexation was described by Robinson et al. [200], who investigated the effect of "microbial metabolites" produced by soil microorganisms on Pu speciation. Depending on the microbial isolate tested, Pu mobility was either enhanced or retarded as compared to the mobility of the Pu-DTPA (diethylenediaminetetraacetic acid) complex. Thus, actinide mobility in the presence of capable organisms can either be enhanced or reduced by extracellular complexation mechanisms.

In contrast to the compounds produced by bacteria in response to a toxic challenge or to promote self-adhesion and agglomeration, organisms secrete specific extracellular complexants for chelation of required metal micronutrients [201]. The most well studied of the metal-specific extracellular chelators are siderophores, compounds produced and released by microorganisms to solubilize solid iron minerals and enhance the transport of iron into the cell, a process known as *biouptake.* Although specifically produced for iron chelation, siderophores form stable complexes with other metals [35, 189, 201, 202]. Raymond et al. [203] observed that, because of their similar ratios of charge to ionic radius, Fe(III) and Pu(IV) behave similarly in biological systems. Building on this work, Hersman et al. [202] qualitatively compared the Pu(IV)

complexing ability of a siderophore isolated from a *Pseudomonas* sp. to EDTA, "desferal" (a commercially available siderophore), and citrate in concentrated carbonate solutions. The two siderophores and EDTA out competed carbonate to form stable Pu(IV) complexes, while the addition of citrate caused no observable change in Pu speciation, indicating that the complexing ability of the siderophores was greater than that of citrate and carbonate/bicarbonate. Similar behavior, although not specifically attributed to the action of siderophores, was observed by Reed et al. [165], who found that viable cells of *C. heintzii* bioaccumulated Pu(IV) in an iron-deficient NTA solution, indicating that the "complexing" ability of live cells was higher than that of NTA. This type of complexation by microbial siderophores may enhance the mobility of actinides in the subsurface, as Brainard et al. [204] showed that siderophores solubilized polymeric Pu(IV) about 1000 times faster than the chelating agents citrate and EDTA.

Obviously, substitution of radionuclides into transport-specific siderophore complexes would likely result in biouptake of the radioactive metal into the cell. Because of the high degree of specificity found in transport proteins, the potential for metal substitution may be linked to the similarity of chemical behavior within groups of elements. For example, Cherrier et al. [205] studied the uptake and biological fate of polonium in a *Pseudomonas* sp and found that Po distributed among the cell walls, cytoplasm, and protein of the cells in a similar manner as sulfur, a common building block of many amino acids. Their hypothesis was that, because Po and S occupy the same column in the periodic table, Po behaves as an S analog in various biochemical processes. Since actinides are known to cross biological membranes in animals [206] and because of the chemical similarity of Pu(IV) to Fe(III) [203, 207], biouptake of actinide elements by microorganisms is likely and has dramatic implications for the potential toxicity of actinides toward bacteria, as we discuss in Section 3.2.

Although biouptake should be critically important for toxicity, it most likely does not have as much impact on actinide speciation and mobility as do biosorption, bioprecipitation (internal or external), and extracellular complexation. The reason for the small impact on speciation and mobility is that biouptake involves a relatively small fraction of the total actinide as compared to the other mechanisms. Detailed measurements of the fate of copper associated with a methanotroph (an aerobic, methane-oxidizing bacterium) showed that at most 1% of the total Cu associated with the organism was internally associated [208].

To conclude this section, we mention the obvious roles of metabolic by-products in actinide complexation. Among the most important by-products are intermediate organic complexants that can be produced as a result of microbial metabolism. Some examples include:

- Production of acetate by sulfate-reducing and other bacteria. Acetate is a moderate organic complexant.
- Production of a wide variety of organic acids and alcohols during fermentation.
- Production of IDA, a moderate complexant, during the first step of the aerobic utilization of NTA.

3. Effects of Actinide Species on Biological Activity

A unique aspect of microbiology-actinide interactions is radiotoxicity, which goes beyond the *chemical* toxicity of the actinide metal to include *radiolytic* toxicity caused by the ionizing radiation associated with the radioactive decay of the actinide. The chemical toxicity of actinides is similar to that of heavy metals, but the radiolytic toxicity is an added feature. Effects of toxicity are certainly significant when the actinide concentration exceeds micromolar levels or the actinide is concentrated into the biomass.

Actinide species in the subsurface will influence the microbiological population present. The three most important effects are: (1) cell death due to the ionizing radiation associated with the radioactive decay of the actinide; (2) cell death due to the chemical toxicity of actinides toward microbes; and (3) limitations in the availability of organic nutrients due to the formation of actinide-organic complexes. These effects are potentially important because the success of bioremediation depends on the ability of the active organisms to tolerate the radiotoxicity of the radionuclide and access the substrates (often the organic contaminants of interest) present.

3.1 Radiation tolerance of bacteria

The interaction of ionizing radiation with bacteria almost always causes harm to the cell. This effect was discovered quite some time ago [209, 210], and the manipulation of this effect in living cells is the basis of using ionizing radiation to treat cancer. A substantial amount of work has been done in this area. We refer the reader to an excellent review of this work by Ewing [211].

The radioactive decay of the transuranics of most interest involves alpha particles, beta particles, and residual/secondary gamma rays. These types of ionizing radiation will not interact with the aqueous medium in the same way due to their differences in linear energy transfer (LET). The differences in LET, therefore, lead to differences in relative effects on cell viability. For example, plutonium-239 primarily undergoes alpha decay (5.155, 5.143, and 5.105 MeV energies). These decays are accompanied by primary low-energy gamma rays (0.05162, 0.0301, and 1.057 MeV) and secondary gamma rays and beta particles associated with the interaction of the alpha particle with the aqueous medium. All these interactions potentially affect the observed radiation tolerance of the bacteria.

3.1.1 Gamma and beta (low LET) radiation

The effect of low LET ionizing radiation (e.g., gamma radiation and beta particles) is best described by survival curves that establish the cellular viability as a function of absorbed dose. The survival curve for the *C. heintzii* bacterium is shown in Fig. 3.1. Survival curves for many bacteria have been measured, and some progress has been made in understanding the mechanism by which ionizing radiation causes cell death [211-215].

Radiation damage [211] occurs when the radiolytic transients generated, either externally in the aqueous medium or internally due to the deposition of energy within the cell, break down key molecules within the cell. A schematic of the most important of these transients is shown in Fig. 3.2. Radiation damage to cell reproduction has been clearly linked to the effect of oxidizing radiolytic products. The key oxidizing transient and molecular products generated are the hydroxyl free radical ($OH^{\bullet}$), the hydroperoxyl free radical ($HO_2^{\bullet}$ and $^{\bullet}O_2^-$), and hydrogen

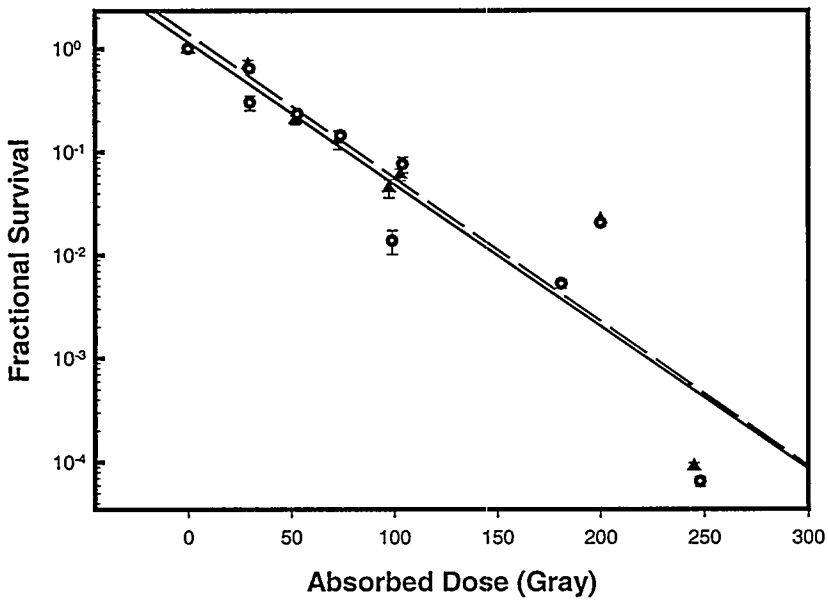


Fig. 3.1 - Fractional survival of *Chelatobacter heintzii* as a function of gamma-absorbed dose in oxygenated high-purity water (HPW) and in 0.01 M PIPES (piperazine-N, N'-bis[2-ethanesulfonic acid]) buffer at pH 6.2. There is a 99% loss of viability at 180 Gray, which is typical of bacteria in oxygen-sensitized systems.

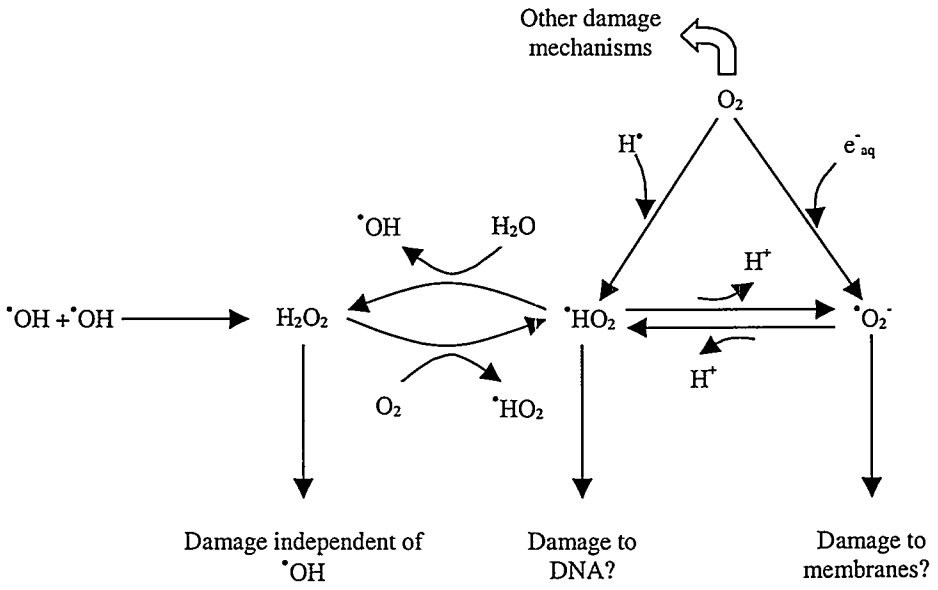


Fig. 3.2 - Schematic representation of cellular damage caused by free radical generation. Adapted from Ewing *[211]*.

peroxide (H_2O_2). All are generated due to the radiolytic breakdown of water. Of these, hydrogen peroxide is currently thought to be the most important in causing radiation damage, although direct contributions from the free radicals and oxidizing transients generated from solutes in water can be significant.

Radiation most affects microbes during growth stages when replication is at its peak. Specific interactions and damage to DNA have been proven. Damage to DNA is most responsible for the observed mutations or loss of reproductive capability. This also explains the observed trend in radiation sensitivity that, in general, increases as the complexity of the cell is increased: eukaryotic cells > procaryotic cells > viruses. The radiation tolerance of microbes is varied, but sometimes very high. The factors that define this tolerance are not fully understood, although in some cases it appears that radiation tolerance may be genetically encoded [216].

The aqueous environment can have a pronounced effect on the radiation sensitivity of a particular bacteria. The most important aqueous constituent is dissolved oxygen. In anoxic systems, radiation damage is caused by long-lived oxidizing free radicals, $^{\bullet}OH$ radicals, and hydrogen peroxide. Oxygen almost universally sensitizes bacteria to radiation damage by increasing the net yield of oxidizing radicals. At low oxygen levels, the mechanism for radiation damage proceeds primarily through the $^{\bullet}OH$ radical and hydrogen peroxide. The presence of free radical scavengers effectively suppresses radiation damage. At high dissolved oxygen concentrations (e.g., air-saturated systems), the radiation damage is more complex and proceeds by the $^{\bullet}OH$ radical and other mechanisms that are not clearly understood. Here, $^{\bullet}OH$ scavenging suppresses radiation damage but does not completely protect against this damage.

Bacteria, especially under environmental conditions, exhibit a wide range of sensitivity to ionizing radiation. There are many factors that contribute to this effect:

- presence/efficiency of free radical scavengers in the groundwater
- oxygen concentrations
- temperature of the groundwater
- energy and type of ionizing radiation
- repair capabilities of the cell
- physiological status of the cell (i.e., growth stage and history)
- nutrients present

In this context, soil isolates of the same bacteria can have quite different radiation tolerances depending on how the cells were grown. More importantly, the culturing of cells in the laboratory, over time, may alter the radiation sensitivity of the bacteria.

As an example of the large differences in radiation sensitivity that exist, the fractional survival of *Halobacter halobium* (a halophile in 5.5 M sodium chloride) as a function of absorbed gamma dose is shown in Fig. 3.3. For *H. halobium*, 99% loss of viability occurred at 8000 Gray (Reed, unpublished data), a radiation level 45 times higher than the radiation tolerance of *C. heintzii*. This enhanced tolerance was attributed to a combination of differences in cell structure and the known effects of the chloride ion [217] as a free-radical scavenger. Procaryotic bacteria with very high radiation tolerances have been discovered. The presence of ionizing

radiation, in and of itself, may help promote mutations that increase the radiation tolerance of bacteria, and radiation-resistant bacteria have been isolated. For example, strains of *Deinococcus radiodurans* can survive gamma radiation levels of 5000-30,000 Gray without loss of viability [218, and references therein]. Very high radiation tolerances such as these are generally attributed to fast and efficient mechanisms that can repair radiolytically damaged DNA as quickly as it is damaged [216].

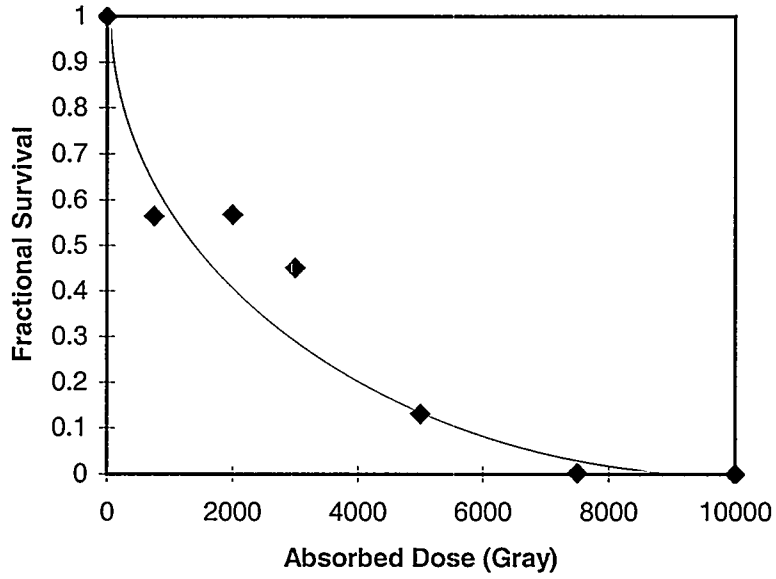


Fig. 3.3 - Radiation tolerance of *Halobacter halobium* towards gamma irradiation. The halophile was irradiated in a 5.5 M sodium chloride medium.

3.1.2 Alpha particle (high LET) effects

Alpha particle interactions with cells or suspended cells also lead to the loss of cell viability in environmental systems. Alpha particles are the major source of ionizing radiation for systems containing actinides. The observed loss of cell viability should come as no surprise given the large body of data that points to the detrimental effects of gamma and beta radiation. Many, if not all, the mechanisms leading to the loss of cell viability just discussed should also extend to the discussion of the effects of alpha particle interactions.

There are, however, two important differences between gamma/beta radiation and alpha particles that bear consideration. First, alpha particles deposit their ionizing radiation much more unevenly throughout the solution due to the high LET nature of the interaction; this issue is discussed in this section. Second, alpha particles arise from the radioactive decay of an actinide, meaning that both chemical and radiolytic toxicity are probable. These effects are often difficult to separate. The chemical toxicity of actinides is discussed in Section 3.2.

The energies of alpha particles emitted by the actinides of most relevance to environmental systems (Pu, Np, and Am) range between 4.7 and 5.5 MeV/disintegration. This

gives them an averaged LET of ~ 130-150 keV/μm (compared to ~ 0.2 keV/μm for gamma radiation and beta particles). Alpha particles deposit their energy in aqueous solution in dense tracks with very high concentrations of radiolytic products. These tracks have a range of ~ 40 μm and a diameter of ~ 1 μm [219]. This deposition pattern leads to a non-homogeneous distribution of radiolytic products and a relative increase in molecular products (e.g., H_2O_2 and H_2) at the expense of the more transient free radical species ($^{\bullet}OH$, $^{\bullet}H$, and e_{aq}).

The inhomogeneity of the energy deposition in aqueous medium means that the proximity of the cell to the alpha particle track is an important consideration in establishing radiolytic effects on microbiological systems (e.g., cell suspensions in aqueous media). The potential extent of cell damage is far less for a cell exposed to the dose-to-solution yield of radiolytic products than a cell that receives a "direct hit" by the alpha particle track. In practice, the radiolytic effects on cell viability would be expected to be lower when a dissolved plutonium complex is the source of ionizing radiation than when the plutonium atom is primarily located in/on the cell, where a much larger probability of direct interaction between the transients in the particle track and the cell mass exists.

The effects of ionizing radiation, as opposed to chemical toxicity, were shown to be the predominant cause of toxicity when bacteria isolated from subsurface environments interact with the higher activity isotopes of plutonium [130, 220, 221]. Wildung et al. [130] examined the effects of 0.1 to 180 μg/g soluble (DTPA-complexed) and hydrolyzed ^{239}Pu and ^{238}Pu on soil isolates of aerobic bacteria, aerobic spore-forming bacteria, anaerobic bacteria, anaerobic spore-forming bacteria, fungi, and actinomycetes. The two plutonium isotopes were used to differentiate between chemical and radiolytic contributions to toxicity. Both growth and longer term (~30 day) static experiments were performed. Cell viability was determined by counts of colony-forming units (CFU) and effort was made to establish the relationship between actinide speciation and the observed effects, and to determine the final distribution of the actinide associated with the biomass.

The most important result reported in Wildung et al. [130] is that, for ^{239}Pu and ^{238}Pu, the loss of cell viability was predominantly caused by radiolytic, rather than chemical, pathways. Different types of organisms also exhibited different abilities to tolerate ionizing radiation. Plutonium(IV) species, which were the predominant oxidation state in the systems studied, became associated with the biomass and were solubilized by exocellular material present. Speciation of the Pu was qualitatively shown to have an effect on the observed toxicity.

A similar conclusion was reached in our investigations of the Pu(IV)-NTA-*C. heintzii* system [165]. When ^{239}Pu was the source of ionizing radiation, the loss of viability was caused by radiolytic, rather than chemical, effects. Loss of cell viability due to exposure to 10^{-5} M ^{239}Pu and ^{242}Pu is shown in Fig. 3.4. The results indicate that the viability effects can be attributed to differences in alpha activity. At the same Pu concentration, loss of viability was much greater for ^{239}Pu than ^{242}Pu. No difference was noted between the 10^{-5} M ^{242}Pu and 10^{-6} M ^{239}Pu samples, which had the same activity but differed in concentration by an order of magnitude. This, as will be discussed later, was not the case when depleted uranium and ^{237}Np were the sources of ionizing radiation.

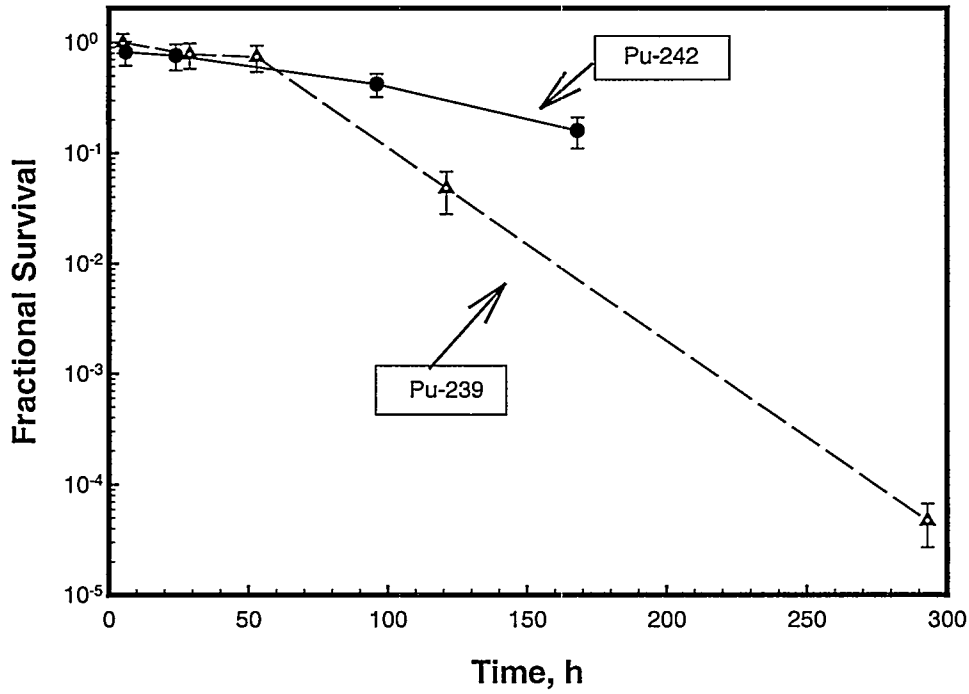


Fig. 3.4 - Loss of cell viability, as a function of time, for *Chelatobacter heintzii* in the presence of 10^{-5} M ^{239}Pu and 10^{-5} M ^{242}Pu. With the isotopic purity of the plutonium used, 10^{-5} M ^{239}Pu has ten times the dose-to-solution as does 10^{-5} M ^{242}Pu.

In the plutonium system, the loss of cell viability could not be accounted for solely by considering the alpha particle dose-to-solution. In other words, the observed loss of cell viability was much greater when plutonium was the source of ionizing radiation than comparable gamma absorbed doses. Reed et al. [165] hypothesized that this enhanced radiolytic effect was caused by the bioassociation of the plutonium in the system. A link between increased bioassociation and the onset of loss of viability was noted. Bioassociation led to a much greater likelihood of a "direct hit" between the alpha particle track and the cell mass (50% probability for Pu on the cell wall, 100% probability for Pu that is within the cell), compared to <<0.1 % based on the cell densities used. This suggests that direct interactions between alpha particle tracks and cell mass are nearly always fatal.

The potential importance of bioassociation and/or actinide distribution in the observed radiolytic contribution to toxicity emphasizes the importance of complexation as it relates to bioassociation (both sorption and uptake) in environmentally relevant biological systems. This process is oxidation-state specific and depends on the availability of exocellular biologically

derived complexants and the strength of specific functional groups on the cell wall relative to complexation in solution. Further work is needed to fully understand these interdependencies.

3.2 Actinide toxicity

With so much focus on the radioactivity of actinides, it is easy to overlook the fact that actinides exhibit similar tendencies toward chemical toxicity as other, much better studied heavy metals. Chemical toxicity, rather than radiolytic toxicity, is likely to predominate when low-activity isotopes are present (e.g., ^{238}U, ^{237}Np, or ^{242}Pu). Under conditions relevant to the environment, chemical toxicity is also likely to be a more important consideration at the lower actinide concentrations expected to be present.

That actinides exhibit chemical toxicity has been demonstrated for ^{237}Np and ^{238}U in our laboratory [116, Reed, unpublished data]. We observed that NpO_2^+, as an aquo species, inhibits the growth of *C. heintzii* at free-ion concentrations exceeding 5×10^{-5} M (see Fig. 3.5). In these experiments, *C. heintzii* was grown on glucose in the presence of the neptunyl species. Complexation of neptunium by nitrilotriacetic acid, even at neptunyl concentrations of 1.25×10^{-4} M, resulted in no effect on growth. Tying up the neptunyl as a phosphate complex or precipitate also eliminated its toxicity toward *C. heintzii.*

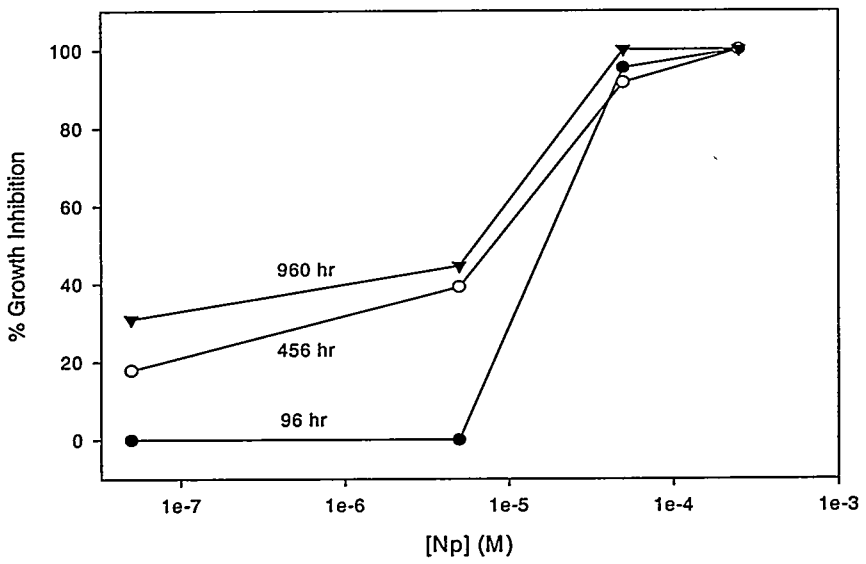


Fig. 3.5 - Relative growth inhibition of *Chelatobacter heintzii* (calculated by comparison of OD_{600} values from sample vessels to uninhibited controls) in response to increasing NpO_2^+ free ion concentrations. Experiments were conducted in a minimal glucose growth medium to prevent Np complexation.

We also showed that uranium exhibits high chemical toxicity toward *P. fluorescens*. Biodegradation of citrate in the UO_2^{2+}-citrate complex led to a loss of viability, as measured by the number of colony-forming units, over time (see Fig. 3.6). The mechanism here appears complicated and is very sensitive to the speciation of the uranyl. No uranium toxicity was noted when the *P. fluorescens* was grown on glucose medium at pH 6-8, even at ~ 1 mM concentrations, indicating that hydrolyzed uranyl was not toxic. The toxicity noted appears to be caused by the UO_2^{2+}-citrate complex itself.

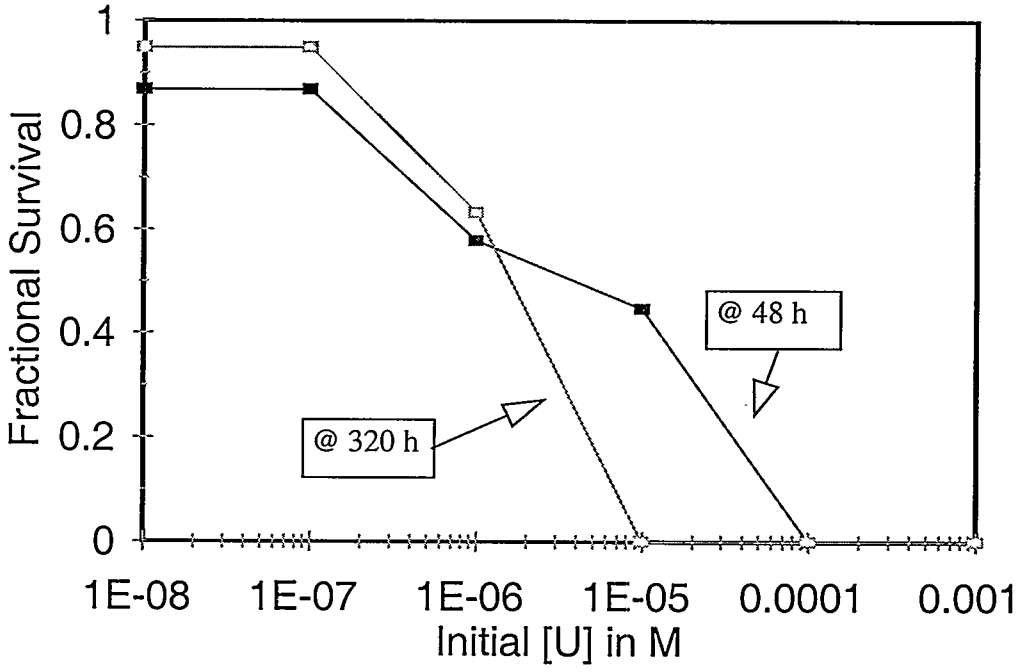


Fig. 3.6 - Relative growth inhibition of *Pseudomonas fluorescens*, based on CFU counts, at two times and in the presence of different concentrations of the uranyl citrate complex. The degradation of citrate between 48 and 320 hours led to increased uranyl toxicity that eventually stopped the biodegradation process.

The mechanisms by which actinides cause chemical toxicity are not well understood. The main reason for this is simply that this issue has not been well studied. Presumably, actinides cause toxicity in ways that are analogous to those observed for heavy metals. The study of heavy metal toxicity continues to be a very active area of research and increasingly diverse and complex mechanisms are being discovered (see earlier discussion in Section 1.4).

The most important, and perhaps best studied, of these mechanisms is the ability of certain actinide species to substitute for metals in metabolic processes [42, 184, 220]. The

critical factor appears to be the charge-to-volume ratio. The similar charge-to-volume ratio of Pu^{4+} and Fe^{3+} has led to the suggestion that plutonium will exhibit similar behavior to iron in biologically active systems in the environment [203, 207]. Most of the evidence available, including our data on Np and Pu, indicates that only the aquo (i.e., bare) actinide species are chemically toxic [222]. That complexation greatly reduces the toxicity of actinides by making them unavailable to the bacteria has been noted by others [157, 223, 224].

The apparently high specificity of actinide toxicity toward microbes again points to the important role actinide speciation plays in actinide-microbiological interactions. In this context, the removal of organic complexants by biodegradation may be self-limiting in that it leads to enhanced levels of toxicity. Complexation, particularly the formation of hydroxide complexes, varies greatly with the oxidation state of the actinide. For this reason, biological effects on the redox status of the actinides also should affect the observed toxicity of actinides in the environment.

3.3 Effect of actinides on organic speciation and bioavailability

In the previous two sections, we discussed how actinides affect contaminant degradation by reducing the *activity* of microorganisms. However, actinides also can act like other metals by reducing the *bioavailability* of substrates, simply because of chemical speciation effects. The rates of microbially mediated reactions are related to the concentrations of their degradable substrates. Because of chemical interactions between metals and potential organic substrates, high metal concentrations can reduce substrate concentrations, especially when the substrates are strong complexants.

Based on our brief description in Section 2 of the properties of bacterial cell membranes, we know that the cell wall and membrane structure provide a highly selective barrier to chemicals entering the interior of the cell. For this reason, proteins embedded in the cell membrane often facilitate the transport of most substrates and nutrients into the cell. Complexation of organic compounds with metals enhances or reduces the ability of microorganisms to uptake the organics as substrates. Enhancement occurs when a specific metal ion is a co-substrate for the transport protein, as in the case of coupled citrate and magnesium transport by *Bacillus subtilis* [225]. When other, non-preferred metals form the most abundant substrate complex, substrate utilization usually is slowed [115, 145, 146, 163, 226, 227].

Although several mechanisms have been proposed to account for the reduction in substrate availability and utilization in the presence of various metals, the most general effect comes about when significant metal concentrations reduce substrate availability. This reduction in availability through chemical speciation effects alone occurs when the organic substrate is a strong ligand. We consider the simplest case, the formation of a single metal-ligand complex (MeLig) when only the uncomplexed ligand (Lig^-) is a usable growth substrate for microorganisms:

$$Me^+ + Lig^- \Leftrightarrow MeLig \qquad \beta = \frac{[MeLig]}{[Me^+][Lig^-]}$$

When the concentration of the ligand greatly exceeds that of the metal (Me^+), complexation has little effect on the concentration of the free ligand species. However, when the ligand and metal

concentrations are nearly equal, or when the total metal concentration exceeds that of the ligand, formation of the MeLig complex reduces the concentration of the degradable substrate, Lig^-. In the absence of competitive complexation of the metal with other ligands, the amount of substrate "lost" to the complex is related to the magnitude of the complex formation constant, as is shown in Fig. 3.7. For comparison, Fig 3.7 identifies values of formation constants for important actinide-ligand complexes. At low β values (Fig 3.7a), the concentration of the free ligand (the degradable substrate) decreases from nearly 100% of the total amount of ligand in solution to only 0.1% when the formation constant increases from 10^{-2} to 10^{5}. As the magnitude of the formation constant increases further, the equilibrium concentration of the ligand substrate falls to vanishingly low levels (Figs. 3.6b and c).

Because of competitive complexation between various metals with organic and inorganic ligands in most real systems, we cannot directly compare our simple example to existing experimental evidence. However, in most cases where detailed chemical speciation modeling has been used to interpret experimental data, the degradation (or lack thereof) of organic complexants has been linked to the concentration of the known or hypothesized degradable species [228, 229]. For example, Francis et al. [146, 156] showed that citrate was degraded by *P. fluorescens* to the point where the ligand-to-metal ratio reached 1:1 in Cu-citrate and 2:3 in U-citrate systems, while degradation of citrate in the presence of other metals proceeded to completion, although at a lower rate than in systems with no significant metal concentrations. Similar results were seen by Bolton et al. [163] for NTA degradation in the presence of metals. In actinide systems, Banaszak et al. [116] showed that NTA degradation in the presence of Np(V) went to completion in the absence of metal toxicity effects, while Reed et al. [165] found that the degradation rate of NTA in the presence of Pu(IV), which has a NTA complex formation constant six to seven orders of magnitude greater than that of Np(V)-NTA, was significantly decreased, but not stopped, as the ligand-to-metal ratio approached 1:1.

Differences in degradation rates of some metal-ligand complexes may be controlled by membrane-transport systems that are speciation-dependent. Joshi-Topé and Francis [115] found that cells initially grown on citrate alone required an induction period before they were able to utilize citrate in the presence of Zn and Ni. However, cells grown in Zn-citrate medium were able to degrade citrate in the presence of Zn and Ni without an induction period. The same researchers correlated this behavior with the lag time required for the organism to transport the complexes across its cell membrane. However, even after acclimation to the presence of the metals, the citrate degradation rate was lower than that of citrate alone.

Equilibrium speciation plays a key role in determining the bioavailability of organic substrates when the kinetics of complexation reactions are much faster than the rate of biodegradation reactions. However, when complexation reactions are slower than biodegradation reactions, the kinetics of ligand exchange reactions may limit substrate availability. For example, Xue [152] found that the exchange of Fe- with Zn-EDTA took hours to days to complete. In real systems, sluggish complexation kinetics will slow down biodegradation, but not the long-term outcome.

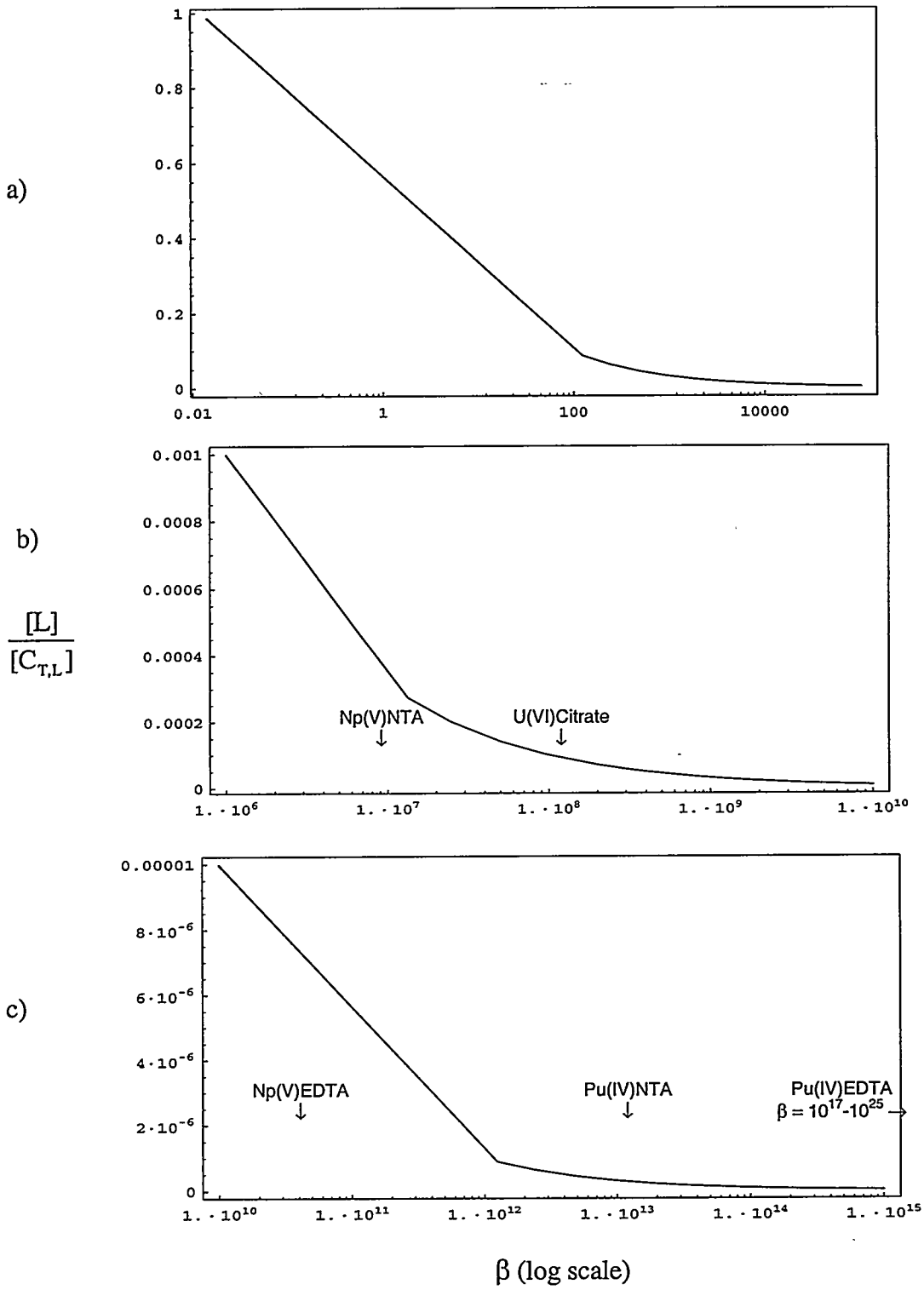


Fig. 3.7 - Free ligand concentration [L] as a percentage of total ligand in solution $[C_{T,L}]$ and as a function of complex formation constant. (a) $\beta = 10^{-2}$ to 10^{5}; (b) $\beta = 10^{6}$ to 10^{10}; (c) $\beta = 10^{10}$ to 10^{15}. Important actinide chelate formation constants are indicated on the figures for comparison.

Because of the range of actinide oxidation states in the environment, the effect of actinides on bioavailability of organic substrates and the activity of subsurface microorganisms is highly variable. We can generalize the effects by focusing on the extreme cases: the +4 and +5 oxidation states. Because of the relatively weak complexation of the An(V) oxidation state in comparison to the An(IV) oxidation state, +5 cations will be present in much more significant concentrations as the free aquo species than are the +4 cations. Thus, metal toxicity effects should be more important than substrate speciation in retarding the growth of subsurface bacteria when actinides are predominantly in the +5 state. The more important effect for the +4 state should be complexation, which reduces the availability of the ligand as a substrate.

The presence of actinides in subsurface groundwaters can also affect the population of bacteria by generating compounds that may promote one species or class of species over another. Radiolytic decomposition of dissolved organics can enhance their biodegradability, thereby increasing the amount and type of substrates available [230]. Radiolytic breakdown of dissolved organics will break bonds, reduce the average molecular weight, and oxidize the organics. This overall process is analogous to the well-studied effects of ozonation, where enhanced biodegradability has been noted [231, and references therein].

The radiolytic decomposition of water is also significant. This, for high LET radiation, leads to the formation of transient and molecular products:

$$3.35\ H_2O \rightarrow 1.6\ H_2 + 1.3\ H_2O_2 + 0.28\ H^{\cdot} + 0.28\ OH^{\cdot} + 0.28\ HO_2^{\cdot}$$

In particular, hydrogen (H_2) formation is significant, since hydrogen provides an electron donor source for anaerobic microorganisms (e.g., methanogens, sulfate reducers). The amount of hydrogen generated could be substantial when significant quantities of radioactive waste exists--as would be the case in a TRU or high-level nuclear waste repository. The molecular yields due to alpha particle decay, which is characterized as a high LET interaction, are largely unaffected by the host environment and solutes [232, 233] and are defined solely by the alpha activity in the groundwater.

4. Mathematical Modeling of Subsurface Biological Systems

Many of the processes we described in Sections 2 and 3 interact in complicated ways that are not understood in isolation from one another. For example, when we see retarded growth in an experiment, is it due to lack of substrate availability, to radiotoxicity, or to chemical toxicity? Because of the inherent complexity of the possible interactions among microorganisms, organic contaminants, and actinide elements, mathematical modeling of the coupled processes involved in these systems is a necessary tool to improve our ability to answer these and other questions. Additionally, the hazards associated with actinide handling limit the number and types of experiments that can be performed safely, increasing the attractiveness of accurate models in predicting the fate of actinides in subsurface environments. We describe modeling tools appropriate for this purpose here.

Like all living things, microorganisms require a source of energy to drive metabolic processes and a source of electrons to reduce carbon and other required nutrients to an appropriate level for use as precursors for biosynthesis. Also, as in all biological processes,

microorganisms use enzymes to catalyze energy- and electron-generating reactions. Enzymatic reactions cannot override thermodynamics; they behave like any other catalyst by lowering the required activation energy, thereby accelerating the kinetics of already thermodynamically feasible, but perhaps not spontaneous, chemical reactions.

We can exploit the fact that microorganisms act as living catalysts and use our knowledge of chemical kinetics and thermodynamics to develop mathematical models that relate their behavior in the environment (or in an engineered reactor) to the rates of consumption or production of chemical compounds in the system. In this section, we focus our attention on the development of models to quantify biological processes.

4.1 Coupling chemical and microbiological reactions

We already described many of the ways that microorganisms interact with their surroundings by catalyzing chemical redox transformations and altering important system parameters such as concentrations of electron acceptors and pH. However, unlike abiotic catalysts, living cells are, in turn, affected by their surroundings by mechanisms like toxicity and bioavailability. To accurately describe the interactions of microorganisms, organic compounds, and actinides in the subsurface (or in a treatment process or in the laboratory), mathematical models must accurately couple biological growth and substrate utilization kinetics to chemical speciation specific phenomena. Coupled models have been described for batch reactions [234], subsurface transport systems [235], and engineered anaerobic bioreactors [236]. In this section we discuss the basics of coupling microbial growth and substrate utilization to equilibrium chemical speciation.

4.1.1 Microbial metabolism

The fundamental principle that couples microbial growth to substrate utilization is that living cells catalyze oxidation/reduction transformations that provide electrons and energy. The primary goal of microbial metabolism is to invest those electrons and energy to produce more microorganisms. Thus, as more of the substrate is transformed, the amount of catalyst in the system, i.e., the biomass, increases in an amount proportional to the amount of substrate utilized.

McCarty [114, 237] systematically showed how the amount of biomass produced per unit mass of substrate consumed is, under most conditions, directly related to the amount of energy available during the transfer of electrons from the electron-donor to the electron-acceptor substrate and inversely proportional to the energy required to synthesize biomass from the available nutrient precursors. As an example of McCarty's methodology, we consider the aerobic utilization of citric acid as the electron-donor primary substrate, i.e., the carbon, energy, and electron source for microbial metabolism. When the fully protonated species, H_3Cit, is selected as the reference level for citric acid, the electron-donor reaction is the half reaction for the oxidation of H_3Cit to H_2CO_3:

$$\frac{1}{18}C_6H_8O_7 + \frac{11}{18}H_2O \rightarrow \frac{1}{3}H_2CO_3 + H^+ + e^- \qquad \Delta G^{0\prime} = -9.68 \text{ kcal/e}^- \text{ eq}$$

The electron-acceptor reaction is the reduction of O_2 to H_2O:

$$\frac{1}{4}O_2 + H^+ + e^- \rightarrow \frac{1}{2}H_2O \qquad \Delta G^{0\prime} = -18.675 \text{ kcal/e}^- \text{ eq}$$

where $\Delta G^{0\prime}$ is the standard free energy in pH 7 water. As we discussed in Section 2.1, a portion of the electrons obtained from the electron donor are shunted through an electron transport chain to the electron acceptor in order to generate energy. However, a fraction of the carbon and electrons derived from the primary electron donor substrate serve as a source of cellular carbon for biosynthesis and electrons for conversion of nutrient elements into the proper redox state for incorporation into cellular material. A simple representation of cell synthesis is:

$$\frac{1}{4}H_2CO_3 + \frac{1}{20}NH_4^+ + \frac{19}{20}H^+ + e^- \rightarrow \frac{1}{20}C_5H_7O_2N + \frac{13}{20}H_2O$$

where $C_5H_7O_2N$ represents the average elemental formula of microbial biomass.

The fraction of electrons shunted to energy generation is defined as f_e^0. The fraction of electrons available for cell synthesis is termed f_s^0 and is proportional to the relative amount of energy available from citric acid oxidation as compared to the energy cost associated with growth. According to the methodology developed by McCarty [114], f_s^0 is calculated as 0.714 for aerobic citric acid oxidation when NH_4^+ is the N source.

The proper proportioning of the carbon and electrons from citric acid between the electron-acceptor and cell-synthesis reactions is given by:

$$R_{overall} = R_{donor} - f_s^0 R_{synthesis} - f_e^0 R_{acceptor}$$

when all the half reactions are written as *oxidations*. The reaction describing the aerobic oxidation of citric acid coupled to biomass synthesis is then given by:

$$C_6H_8O_7 + 0.073\ H_2O + 0.642\ NH_4^+ + 1.287\ O_2 \rightarrow$$
$$0.642\ C_5H_7O_2N + 0.642\ H^+ + 2.788\ H_2CO_3$$

Proper development of the citric acid degradation stoichiometry allows for the direct calculation of the relationship between substrate utilization, the consumption of oxygen and ammonia, and the production of biomass, acidic hydrogen, and carbonic acid. For example, in the case of aerobic citric acid utilization, 0.642 moles of cells are produced for every mole of H_3Cit oxidized. This corresponds to 71.4% of the 18 electrons in $C_6H_8O_7$ being converted to biomass, while 53.5% of the C ends up in biomass. This reaction also produces 0.642 equivalents of acidic hydrogen (H^+) and 2.788 moles of H_2CO_3 for each mole of H_3Cit utilized.

In Section 2.1, we discussed how the energy available to microorganisms varies depending on the combination of electron donor and acceptor reactions. Organisms growing via aerobic respiration have the highest growth rates because the energy available per electron transferred from the donor to the acceptor is greatest when oxygen is used as the terminal electron acceptor. We can now relate the growth rate of microorganisms performing various chemical transformations to the amount of free energy available from the combination of electron-donor and -acceptor reactions. As the amount of energy available from the electron-

donor and -acceptor substrates decreases per electron transferred, less energy is available for biomass synthesis, and f_s^0 decreases, increasing the proportion of electrons shunted to energy requirements and reducing the amount available for producing new biomass. In fermentation, no external electron-donor substrate is available to the cell; thus, a portion of the electron donor acts as the ultimate electron acceptor, resulting in the formation of reduced by-products.

A comparison of the cellular yields calculated for citric acid utilization using different electron-acceptor substrates is shown in Table 4.1. Although not all of the reactions have been observed in practice, the biomass yields shown in Table 4.1 illustrate the relationship between available thermodynamic energy and cellular growth. The table also illustrates the wide range of stoichiometries in terms of acid and H_2CO_3 production. For example, aerobic oxidation and fermentation produce acidic hydrogen, but nitrate, sulfate, iron, and manganese reduction consume acidic hydrogen in very different amounts.

In addition to the effect of substrate energy availability, biomass yield can also be decreased due to increased energy costs during cell biosynthesis. McCarty [237] found that the amount of energy required for synthesis of cell components was fairly constant. However, the amount of energy required to reduce the precursors of biosynthesis to the appropriate redox level varies widely. For example, in the case of autotrophic growth, organisms fix H_2CO_3 as a source of cellular carbon. The average redox state of cellular carbon in biomass ($C_5H_7O_2N$) is zero. When a common organic compound, like citric acid (C redox state equals +1), is used as the carbon source, it must be reduced to the level of cellular carbon, consuming some energy and electrons in the process. When H_2CO_3 is used as the carbon source, the carbon redox state is +4; thus, more energy and electrons must be invested by the cell to utilize H_2CO_3 as a carbon source than citric acid.

We consider the growth of aerobic, autotrophic, hydrogen-oxidizing bacteria to illustrate the effects of autotrophy on biomass yields. The electron donor substrate half reaction is:

$$\frac{1}{2}H_2 \rightarrow H^+ + e^- \qquad \Delta G^{0\prime} = -9.67 \text{ kcal/e}^- \text{ eq}$$

The free energy available from hydrogen oxidation is nearly identical to that released during citric acid oxidation. However, because hydrogen oxidation requires fixation of CO_2 as a carbon source, the cell must invest more energy to incorporate carbon into its biosynthetic pathways. The resulting f_s^0, 0.244 (as compared to 0.714 for citric acid!), leads to an overall reaction stoichiometry of:

$$H_2 + 0.122\ H_2CO_3 + 0.0244\ NH_4^+ + 0.378\ O_2 \rightarrow$$
$$0.0244\ C_5H_7O_2N + 0.0244\ H^+ + 1.073\ H_2O$$

Because of the additional energy requirement for carbon fixation, the cellular yield on an electron-equivalent basis of an organism growing autotrophically is about one-third that of an organism using an organic carbon source with the same amount of available energy per electron. Anaerobic, autotrophic organisms can have f_s^0 values as low as 0.05, and their specific growth rates are correspondingly lower.

Table 4.1 - Citric acid degradation stoichiometry using a variety of electron acceptors, as determined from energetic calculations developed by McCarty [114]. H_3Cit is chosen as the reference level for citric acid.

Energy Generation Mechanism	*Electron Acceptor Couple (ox/red)*	f_e^0 *(e⁻ cells/ e⁻ citrate)*	*Biomass Yield (mole cells/mole citrate)*	*Electron Acceptor (mole consumed/mole citrate)*	*Acid Equivalents Produced (+) or Consumed (-)/ mole citrate*	*Carbonic Acid (mole produced/mole citrate)*	NH_4^+ *(mole consumed/ mole citrate)*
Aerobic Respiration	O_2/H_2O	0.714	0.64	1.29	+0.64	2.79	0.64
Nitrate Reduction	$NO_3^-/N_{2(g)}$	0.702	0.63	1.07	-0.44	2.84	0.63
Manganese Reduction	$MnO_{2(s)}/Mn^{2+}$	0.670	0.60	2.97	-5.34	2.99	0.60
Iron Reduction	$Fe(OH)_{3(s)}/Fe^{2+}$	0.493	0.44	9.13	-17.8	3.78	0.44
Sulfate Reduction	SO_4^{2-}/H_2S	0.288	0.26	1.60	-2.95	4.70	0.26
Fermentation to $H_{2(g)}$ and Acetate	Citrate/Acetate and H_2	0.153	0.14	1.27 mole acetate produced/mole citrate	+1.41	2.77	0.14

Energetic calculation is a powerful tool for predicting combinations of electron-donor and -acceptor substrates that provide sufficient thermodynamic energy to grow new biomass. For example, we return our discussion of the Rusin et al. [79] observation of enhanced plutonium solubilization by iron reducing bacteria and investigate if the reduction of $PuO_2(s)$ to Pu^{3+} provides enough energy to support microbial growth. The primary electron-donor substrate for the Pu-solubilization experiments was glucose:

$$1/24\ C_6H_{12}O_6 + 1/2\ H_2O \rightarrow 1/4\ H_2CO_3 + H^+ + e^- \quad \Delta G^{0\prime} = -10.02\ kcal/e^-\ eq$$

Rusin et al. [79] proposed that the electron-acceptor half reaction was the reductive solubilization of hydrated $PuO_2(s)$ to Pu^{3+}, written here without the water molecules associated with the solid:

$$PuO_2(s) + 4\ H^+ + e^- \rightarrow Pu^{3+} + 2\ H_2O \qquad \Delta G^{0\prime} = 6.23\ kcal/e^-\ eq$$

The f_s^0 value calculated based on these two half reactions, 0.256, shows that, from a thermodynamic standpoint, reductive Pu solubilization could provide energy to support microbial growth. The calculated stoichiometry based on this f_s^0 value is:

$$C_6H_{12}O_6 + 53.3\ H^+ + 0.307\ NH_4^+ + 17.9\ PuO_2(s) \rightarrow$$
$$0.307\ C_5H_7O_2N + 4.46\ H_2CO_3 + 27.7\ H_2O + 17.9\ Pu^{3+}$$

If this were the stoichiometry of the actual reaction, we would expect an increase in alkalinity and, depending on the presence of other buffers, an increase in pH during Pu solubilization, because the amount of acidic hydrogen consumed overwhelms the small amount of carbonic acid production.

Rusin et al. [79] found that addition of NTA to the growth medium significantly enhanced the rate of plutonium solubilization. Similar results were observed by Lovley et al. [86, 155], who showed that the growth rate of iron-reducing bacteria using solid Fe-containing minerals as an electron-acceptor substrate was enhanced by the addition of NTA (and other chelates) to the growth medium. Several hypotheses have been made as to the mechanism by which chelating agents enhance the growth rate of organisms using solid electron-acceptor substrates, including increased bioavailability of the oxidized metal in a soluble form, stabilization of the reduced form of the metal in a complex, and acceleration of the electron transfer reaction by the chelate [79, 86, 155]. Another potential mechanism that can explain the growth enhancement of organisms using metal electron acceptors is the differential effect of complexation on the redox potential of the oxidized and reduced metal species. Returning to the glucose-Pu-NTA system, instead of representing Pu reduction as the reductive solubilization of the Pu(IV) oxide to Pu^{3+}, we can assume that the actual electron transfer occurs between two soluble species, Pu(IV)NTA^+ and Pu(III)NTA. In this case, we can determine the overall free-energy change for the electron transfer between the two complexes by combining the formation reactions of the complexes with the electron-transfer half reaction between Pu(IV) and Pu(III):

$Pu^{3+} + NTA^{3-} \rightarrow PuNTA$	$\Delta G^{0\prime} = -14.45$ kcal/mole
$PuNTA^{+} \rightarrow Pu^{4+} + NTA^{3-}$	$\Delta G^{0\prime} = +17.53$ kcal/mole
$Pu^{4+} + e^{-} \rightarrow Pu^{3+}$	$\Delta G^{0\prime} = -23.29$ kcal/e eq
$PuNTA^{+} + e^{-} \rightarrow PuNTA$	$\Delta G^{0\prime} = -20.21$ kcal/e eq

Under the stated conditions, the reduction of $Pu(IV)NTA^{+}$ to Pu(III)NTA can potentially supply the organism more energy than the 18.7 kcal available when oxygen is used as an electron acceptor! Finishing the calculation, we find that the f_s^0 value based on the reduction of $Pu(IV)NTA^{+}$ to Pu(III)NTA as the electron acceptor half reaction is 0.733, resulting in the following predicted stoichiometry:

$$C_6H_{12}O_6 + 0.569\ H_2O + 0.879\ NH_4^{+} + 6.41\ PuNTA^{+} \rightarrow$$
$$0.879\ C_5H_7O_2N + 7.29\ H^{+} + 1.60\ H_2CO_3 + 6.41\ PuNTA$$

The fact that this reaction predicts a net production of acidic hydrogen is misleading; recall that 6.41 x 4 = 25.6 protons are consumed during the dissolution of $PuO_2(s)$ to aqueous Pu(IV). If we assume that the reductive dissolution of $PuO_2(s)$ to the $PuNTA^0$ complex is the electron-acceptor half reaction, the f_s^0 is calculated based on a $\Delta G^{0\prime}$ value of 6.23 - 14.45 = -8.22 kcal/e eq, yielding a predicted stoichiometry of:

$$C_6H_{12}O_6 + 26.4\ H^{+} + 0.748\ NH_4^{+} + 9.04\ NTA^{3-} + 9.04\ PuO_2(s) \rightarrow$$
$$0.748\ C_5H_7O_2N + 15.81\ H_2O + 2.26\ H_2CO_3 + 9.04\ PuNTA$$

While we have no direct evidence that any of the three calculated reactions is correct, or that the bacteria in the experiments conducted by Rusin et al. [79] were able to grow by the reductive solubilization of Pu, this analysis shows that sufficient energy could be conserved by the reduction of Pu(IV) to Pu(III) in any of several forms to support microbial growth. It highlights this area as one where more detailed studies are warranted.

So far we have discussed the utilization of common organic compounds, like citrate and acetate, as primary electron-donor substrates. These simple organic substrates are readily degraded by many respiring organisms using the enzymes of the tri-carboxylic acid (TCA) cycle, a common metabolic pathway [Consult Madigan [37] for a detailed description of the TCA cycle]. For example, citric acid is an intermediate compound produced during the operation of the TCA cycle; any organism with an operational TCA cycle possessing the ability to transport citric acid into the cell will be able to use it as a primary electron-donor substrate. However, many organic compounds, including most organic contaminants found in the subsurface, cannot initially be degraded by the same enzymes used in normal metabolic pathways. In these cases, many microorganisms possess specialized enzymes that catalyze chemical transformations of complex organic compounds into simpler molecules. Often, the specialized enzymes are only required to perform an initial chemical transformation to make the organic compound a suitable substrate for common metabolic enzymes. In some cases, organic contaminants can only be fully degraded by a consortia of microbial species, with each group of organisms catalyzing one or a set of chemical transformations of the contaminant [16, 66, 101, 134-137, 149, 238-242].

A common, nonspecific enzyme that catalyzes the first step in the breakdown of many complex organic contaminants is the monooxygenase (MO) enzyme. The role of the MO enzyme is to "activate" an organic compound via substitution of an -OH for an -H on the molecule. In Section 2.3.2, we discussed the role of the NTA-MO enzyme in catalyzing the first step in the aerobic breakdown of NTA by the *C. heintzii* bacterium. The stoichiometry of this monooxygenation reaction is [234]:

$$N(CH_2COO_3)_3 + O_2 + NADH + H^+ \rightarrow (HOOCCH_2)_2N(CHOHCOOH) + H_2O + NAD^+$$

The key feature of any monooxygenation is that O_2 acts in a dual role during the degradation of the compound. First, oxygen serves as the terminal electron acceptor for the energy generating reaction. Second, O_2 is a direct co-substrate for the MO enzyme: One atom from O_2 becomes the -OH group inserted onto the electron-donor substrate, and the other O is reduced to H_2O by NADH during the reaction. Because of the stoichiometric requirement for oxygen as a co-substrate, the kinetics of MO reactions are particularly sensitive to dissolved oxygen concentrations [243], as we discuss in more detail in the next section.

The MO enzyme is only one example of the various specialized enzymes that microorganisms use to initiate the breakdown of anthropogenic compounds. Often, the initial breakdown steps result in transformation of the original organic contaminant into two or more smaller molecules, some of which are readily degradable by the organism and others that are released into the environment as intermediates. The release of intermediates from the cell constitutes a loss of carbon, electrons, and energy that otherwise could be used to produce new biomass. This results in a slower growth rate than if the cell were able to completely mineralize the substrate. As an example of the effect of intermediate formation on cellular growth, we continue with our description of the aerobic degradation of NTA. After the "activation" of NTA by NTA-MO, 2 NTA molecules are cleaved to form iminodiacetic acid (IDA) and glyoxalate [161]:

$$2\ C_6H_9O_6N + 2\ NAD^+ + 2\ H^+ \rightarrow 2\ C_4H_7O_4N + 2\ C_2H_2O_3 + 2\ NADH$$

The glyoxalate produced during this reaction enters normal metabolic pathways and is oxidized to form precursors for biosynthesis. The two IDA molecules are sent to a separate membrane-bound pathway, where they are broken down into two glyoxalate molecules that are used for biosynthesis and two glycine molecules. One glycine is incorporated into biosynthetic pathways as a carbon and nitrogen source, and the other is mineralized to CO_2 and NH_3, which are released from the cell [161].

As opposed to the degradation of a simple organic compound, like citric acid, via a single common biochemical pathway, such as the TCA cycle, the operation of separate biochemical pathways for NTA degradation provides multiple opportunities for the formation of intermediate compounds. If any of the three pathways necessary for NTA degradation is inhibited, intermediate organic compounds may be excreted from the cell, with the corresponding reduction in cellular yield. As an illustration of this point, the cellular yield of *C. heintzii* was measured as 0.41 gm VSS/gm ThOD in a chemostat supplied with a nutrient growth medium [244], which is within 10% of the yield we would estimate based on thermodynamic calculations. However, carbon mass balance experiments [163, 245] in nutrient-limited growth media showed that up to

50% of the carbon originally present in NTA was transformed into soluble organic carbon intermediates, resulting in a decrease in cellular yield to 0.20 gm VSS/gm ThOD. Since intermediate production has such a significant impact on the growth of bacteria, accurate laboratory investigations must always include the tracking of carbon mass balance in the system to provide accurate parameters for kinetic models.

So far, we discussed how microorganisms release soluble organic compounds into the environment through incomplete oxidation (e.g., of pyruvate by sulfate reducers), fermentation, and inhibition of biochemical pathways. In addition to these particular cases, microorganisms release a small fraction of normal metabolic intermediates, called soluble microbial products (SMP), as part of their routine metabolism [246-248]. Some of the SMP (termed utilization-associated products, or UAP) is produced in proportion to the amount of substrate that is degraded, and the remainder (termed biomass-associated products, or BAP) is formed in proportion to the amount of biomass in the system [248, 249]. Although SMP has never been fully characterized, it does have metal complexing abilities and is slowly biodegraded [247-251]. The formation constants for ligand groups on SMP are not nearly as large as for chelators. However, in subsurface systems with high substrate loading rates or large biomass populations, SMP may have an important effect on actinide speciation.

Before turning our attention to the development of kinetic descriptions of biomass growth and substrate utilization, we need to introduce two mechanisms that result in the removal of organic contaminants, but provide no support for growth of microorganisms [252]. The first, *secondary utilization*, is the degradation of an organic contaminant that *could* serve as a substrate for growth, but is present in concentrations too low to support net growth. Secondary utilization is possible when capable organisms are present in the system due their utilization of another, primary substrate. Secondary utilization should be contrasted with *cometabolism*, which is the fortuitous removal of a contaminant that *could never* serve as a growth substrate during the degradation of a primary substrate. The distinction is important, because some aspects of the kinetics of the two processes are inherently different.

4.1.2 Biomass growth and substrate utilization kinetics

The most widely used rate expression for the biodegradation of primary electron donor substrates is the classical Monod relationship [253]:

$$r_d = -q_m \frac{S_d}{K_d + S_d} X$$

where r_d is the rate of accumulation of the electron-donor substrate (mass-donor vol^{-1} $time^{-1}$); q_m is the maximum specific rate of donor utilization (mass-donor mass-biomass^{-1} $time^{-1}$; K_d is the donor half-maximum rate concentration (mass-donor vol^{-1}); S_d is the donor concentration (mass-donor vol^{-1}); and X is the concentration of active biomass degrading the electron-donor substrate (mass-biomass vol^{-1}). When $S_d >> K_d$, the Monod expression reduces to $r_d = -q_{md} X$, which is the maximum rate at which the organisms can metabolize the substrate. McCarty [237] found that the maximum utilization rate of simple substrates is limited by the rate at which electrons can be transferred from the electron donor to the electron acceptor. On the other hand, other mechanisms, such as the rate at which the substrate can be transported into the cell, can reduce q_{md} for more complex substrates.

The donor half-maximum rate concentration, K_d, is a qualitative measure of the cell's affinity for a particular substrate. K_d values vary widely depending on the organism/substrate combination. High K_d values mean that the cell has a low affinity for the substrate, and vice versa. Examination of the interaction between q_{md} and K_d can help explain why the fastest growing organism is not always the dominant population in substrate-limited subsurface environments. In Fig. 4.1, we show the substrate utilization rate (normalized by biomass concentration) of two hypothetical organisms as a function of substrate concentration. Organism 1 has q_{md} and K_d values twice and 10 times as high as Organism 2, respectively. In the case of high substrate availability, Organism 1 consumes substrate twice as fast as Organism 2. However, at low substrate concentrations, substrate utilization is controlled by K_d, not q_{md}, allowing the "slower" growing organism to have a much faster utilization rate.

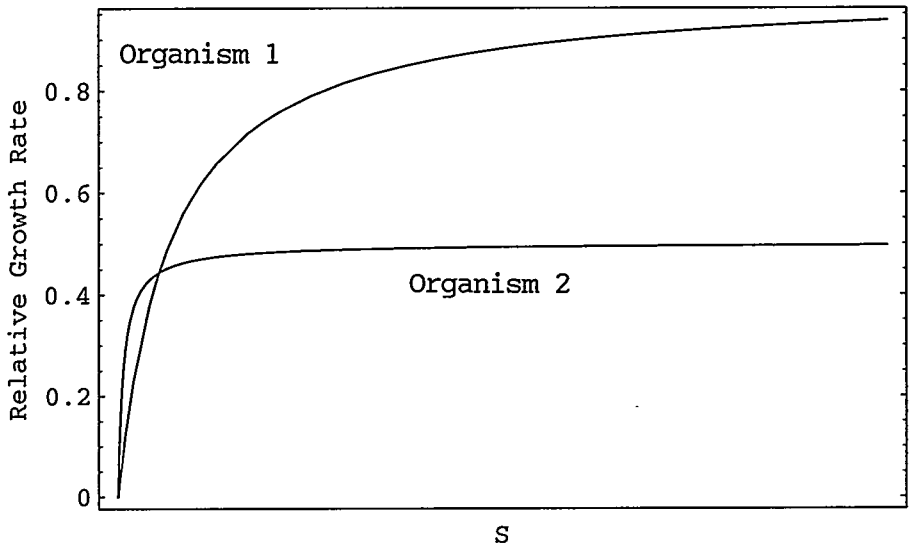


Fig. 4.1 - Relative substrate utilization rate (fraction of maximum rate) per unit biomass for two hypothetical organisms, showing the dependence of the Monod rate expression on the parameters q_{md} and K_d. At high substrate concentration, the rate of substrate utilization is dominated by the maximum rate of substrate utilization, q_{md}, while at low substrate concentrations the rate is more dependent on the affinity for the substrate, K_d.

The Monod expression is useful for calculating substrate utilization rates in systems limited only by the availability of electron-donor substrates. However, in many cases the metabolic rate of a microorganism is also limited by the availability of electron acceptors. Bae and Rittmann [91] showed that dual donor/acceptor limitation can be described through the addition of another term to the basic Monod expression:

$$r_d = -q_{md}\frac{S_d}{K_d + S_d}\frac{S_a}{K_a + S_a}X$$

where the additional coefficients S_a and K_a represent the concentration (mass vol^{-1}) and half-maximum concentration of the electron-acceptor substrate, respectively. The value of K_a is dependent on the role of the electron acceptor plays in contaminant degradation. Typical K_a values for electron acceptors only active in the terminal stage of respiration are much less than 1 mg/l. However, as we discussed earlier, organic degradation reactions catalyzed by the monooxygenase enzyme require O_2 as a direct co-substrate and are much more sensitive to dissolved oxygen concentrations. This is reflected in higher K_a values measured for these reactions [243]. Figure 4.2 compares the magnitude of the electron acceptor-dependent portion of the dual-limitation Monod expression at dissolved oxygen concentration up to saturation using typical K_a values measured for aerobic respiration and monooxygenation reactions [243]. Above 1 mg/l, increasing dissolved oxygen concentrations have no effect on the rate of substrate utilization when O_2 is used solely as an electron acceptor. However, the rate of substrate utilization for a monooxygenase-catalyzed reaction is only 30% of the maximum rate at the same dissolved oxygen concentration.

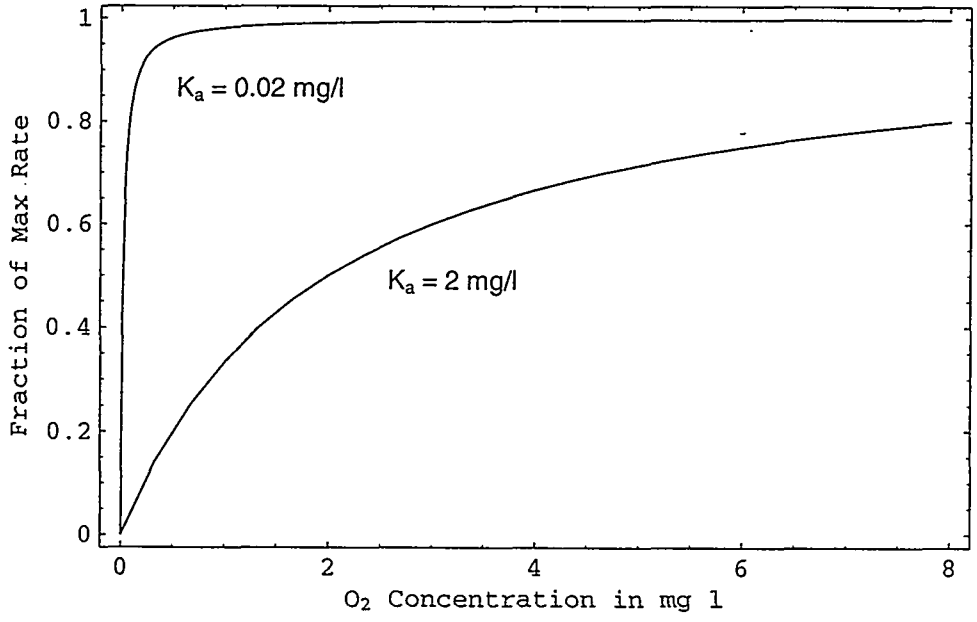


Fig. 4.2 - Value of the electron-acceptor substrate limitation term in the dual-Monod rate expression as a function of dissolved oxygen concentration, based on the values of K_a reported by Malmstead *[243]* for terminal respiration (K_a = 0.02 mg/l) and monooxygenation (K_a = 2 mg/l).

Regardless of the possible limitation of substrate utilization by electron-donor and/or -acceptor concentrations, the rate of substrate utilization is controlled by the concentration of active biomass degrading the substrate, X. Thus, high contaminant degradation rates can be achieved in systems with low substrate concentrations if a large population of active microorganisms is present. The previous section outlined how to determine the relationship between biomass synthesis and substrate utilization through the use of energetic calculations. The true yield, Y, can be directly calculated from f_s^0. For example, in the case of aerobic citric acid utilization:

$$Y = 0.714\frac{e^- \text{ cells}}{e^- \text{ citrate}} \times 18\frac{e^- \text{ citrate}}{\text{mole citrate}} \times \frac{\text{mole cells}}{20\, e^- \text{ cells}} = 0.64\frac{\text{mole cells}}{\text{mole citrate}}$$

Once we know Y, the rate of biomass synthesis is proportional to the amount of substrate utilized:

$$r_{syn} = -Y\, r_d$$

In addition to the growth of new cells, biomass is also lost to cell death and endogenous respiration, the oxidation of biomass to fulfill the energy requirements of cell maintenance. The rate of biomass loss is represented for these phenomena as:

$$r_{decay} = -\, b\, X$$

where b is the biomass decay coefficient ($time^{-1}$). Combination of these two rate expressions gives the overall rate of biomass accumulation:

$$r_{net} = r_{syn} + r_{decay} = -Yr_d - bX$$

Often, biodegradation experiments are performed under "non-growing" conditions to determine the necessary kinetic parameters for the Monod expression by eliminating the complication of changing X due to biomass synthesis. In these situations, "non-growing" means that the net rate of synthesis (or loss) of new cells is small compared to the amount of biomass in the system. In reality, any time primary substrates are degraded, new biomass is being synthesized, even under "non-growing" conditions in which the effect of new growth on the total biomass concentration is negligible.

By coupling the rate expressions for substrate utilization and biomass synthesis, we can predict the rate of substrate disappearance and biomass growth directly as a function of time. The rate of change of concentrations of other compounds consumed or produced during substrate degradation can be related to the rate of substrate utilization through the stoichiometry of the degradation reaction. For example, in the citric acid degradation reaction presented earlier, the rate of ammonia consumption is:

$$r_{NH_4^+} = 0.642\, r_d$$

Similar rate expressions can be written for each component included in the stoichiometry, effectively coupling the biochemistry of substrate utilization to the chemistry of the solution. For example, the rates of production or consumption of acidic hydrogen, organic and inorganic complexing ligands, and intermediates can be related to the rate of substrate utilization.

Rittmann and VanBriesen [234] described how to construct a model linking biochemical substrate degradation, biomass synthesis, and equilibrium chemical speciation. In brief, mass-balance equations are required for all components that must be tracked. These equations are tightly coupled through the stoichiometric relationship outlined above, are highly nonlinear, and normally are "stiff," which means that the time scales over which the different reactions occur

are very different. For example, biomass growth/loss reactions typically occur over hours to days, substrate utilization time scales are seconds to hours, and acid/base and many complexation reactions are essentially instantaneous. These attributes preclude analytical solutions, and sophisticated numerical methods must be solved with high-speed computers.

In many cases, particularly in subsurface environments, the bacteria are not free to move about in solution but are attached to a solid surface in the form of a biofilm, a layer-like agglomeration of microorganisms [254]. Due to transport limitations within biofilms and along the flow paths in the subsurface, all microorganisms attached to solid surfaces do not "see" the same substrate concentrations. For example, organisms near the attachment surface of the biofilm are exposed to lower substrate concentrations than bacteria attached at the liquid/biofilm interface [255]. Likewise, as contaminated water moves through a subsurface aquifer and organic compounds are degraded, organisms far away from the source of contamination are exposed to lower substrate concentrations than those near the source.

The key difference between modeling the growth kinetics of microorganisms in suspended growth versus bacteria found in biofilms is that the immobilization of organisms in a biofilm can create substrate (and by-product) concentration gradients within and outside the biofilm. In the case of a biofilm having a high rate of substrate utilization, but limited transport diffusion, substrate utilization within the biofilm results in concentration gradients of substrates (and other nutrients) just outside and inside the biofilm. In order to model substrate removal in biofilms of this type, the expressions for substrate utilization and biomass synthesis have to be coupled to equations describing the mass transport of substrate into and within the biofilm [255]. However, the usually small accumulation of biomass per unit surface area in subsurface situations frequently gives slow enough utilization rates inside the biofilm that substrate gradients within the biofilm are negligible; in these cases mass transport limitations within the biofilm can be neglected during modeling [256]. On the other hand, substrate utilization by immobile bacteria often causes significant concentration gradients along the flow path within the aquifer; thus, the expressions describing substrate utilization and biomass synthesis must be coupled to equations describing the flow of water past the stationary organisms [234, 257].

4.1.3 Speciation and complex-specific behavior

Recent evidence suggests that the degradation rate of many organic compounds, including organic chelates, is affected by the presence of specific metal cations [146, 226, 227, 258], indicating that the degradation of many organic compounds is limited to a single chemical species [163, 259] or is dependent on the presence of certain metals as co-substrates for key enzymes [166, 225, 260]. In Section 3.3, we discussed how the bioavailability of organic compounds is affected by complexation with metals and actinides. In this section, we focus on how to model the utilization of specific chemical species as substrates.

Many organic compounds that are suitable substrates for microorganisms contain ionizable functional groups that accept or donate protons, depending on pH. Citric acid, which can exist in any of four species, is a good example. At zero ionic strength and a temperature of 25°C, equilibria between the four citric acid species are [113]:

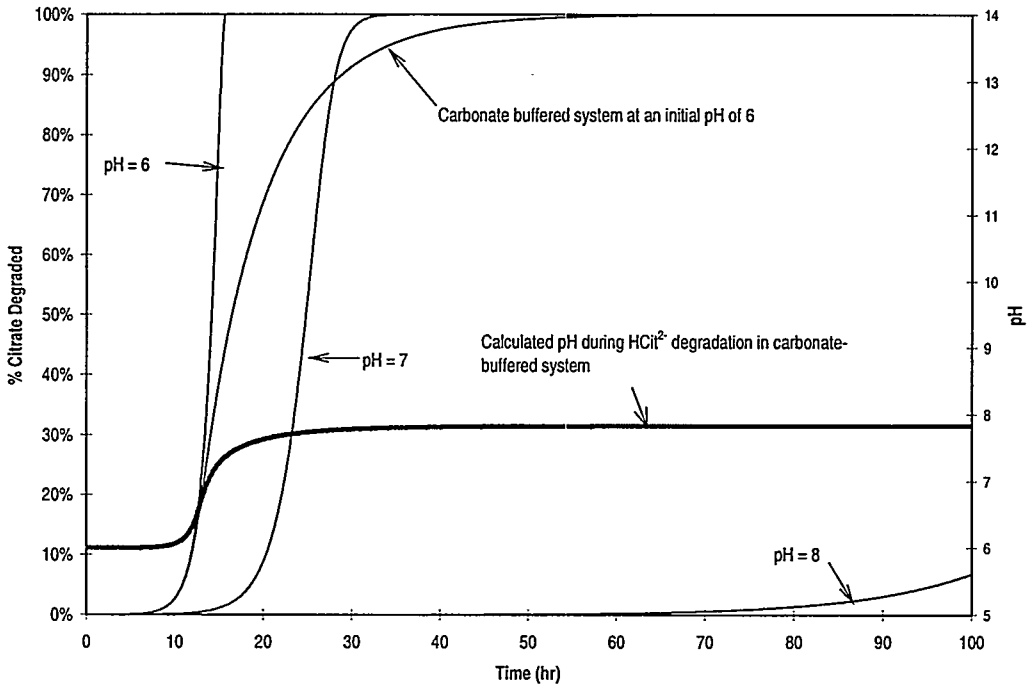
$$H_3Cit \Leftrightarrow H^+ + H_2Cit^- \qquad pK_{a,1} = 3.13$$
$$H_2Cit^- \Leftrightarrow H^+ + HCit^{2-} \qquad pK_{a,2} = 4.76$$
$$HCit^{2-} \Leftrightarrow H^+ + Cit^{3-} \qquad pK_{a,3} = 6.40$$

In the pH range of natural waters, citric acid speciation is dominated by the $HCit^{2-}$ and Cit^{3-} species. However, detailed experiments by Cachon et al. [259] suggested that substrate utilization rates were limited by the concentration of $HCit^{2-}$ alone. Because the $pK_{a,\,3}$ for deprotonation of $HCit^{2-}$ is 6.4, at near neutral pH the limitation of degradation to the $HCit^{2-}$ species should cause the rate of citric acid utilization to be strongly affected by small changes in pH. Additionally, the degradation stoichiometry presented earlier in Section 4.1.1 and Table 4.1 shows that the aerobic utilization of citric acid consumes acidic hydrogen: At near-neutral pH, 2 to 3 acid equivalents are removed due to citric acid utilization for every 0.64 moles of acid equivalents produced. Thus, the biodegradation of citric acid not only removes some of the substrate from solution, it also affects the concentration of the degradable form of the substrate in solution.

Figure 4.3 illustrates several of the possibilities for citric acid degradation when the degradable form is limited to a single species. Using the modeling methodology described by Rittmann and VanBriesen [234], we show in Fig 4.3 the calculated degradation of citric acid at fixed pHs of 6, 7, and 8, compared to the degradation of citric acid in a carbonate-buffered system with an initial pH of 6, based on the assumption that only $HCit^{2-}$ is the form utilized as a substrate. If $HCit^{2-}$ alone is the degradable species, citrate degradation should be highly pH dependent (Fig. 4.3a): As pH increases, the relative amount of the substrate species, $HCit^{2-}$, decreases, resulting in slower degradation at pH 7, and incomplete degradation within the time scale of the simulation occurs for pH 8. In the case of a variable-pH system, such as a natural water buffered by carbonate equilibria, the pH increase caused by citric acid degradation results in a continually slower rate of substrate utilization. On the other hand, if only Cit^{3-} is the degradable form of the acid (Fig. 4.3b), we see no effect of pH on degradation in the same pH range, since at the lowest pH of 6 the Cit^{3-} species accounts for approximately 75% of the total amount of citric acid in solution. In this case, the pH increase during degradation has little effect on substrate concentration because the availability of the Cit^{3-} species increases with rising pH.

Figure 4.3b highlights some of the caveats that must be considered when modeling biological data. As we showed in Fig. 4.3a, the calculated pH during the degradation of 0.5 mM citric acid in a carbonate-buffered system changes from 6.1 to 7.9. If we account for the change in citric acid speciation caused by the varying pH, we can calculate similar modeling results for the degradation of citric acid by assuming either $HCit^{2-}$ or Cit^{3-} is the degradable form of citric acid, as shown in Fig. 4.3b. However, Fig. 4.3a shows that citrate biodegradation at fixed pH should behave quite differently, depending on the actual form of the substrate: If Cit^{3-} is the degradable form of the acid, degradation *accelerates* as the pH increases, whereas if degradation is limited to the $HCit^{2-}$ species, degradation *decelerates* with increasing pH. Recent evidence [261] shows that the degradation of citric acid is faster in buffered systems at pH 6 than at pH 8, suggesting that $HCit^{2-}$ is the substrate for *P. fluorescens*. Thus, inferences as to the degradable

a)



b)

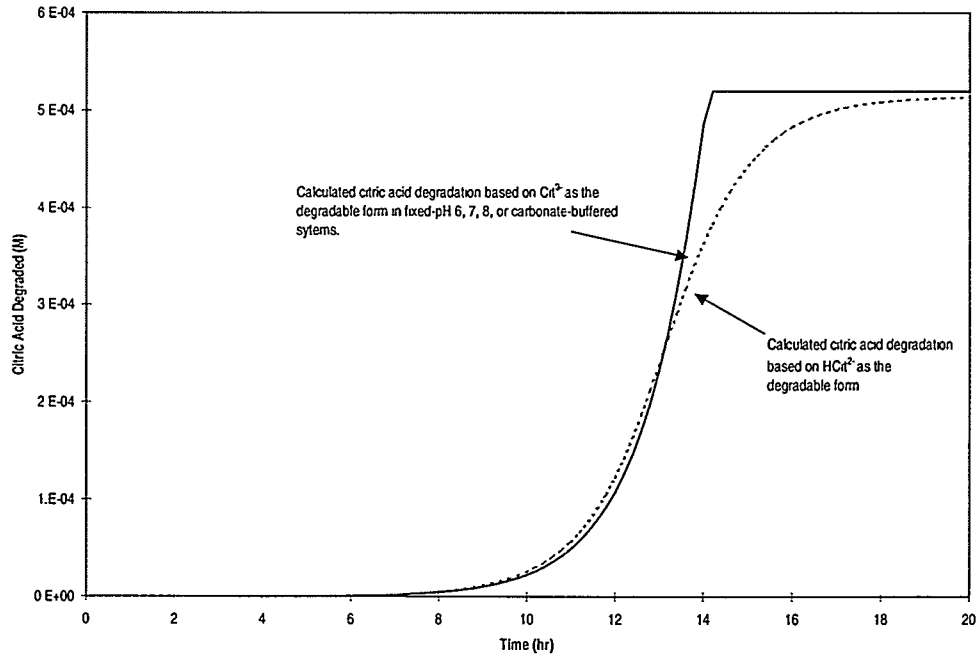


Fig. 4.3 - (a) Calculated citric acid degradation at fixed and variable pH when $HCit^{2-}$ is modeled as the degradable form of citric acid. The rate of citrate degradation decreases with increasing pH due to the shifting of citrate equilibria from the $HCit^{2-}$ to the Cit^{3-} species. (b) Comparison of calculated degradation rates when $HCit^{2-}$ and Cit^{3-} are modeled as the degradable forms of citric acid. Degradation accelerates as pH increases if Cit^{3-} is the degradable form of the acid and decelerates with increasing pH if the substrate utilization rate depends only on the concentration of $HCit^{2-}$. The model system was assumed to maintain equilibrium with atmospheric CO_2.

form of specific substrate species can only be made based on controlled experimentation combined with modeling.

Other speciation-specific phenomena have been reported for various metal-ligand systems. Bolton et al. [163] suggested that the degradation of NTA by *C. heintzii* is dependent on the concentration of the $HNTA^{2-}$ species, but VanBriesen and Rittmann [228, 229] reanalyzed their data using a comprehensive model that links equilibrium speciation with the kinetics of biodegradation. They concluded that the biodegradable form of NTA is NTA^{3-} for *C. heintzii*. Joshi-Topé and Francis [115] reported that Ni-citrate and Zn-citrate complexes could be transported across cell membranes and degraded by *P. fluorescens* after an adaptation period, but at a slower rate than free citric acid. Van Ginkel et al. [173] found that EDTA was only degraded in a wastewater treatment plant at pH values greater than 8, implying that EDTA degradation may also be speciation dependent. Clearly, the application of coupled models will be required to increase our understanding of speciation-specific substrate utilization.

4.2 Coupling of microbiological and chemical reaction modeling to subsurface actinide-specific phenomena

In the previous section, we described the use of several modeling tools under development to help us predict the fate of actinide elements as they interact with subsurface microorganisms. The key is linking kinetic models for microbial growth and substrate utilization kinetics with equilibrium chemical speciation. As these coupled models improve to include other mechanisms--such as sorption, precipitation, and redox effects--so too will our ability to accurately describe key subsurface interactions. One current weak link in model development is the availability of accurate, representative, actinide-specific data. In this section, we discuss some of the limitations of current actinide data in the context of describing actinide-microbe interactions and suggest key areas for future research that would improve our modeling capabilities.

4.2.1 Actinide equilibrium speciation

This review highlights that chemical speciation strongly affects actinide/microorganism interactions. Thus, a fundamental requirement for the successful modeling of actinide-microbe interactions is the ability to accurately describe chemical speciation in the system. Unfortunately, because of the inherent difficulties associated with actinide handling, the complexity of actinide chemistry, and the initial emphasis on research related to actinide separations processes (i.e., low pH chemistry), complete databases are not available for many environmentally relevant actinide systems.

The accuracy of current thermodynamic databases is dependent on the actinide element under study. Nitsche [262] compared the calculated chemical equilibrium speciation of U, Np, Pu, and Am to the measured actinide speciation in groundwater. Based on the assumption that the measured actinide oxidation state distributions approximated equilibrium values because they were consistent with results measured in similar groundwaters, Nitsche [262] found that model calculations accurately reflected the environmental distribution of Np and Am. On the other hand, the calculated Pu and U distributions were several orders of magnitude different than their actual speciation in the subsurface. Nitsche [262] concluded that the discrepancies between the measured and calculated Pu and U distributions were most likely caused by limitations in their

corresponding thermodynamic databases. However, because Np(V) and Am(III) are stable under a wide range of environmentally relevant conditions (and in the laboratory), their complexation behavior with common inorganic ligands is relatively well known. Thus, the calculated oxidation state distributions of Np and Am more closely matched their measured values.

As an example of a relatively well-defined actinide system, we investigated the calculated and experimentally determined speciation of Np(V) at near neutral pH. Table 4.2 shows the calculated equilibrium chemical speciation of 0.51 mM Np in a 0.51 mM NTA solution at pH 6.1 and in equilibrium with atmospheric carbon dioxide. These data are based on published formation constants for the appropriate Np complexes [153, 263-270]. According to equilibrium calculations, about 68% of the total Np in solution should be found as the aquo NpO_2^+ ion. We can compare this result to a UV-vis absorption spectrum obtained on the same solution, shown in Fig. 4.4. The spectrum shows the coexistence of the free NpO_2^+ ion, represented by the 980.2 nm absorption band, with the NpO_2NTA^{2-} complex, visible as the 990.2 nm shoulder on the larger 980.2 nm peak. Using an extinction coefficient of 391 for the aquo absorption band results in a calculated NpO_2^+ concentration of 3.48×10^{-4} M, or 68.2% of the total Np in solution. In this case, the calculated Np speciation agrees well with the observed Np distribution in actual samples. Since Np(V) is stable in air at a wide range of pH values, the study of Np(V) complexation is relatively straightforward, and experiments can be conducted at conditions that are relevant to environmental systems. For example, the formation constant for the Np-NTA complex was determined over the pH range of 2.37-6.79 [266].

Table 4.2 - Calculated chemical speciation of 0.51 mM Np in 0.51 mM NTA solution at pH 6.1 and in equilibrium with atmospheric CO_2. Free NpO_2^+ accounts for 68% of the total Np in solution, while NTA-complexed Np is 32%, while NTA-complexed Np is 32%.

Species	Concentration (M)	% of NTA	% of Np
NTA^{3-}	7.41×10^{-8}	0.015	0
NpO_2^+	3.47×10^{-4}	0	68.0
H_3NTA	3.02×10^{-12}	0	0
H_2NTA^-	1.12×10^{-7}	0.022	0
$HNTA^{2-}$	3.46×10^{-4}	67.8	0
NpO_2OH^0	1.73×10^{-9}	0	≈ 0
$NpO_2(OH)_2^-$	1.28×10^{-15}	0	≈ 0
$NpO_2CO_3^-$	7.20×10^{-9}	0	≈ 0
$NpO_2(CO_3)_2^{3-}$	1.02×10^{-16}	0	≈ 0
$NpO_2(CO_3)_3^{5-}$	6.85×10^{-25}	0	≈ 0
NpO_2NTA^{2-}	1.63×10^{-4}	32.0	32.0
NpO_2HNTA^-	1.54×10^{-14}	≈ 0	≈ 0
$NpO_2(OH)NTA^{3-}$	5.72×10^{-10}	≈ 0	≈ 0

Unlike the Np(V) system, Pu chemistry, in general, and the behavior of Pu(IV), in particular, is clearly more complicated. In many subsurface and other natural systems, the total Pu speciation is dominated by the +4 oxidation state, the majority of which is often present as a solid or colloidal phase [75, 97, 99, 110]. However, in dilute solutions at near neutral pH, the soluble Pu fraction can exist in four oxidation states simultaneously, depending on the redox conditions and presence of ligands in the system [75, 105, 107, 117, 118]. Although complexation tends to stabilize the Pu(IV) oxidation state, Pu(IV) solubility can be controlled by the $Pu(OH)_4$ and PuO_2 solid phases or by the Pu(IV) polymeric colloid in the absence of chelators [60, 117, 118]. Thus, at near neutral pH, the concentration of the free Pu^{4+} ion never reaches a level at which it can be directly quantified, and the oxidation state distribution in any Pu solution is often difficult to define. To circumvent these problems in the laboratory, researchers have studied Pu(IV) complexation at pH $\leq$ 3 to prevent hydroxide complexation and polymer formation.

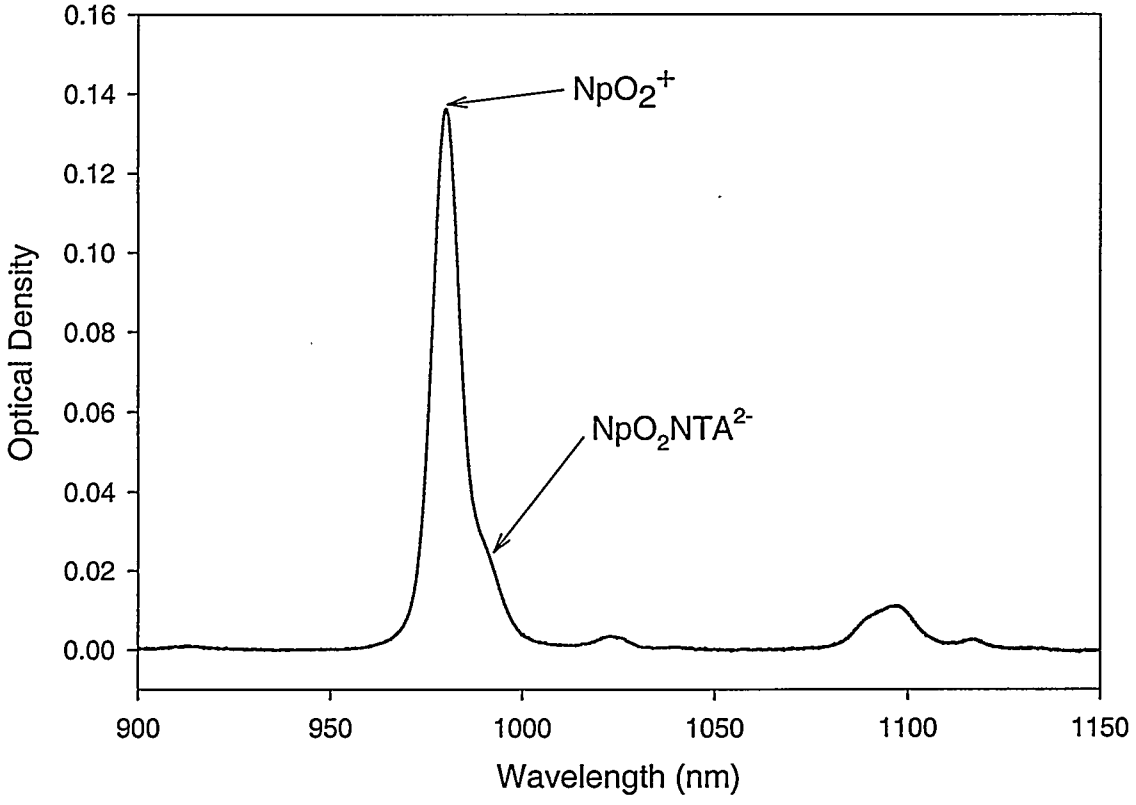


Fig. 4.4 - UV-vis absorption spectrum of 0.51 mM Np in 0.51 mM NTA solution at pH 6.1. The aquo NpO_2^+ ion and NpO_2NTA^{2-} complex are shown at 980.2 and 990.2 nm, respectively. The aquo NpO_2^+ ion accounts for about 68% of the total Np in solution based on an extinction coefficient of 391.

As an example of the difficulties associated with modeling the aqueous speciation of Pu(IV) systems, we return to our discussion of the competition between chelates and common inorganic ligands for complexation with Pu(IV). In Section 2.3, we showed how the calculated speciation of Pu(IV) is affected by relatively small changes in the magnitude of the Pu(IV)-NTA formation constant, because its value is not significantly larger than the values for other inorganic complexants in the system. The most recent value for the Pu(IV)-NTA formation constant was determined at pH 1.1 [266]. Absorption spectra measured in Pu(IV)-NTA solutions at near neutral pH indicate that the species formed at low pH do not match those found in higher pH systems [81], suggesting that the formation constant may not be for the relevant species. Additionally, the dominant Pu(IV) species formed with inorganic ligands in natural waters remain a subject of debate. Initially, Pu(IV) equilibria in aqueous solutions were interpreted as involving the stepwise formation of $Pu(OH)_n^{4-n}$ and $Pu(CO_3)_n^{4-2n}$ complexes [271, 272]. More recently, $Pu(CO_3)_5^{6-}$ was recently described as the limiting Pu(IV) carbonate complex [273]. On the other hand, Hobart et al. [274] and Yamaguchi et al. [275] showed that Pu(IV) speciation at near neutral pH is described by the formation of mixed hydroxide carbonate complexes. In a critical review on actinide carbonate speciation, Clark et al. [153] concluded that $Pu(OH)_x(CO_3)_y$ species are likely to form, although x and y remain to be accurately determined.

We used the formation constants of Yamaguchi et al. [275] and Nitsche and Becraft [266] to construct the diagrams shown in Figs. 2.6a, b, and c. At pH values of 5 or below, the calculations indicate the expected equilibrium behavior: As the ligand-to-metal ratio exceeds one (i.e., L is in excess), the metal speciation is dominated by the chelated species. However, at pH 6, the $Pu(OH)_4^0$ species accounts for more than 90% of the total Pu in solution. The calculated speciation is contrary to the results observed by Al Mahamid et al. [81] and Reed et al. [165], who showed that NTA chelation dissolved the Pu polymer to form a stable Pu(IV)-NTA complex at pH values 5 and above. Because the experiments conducted by Al Mahamid et al. [81] were performed in an inert atmosphere (argon), they provide an excellent source of data to evaluate the applicability of existing Pu(IV)-NTA formation constants to near-neutral pH systems, since the effects of carbonate complexation (and the uncertainty as to the type of carbonate complex formed) can be neglected. Table 4.3 shows the calculated concentrations of the two dominant Pu(IV) species as a function of the magnitude of the formation constant for the Pu(IV)-NTA complex and under conditions similar to those used by Al Mahamid et al. [81]: an NTA/Pu ratio of 45/1, a total Pu concentration of 10^{-3} M, and pH 7. The results in Table 4.3 support the finding of Al Mahamid et al. [81] that the complex formed between Pu(IV) and NTA at neutral pH is different from the complex observed at low pH, because the formation constant measured by Nitsche and Becraft [266] is at least five orders of magnitude too low to correctly predict the domination of Pu speciation by NTA at neutral pH if $PuNTA^+$ is the major complex at all pH values.

Although our analysis of the Pu(IV)-NTA system is rather crude, it is interesting to note that the log β value in Table 4.3 where NTA complexation first becomes significant at pH 7, 16.89, is similar to the value of 16.9 measured for the formation of $ThNTA^+$ [276] and to the value of 17.9 for $FeNTA^0$ [113]. However, this assumes that the complex formed between Pu(IV) and NTA has 1:1 stoichiometry. Modeling and laboratory studies of NTA biodegradation in systems containing Pu(IV) suggest that a species with different stoichiometry may dominate Pu(IV) speciation at near-neutral pH [277].

Table 4.3 - Dominant calculated Pu(IV) speciation in pH 7, carbonate-free water with $C_{T,Pu}$ = 10^{-3} M and $C_{T,\ NTA}$ = 0.045 M, as a function of increasing $PuNTA^+$ formation constant. Pu(IV)-hydroxide formation constants taken from Kim et al [272]. Calculated Pu speciation based on the formation constant measured by Nitsche and Becraft at pH 1.1 is shown in bold type. Reed [165] and Al Mahamid et al. [81] showed that NTA will dissolve the Pu polymer, suggesting that the Pu-NTA complex formed at near-neutral pH has a formation constant 5 or more orders of magnitude higher than the complex observed at pH 1.1.

Log β formation of the Pu(IV)-NTA Complex	$[PuNTA^+]$ (M)	$[Pu(OH)_4^0]$ (M)
12.86	**1.63×10^{-8}**	**9.97×10^{-4}**
13.86	1.63×10^{-7}	9.97×10^{-4}
14.86	1.63×10^{-6}	9.96×10^{-4}
15.86	1.60×10^{-5}	9.82×10^{-4}
16.86	1.40×10^{-4}	8.58×10^{-4}
17.86	6.16×10^{-4}	3.83×10^{-4}
18.86	9.41×10^{-4}	5.88×10^{-5}

Similar discrepancies are found in other actinide-ligand systems. For example, the formation constant for $PuEDTA^0$ varies by about eight orders of magnitude [266]; there is considerable disagreement over the structure of the initial Pu(VI)-hydroxide species [278-282]; and a 2:3 U(VI):citrate complex has been proposed, but the formation constant has never been determined [283]. In order to predict the interactions between microorganisms and actinide elements in natural systems with confidence, actinide speciation calculations should be accurate to within an order of magnitude. Thus, determination of formation constant data under a wide range of conditions is a fundamental research need to enable the development of accurate models for actinide-microbe interactions.

4.2.2 Actinide redox stability in the presence of organic compounds

The key to accurate prediction of actinide speciation in subsurface environments begins with an accurate representation of the oxidation state distribution of the metals. In order to develop coupled models able to predict the effect of microbial activity on the redox distribution of actinides, we need to improve our understanding of the chemical, or *abiotic*, redox transformations of actinides by organic compounds. As we discussed in Section 2, organic and inorganic ligands can affect the oxidation state stability of multivalent actinides by shifting the equilibrium redox potential toward the oxidation states that form the most stable complexes. In addition to the preferential stabilization of certain oxidation states, organic compounds can alter actinide redox speciation by directly serving as electron donors in redox transformation reactions.

The role of organic compounds in the reduction of Np(VI) was reviewed by Choppin and Rao [284], who investigated the kinetics of actinide reduction by known dicarboxylic acids and hydroxylic compounds as analogues to the humic acids normally encountered in the environment. Choppin and Rao represented the reduction of NpO_2^{2+} by a dicarboxylic acid ligand, L, as:

$$NpO_2L \rightarrow NpO_2^+ + \bullet L^-$$

where $\bullet L^-$ is the dicarboxylate free radical anion, and NpO_2^+ is the final Np oxidation state. Although these researchers did not identify the end product of the free radical decomposition, they did relate the pH-dependent rate of Np reduction to the loss of a methylene proton from the ligand, and they concluded that Np(VI) reduction resulted from NpO_2L formation and was inhibited by NpO_2L_2 formation [284]. Similar results were found for the reduction of Np(VI) by hydroxylic compounds: pH-dependent reduction of the actinide and production of unidentified oxidation products [284].

In the Pu(VI) system, reduction of the actinide by organic compounds initially results in formation of Pu(V), with the further production of Pu(IV) as the stable oxidation state. Reed et al. [285, 286] investigated the reduction of Pu(VI) by various chelating agents at moderate to high pH. Fig. 4.5 shows the UV-vis absorption spectra of Pu(VI) as a function of time, in the presence of excess citrate and EDTA at moderate pH values of 4-7. Within 10 days, most of the Pu was reduced to the +4 oxidation state. However, at pH > 8, Reed et al. [285, 286] found that Pu(VI) was stable for months in the presence of high citrate concentrations.

Organic chelates are particularly effective reductants because most form the strongest complexes with actinides in the +4 oxidation state. Figure 4.6 shows the Np(IV) region of UV-vis spectra obtained from Np(VI) solutions that underwent abiotic reduction by excess PIPES (piperazine-N, N'-bis[2-ethanesulfonic acid]), an organic pH buffer, and NTA. Although most of the Np in the solution was reduced to Np(V), a sharp band in the 740 nm region of the Np-NTA solution indicates the formation of an Np(IV)-NTA complex, even though the solution remained aerobic. We found that the Np(IV)-NTA complex is stable in contact with air for many months, suggesting that in the long term, Np(IV) may be a stable oxidation state in aerobic solutions with excess strong ligand concentrations.

Because there is relatively poor understanding of the mechanisms involved in abiotic redox reactions between actinides and organic compounds prevalent in natural systems, most attempts at modeling these reactions have been limited to the determination of "pseudo first-order" rate constants for the overall reactions. Similarly, the end products of organic oxidation vary or have not been identified, thus few data are available to allow formulation of electron-donor half reactions for organic reductants. For these reasons, modeling abiotic redox transformations of actinides is currently a weak link in the prediction of actinide chemical speciation in natural systems.

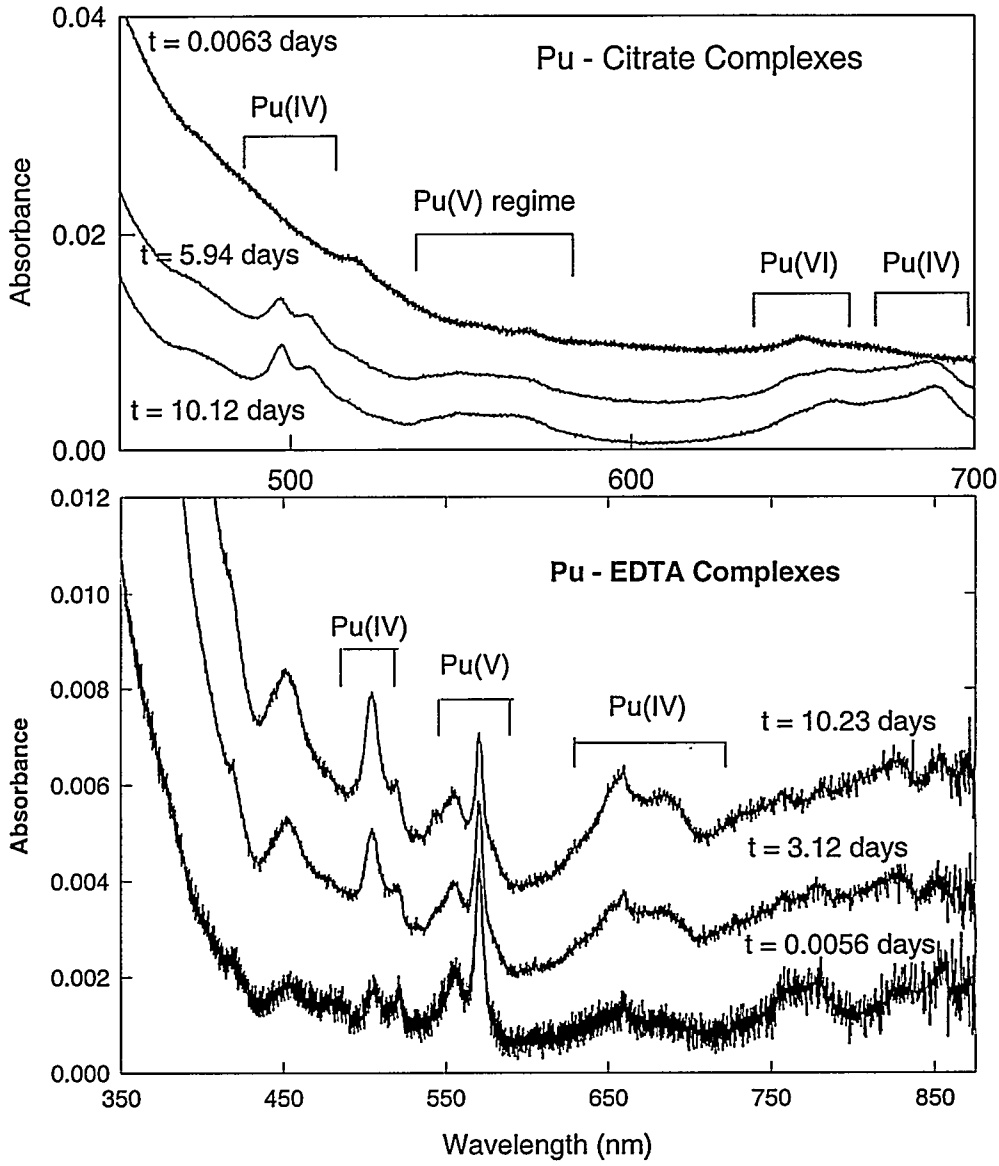


Fig. 4.5 - Redox stability of Pu(VI) in the presence of EDTA and citrate (similar results were found for oxalate). The organic acids cause reduction of the Pu to the IV valence state. The final stable species is the Pu(IV)-organic complex.

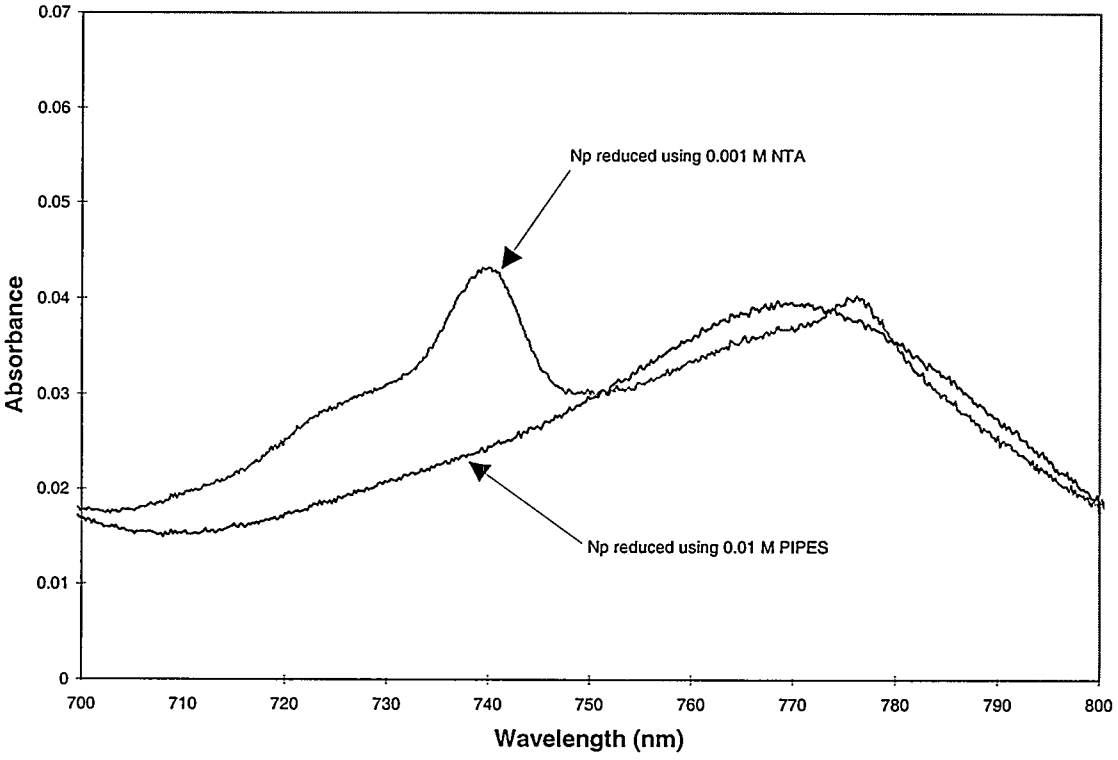


Fig. 4.6 - Comparison of spectra obtained from Np(VI) samples chemically reduced using 0.01 M PIPES and 0.001 M NTA. The Np reduced using NTA showed evidence of formation of the Np(IV)-NTA complex (740 nm and 777 nm bands).

Before we can attempt to develop improved models describing abiotic redox transformations involving actinides and organic compounds, we must overcome many research challenges. We have only partial understanding of the mechanism and kinetics of the actual redox transformation. Although we know oxidation of the organic compound initially results in the formation of an "activated" product or free radical, we have not yet identified with any confidence many of the final organic products. Additionally, the breakdown of complex organic molecules into simpler structures usually enhances the biodegradability of organic compounds. This decomposition certainly affects the chemical properties, especially the complexing ability, of the compound. We also can speculate that natural organic matter and organic compounds produced by microorganisms may participate in the abiotic reduction of actinides. It is obvious that quantification of abiotic subsurface interactions provides exciting research opportunities.

4.2.3 Radiotoxicity effects

Models of microbial growth and substrate utilization are well developed and can accurately predict the operational behavior of natural and engineered systems when appropriate kinetic parameters are available. Modeling techniques are also available to describe the effect of various types of inhibition on the growth rate of microorganisms, including the effects of metal chemical toxicity [252]. Although no mathematical models can yet fully describe the effects of

radiotoxicity on microorganisms, we suggest how the various forms of Pu toxicity proposed by Reed [165] can be represented:

1. Metal toxicity. Metal chemical toxicity often exhibits saturation-type behavior. In these cases, metal toxicity can be described by addition of the term $\frac{K_{Me}}{[Me]+K_{Me}}$ to adjust q_m in the Monod expression. As the concentration of the actual toxic metal species, [Me], becomes much greater than the constant K_{Me} (usually determined experimentally), microbial growth and substrate utilization slows and eventually are totally inhibited.
2. Radiation dose-to-solution. Radiation effects are modeled as linear dose-response relationships. In principle, the effect on microbial growth can be described by adjusting the endogenous decay constant, b, for the accumulated radiation dose present. However, the accounting of accumulated dose may be difficult for cells that are multiplying.
3. Radiotoxicity caused by bioassociation. As an actinide becomes associated with a microorganism, either as a result of biouptake or physical adsorption, the radiation effects can no longer be described by the average dose-to-solution, because the close proximity of the alpha-emitting atom to the organism increases the likelihood of cellular damage. Development of models for this type of interaction requires knowledge of the amount and type of actinide association with the organism; information that must be determined empirically.

Modeling each of the three effects described above presents significant research challenges. Number (1) requires identification of the toxic metal species and controlled experiments that separate radiation toxicity effects from chemical inhibition; number (2) requires detailed radiation tolerance studies on the organism or microbial consortia of interest; and number (3) requires determination of the actinide association capacity of the organism and how association is related to the presence of other ligands in the system, characterization of the amount of inhibition caused by various types of association, separation of the effects of (3) from those of (1) and (2), and incorporation of this information into existing models. Although difficult to develop, models that accurately describe the inhibition of microbial activity by actinides are critical to the application of bioremediation techniques to the cleanup of contaminated sites.

5. Bioremediation of Organic-Actinide Contaminants

The ideal bioremediation scenario is an *in situ* one, which minimizes exposures and greatly reduces cleanup costs. Microbial processes offer significant potential, although many technical questions are unanswered. *In situ* bioremediation can be used directly as a means to control the mobility of actinides. In addition, approaches taken to bioremediate the organic co-contaminants will affect the fate of the actinides. For both situations, we summarize by identifying promising paths and critical research needs.

5.1 Bioremediation directed to the actinides

The most promising strategy for direct action towards the actinides is to immobilize them. Without strong chelators, the least mobile form of the actinides is An(IV), such as PuO_2(s) and

$UO_2(s)$. On the other hand, the An(IV) species also are the most strongly complexed by the organic chelators. Although the chelates are adsorbable to subsurface solids, the actinides are less mobile when precipitated then when they are adsorbed as complexes. Therefore, the ideal scenario for immobilizing the actinides is conversion to An(IV) *and* biodegradation of the organic chelates.

Biodegradation of all the key chelates can occur under aerobic conditions, although EDTA biodegradation may be highly specialized. Aerobic biodegradation of the chelates tends to increase solution pH, and this factor usually increases the rate and extent of actinide precipitation. On the other hand, increasing pH can slow the biodegradation kinetics by shifting chelate speciation away from the species transported into the cells. Substantial research is needed to define the scope of EDTA biodegradation and the species-dependent kinetics for the biodegradation of all the chelates.

Sustaining the oxic conditions needed to drive aerobic biodegradation of the chelates may affect the redox status of an actinide. If the actinide already is present in its oxidized (V or VI) form, maintaining strongly oxic conditions should prevent reduction to the An(IV) form. In general, the An(V and VI) forms are the most soluble, but the least strongly complexed. If the actinide is present in its reduced (III or IV) state, it might serve as electron donor for aerobic microorganisms. In the case of plutonium, oxidation of Pu^{3+} to $PuO_2(s)$ would benefit immobilization. Conversely, oxidation of $UO_2(s)$ to UO_2^{2+} would increase mobility for uranium.

In cases for which precipitation is feasible, the microorganisms may accelerate the process through bioprecipitation related to localized production of precipitating anions (e.g., OH^-, PO_4^{3-}, and CO_3^{2-}) or by being a site for nucleation. In such cases, radiotoxicity becomes an issue unique to bioremediation involving radionuclides.

Many complexants (e.g., citrate and NTA) biodegrade anaerobically, although denitrification is the only path for NTA degradation so far. Because the redox potential of nitrate reduction is close to that of O_2 reduction, biodegradation through denitrification should similarly affect actinide redox speciation. One notable difference between denitrification and O_2 respiration is the much greater generation of base during denitrification. Thus, pH increases should be greater with denitrification, and the positive effect on the precipitation rate and the negative effect on the biodegradation kinetics should be accentuated.

Anaerobic biodegradation of citrate and other natural organic acids is possible, although we have limited understand of the kinetics. Furthermore, most sites co-contaminated with actinides and chelates are naturally anaerobic. Strongly reducing conditions associated with fermentation, sulfate reduction, methanogenesis, and iron reduction make oxidized actinides thermodynamically favorable electron acceptors. If microorganisms are capable of catalyzing the reactions, reduction of PuO_2^{2+} and PuO_2^{+} to $PuO_2(s)$ is most favorable, while reductions of Np(V) to $Np(OH)_4/NpO_2(s)$ and UO_2^{2+} to $UO_2(s)$ are possible; microbially catalyzed uranium reduction already is documented. In each case, the resulting actinide is in the An(IV) state, which is the least soluble form at near-neutral pH once the chelate is depleted. On the other hand, anaerobic respiration of $MnO_2(s)$, $Fe(OH)_3(s)$ or FeOOH(s) can release precipitated or adsorbed actinides.

5.2 Effects on actinides of bioremediation directed to other contaminants

Chlorinated solvents and fuel hydrocarbons frequently are co-contaminants with the actinides, and their greater mobility may make them the prime target for bioremediation in some circumstances. The conditions created by bioremediation directed towards the organic co-contaminants can affect the fate of the actinides.

Fuel hydrocarbons are routinely bioremediated by creating strongly aerobic conditions. Co-metabolism of TCE also requires aerobic conditions. The presence of ample O_2 should have the following impacts on the actinides:

- likely to enhance biodegradation of the organic chelates, thereby decreasing actinide mobility.
- likely to solubilize immobilized actinides by oxidation.
- likely to alter speciation through loss of chelates, formation of carbonates, and release of HCl (TCE only).

The chlorinated solvents can be reductively dechlorinated, and the ample presence of biologically available electron donors accelerates the full dechlorination. Creating strongly reducing conditions can affect the fate of actinides in these ways:

- may allow reduction of oxidized forms of the actinides to the An(IV) state, which is the least soluble.
- may or may not allow biodegradation of the organic chelates; it is most likely for citrate.
- may cause dissolution of iron-oxide solids, thereby releasing precipitated or adsorbed actinides.

5.3 Future needs

Immobilization of the actinides through bioremediation depends on several phenomena that we understand poorly today. The biodegradation capabilities of microorganisms present in subsurface sites are most fundamental. Anaerobic biodegradation of the chelates and the microbially catalyzed oxidation or reduction of the actinides are of paramount importance. We must elucidate the kinetics describing the biotransformation reactions. Keys among the kinetics issues are the speciation effects for the chelates and inhibition effects from the actinides. Inorganic speciation of the actinides remains hazy, and it can profoundly affect the fate of the actinides and the way they interact with microorganisms. We need to ascertain the actual species of importance for conditions relevant to bioremediation, and the thermodynamic database must be expanded and made more accurate. The chemical, microbiological, and geological complexity of co-contaminated sites makes mathematical modeling the essential tool for linking all the processes, testing our understanding, and applying the knowledge in the laboratory, for pilot studies, and for field applications.

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