CHAPTER THIRTY-THREE

SUBSURFACE INTERACTIONS OF ACTINIDE SPECIES WITH MICROORGANISMS[1]

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33.1 INTRODUCTION

Subsurface microbiological processes have an important role in defining the speciation and mobility of actinide contaminants in groundwater. The relative importance of these processes, especially when groundwater conditions support high microbiological activity, has, however, only been recognized by researchers in the field since the early 1990s. The need to mechanistically understand the key interactions between actinide species and microbial processes becomes greater as we increasingly rely on more passive, long-term containment strategies, such as natural attenuation, where microbial processes are likely to predominate (NRC, 2000a).

The effects and interactions of microbiological processes with subsurface actinide species are complex and often not fully understood. Overall, the subsurface

processes that are influenced and, under many circumstances, controlled by microbial activity have been identified. The most important of these are the ability of microorganisms to influence localized redox and pH conditions, their ability to indirectly and/or directly reduce or oxidize multivalent actinides, the bioassociation by surface complexation or uptake of dissolved actinides that can lead to the formation of bio-colloids, and the biodegradation and utilization of inorganic and organic nutrients present in the subsurface that also form actinide complexes and affect their subsurface speciation and mobility. Conversely, the presence of actinide contaminants in the subsurface can also affect the microorganisms present. Actinide species are radiolytically and chemically toxic towards many microorganisms, and their presence can alter the indigenous microbial ecology. Actinides can also be substrates that provide energy for growth.

In this chapter, we present subsurface microbial effects from the perspective of their potential influence on the speciation, fate, and transport of actinide contaminants. This is discussed in two parts: Section 33.2 discusses the effects of actinide contaminants on key microbial processes in the subsurface; Section 33.3 discusses the effects of microbiological processes on actinide speciation and, correspondingly, actinide subsurface mobility. Section 33.4 provides a brief overview of biogeochemical modeling approaches. Lastly, Section 33.5 summarizes existing observations in natural systems centered on bioremediation and bio-containment field studies to provide some perspective on important microbial issues related to long-term containment of actinide contaminants.

This chapter complements Chapter 32 which focuses on geochemical and chemical actinide subsurface chemistry. It also complements Chapter 31 where the biological interactions of actinides with mammals were reviewed.

33.1.1 Historical perspective

The potentially significant effects of microbiological process on the speciation of actinides in the subsurface were explicitly recognized in the early 1990s. The recognition that actinide speciation is influenced by microorganisms is a natural extension of the analogous observation that many subsurface metal reactions (e.g., Fe and Mn along with contaminants Pb, Ni, Cr, and As), formerly thought to be mostly defined by the site geochemistry, are also predominantly microbially mediated. In particular, the biogeochemical cycling of iron and manganese, along with the many inorganic and organic species that can be microbially generated, combine to define many of the key subsurface reactions where actinide contaminants are often co-located.

Although there are a scattering of papers on actinide interactions with organisms prior to 1990, these are largely focused on health and safety issues related to actinide exposure and uptake. Wildung and coworkers investigated the radiolytic toxicity of plutonium towards soil bacteria in the early 1980s (Wildung and Garland, 1980, 1982; Wildung *et al*., 1987). The discovery of

microorganisms that can couple growth to uranium reduction (Lovley *et al*., 1991) provided an alternative and now-accepted explanation for the accumulation of uranium in anoxic sediments. Since then, over 600 peer-reviewed publications on the subsurface biogeochemistry of uranium have been published. This increased emphasis on subsurface biogeochemical processes has extended to a much lesser extent to the transuranic actinides because of the greatly increased difficulty in the safety aspects of working with these higher activity isotopes. Nevertheless, nearly 100 publications report on microbial processes as they relate to various aspects of thorium, neptunium, plutonium, americium, and curium subsurface chemistry – as are discussed in later sections of this chapter.

Radionuclide/actinide microbial interactions have been an ongoing feature of the biennial international symposia on the Migration of Actinides in the Geosphere (1987–present) and were featured extensively in the first International Conference on Bacterial Metal/Radionuclide Interactions (FZR Rossendorf, Dresden, Germany in 1998). The subsurface interactions of actinides in relation to the prospect of their bioremediation were reviewed by Banaszak *et al*. (1999a). An extensive and detailed update of the principles and fundamental mechanisms by which microbes and radionuclides interact under subsurface conditions was also published as a book review with several contributing authors (Keith-Roach and Livens, 2002). More specific to the actinides are reviews by Anderson and Lovley (2002) on microbial interactions with uranium in the environment and by Neu *et al*. (2002) on plutonium–microbial interactions.

The increased reliance on natural attenuation (NRC, 2000a) as a site containment strategy involves the natural immobilization of actinide contaminants in the subsurface. In many cases (NRC, 2000b), the problems at DOE sites are intractable in that complete and total restoration of contaminated lands to pristine conditions is not achievable. This realization has occurred in parallel with renewed efforts within the DOE complex to clean up sites as they are decommissioned. The recent success of the DOE cleanup of the Rocky Flats plutonium complex in central Colorado (Clark *et al*., 2006, 2007) is the best example of a successful cleanup of near-surface contamination although a significant amount of contamination remains in the deeper subsurface. The long-term immobilization and or mobilization of the residual actinide contamination will likely be influenced by microbial processes. Similar conditions likely exist at other sites throughout the nuclear complexes worldwide.

33.1.2 Overview of microbial ecology and its relationship to actinide speciation

Microbes are ubiquitous in the subsurface. The pronounced effects that microbial processes have on actinide speciation are based on the extensive overlap between the organic and inorganic species that define the environmental chemistry of the actinides, but also function as nutrients needed or byproducts formed in the growth of microorganisms. In this section, we give an overview

of the biogeochemical zones and associated reactions along with the relevant actinide environmental chemistry.

(a) Microbial ecology and diversity in the subsurface

Our focus is on *Bacteria* and, to a lesser extent, *Archaea*, which comprise the prokaryotic microorganisms found in the near-surface groundwaters and soils where actinide contamination is typically found. Eukaryotic microorganisms (e.g., algae and fungi) are not considered here, although it is conceivable that they may influence some contaminant plumes in special cases.

The prokaryotes are usually present in the subsurface as highly diversified microcosms. The magnitude and degree of complexity generally found is enormous with over 10^9 different species of bacteria (Dykhuizen, 1998) estimated with a total of 2.6×10^{29} prokaryotes (Whitman *et al*., 1998) found globally in the soils corresponding to over a biomass of 1×10^{18} kg. Only a small percentage of these bacteria have been characterized (estimated to be $<0.3\%$), and even fewer have been successfully cultured and grown under laboratory conditions.

Bacteria and *Archaea* are very adaptable and can survive under highly stressed and nutrient-depleted conditions for millions of years. Ancient *Archaea* also have been cultured from salt inclusions that were over 200 million year old (Vreeland, 2000). Many bacteria form spores that can have extremely long survival times (over at least known human history) and can survive climate cycles. Microorganisms have been shown to have very high tolerance to ionizing radiation (e.g., *Deinococcus radiodurans*) due to their ability to repair radiation-damaged DNA very rapidly (Liu *et al*., 2003; Shukla *et al*., 2007). In this context, microorganisms will prevail even in the presence of the ionizing radiation typically associated with long-lived actinide contaminants.

The diversity of a microbial community also can be affected by the contaminants themselves. This effect is well established for heavy metals that exhibit toxicity towards microorganisms (Silver 1996; Smit *et al*., 1997; Sandaa *et al*., 1999). This, as discussed in Section 33.2.1, extends to the actinides. The overall effect of contaminant interactions is to narrow, and in some cases greatly narrow, the diversity of the microbial population. Microorganisms reduce oxygen, oxyanions (e.g., nitrate, sulfate and carbonate), and redox-active metals (Fe and Mn) that are electron acceptors, to generate energy for growth. These reactions tend to occur sequentially according to the amount of energy that the microorganisms can obtain by reducing the acceptors (Claypool and Kaplan, 1974; Froelich *et al*., 1979). This sequence leads to biogeochemical zones that vary spatially in the subsurface as acceptor availability changes. In nature, these zones may overlap considerably, and correspondingly the microbial processes occur concurrently. The most important of these zones are (see also Fig. 33.1):

- Aerobic respiration: utilizing oxygen as the terminal electron acceptor to form water

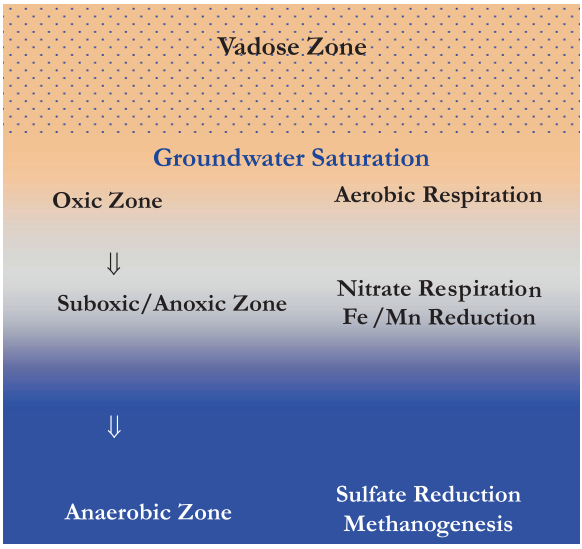

Fig. 33.1 *Relationship between biogeochemical zones and the prevalent microorganism present in the subsurface.*

- Nitrate respiration: utilizing nitrate as the terminal electron acceptor to form nitrogen gas
- Reduction of Fe and Mn: utilizing oxidized metal species as the terminal electron acceptor to form lower-valent metals
- Sulfate reduction: using sulfate as the terminal acceptor to form sulfides
- Methanogenesis: using carbonate as the terminal acceptor to form methane

(*i*) *Oxic conditions: aerobic respiration*

Aerobic respiration occurs when oxygen is used by microorganisms as the terminal electron acceptor. Because this is the most energetically favorable metabolic option, it occurs preferentially under most subsurface environments when oxygen is present and also allows the bacteria to oxidize a wide range of organic and inorganic electron donors. The result of this metabolism of organic donors is the formation of carbon dioxide, water, and biomass:

$$\text{Aerobic respiration} : \text{organics} + \text{oxygen} \rightarrow CO_2 + H_2O + \text{biomass} \quad (33.1)$$

The depletion of oxygen due to aerobic respiration in soils can occur over a very small spatial distance (a few millimeters). This also can occur over as much as a meter if the availability of oxygen is high relative to the availability of biodegradable organic matter. The depletion of oxygen in the subsurface helps establish reducing conditions that is a key to the immobilization of multivalent actinides (see Section 33.3.1).

(*ii*) *Suboxic/anoxic conditions: nitrate- and metal-reducing bacteria*

The depletion of oxygen forms a suboxic/anoxic zone that leads to nitrate reduction, or denitrification:

$$\text{Organics} + \text{nitrate} \rightarrow N_2 + CO_2 + H_2O + \text{biomass} \qquad (33.2)$$

The microorganisms gain almost as much energy with nitrate reduction as with oxygen reduction, and the reduction of nitrate typically occurs very rapidly over relatively short distances in the subsurface.

The depletion of oxygen and subsequently nitrate establishes the reduction of oxidized metals as the next most energy-efficient respiratory pathway for microorganisms. This anoxic biogeochemical zone is centered on the reduction of manganese and iron by metal-reducing bacteria that are often facultative, meaning that they can switch from aerobic to anaerobic respiration. Iron and manganese reduction is especially important in subsurface environments contaminated with multivalent actinides since reduced iron and manganese, as aqueous, sorbed, or solid species, reduce higher-valent actinides (Reed *et al*., 1998, 2006), leading to their subsurface immobilization. Reduced iron and manganese minerals are also highly sorptive towards dissolved actinide species.

The bioreduction of manganese dioxide (Santschi *et al*., 1990), which is the most common form of manganese in soils, is described by the following overall reaction:

$$Mn^{IV}O_2 + \text{Organic material} \rightarrow Mn^{2+}(aq) + CO_2 + \text{biomass} \qquad (33.3)$$

The divalent manganese forms reduced metal phases with carbonate or sulfide and migrates upwards into more oxic zones to reform manganese oxides. This overall reaction does not contribute greatly to the overall degradation of organic materials (typically $<5\%$), since it is not very prevalent in the subsurface. But, when present, it is relatively reactive and can have a strong influence on the speciation of other metals.

The microbial reduction of iron(III), present as an aqueous species or incorporated in a mineral phase, is the next energetically favorable respiratory pathway by which microorganisms derive energy. This is an important subsurface process because of the relatively large amount of iron present in most subsurface environments, the use of iron for nuclear waste storage or disposal, and the microbial redox-cycling of iron. This redox cycling leads to the formation of many iron minerals (e.g. $FeCO_3$ – siderite, Fe oxyhydroxides, FeS – pyrite, and vivianite – $Fe_3(PO_4)_2 \cdot 8H_2O$) depending on the aqueous environment present. This reaction is represented as the following:

$$Fe^{III}(\text{aq or s}) + \text{organic material} \rightarrow Fe^{2+}(aq) + CO_2 + \text{degraded organic} \qquad (33.4)$$

(*iii*) *Anaerobic conditions: sulfate reduction and methanogenesis*

Complete depletion of oxygen, nitrate, and metals establishes two anaerobic biogeochemical zones: sulfate reduction and methanogenesis.

Sulfate reduction:

$$SO_4^{2-} + \text{organic matter} \rightarrow S^{2-}(\text{or } H_2S) + CO_2 + \text{biomass} \quad (33.5)$$

Methanogenesis reactions:

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O + \text{biomass} \quad (33.6)$$

$$CH_3COO^- \rightarrow CH_4 + CO_2 + \text{biomass} \quad (33.7)$$

Sulfate reduction occurs sequentially when other, more energetically favorable, terminal electron acceptors are depleted. These microorganisms tend to be obligate anaerobes in that they can only grow in the absence of oxygen and account for a relatively small (typically $<10\%$) of the overall metabolism of organics in freshwater environments, although this percent can be much higher in marine sediments where sulfate concentrations are higher and oxygen availability is lower. This process helps drive iron reduction by precipitating Fe(II) sulfide phases and in this way affects the iron mineralogy of the site. Consequently, by analogy, it can greatly alter actinide speciation by forming sulfide phases with the reduced actinide species present. This overall process is thought to be predominant with respect to actinide immobilization in deeper subsurface environments where oxygen is very limited while sulfate minerals are abundant.

Methanogenesis, leading to the formation of methane, is often the terminal step in the anaerobic degradation of organic matter. Methanogens are highly specific *Archaea* that fall into two distinct groups (Madigan *et al.*, 1997). One group oxidizes H_2 and reduces CO_2 to form CH_4. The second group ferments acetate into CO_2 and CH_4.

Under strictly anoxic conditions, sulfate-reducing bacteria tend to outcompete methanogens and will predominate so long as sulfate is available. Methanogenesis is more readily observed in groundwater systems, rather than marine systems, because sulfate is not so prevalent in freshwater. Methanogenesis occurs under strongly reducing conditions, which can affect actinide speciation indirectly and abiotically in subsurface groundwaters.

(b) Microorganisms at actinide-contaminated sites

Microorganisms are generally detected at most actinide contaminated sites but their importance and prevalence varies greatly depending on the geochemical conditions and availability of nutrients and organic matter.

Microorganisms were detected at low-level radioactive sites containing transuranic (TRU) wastes and Pu contaminated soils (Weiss *et al.*, 1979; Cleveland and Rees, 1981; Francis, 1990, 2001; Kaiser and Bollag, 1990; Hussain *et al.*, 2001). Several aerobic and anaerobic bacteria were isolated from the leachate samples collected from the low-level radioactive-waste disposal sites and include

Bacillus sp., *Pseudomonas* sp., *Citrobacter* sp. and *Clostridium* sp. (Francis *et al*., 1980a). The radioactivity and the organic chemicals present in the leachate were not toxic to the bacteria and were metabolized producing-tritiated and ^{14}C-methane (Francis *et al*., 1980b; Francis, 1990).

Microorganisms are also present at the nuclear repository sites under consideration or currently in use, and the potential effects of their presence have impacted the performance assessment and engineering decisions related to these sites. The currently operating Waste Isolation Pilot Plant transuranic repository in southeastern New Mexico predicts significant microbial effects over time due to the high nutrient and organic content of the emplaced TRU waste. Indigenous aerobic and anaerobic microbes, although not present in great amounts, were identified (Francis *et al*., 1998; Vreeland, 2000). Similarly, a detailed study of the Yucca Mountain site in Nevada showed a diverse microbial community even though this is a relatively dry site.

Microorganisms have also been identified, characterized, and linked to the subsurface migration of actinides at many sites with known actinide contamination. These are highly variable given the wide diversity of sites where contamination is present. Viable, metabolically active microbes were also detected at the Los Alamos National Laboratory (LANL) TRU waste burial site containing ^{239}Pu-contaminated soil and flammable waste (Barnhart, 1980). Similarly, viable and diverse microbial communities have been established at the Hanford site (White and Ringelberg, 1991; Kieft *et al*., 1993; Fry *et al*., 1997; Chandler *et al*., 1998; Fredrickson *et al*., 2004), Idaho National Engineering site (White and Ringelberg, 1991; Chauhan *et al*., 2002), TRU-contaminated sites at Savannah River (Christensen and Gordon, 1983; Fliermans and Balkwill, 1989; Santo Domingo *et al*., 1998; Scala *et al*., 2006), uranium contamination sites at Oak Ridge (Balkwill *et al*., 1998; Gu *et al*., 2002; Sinsabaugh *et al*., 2003) and the Western Colorado UMTRA (Uranium Mill Tailings Remedial Action) site (Nuttall *et al*., 1997; Yabusaki *et al*., 2007), and the TRU-contaminated Rocky Flats site in central Colorado. Microbial influences on actinide speciation are also under investigation at the Sellafield site in Great Britain (Lloyd *et al*., 2002; Lloyd, 2003; Lloyd *et al*., 2005), the Aespoe underground laboratory in Sweden (Moll *et al*., 2007) to simulate a granitic repository site, and in connection with the nuclear accident at Chernobyl (Zavilgelsky *et al*., 1998).

(c) Overview of key actinide-microbiological processes

The most important factors that determine the fate and transport of actinides in the subsurface are:

- Redox conditions and actinide oxidation state
- Formation of inorganic and organic complexes
- Tendency toward aggregation and/or formation of colloids

We introduce each briefly here. The environmental chemistry of actinides is reviewed more comprehensively in Chapter 32.

(*i*) *Actinide redox chemistry and oxidation state distribution*

The possible oxidation states in groundwater for the various actinides are given in Table 33.1. Thorium, americium, and curium exist in one oxidation state as Th^{4+}, Am^{3+}, or Cm^{3+} species over the range of redox conditions typically observed in groundwater. Uranium, neptunium, and plutonium are multivalent under subsurface conditions and exist as An^{3+}, An^{4+}, AnO_2^{+} or AnO_2^{2+} species. Protactinium is also multivalent but can only exist in the IV and V oxidation state. The actinides that are multivalent can be reduced to their lower oxidation states by microbial processes under anoxic and anaerobic biogeochemical zones.

The linkage between microbial processes and actinide speciation in groundwater is very important in defining the potential mobility of the multivalent actinides. In general, the direct or indirect reduction of higher-valent actinides to An^{3+} or An^{4+} species leads to much lower solubility and a stronger tendency toward sorptive interactions with the minerals present. In this context, the somewhat sequential progression of microbial processes through the various biogeochemical zones and the ability of microbial processes to establish the redox conditions for a given subsurface environment are key factors in defining the predominant form of the actinide in solution in microbiologically-active

Table 33.1 *Most likely actinide oxidation states as a function of microbial activity and the corresponding biogeochemical zone.*

	Actinide							
Biogeochemical zone	*89 Ac*	*90 Th*	*91 Pa*	*92 U*	*93 Np*	*94 Pu*	*95 Am*	*96 Cm*
Oxidation states observed under all conditions	3	(3) **4**	(3) 4 **5**	3 4 5 **6**	3 4 **5** 6 7	3 **4** 5 6 (7)	3 4 5 6 7?	3 4 5? 6?
⇓								
Oxic conditions in groundwater	**3**	**4**	**5**	**6**	**5**	**4** **5**	**3** (5)	**3**
⇓								
Microbially active suboxic zone	**3**	**4**	**4**	**4** **6**	**4** 5	**3** **4**	**3**	**3**
⇓								
Microbially active anaerobic zone	**3**	**4**	**4**	**4**	(3) **4**	**3** **4**	**3**	**3**

( ) = unstable, ? = claimed but unsubstantiated, bold/blue = most stable.

subsurface environments. This is accomplished directly by enzymatic pathways (as is shown in Section 33.3.1) and indirectly by abiotic reaction with reduced species (e.g., Fe^{2+} and Mn^{2+}) that also are generated by microbial pathways.

(*ii*) *Actinide complexation and microbial activity*

The second important interaction between microbial processes and actinide speciation is that many of the inorganic and organic species that are either byproducts of microbial activity or utilized as nutrients for growth are also important factors in defining the speciation of actinides. Inorganic and organic species that are typically associated with microbial activity include:

Inorganic species:	HCO_3^-/CO_3^{2-}, SO_4^{2-}, HS^-/S^{2-}, HPO_4^{2-}, Fe^{2+}/Fe^{3+}, Mn^{2+}/Mn^{4+}, H^+/OH^-, NH_4^+/NH_3, and NO_2^-
Organic species:	Natural (humics/fulvics) organics, citrate, acetate, succinate, methylated compounds, oxalate, and many others
Minerals:	Fe minerals (oxides and oxyhydroxides), Mn minerals, carbonate/sulfide precipitates

The majority of these inorganic, organic, and mineral species are known to have very predominant roles in establishing the fate and mobility of actinides in the subsurface. These lead to extensive and significant coupling between microbial processes and actinide speciation when microbial activity is present in the subsurface.

The sorption of actinide species also is strongly linked to their oxidation state. In general, the following order is observed for their relative complexation strength:

$$An^{4+} > An^{3+} > AnO_2^{2+} \gg AnO_2^+ \tag{33.8}$$

The cell walls of bacteria consist of peptidoglycans, and many carboxyl, phosphate, and hydroxide sites exist, leading to strong bioassociation (Haas *et al.*, 2001; Songkasiri *et al.*, 2002; Songkasiri, 2003; Gorman-Lewis *et al.*, 2005). Additionally, many bacteria utilize sequestering agents (Neu *et al.*, 2003) to obtain essential metals in environments where these metals are often scarce. Sequestering agents usually are strongly complexing and tend to bind to lower-valent actinide species. For these reasons, strong bioassociation between actinide species and microorganisms are often observed, and this interaction can have a significant impact on the speciation of the actinides and their overall mobility in groundwater systems.

33.2 EFFECTS OF ACTINIDES ON MICROORGANISMS

The presence of actinides in the subsurface influences the microorganisms present. This can be especially significant at the source of contamination

where actinide concentrations are relatively high. The three most important potential effects are: (1) loss of cell viability caused by the ionizing radiation associated with the radioactive decay of the actinide; (2) cell death as a result of the chemical toxicity of actinides toward some microorganisms; and (3) limited availability of organic/inorganic nutrients when actinide complexes and precipitates are formed. Once actinide migration is underway, the conditions generally encountered in the subsurface where very low (sub-micromolar and often sub-nanomolar) actinide concentrations are present are not likely to have a significant effect on microbial processes.

33.2.1 Radiotoxicity of actinides towards bacteria

The interaction of ionizing radiation with bacteria almost always causes harm to the cell. The potential loss of cell viability caused by ionizing radiation was discovered quite some time ago (Grubbé, 1933; Puck and Marcus, 1956), 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 and is more extensively summarized in Chapter 31 of this work. Overviews have been published on the general effects of ionizing radiation on matter (Spinks and Woods, 1990), aqueous systems (Draganic and Draganic, 1971), and cell viability (Ewing, 1987).

The radioactive decay of the transuranic actinides of most interest involves alpha particles, beta particles, neutrons, and residual/secondary gamma rays. These types of ionizing radiation do not interact with the aqueous medium in the same way due to their differences in linear energy transfer (LET), which leads to differences in relative effects on cell viability. For example, plutonium-239 primarily undergoes alpha decay (5.157, 5.144, and 5.106 MeV energies). These decays are accompanied by primary low-energy gamma rays (0.0516, 0.0301, 0.129 MeV) and secondary gamma rays and beta particles associated with the interaction of the alpha particle with the aqueous medium. The interactions of all these forms of ionizing radiation potentially affect the observed radiation tolerance of the bacteria.

(a) Gamma and beta (low LET) radiation

The effect of low-LET ionizing radiation (e.g., gamma radiation and beta particles) on the loss of cell viability is described by survival curves that establish the cellular viability as a function of absorbed dose. As an example, the survival curve for the *C. heintzii* bacterium is shown in Fig. 33.2 (Banaszak *et al.*, 1999a). Survival curves for many bacteria have been established, and progress has been made in understanding the mechanism by which ionizing radiation causes cell death (Johansen and Howard-Flanders, 1965; Ewing, 1973; 1982a, b, 1987).

Radiation damage (Ewing, 1987) occurs when the radiolytic transients generated, either externally in the aqueous medium or internally due to the

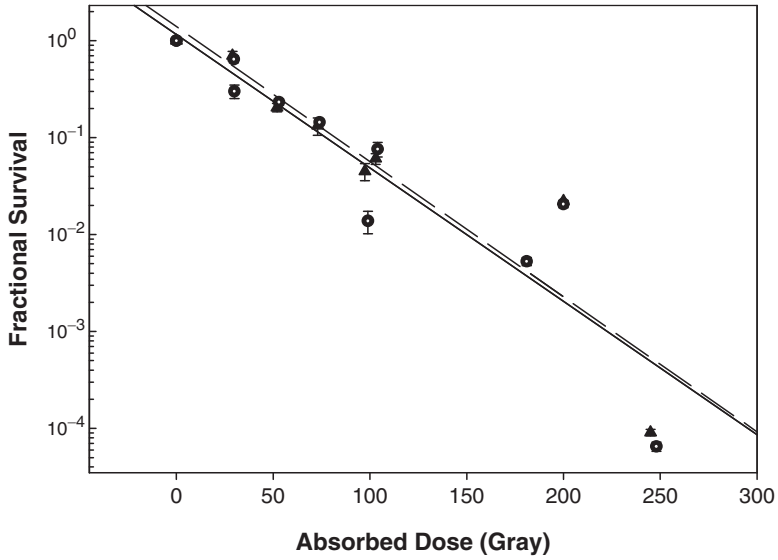

Fig. 33.2 *Fractional survival of Chelatobacter heintzii as a function of gamma-absorbed dose in oxygenated high-purity water (HPW) and in* 0.01 *M PIPES (piperazine-N, N′-bis* [2*-ethanesulfonic acid*]) *buffer at pH* 6.2. *There is a* 99*% loss of viability at* 180 *Gray, which is typical of bacteria in oxygen-sensitized systems.*

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. 33.3. Radiation damage to cell reproduction is linked to the effects of the oxidizing radiolytic products. These are the hydroxyl free radical (OH), the hydroperoxyl free radical (HO_2 and O_2^-), and hydrogen peroxide (H_2O_2). In high-chloride and bromide-containing brines, these transients are converted to hypochlorite, oxychlorides, and hypobromite. Of these, the molecular products (hydrogen peroxide and oxychlorides) are thought to be the most important in causing radiation damage, although some direct contributions from the free radicals and oxidizing transients generated from solutes in water are also possible.

Ionizing radiation most affects microbes during growth stages when replication is at its peak. Specific interactions and damage to DNA are 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 > prokaryotic cells > viruses. The radiation tolerance of microbes varies, but sometimes is very high. The specific factors that define this tolerance are not fully understood, although in some cases it appears that radiation tolerance may be genetically encoded (Mattimore *et al.*, 1995).

Solutes present in the aqueous media can have a pronounced effect on the radiation sensitivity of particular bacteria. The most important aqueous

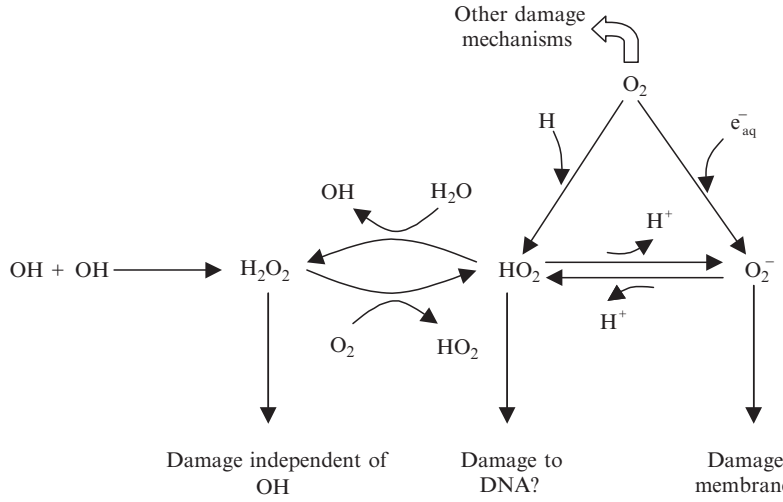

Fig. 33.3 *Schematic representation of cellular damage caused by the radiolytic decomposition of water* (*adapted from Ewing*, 1987).

constituent is dissolved oxygen. In anoxic systems, radiation damage is caused by long-lived oxidizing free radicals, 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 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 OH radical and other mechanisms that are not clearly understood. Here, 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. The key factors that contribute to this effect are:

- Presence/efficiency of free radical scavengers in the groundwater
- Dissolved oxygen concentrations
- Ambient temperature of the groundwater
- Energy, LET, and type of ionizing radiation
- Genetic predisposition to repair cellular damage
- 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) is much higher for a given absorbed gamma dose. For *H. halobium*, 99% loss of viability occurred at 8,000 Gy (Banaszak *et al.*, 1999a), a radiation dose 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 (Spinks and Woods, 1990) as a free-radical scavenger. Prokaryotic bacteria with very high radiation tolerances also exist. 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 5,000–30,000 Gy without loss of cell viability (Binks, 1996). Very high radiation tolerances such as these are generally attributed to fast and efficient mechanisms that can repair radiolytically damaged DNA (Mattimore *et al.*, 1995).

(b) Alpha particle and neutron (high LET) effects

Alpha particle interactions with cells also lead to the loss of cell viability in environmental systems. Alpha particles and neutrons, both of which interact as high LET radiation, are the major types of ionizing radiation for actinides. The observed loss of cell viability occurs with the same mechanisms just discussed for low LET radiation in Section 33.2.1 (a).

There are, however, two important differences between gamma/beta radiation and alpha/neutron particles to consider. First, these particles deposit their ionizing radiation in dense tracks that are much more unevenly distributed throughout the solution and correspondingly the cellular material due to the high LET nature of the interaction; this issue is discussed in this section. Second, because alpha particles are emitted in close proximity to the actinide, chemical and radiolytic toxicity will coexist. The chemical toxicity of actinides, which is often the more predominant form of toxicity in the laboratory, is discussed in Section 33.2.2.

The alpha particle energy of the actinides of most relevance to environmental systems (Pu, Np, and Am) ranges between 4.7 and 5.5 MeV per disintegration. This gives them an averaged LET of ~130–150 keV $(\mu m)^{-1}$ (compared to ~0.2 keV $(\mu m)^{-1}$ 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 (Draganic and Draganic, 1971). 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 (OH, 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 are expected to be lower when a dissolved plutonium complex is the source of ionizing radiation than when the plutonium is primarily located in/on the cell, as in the case of bioassociation or biouptake, 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, rather than chemical toxicity, were shown to be the predominant cause of toxicity when bacteria isolated from subsurface environments interacted with the higher activity isotopes of plutonium (Wildung and Garland, 1980, 1982; Robinson *et al*., 1986). Wildung *et al*. (1987) examined the effects of 0.1–180 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. Growth and longer term (~30 days) static experiments were performed. Cell viability was determined by counts of colony-forming units (CFU), and efforts were 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*. (1987) is that, for ^{239}Pu and ^{238}Pu, the loss of cell viability was predominantly caused by radiolytic, rather than chemical, pathways for the microorganisms investigated. Different types of organisms also exhibited different abilities to tolerate ionizing radiation. Plutonium(IV) species, which were predominantly in that oxidation state in the systems studied, became associated with the biomass and were solubilized by the exocellular material present. Plutonium speciation was qualitatively shown to have an effect on the observed toxicity.

A similar conclusion was reached by Reed *et al*. (1999) in the investigation of the Pu(IV)-NTA-*C. heintzii* system. 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. 33.4. 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.

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

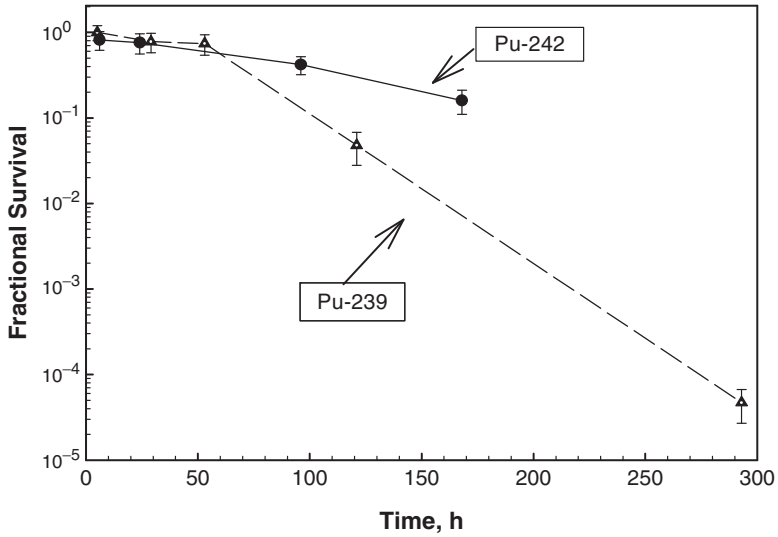

Fig. 33.4 *Loss of cell viability, as a function of time, for Chelatobacter heintzii in the presence of* 10^{-5} *M* ^{239}*Pu and* 10^{-5} *M* ^{242}*Pu. With the isotopic purity of the plutonium used,* 10^{-5} *M* ^{239}*Pu has ten times the dose-to-solution as does* 10^{-5} *M* ^{242}*Pu leading to much greater loss of cell viability* (*Reed et al.*, 1999).

observed loss of cell viability was much greater when plutonium was the source of ionizing radiation than comparable gamma absorbed doses. It was hypothesized that this enhanced radiolytic effect was caused by the bioassociation of the plutonium in the system, establishing a link between increased bioassociation and the onset of loss of viability. 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 nearly always lead to the loss of viability.

33.2.2 Chemical toxicity of actinides towards microorganisms

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). In the environment, chemical toxicity is the potentially more important effect for all actinides, regardless of activity, due to the lower actinide concentrations typically present.

Although actinide toxicity toward microorganisms is often observed, the mechanisms by which actinides cause chemical toxicity are not well understood

and have not been investigated. Empirically, as discussed in more detail later in this section, the following trends are generally observed:

- Chemical toxicity is present at very high (>1 mM) actinide concentrations for almost all actinides.
- Actinide toxicity depends on the speciation of the actinide in solution and is generally mitigated by complexation, and significantly increased toxicity (at <1 μM concentration) is observed when little/no complexation exists (e.g., pentavalent actinides that can persist as aquo species).
- Complex-specific toxicity can be present for chelating agents known to facilitate transport into the cell leading to biouptake.

Actinides cause toxicity in ways that are analogous to those observed for heavy metals (Beveridge and Doyle, 1989). Chemical toxicity generally results from binding to cell membranes, non-specific binding to proteins, or metal substitution into the active center of metalloproteins. The study of heavy-metal toxicity itself continues to be a very active area of research, and increasingly diverse and complex mechanisms are being discovered. The most important and perhaps best studied of these mechanisms is the ability of certain actinide species to substitute for metals in metabolic processes (Wildung and Garland, 1980; Collins and Stotzky, 1989; Plummer and Macaskie, 1990). 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 (Raymond *et al*., 1982; Durbin *et al*., 1984). Most of the evidence available indicates that predominantly the aquo (i.e., uncomplexed) actinide species are chemically toxic, and complexation greatly reduces the toxicity of actinides by making them unavailable to the bacteria (Francis *et al*., 1996; Markich *et al*., 1996).

(a) Chemical toxicity of actinium, protactinium, thorium, uranium, and americium towards microorganisms

Actinium, protactinium, thorium, uranium, and americium are highly complexed by the hydroxyl ion (or hydrolyzed) in the pH range typical of groundwater systems. For this reason, they tend not to exhibit high chemical toxicity towards microorganisms in the environment. Some toxicity, however, was noted for uranyl (Lovley and Phillips, 1992a, b) when present at ~3 mM concentration in high-carbonate systems. But measurable toxicity for uranyl, even at millimolar concentrations, is not routinely reported.

The one exception to this general observation was reported by Banaszak *et al*. (1999a) for the uranyl-citrate-*P. fluorescens* system (Francis and Dodge, 1993). Biodegradation of citrate in the UO_2^{2+}-citrate complex led to a rapid loss of viability, as measured by the number of colony-forming units, over time. 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. Uranium uptake, in the U-citrate system, appears to be facilitated, rather than inhibited, by the citrate complex. In this context, the role of citrate complexation appears to be analogous to that observed in the transport of Fe^{3+} where the complex is able to mimic the structure of metal–ligand complexes that are specifically recognized and transported by metal uptake proteins (e.g. Fe-siderophores and Zn-specific transporters). This observed mechanism illustrates the highly specific and selective mechanisms for toxicity that can exist.

(b) Chemical toxicity of neptunium and plutonium towards microorganisms

Neptunium and plutonium can have relatively high chemical toxicity towards microorganisms. The difference in these actinides relative to the other actinides just discussed is that they can form the relatively non-complexing PuO_2^+ and NpO_2^+ cations under environmentally relevant conditions. Both of these cations do not undergo significant hydrolysis until pH > 8 and form very weak and highly labile complexes with many inorganic and organic complexants typically found in groundwater.

NpO_2^+, an aquo species, inhibited the growth of *C. heintzii* at free-ion concentrations exceeding 5×10^{-5} M (Banaszak *et al.*, 1998b). In these experiments, *C. heintzii* was grown on glucose in the presence of the neptunyl species. The toxicity noted was mitigated by the complexation of neptunium with a strong complexant such as nitrilotriacetic acid (NTA). Even at neptunyl concentrations of 1.25×10^{-4} M, no effect on growth was noted. Tying up the neptunyl as a phosphate complex or precipitate also eliminated its toxicity toward *C. heintzii*. In this system, toxicity response was postulated as the mechanism for the bioreduction of Np(V) to Np(IV), which was not toxic.

A comparative study (Ruggiero *et al.*, 2005) of Np(V), Pu(V/VI) and U(VI) on *D. radiodurans* is shown in Fig. 33.5. Significantly enhanced toxicity was noted for neptunium which was only present in the V oxidation state. Because Np does not bind strongly to cell membranes, this is unlikely to be a big factor in the observed Np chemical toxicity. The observed toxicity was presumably due to the high uptake of uncomplexed metals relative to complexed metals.

High chemical toxicity of plutonium towards microorganisms also has been noted. Reed *et al.* (2007) established toxicity effects on *Shewanella alga BrY*, a metal-reducing facultative bacteria, at plutonium concentrations <0.1 μM when present as an uncomplexed PuO_2^+ aquo species. Plutonium (VI), as PuO_2^{2+}, and PuO_2^+ complexed with acetate, citrate, oxalate and NTA did not exhibit any toxicity under similar conditions. In other investigations (Boukhalfa *et al.*, 2007), plutonium (IV) or plutonium (III), present as either an EDTA complex or excess-oxyhydroxide precipitates, did not exhibit any toxicity towards *Shewanella oneidensis* or *Geobacter metallireducens*, both metal-reducing facultative bacteria.

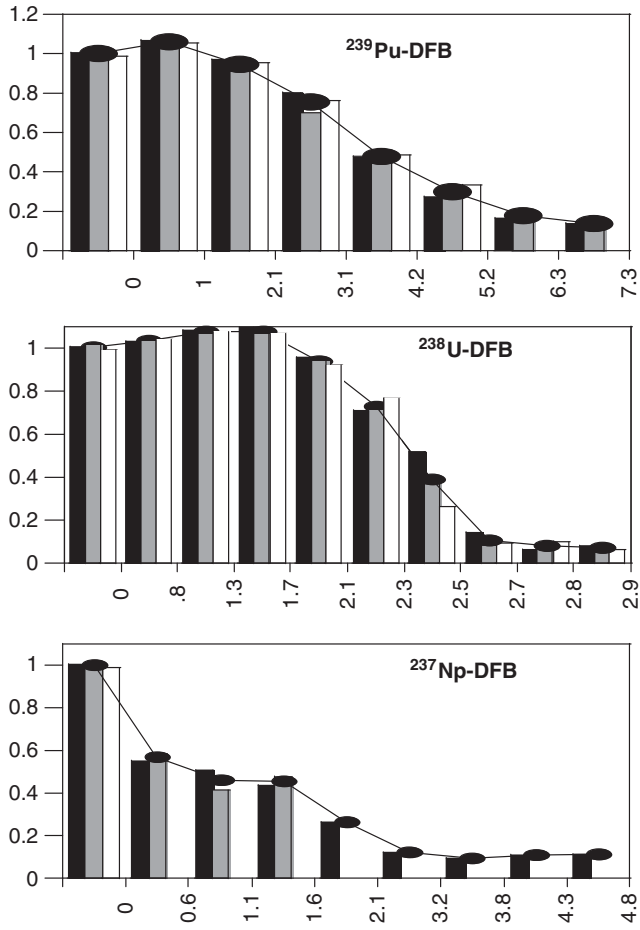

Fig. 33.5 *Comparative toxicity of* ^{237}*Np-DFB* (*DFB = desferrioxamine B*), ^{238}*U-DFB and* ^{239}*Pu-DFB towards D. radiodurans. The actinide concentrations are in mM with the toxicity expressed in terms of the ratio of the growth in cultures with and without the actinide metal complex* (*based on Ruggiero et al*., 2005).

The plutonium results continue to point toward the important role of the more readily bioavailable PuO_2^+ species. Because plutonium redox cycling is an important subsurface process, there will always be some bioavailability of Pu(V) species. Beyond this empirical observation, however, the mechanism of plutonium chemical toxicity is not well understood.

33.2.3 Effect of actinides on organic speciation and bioavailability

The third effect of actinides on microbial processes is the potential for actinides to influence the bioavailability of organic substrates. Actinides, similar to other

metals, form complexes with organic substrates that may reduce their bioavailability. Conversely, the ionizing radiation associated with actinide contaminants can increase bioavailability as the result of the radiolytic breakdown of larger, less biodegradable organic contaminants, e.g., cellulosics and plastics, as well as natural humics and fulvics. The rates of microbially mediated reactions are related to the concentrations of their degradable substrates which are often species-specific.

(a) Complexation effects of actinides on bioavailability

Bacterial cells have cell-wall and membrane structures that 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 take up the organics as substrates. This process is in many ways analogous to the chemical toxicity issue discussed in Section 33.2.2. 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* (Willecke *et al*., 1973). When other, non-preferred metals form the most abundant substrate complex, substrate utilization usually is slowed (Brynhildsen and Rosswall, 1989; Francis *et al*., 1992; Francis and Dodge, 1993; Brynhildsen and Allard, 1994; Joshi-Tope and Francis, 1995; Bolton *et al*., 1996).

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 occurs when metal concentrations reduce substrate availability through the formation of complexes. 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:

$$Me^+ + Lig^- \Leftrightarrow MeLig \qquad \beta = \frac{[MeLig]}{[Me^+][Lig^-]} \tag{33.9}$$

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. At lower ligand to metal ratios, the formation of the MeLig complex reduces the concentration of the degradable substrate, Lig^-, and can greatly slow the rate of biodegradation.

When detailed chemical speciation modeling was used to interpret experimental data, the degradation (or lack thereof) of organic complexants was linked to the concentration of the known or hypothesized degradable species (VanBriesen and Rittmann, 1999, 2000). For example, Francis *et al*. (1992) and Francis (1994) 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.* (1996) for NTA degradation in the presence of metals. In actinide systems, Banaszak *et al.* (1998b) showed that NTA degradation in the presence of Np(V) went to completion in the absence of metal toxicity effects, while Reed *et al.* (1999) found that the degradation rate of NTA in the presence of Pu(IV), which has an 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 (1995) 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 in the presence of a metal 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 *et al.* (1995) 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 will not change the long-term outcome.

Linkages between organic complexation, hence their bioavailability, and the biogeochemical cycling of multivalent actinides (U, Np and Pu) are also important since the strength and lability of actinide complexes is oxidation-state specific. This can be illustrated by focusing on the extreme cases: the IV and V oxidation states for Pu or Np. Because of the relatively weak complexation of the An(V) oxidation state in comparison to the An(IV) oxidation state, AnO_2^+ cations will be present in much more significant concentrations as the free aquo species than are the An^{4+} cations. Thus, metal toxicity effects are more important than substrate speciation in retarding the growth of subsurface bacteria when actinides are predominantly in the V state. The more important effect for the IV state should be complexation, which reduces the availability of the ligand as a substrate.

(b) Radiolytic degradation of organic species

The presence of actinides in subsurface groundwaters, especially at or near the source of contamination, will lead to the radiolytic breakdown and

decomposition of the organics whether present in solution or in solid form. The interaction of ionizing radiation with water also can produce molecular products that are utilized by microbes for growth. This can indirectly affect the population of bacteria by generating breakdown products and radiolytic molecular products 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 (Bosma, 1994). Radiolytic breakdown of dissolved organics will break bonds, reduce the average molecular weight, and oxidize the organics. In vadose-zone contamination, ionizing radiation will break down organic constituents of the contamination (Reed *et al.*, 1993), leading to the solubilization of organics in the groundwater below. This overall process is analogous to the well-studied effects of ozonation, where enhanced biodegradability has been noted (van der Kooij, 1995).

The radiolytic decomposition products of water are also significant. This, for high LET radiation, leads to the formation of transient and molecular products (Draganic and Draganic, 1971):

$$3.6\ H_2O \rightarrow 0.9\ H_2 + 1.1\ H_2O_2 + 2.0\ H + 1.2\ OH + 0.1\ HO_2 \qquad (33.10)$$

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 exist – 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 (Reed *et al.*, 1993, 1994) and are defined solely by the alpha activity in the groundwater.

33.3 IMPACT OF MICROORGANISMS ON SUBSURFACE ACTINIDE SPECIATION

The speciation of actinides in a subsurface environment will depend on the pH, E_h, complexation, and extent of aggregation (Choppin, 2003). All of these key processes are potentially influenced, and in many cases, defined by microbial activity (Lieser, 1995; Silva and Nitsche, 1995; Von Gunten and Benes, 1995; Banaszak *et al.*, 1999a; Neu *et al.*, 2000).

In this section, the key microbial mechanisms and associated actinide-specific studies, are summarized in the following three areas:

- Bioreduction of multivalent actinides
- Bioassociation of actinides
- Biodegradation of organic chelating agents

A schematic of these processes is shown in Fig. 33.6. These mechanisms directly impact the fate and transport of actinides in groundwater and are

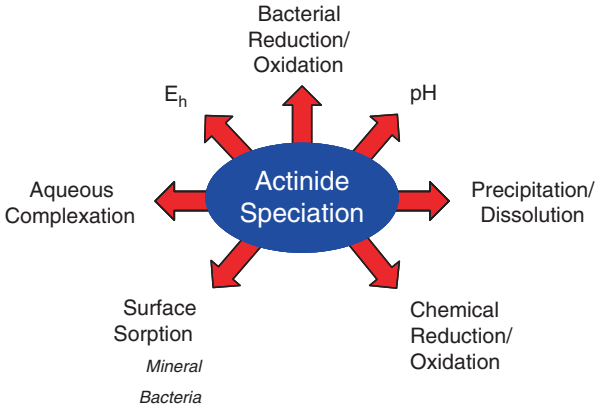

Fig. 33.6 *Schematic of biogeochemical processes that affect actinide speciation*

often strongly coupled with abiotic geochemical and chemical processes. The vast majority of the microorganisms that have been investigated are soil bacteria, since most of the actinide subsurface contamination exists at or near the surface, so this is also a focus of this section.

33.3.1 Bioreduction of multivalent actinides

The bioreduction of multivalent actinides under a wide range of subsurface conditions is well established and is the most important microbiological impact on the subsurface speciation of actinides (Zajic, 1969; Lovley, 1991; Lovley *et al.*, 1991, 1993; Francis, 1994a; Nealson and Saffarini, 1994; Anderson and Lovley, 2002). The reduction of actinides, in many cases, leads to much lower solubilities and is a critical step in the subsurface immobilization of the actinide.

Bioreduction specifically pertains to the multivalent actinides (see Table 33.1) uranium, neptunium, and plutonium, which are the most important actinide subsurface contaminants. The overall relationship between microbial processes and the predicted actinide oxidation state are given in Fig. 33.7. The overall mechanism for bioreduction and its observed effects on the oxidation state of uranium, neptunium, and plutonium are given in this section.

(a) Mechanism of bioreduction

In order to harness energy for growth, microorganisms catalyze reduction/oxidation (redox) reactions. While sources of trace micronutrients (like Cu, Mn, Fe, and Zn) and macronutrients (like N, P, and S) are important, microorganisms most essentially require sources of cellular carbon and usable electron-donor and -acceptor substrates. Oxidation of an available, electron-donor substrate provides electrons to use in energy generating and biosynthesis reactions, and

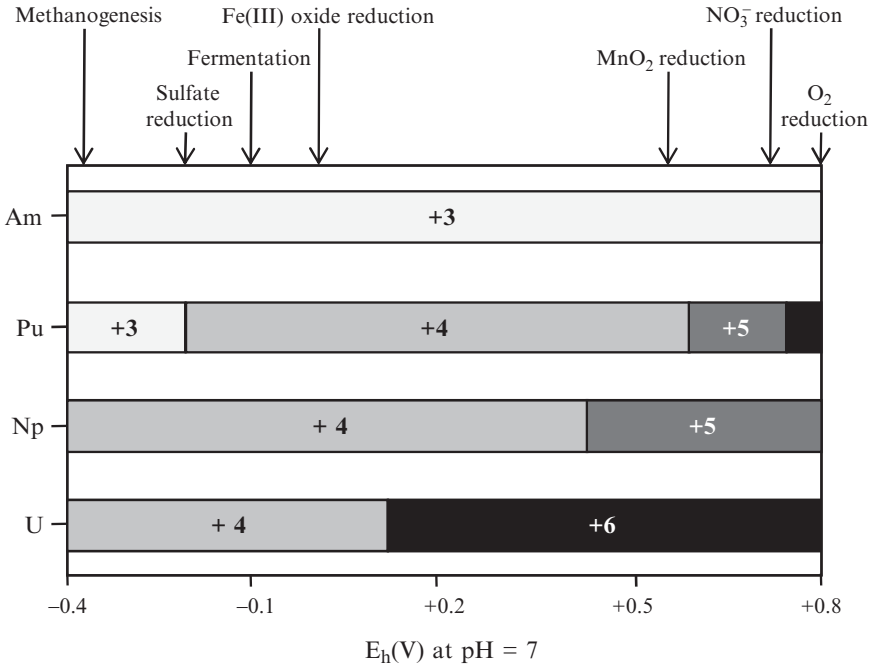

Fig. 33.7 *Expected dominant actinide oxidation states as a function of the standard reduction potential at pH = 7 in water that is in equilibrium with atmospheric CO_2. The linkages between the redox potentials associated specific oxidation states and microbial electron acceptor couples are also shown* (*adapted from Banaszak et al.*, 1999a).

reduction of the electron-acceptor substrate provides a sink for the electrons used in energy-generating reactions. In many cases, the electron donor is an organic compound that also can be the carbon source; this is called heterotrophy. Should the electron donor be inorganic, such as H_2 or H_2S, the C source usually is inorganic carbon, or CO_2; this is called autotrophy.

Bacteria derive the maximum amount of free energy by combining appropriate electron-donor and -acceptor half reactions. Just like humans and animals, aerobic respiration (i.e., reducing O_2) provides the maximum amount of energy to microorganisms. Since bacteria are ubiquitous, they either can or must grow by anaerobic respiration, the reduction of an electron acceptor other than oxygen. Facultative microorganisms help transition to strictly anaerobic conditions where obligate anaerobes predominate.

Common alternative electron acceptors in the environment include nitrate, nitrite, and sulfate, which are common constituents of nuclear wastes. When these common electron acceptors are rapidly depleted, solid metal oxides such as FeOOH and MnO_2 appear as energetically attractive alternative terminal electron acceptors (Myers and Nealson, 1988). Many species of bacteria are able

to use metal ions as electron acceptors, coupled to oxidation of organic and inorganic electron donors (Lovley *et al*., 1989; Nealson and Saffarini, 1994). In this case, the best-studied example is of the dissimilatory metal-reducing bacteria that reduce Fe(III) and Mn(IV) oxides to soluble free reduced Fe(II) and Mn(II) ions. This enzymatic transfer of electrons to metal ions is not only linked to energy conservation (Luu and Ramsay, 2003) but is critical in the cycling of trace metals in the geochemical environment (Zajic, 1969; Lovley, 1991, 1993; Nealson and Saffarini, 1994; Banfield and Nealson, 1997).

In order to connect oxidation of electron donor substrate to reduction of a wide variety of electron acceptors, electrons removed from the donor are conserved in intracellular electron carriers, usually nicotinamide-adenine dinucleotide (NAD^+), creating its reduced form (NADH):

$$NAD^+ + 2e^- + H^+ \rightarrow NADH \qquad (33.11)$$

Electron-donor oxidation is illustrated with acetate, a common electron-donor substrate in microbiological reactions. Complete mineralization of acetate releases eight electrons, forming four NADH and two H_2CO_3 molecules:

$$CH_3COOH + 4NAD^+ + 4H_2O \rightarrow 2H_2CO_3 + 4NADH + 4H^+ \qquad (33.12)$$

Although in reality microorganisms carry out this reaction in a more elaborate fashion using intermediates via the tricarboxylic acid cycle, the end result is the same as shown above (Madigan *et al*., 1997).

The electrons contained in NADH are then invested in either energy generation or cell synthesis. For simplicity, we focus here only on energy generation. Thus, the reduced form of NADH transfers two electrons to membrane-bound redox-active coenzymes, such as flavoprotein (a derivative of riboflavin), and in the process regenerates NAD^+ and reduces the membrane-bound coenzyme. The electrons are transported further along a redox ''chain'' of coenzymes, including cytochromes. Due to redox-potential difference between each pair of cytochromes, transfer of electrons along the redox chain produces an electrochemical gradient across the cell membrane, which drives energy generation and membrane transport processes in the organism. Finally, after a maximum amount of useful energy has been gained, the electrons are transferred to the terminal electron acceptor by a specialized enzyme, a reductase.

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

$$O_2 + 2NADH + 2H^+ \rightarrow 2H_2O + 2NAD^+ \qquad (33.13)$$

Although NAD^+ is not directly involved in the transfer of electrons to the terminal electron acceptor, it plays a significant role in transferring electrons to the redox ''chain'' of coenzymes. Combination of the electron-donor and electron-acceptor reactions therefore yields, for acetate and oxygen:

$$CH_3COOH + 2O_2 \rightarrow 2H_2CO_3 \qquad (33.14)$$

Although an organic electron donor is used as an example, microorganisms are not limited to organic electron donor substrates. For example, autotrophic bacteria utilize inorganic electron donors, including reduced metals and H_2, as sources of electrons, and inorganic carbon as their source of carbon for biosynthesis of cell components (Madigan *et al.*, 1997). Although different species of microorganisms have documented a variety of electron acceptors and their electron transport systems, all of them obey the fundamental laws of thermodynamics, i.e., the spontaneity in electron transfer to NAD^+ is only possible if the reduction potential of the electron donor is higher than the NAD/NADH couple. Similarly, the reduction potential of the terminal cytochrome in the electron transport chain must have a higher redox potential than the redox potential of the terminal electron acceptor. This phenomenon is comprehensively illustrated in Fig. 33.8, which shows the standard reduction potentials

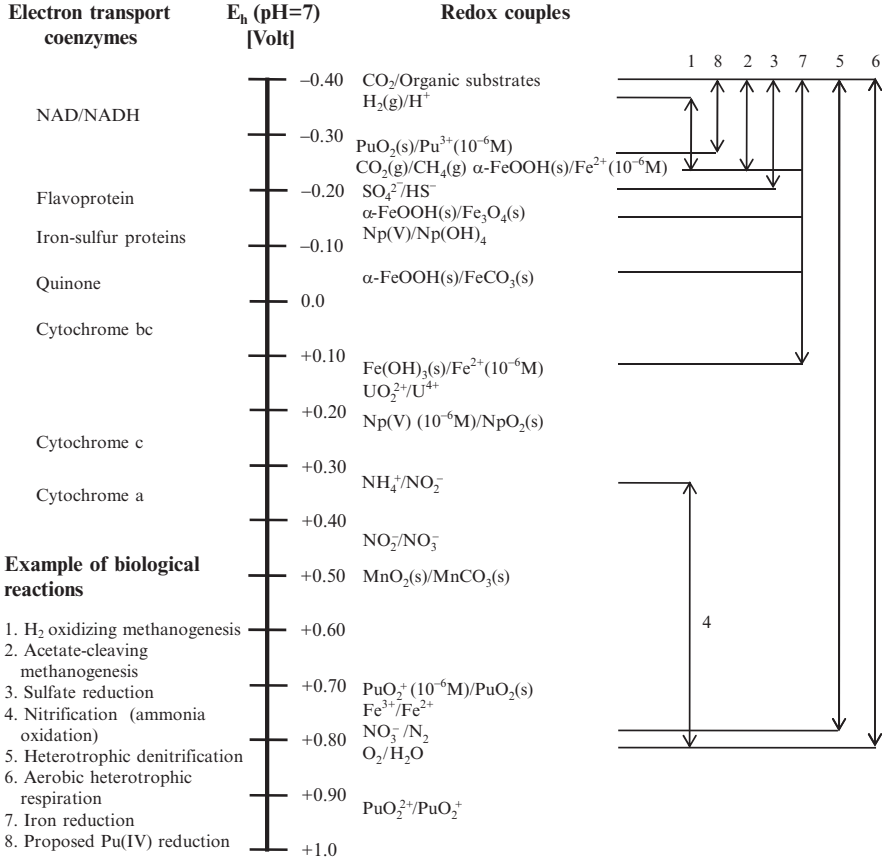

Fig. 33.8 *Conceptual electron transport tower, compared to important biological electron acceptor and actinide redox couples* (*adapted from Banaszak et al.*, 1999a).

(at pH 7) of common electron transport coenzymes and potentials of key electron donors, electron acceptors, and actinide redox couples. Figure 33.8 also shows that the organisms capture different amounts of energy from their choice of electron-donor and acceptor substrates. Thus, sulfate-reducing bacteria (SRB) and methanogens capture a very small amount of energy as a result of the small potential difference between electron-donor and acceptor substrates (electron-transport chains for 1, 2, and 3), while aerobic or denitrifying heterotrophs have long chains, which provide much more energy per electron pair extracted from the donor (electron-transport chains 4–6).

Besides having substrates with a thermodynamically favorable redox couple, microorganisms must possess the necessary enzymes to effectively interact with the electron-donor and -acceptor substrates, where the substrates also must be in suitable bioavailable forms (Nealson and Saffarini, 1994).

Using the information in Fig. 33.8, we can infer some thermodynamically feasible redox couples involving actinides and their multi-valence phases. For example, the Pu system has +3, +4, +5, and +6 oxidation states. At high pH and oxic conditions, the higher valent Pu(V) and Pu(VI) species are favored (Penrose *et al*., 1990; Cleveland and Mulhn, 1993; Silver, 1994; Bryan *et al*., 1994; Lieser, 1995; Silva and Nitsche, 1995).

In the presence of a high concentration of electron donor substrate, the most abundant electron acceptors, oxygen and nitrate, are rapidly depleted by aerobic and denitrifying heterotrophs, respectively. This then allows metal reduction to be the dominant energy-generating mechanism in the presence of capable organisms. Since the potential for the PuO_2^+/PuO_2 couple is less positive than that of O_2/H_2O, we would expect that Pu(V) could not be reduced to Pu(IV) until oxygen depletion. Although reduction of Pu(IV) to Pu(III) (chain 8) is thermodynamically less favorable, it is energetically favorable for microorganisms that oxidize organic compounds or H_2. Similar arguments can be made for Np(V) and U(VI), in the forms of NpO_2^+ and UO_2^{2+}, respectively, since these are also potential electron acceptors with energy-generating potentials superior to Fe(III) and SO_4^{2-}, but substantially less than NO_3^- and O_2.

In addition to the direct enzymatic reduction of multivalent actinides, some microorganisms can indirectly reduce actinides in sedimentary and subsurface environments. This is accomplished via chemical reduction of actinides by reduced species (Behrends and Cappellen, 2005; Tabak *et al*., 2005; Reed *et al*., 2007), such as ferrous iron (Fe(II)) and sulfide (S(−II)), which are produced by metal-reducing and sulfate-reducing bacteria, respectively, as well as many biogenic organic species.

Ferrous ions have been reported to chemically reduce multivalent metals such as uranium, chromium, and technetium (Tabak *et al*., 2005). Recently it was hypothesized that PuO_2^+ reduction in the presence of Fe^{3+}-NTA and *Shewanella alga* was most likely due to chemical reduction by biogenically produced ferrous iron (Reed *et al*., 2007). Similarly, sulfide was reported to chemically

reduce other toxic metals in the biofilm and, in the process, decrease their migration (Tabak *et al*., 2005).

Furthermore, as more of the abundant, thermodynamically favored, terminal electron acceptors (O_2, NO_3^-, Mn^{4+}, Fe^{3+}, SO_4^{2-}, CO_2) are used up, the overall redox potential of the subsurface changes from oxidizing to more reducing conditions. This situation may indirectly influence the actinide oxidation state and, therefore, its speciation. Because many actinides are multivalent, changes to oxidation state re-equilibrates actinide complexation (aqueous and surfaces) with competing ligands.

(b) Bioreduction of uranium

Uranium exists as either the U^{4+} or UO_2^{2+} species in groundwater (Table 33.1). Uranium (VI), as UO_2^{2+}, is stable towards reduction under oxic conditions and is the species responsible for the relatively high mobility and solubility of uranium in near-surface groundwaters. Under reducing conditions, uranium(VI) can be reduced abiotically, although this is relatively difficult. The bioreduction of uranium under anaerobic conditions by metal-reducing and sulfate reducing bacteria is the most studied actinide bioreduction reaction and was recently reviewed by Anderson and Lovley (2002).

Direct reduction of uranium under anaerobic conditions has been documented for a wide variety of bacteria, including the genera *Shewanella* and *Geobacter*, using reductase enzymes (Gorby and Lovley, 1992; Caccavo *et al*., 1992; Lovley and Phillips, 1992a, b; Lovley *et al*., 1993; Francis, 1994b; Nealson and Saffarini, 1994; Truex *et al*., 1997; Haas and DiChristina, 2002; Liu *et al*., 2002). *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:

$$CH_3COOH + 4UO_2^{2+} + 4H_2O \rightarrow 4UO_2(s) + 2H_2CO_3 + 8H^+ \qquad (33.15)$$

Other organisms are capable of reducing UO_2^{2+}. *Shewanella putrefaciens* and several *Desulfovibrio* species couple U(VI) reduction to H_2 oxidation (Lovley, 1991, 1993; Nealson and Saffarini, 1994):

$$H_2 + UO_2^{2+} \rightarrow 2H^+ + UO_2(s) \qquad (33.16)$$

Francis *et al*., (1994) showed uranium reduction by a *Clostridium sp*. growing on glucose.

Among the metal-reducing bacteria, the genus *Shewanella* has recently received much attention due to its remarkable adaptability to utilize a wide variety of electron acceptors (DiChristina *et al*., 2006). Being facultative, *Shewanella* grow aerobically when oxygen is available and anaerobically using nitrate, nitrite, oxidized Mn and Fe, sulfite, thiosulfate, and higher-valent actinides. Under anaerobic conditions, *Shewanella* generates enzymes (reductases and hydrogenases) used to enzymatically reduce uranium.

Although enzymatic reduction of uranyl has been established under anaerobic conditions, the extension of this to real-system subsurface problems remains problematic. In bio-augmentation experiments to induce anaerobic growth, dissolved uranium(VI) can be bioreduced, leading to its precipitation from groundwater. This process, however, is very susceptible to reoxidation and redox cycling, leading to the solubilization of uranyl. Attempts to bio-immobilize uranium in real-system studies have not been successful (see Section 33.5).

(c) Bioreduction of neptunium

Neptunium potentially can exist as Np^{4+}, NpO_2^+, and NpO_2^{2+} species in groundwater. Np(VI), as NpO_2^{2+}, is not stable in the presence of organic species even under oxic conditions, and its microbially-induced reduction, for this reason, is not considered. Np(V), as the NpO_2^+ species, is relatively stable in groundwater under both oxidizing and reducing conditions and is abiotically very difficult to reduce to Np(IV). The redox half reaction is given by the following:

$$NpO_2^+ + e^- \rightarrow NpO_2(s) \quad E_o = 0.604\,V \tag{33.17}$$

Based on the redox potential of this reaction, it is more energetically favorable (see Fig. 33.8) than iron reduction, meaning that reduction pathways should exist for metal-reducing bacteria and sulfate-reducing bacteria. As will be described later in this section, the bioreduction of Np(V) to Np(IV) under reducing conditions is observed under most conditions investigated and leads to the precipitation of Np(IV) phases. In this context, the biotic pathways for the reduction of neptunium(V) are very important subsurface reactions that lead to neptunium immobilization.

The reduction of Np(V) to Np(IV) by *Desulfovibrio vulgaris*, a sulfate reducing bacterium (SRB), and a mixed methanogenic and sulfate-reducing consortium of microorganisms was investigated (Banaszak *et al.*, 1998b, 1999b; Rittmann *et al.*, 2002a). In all cases, the reduction of Np(V) to form Np(IV) was noted and confirmed by XANES analysis and led to greatly reduced solution concentrations. In the presence of phosphate, an Np(IV)-phosphate phase was identified as the precipitate. The mechanism for this redox process was not established, and electron-flow calculations suggested that the neptunium was being reduced via co-metabolic pathways rather than direct enzymatic pathways. It was proposed that the reduction was occurring as a toxicity response due to the high toxicity of the Np(V) species in solution. The reduction of Np(V) by *Desulfovibrio desulfuricans* (an SRB) also was reported (Soderholm *et al.*, 2000), and the production of amorphous Np(IV) species as a precipitate was noted.

The reduction of Np(V) to Np(IV) was also established for metal-reducing bacteria *Geobacter metallireducens* and *Shewanella oneidensis* (Icopini *et al.*, 2007).

It was demonstrated that these microorganisms could reduce Np(V) directly and indirectly by reaction with biogenic Fe(II). There was, however, not a clear explanation for the mechanisms of reduction. Overall, the rate of Np(V) reduction was observed to be very slow when compared to other metals, which was attributed to evidence of Np(V) toxicity effects on the bioreduction process. Complexation with citrate appeared to mitigate the toxicity effect and increased the overall rate of bioreduction. The reduction of Np(V) by *Citrobacter* sp. and *Shewanella putrefaciens*, also metal-reducing bacteria, was determined (Lloyd *et al*., 2000). Here, reduction was also noted to be complex and the presence of phosphate was needed to precipitate Np from solution. Np(IV) phosphate phases were observed as the end product of the bioreduction process and a co-metabolic process was proposed.

Overall, the bioreduction of Np(V) occurs quite readily with metal reducers and SRB under anaerobic reducing conditions. However, questions remain as to the exact mechanism of the bioreduction process and specifically the ability of microorganisms to utilize Np(V) to derive energy through direct metabolic pathways. Regardless of this uncertainty, this established a key subsurface pathway for the removal of highly soluble Np(V) species from groundwater and its subsequent immobilization in the subsurface.

(d) Bioreduction of plutonium

Plutonium can exist as the Pu^{3+}, Pu^{4+}, PuO_2^{+}, and PuO_2^{2+} species in groundwater (Table 33.1). Microbiological activity and the associated organic species that will coexist destabilize the Pu(VI) oxidation state under oxic and anoxic conditions; hence, Pu(VI) complexes are not expected to be important plutonium species when biological activity is present. Pu(V) is also destabilized towards reduction under anoxic conditions, but can persist under oxic conditions, even when microbial activity is present. Strong abiotic–biotic coupling of plutonium reduction occurs under anoxic/anaerobic conditions. An important distinction from the biogeochemistry of neptunium and uranium is the potential for the formation of Pu(III) species under anaerobic conditions.

The reduction of Pu(VI) was investigated under aerobic growth conditions for *Pseudomonas stutzeri* and *Bacillus sphaericus*, two microorganisms that typify near-surface aerobic soil bacteria (Panak and Nitsche, 2001). Strong bioassociation was noted between the Pu(VI) and the bacteria, but ultimately reduction to Pu(V) was observed. In time, some Pu(IV) was also formed, but this was attributed to the disproportionation of the Pu(V) as its concentration increased. The reduction of Pu(VI) to Pu(V) under aerobic growth conditions for *Shewanella alga BrY* was also reported (Reed *et al*., 2007), but here the Pu(V) formed was stable for several weeks and showed no tendency to form Pu(IV). Under abiotic, but anoxic conditions, Pu(VI) is very difficult to stabilize and generally leads to the formation of Pu(V) species when organics are present. It is not clear if the reduction noted under aerobic conditions is due to enzymatic

activity or coexisting abiotic pathways coincident to the microbial activity present.

Under anaerobic conditions, SRB and metal reducers can reduce Pu(V/VI) to Pu(IV) species, and, when solubilization mechanisms exist, the formation of Pu(III) species can be observed. The reduction of Pu(V/VI) by the metal-reducing bacteria *Geobacter metallireducens* and *Shewanella oneidensis* MR-1 was investigated under anaerobic growth conditions (Icopini *et al*., 2009). Transmission electron microscopy showed that Pu(IV) aggregate nanoparticles were formed and in some cases were crystalline. These were precipitated on the cell wall surface or to a lesser extent within the cell wall membrane. In the absence of chelating agents, no plutonium(III) was formed, and Pu(IV) was the predominant oxidation state.

The reduction of Pu(V) was also observed for *Shewanella alga BrY* when NTA-complexed Fe(III) was utilized as the electron acceptor leading to the formation of mM concentrations of Fe^{2+}. Bioreduction to form Pu(IV) was due to reaction with the biogenic Fe(II) being formed and led to the formation of bioassociated Pu(IV) and Pu(III) phases (Reed *et al*., 2007). The bioreduction of Pu(V) to form Pu(IV) precipitates also was observed when no iron was present and may be due to direct enzymatic pathways. The reduction of Pu(V/VI) to Pu (IV) is not surprising, since dissimilatory metal-reducing bacteria are known to utilize Fe(III) and Mn(IV) species as terminal electron acceptors and can couple this to the oxidation of H_2 or organic substrates to derive the energy for their growth. These results with plutonium are consistent with the results reported for other radionuclides such as U(VI), Np(V), Tc(VII) (Lovley, 1993; Lovley *et al*., 1993; Francis *et al*., 1994; Tebo and Obraztsova, 1998; Fredrickson *et al*., 2000; Suzuki *et al*., 2004, 2005).

The reduction of Pu(VI) to Pu(V) and Pu(IV) was also established for *Desulfovibrio aspoensis* DSM 10631, an SRB, under anaerobic conditions (Moll *et al*., 2006). Reduction to Pu(V) occurred rapidly followed by a slower reduction to form Pu(IV) species. Significant Pu uptake was noted under the conditions of the experiments performed and phosphate complexation was suggested by the EXAFS performed. Lastly, the reduction of Pu(V) was also observed due to abiotic reaction with bio-derived exopolymeric substances (Roberts *et al*., 2008) under anoxic conditions providing a biogenic, but largely abiotic pathway, for the reduction of Pu(V) in the subsurface.

Microbial processes are also potentially important in establishing the relative importance of the Pu(III) and Pu(IV) oxidation-state distribution under reducing conditions in the subsurface. When Pu(IV) is the initial oxidation state, either as a soluble complex or PuO_2 solid, it is possible to generate Pu(III) species leading to increased solubilization of plutonium. The bioreduction of PuO_2 by Fe-reducing strains of *B. polymyxa* and *B. circulans*, in the presence of NTA, led to the solubilization of plutonium as a Pu(III) complex that then was re-oxidized by the NTA to form a Pu(IV)-NTA complex (Rusin *et al*., 1994).

Analogous experiments with non-iron reducing *Escherichia coli* did not lead to the reduction/solubilization of the PuO_2. The reduction of EDTA-complexed Pu(IV) to form Pu(III) complexes by the metal-reducing bacteria *Geobacter metallireducens* GS15 and *Shewanella oneidensis* MR1 was investigated under anaerobic growth conditions (Boukhalfa *et al*., 2007). Rapid reduction of the Pu (IV) complexes were noted and the presence of Pu(III) was established by uv-vis spectroscopy. Pu(IV) introduced as freshly precipitated PuO_2 also led to the formation of soluble Pu(III) EDTA complexes. Little or no dissolution was noted when the EDTA was not present as a solubilization mechanism. The biogenic formation of Pu(III) was also observed for *Shewanella alga* BrY in the presence of NTA, where Fe(II) is also being formed (D. T. Reed, unpublished data). The formation of Pu(III) was also reported for *Clostridium* sp. when solubilized Pu(IV)-nitrate was initially present under anaerobic growth conditions (Francis *et al*., 2007, 2008).

The bioreduction of Pu(V/VI) to Pu(IV) and potentially Pu(III) species is an important factor in establishing the fate and transport of plutonium in biologically active subsurface systems. This reduction proceeds along direct enzymatic pathways, but is also strongly coupled with biogenic, but essentially co-metabolic, processes by reaction with the reduced metals and species generated. Under most conditions expected in the subsurface, the bioreduction will proceed to form Pu(IV) phases, leading to greater immobilization of the plutonium. When a solubilization mechanism is present, such as the presence of NTA or EDTA chelators, further reduction of solubilized Pu(IV) to Pu(III) species is possible. This is a potentially key process that can lead to solubilization mechanisms for PuO_2 in the subsurface. Many questions, however, remain about the role of biogenically produced Pu(III). The long-term stability of the Pu(III) phases generated, as well as the mechanisms of bioreduction, are not yet fully understood.

33.3.2 Bioassociation of actinides with microorganisms

Actinide speciation also can be influenced by direct bioassociation with microorganisms (Haas *et al*., 2001; Panak *et al*., 2002; Songkasiri *et al*., 2002; Songkasiri, 2003; Gorman-Lewis *et al*., 2005). This is one of the most prevalent and well-studied interactions between metal ions and microorganisms. 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 to mitigate toxicity of specific metal ions. Except in cases where the cells are permanently immobilized, all of the mechanisms described above can enhance the mobility of the sorbed or complexed metal.

Despite promoting mobility, bioassociation has important implications for recovery and isolation of actinides from contaminated receptors (Banaszak *et al*., 1999a; Tabak *et al*., 2005). For example, use of microbial cells as biosorbants for heavy metals or actinides offers a potential alternative to existing methods for decontamination or recovery of heavy metals from waste streams and contaminated groundwaters, since standard physical and chemical treatments such as ion exchange, solvent extraction, and conventional precipitation are usually expensive and produce high quantities of sludge. Several recent studies have addressed the bioassociation of radionuclides, especially uranium, plutonium, strontium, and technetium (McCready and Lakshmanan, 1986; Yakubu and Dudeney, 1986; Andres *et al*., 1993; Peretrukhin *et al*., 1996; Piron *et al*., 1997; Tsezos *et al*., 1997a, b; Small *et al*., 1999; Panak and Nitsche, 2001). These studies have established that bioassociation depends on several factors, such as groundwater pH, temperature, actinide speciation (e.g., oxidation state, complexation, and aggregation), and type of microorganisms.

While a significant body of literature reports different aspects of interaction of microorganisms and cell membranes with metal ions, our goal in this section is to focus attention on the possible effects of bioassociation processes on actinide chemistry. In general, we discuss two mechanisms of bioassociation: (1) surface complexation; and (2) formation of bio-colloids and complexation to extracellular substances.

(a) Mechanisms of bioassociation

The structure of single-celled microorganisms is key to understanding the mechanism of bioassociation. 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 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 and external membrane surfaces exposed to the cell cytoplasm and outside environment, respectively. Such chemical properties of the cell membrane make it a highly selective barrier to solute transport; for example, only nonpolar and fat-soluble molecules and water can penetrate the cell membrane. This also explains the effectiveness of alcohol disinfection towards destroying the integrity of microbial cell membranes (Madigan *et al*., 1997).

Since the cell membrane has a semi-fluid nature, bacteria maintain cell rigidity against environmental exposure through their cell walls, which fall into two distinct categories: Gram-positive and Gram-negative (Fig. 33.9), based on the cell walls reaction to a stain used for light microscopy (Schultze-Lam *et al*., 1996). 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

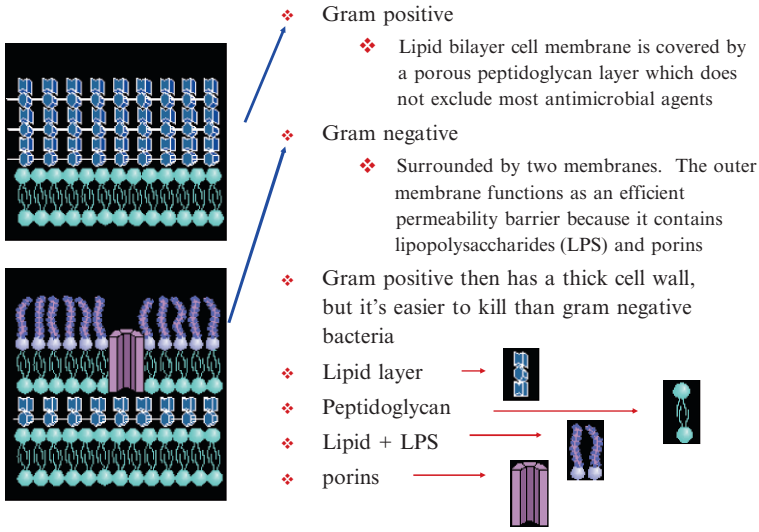

Fig. 33.9 *Comparison of cell wall structure of Gram- positive and Gram negative bacteria. Bacterial cell walls can strongly adsorb actinide species leading to extensive bioassociation in microbiologically-active systems.*

(as compared to the membrane thickness) uniform layer of peptidoglycan. Attached on the outer surface of peptidoglycan are acidic polysaccharides (also called teichoic acids) that contain glycerophosphate or ribitol phosphate residues. Since teichoic acids are negatively charged, they are partially responsible for the negative charge of the cell surface and may affect passage of cations such as Mg^{2+} through the cell wall (Seltmann and Holst, 2002). The presence of teichoic acids in the wall also affects the expansion and contraction of cell walls that occur with alterations of pH and ionic strength in the environment (Rogers, 1983).

Gram-negative cells, on the other hand, do not have teichoic acids. Instead, the peptidoglycan layer is encased in an outer lipid bilayer containing polysaccharides and proteins called porins, resulting in a three-component outer structure. Since outer-lipid polysaccharides, also known as lipopolysaccharides, are attached to the peptidoglycan layer by branched sugar moieties, the cell wall of Gram-negative bacteria appears quite "rough" as compared to the Gram-positive cell wall. Additionally, the outer cell structure is not highly selective due to trans-membrane proteins, such as the amphiphilic nature of lipopolysaccharides, which allows permeation to compounds with molecular weights of less than 5,000 Da. 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 (Madigan *et al.*, 1997).

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, much like their counterparts in dissolved complexes (Morel and Hering, 1993; Banaszak *et al*., 1999a). The functional groups that are most likely to be involved in surface complexation are carboxyl, organic phosphate, phosphodiester, amino, and hydroxyl groups (Macaskie and Basnakova, 1998; Cox *et al*., 1999). Needless to say, these properties should make surface complexation of actinides on bacteria dependent on pH (McCarthy and Zachara, 1989; Kim *et al*., 1992; Lieser, 1995; Schiewer and Volesky, 1997; Zouboulis *et al*., 1999), which may, in turn, be manipulated by bacterial degradation of organic ligands (Banaszak *et al*., 1999a).

(b) Surface complexation of actinides

As we just noted, bacterial cell surfaces have many of the surface functional groups that establish a wide variety of binding sites. The functional groups typically present on bacterial cell surfaces include carboxylic, phosphoric, amino, and hydroxyl groups. These functional groups can form strong surface complexes with many actinide species, and the strength of these interactions are specific to the oxidation state. In the subsurface environment, bioassociation 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 (Reed *et al*., 1991; Bellin and Rao, 1993; Dozol *et al*., 1993; Spor *et al*., 1993; Tanaka and Nagasaki, 1997).

Another type of biomass that can complex to actinides is the extracellular polymeric substances (EPS), which are secreted to help bacteria adhere to each other in flocks or to attach themselves to solid surfaces in the form of a biofilm (Mittelman and Geesey, 1985). EPS mediate the transport of chemicals within the aggregate matrix and provide physical protection for the cell to defend against toxicity from intracellular intake or direct cell surface adsorption (Birch and Bachofen, 1990; Appanna *et al*., 1995a, b; Decho, 2000; Rittmann and McCarty, 2001).

EPS contain a high amount of negatively charged functional groups (i.e., carboxyl, amine, or phosphoryl) that can generate an electrostatic interaction with metals (Muzarelli, 1977; Brown and Lester, 1980; Sutherland, 1984; Rudd *et al*., 1984; Mittelman and Geesey, 1985). Thus, the complexation of the metal causes a reduction in its chemical toxicity since it converts the toxic metal to a non-bioavailable form that is either retained in solution as a mobile metal–ligand complex or immobilized in a polymeric "slime" layer outside the cell wall (Birch and Bachofen, 1990; Appanna *et al*., 1995a, b).

EPS that are extracted from bacteria cultures can complex with actinides or other metal ions, such as uranium, cadmium, cobalt, zinc, and chromium

(Sterritt and Lester, 1986; Scott *et al*., 1986; Ferris *et al*., 1989; McLean *et al*., 1996; Chen *et al*., 1995; Beech, 1996; He *et al*., 2000; Liu *et al*., 2001). Such complexation can either enhance or reduce actinide mobility. For example, investigation of the effect of "microbial metabolites" produced by soil microorganisms on Pu speciation showed that 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 (Robinson *et al*., 1986).

For redox-invariant actinides (e.g. Ac, Th, and Am), the most important microbial interaction is their bioassociation. Strong bioassociation has been reported for Th^{4+} under a wide range of environmental conditions (Hirose and Tanoue, 2001; Hafez *et al*., 2001; Nakajima and Tsuruta, 2003; Tsuruta, 2004; Quiroz *et al*., 2006) for many microorganisms. This strong tendency toward bioassociation is being proposed as a mechanism for bioremediation and recovery of thorium from waste streams. By analogy, strong bioassociation of Np (IV), Pu(IV) and U(IV) should also be observed, although these are more difficult to measure directly due to redox uncertainty.

Uranium(VI), under oxic conditions, where its reduction does not occur, also tends to strongly bioassociate with microorganisms (Nakajima and Tsuruta, 2003; Tsuruta, 2004, 2006). The bioassociation of protactinium, initially as Pa(V), was also established in mixed anaerobic consortia (Sasaki *et al*., 2001) and in the presence of sulfate reducing bacteria (Kudo *et al*., 1998). In both cases, the degree of bioassociation was much stronger than that observed for Np(V). When plutonium was included in parallel studies (Kudo *et al*., 1998), the Pa tracked the plutonium bioassociation confirming consistency with the presence of strong bioassociation.

Under oxic conditions, neptunium is not reduced, and the sorption of neptunyl, as NpO_2^+, was established with metal-reducing bacteria (Songkasiri *et al*., 2002; Gorman-Lewis *et al*., 2005). Under these conditions, the surface complexation observed could be explained by the weak complexes formed with the carboxylate and phosphate groups known to be present on the microorganisms. Overall, microorganisms have a low affinity for Np(V). Results from recent examinations of Np(V) biosorption onto *Pseudomonas fluorescens* and *Bacillus subtilis* agree with the reduced reactivity of pentavalent neptunium. Np(V) binds preferentially to phosphoryl groups at lower pH, and progressively binds to carboxyl binding groups at higher pH. Although there is no spectroscopic evidence to support these observations, this behavior is consistent with the behavior of uranyl, which has been shown through X-ray absorption fine structure (EXAFS) studies to bind exclusively to phosphoryl groups at low pH, with an increasing contribution from carboxyl binding with increasing pH. XANES analysis of bio-sorbed neptunium samples shows that neptunium adsorbed onto *P. fluorescens* remains in the pentavalent oxidation state (Fig. 33.10).

Plutonium bioassociation with microbes is difficult to interpret because of the high likelihood of bioreduction on the cell surface and correspondingly the

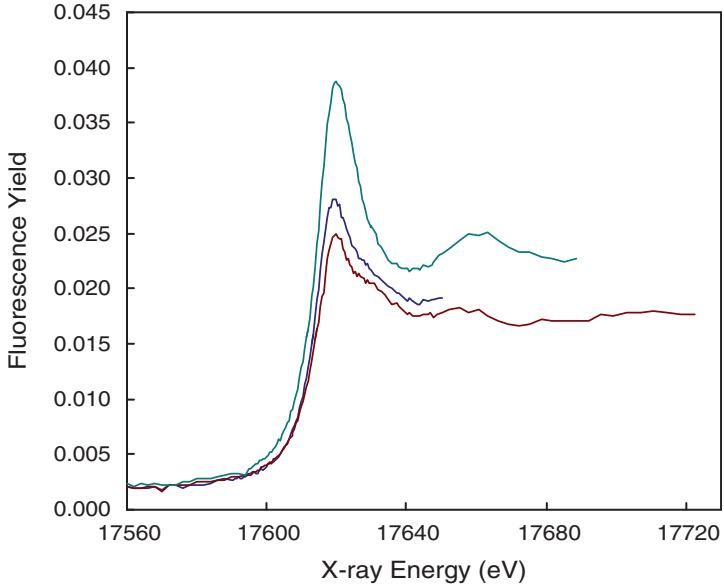

Fig. 33.10 *XANES analysis showing bio-reduced neptunium(IV) sorbed onto S. alga (top), sorbed as Np(V) onto P. fluorescens (middle), and sorbed as Np(V) onto S. alga (bottom).*

accumulation of plutonium phases on the surface of the cell. Bioassociation can occur through metabolically active processes and by non-metabolic pathways. Active bioaccumulation of plutonium occurs when Pu is taken up into the cells through an energy-dependent transport system. Plutonium internalized by active accumulation is sequestered intra-cellularly by specific and non-specific complexation with metal-binding cell components. Most studies examined plutonium bioassociation with microorganisms and provide little details on metabolic pathways involved in the processes.

Although it is somewhat problematic to control the oxidation state of plutonium, studies to establish the bioassociation of Pu(V) and Pu(IV) were performed (Fisher *et al*., 1985; Gillow *et al*., 2000a; Kihara-Negishi *et al*., 2001). For trace-level plutonium concentration (subnanomolar), a high degree of bioassociation was observed. In general, strong bioassociation of plutonium is expected for Pu(VI), Pu(IV), and Pu(III) based on analogy with their complexation chemistry, comparisons with uranium(VI), and what was established using redox-invariant analogs (e.g., thorium, and neodymium) where redox issues are suppressed. Initial spectroscopic studies of Pu binding to cell walls by X-ray absorption fine structure (XAFS) show that Pu(VI) adsorbed to the surface of bacteria suggest that this binding is predominantly with the phosphate groups with little/no carboxylate complexation detected. Similar

results were observed for U(VI) absorption to bacteria (Fowle *et al*., 2000; Haas *et al*., 2001).

(c) Bio-uptake of actinides

Actinide bio-uptake is the accumulation of actinides into the microorganism and can have a significant impact on potential migration pathways as the microorganisms degrade and release dissolved organic matter. Actinides are accumulated by microorganisms through metabolic pathways and surface complexation. Irreversible surface complexation due to binding with the cell outer membrane is the predominant form leading to long term bioassociation and is dependent on the many factors that contribute to this process (e.g., pH, oxidation state, competition with dissolved complexing agents) as discussed in the previous section. Much less is known about metabolic pathways that lead to the uptake of the actinide into the cell.

The uptake of plutonium was established for *Microbacterium flavescens* (JG-9) and *Pseudomonas putida* (John *et al*., 2001). Here siderophore-mediated uptake of Pu by *Microbacterium flavescens* (JG-9) was demonstrated, and uptake into the cell through a metabolic pathway was observed. This uptake was analogous to that observed for Fe(III)-DFB and appears to follow the same metal transport system. Similar results were obtained (Neu *et al*., 2000), where the Pu(IV)-desferrioxamine E (DFE) structures were resolved by single crystal X-ray diffraction studies showing that Fe(III)-DFE and Pu(IV)-DFE have strong similarities.

33.3.3 Biodegradation of organic chelating agents

The presence of organic chelating agents [e.g., NTA, EDTA, citric acid, oxalic acid, and organic phosphates] in the subsurface defines the chemical speciation and mobility of actinides (Banaszak *et al*., 1999a). Being multi-dentate, they have strong affinity for complexation with radionuclides; thus, they were used frequently as actinide extractants and for decontaminating nuclear reactors at weapons production facilities (Means *et al*., 1978; Riley *et al*., 1992; Jardine *et al*., 1993). In some cases, chlorinated and petroleum hydrocarbons were also co-disposed with the actinide contaminants (Riley *et al*., 1992). Since these hazardous organic species are the targets for in situ bioremediation, it is important to account for their role in defining the speciation and mobility of actinides, both before and after their biodegradation.

Organic chelating agents present in groundwater solubilize actinides and enhance their mobility (Means *et al*., 1978; Dozol *et al*., 1993; Hering and Kraemer, 1994). Because mobile actinides pose potential threats to populations near contaminated sites (Riley *et al*., 1992; Marley *et al*., 1993), bacterial degradation of organic chelating agents may seem like a workable immobilization

strategy (VanBriesen *et al*., 2000). However, actinide mobility may be enhanced or retarded by the biodegradation products (VanBriesen and Rittmann, 1999), which include organic acids and intermediates. In this section, we address the effects of biodegradation of organic chelating agents on actinide speciation and mobility.

We open our discussion by considering the effect of organic-chelate removal (due to biodegradation) on the actinide oxidation state. Besides actinide solubilization via complexation, organic chelates tend to stabilize the +4 actinide oxidation state (Reed *et al*., 1997**)** due to high complexation strength. For example, Pu oxidation states (+3, +5, or +6) complexed with NTA were eventually changed/stabilized as the +4 oxidation state (Al Mahamid *et al*., 1996). The stabilization mechanism was suggested to be participation of organic chelating agents (EDTA, citrate, and oxalate) in chemical or biological electron-transfer reactions (Lovley and Woodward, 1996; Reed *et al*., 1997, 2007). Because actinide-organic complexation depends on actinide oxidation state (Silva and Nitsche, 1995; Choppin and Bond, 1996), removal of organic chelates may affect actinide speciation and alter the ability to stabilize the +4 oxidation state. Since actinides with different oxidation states have different strengths of complexation with commonly available complexants, which include soil particles and biomass (Banaszak *et al*., 1999a), the actinide oxidation state can affect the speciation in ways that go beyond their direct complexation with the chelating agents themselves.

Additionally, biodegradation products may complex with the actinides. This was illustrated by an anaerobic, sulfate-reducing consortium that reduced Np(V) to Np(IV) when using pyruvate as the electron donor substrate (Rittmann *et al*., 2002a). Although Np(IV) eventually precipitated, the precipitation reaction was delayed until well after the formation of Np(IV). The retardation was most probably due to an intermediate pyruvate-fermentation product, succinate, that complexed with Np(IV) and kept it soluble.

Similarly, biodegradation of organic chelating agents that proceed through intermediates may affect actinide speciation by complexation by the intermediates. One such example is NTA, for which degradation is widespread among aqueous and soil microorganisms (Egli, 1990, 1994; Egli *et al*., 1990; Bolton *et al*., 1993). NTA biodegradation occurs in aerobic and anaerobic systems by facultative denitrifying bacteria (Egli, 1990, 1994; Egli *et al*., 1990) and in the presence of heavy metals (Bolton *et al*., 1996) and the actinides U, Np, and Pu (Banaszak *et al*., 1998a). NTA biodegradation produces the intermediates glyoxalate and iminodiacetate (IDA), which complex with actinides and affect their speciation.

In addition to generating biodegradation products that have actinide-complexing capability, microbial activity may influence subsurface pH through production and consumption of acidic hydrogen and carbonic acid. We illustrate the pH effects for open, closed, or buffered systems and for different electron donor/acceptor combinations.

We first explore the effects of using acetate degradation as a hypothetical example. Aerobic biodegradation of acetate is represented by the following stoichiometry:

$$\begin{aligned} CH_3COOH + 0.234\,NH_4^+ + 0.828\,O_2 \rightarrow\ & 0.234\,C_5H_7O_2N \\ & + 0.828\,H_2CO_3 + 0.704\,H_2O + 0.234\,H^+ \end{aligned} \tag{33.18}$$

where $C_5H_7O_2N$ represents microbial biomass composition (Rittmann and McCarty, 2001). All acid/base species are represented in their most protonated form. If we consider an initial acetate concentration of 0.5 mM, acetate consumption produces 1.17×10^{-4} M acid equivalents (H^+), but also consumes two weak acids (CH_3COOH and NH_4^+). The carbon dioxide produced is shown as carbonic acid, H_2CO_3. For an open system in equilibrium with normal atmospheric CO_2, the H_2CO_3 evolves as CO_2, and the combined effect of the other acid/base species causes a pH increase from 7.0 to 7.9. In a closed system, however, carbon dioxide produced is retained in the system as H_2CO_3, and the final pH rises to only 7.25. Subsurface environments usually have a buffering capacity beyond the species involved in the reaction, and this attenuates pH changes. As an example, in the presence of 1.0×10^{-2} M phosphate buffer, the pH in our hypothetical reaction only increases to about 7.07 and 7.01 in open and closed systems, respectively.

Different combinations of electron-donor and -acceptor substrates can affect pH differently and sometimes more strongly. We use partial oxidation of pyruvate to acetate by sulfate-reducing bacteria as an example:

$$\begin{aligned} CH_3COCOOH + 0.858\,H_2O + 0.0472\,NH_4^+ + 0.132\,SO_4^{2-} & \\ + 0.216\,H^+ \rightarrow\ 0.0472\,C_5H_7O_2N + 0.764\,H_2CO_3 & \\ + 0.132\,H_2S + CH_3COOH & \end{aligned} \tag{33.19}$$

In contrast to our previous hypothetical example, 0.216 mol of acidic hydrogen is consumed, but 0.132 mol of hydrogen sulfide and 0.764 mol of carbonic acid are produced. To quantify the effect of these on pH, we assume similar initial conditions; pyruvate concentration of 0.5 mM and pH of 7.0. Pyruvate degradation changes the pH in unbuffered open and closed systems to 7.34 and 6.12, and buffered open and closed systems to 7.01 and 6.96, respectively. While the trends among the cases are similar to the previous example with aerobic oxidation of acetate, all pH values are substantially lower for this example of partial oxidation of pyruvate. The main reason is that the reaction releases three weak acids (acetic, carbonic, and hydrogen sulfide).

Microbial activity that affects pH can control the amount and rate of precipitation of actinides. Similar to the generally observed metal-hydroxide/oxide precipitation due to an increase in pH (Joshi-Tope and Francis, 1995), the pH increase associated with biodegradation of NTA by *Chelatobacter heintzii* resulted in precipitation of Np(V) (Banaszak *et al.*, 1998b). On the other hand, a citric-acid-degrading anaerobic consortium retarded Np precipitation

because it caused an increased pH; this was due to strong complexation with the produced carbonate. Similar to carbonates, bacterially caused changes in pH may affect concentrations of other precipitating ions, including OH^-, S^{2-}, PO_4^{3-} and Fe^{3+} (Francis, 1990; Francis and Dodge, 1990; Morel and Hering, 1993; Nealson and Stahl, 1997; Fortin *et al.*, 1997).

Changes in subsurface pH also can affect actinide complexation on surfaces of soil particles, colloidal particles, and bacteria. In Section 33.3.2(b), we showed that the underlying mechanism for surface complexation is the presence of surface functional groups, which ionize depending on pH. The pH effect on surface complexation was shown for Np(V) sorption onto whole cells of *Shewanella alga* strain BrY, along with its components, cell wall and EPS (Songkasiri, 2003). pH affected speciation of surface functional groups – namely carboxyl, phosphoryl, and amine – and this controlled the amount of Np(V) sorbed onto the surfaces of *S. alga* and its components. This is illustrated by considering the effect of pH on net charge of carboxyl and amine groups, as shown in Fig. 33.11.

Similar pH effects occur for actinide complexation with mineral particles and their surface ligands (Bond *et al.*, 1991; Murray and Coughlin, 1992; Lieser, 1995; Murphy and Zachara, 1995).

In addition to changes in the protonation of surface complexes, pH affects the speciation of actinide at a given oxidation state. This is demonstrated in Fig. 33.12 which shows the effect of pH on speciation of Np(V) that is open to the atmosphere (Songkasiri, 2003). For pH < 8, Np(V) is present as the free aquo ion, NpO_2^+. However, as pH increases above 8, NpO_2^+ complexes with carbonate, giving rise to Np(V)-carbonate species, $NpO_2(CO_3)^-$, $NpO_2(CO_3)_2^{3-}$, $NpO_2(CO_3)_3^{5-}$. While carbonate is shown here to control actinide(V) speciation, hydroxide complexation dominates actinide (VI), (III), and (IV) speciation in the pH ranges 5–7, 6–9, and at all pHs greater than 2–4, respectively (Silva and Nitsche, 1995).

Furthermore, Np(V) complexation on *S. alga* depended on pH-specific speciation of Np(V) and bacterial surface functional groups. Below pH 8, the predominant NpO_2^+ species complexed with carboxyl- (pK_a of $\sim$2.4 and $\sim$5), and phosphoryl-sites ($pK_a \sim 7.2$). However, above pH 8, Np(V), speciation shifts

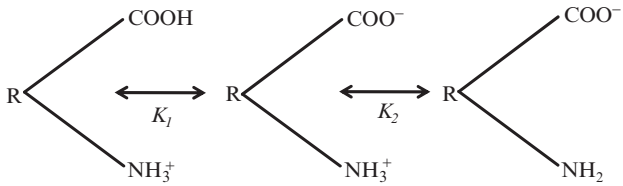

Fig. 33.11 *Effect of change in pH on speciation of carboxyl and amine groups that facilitates shift in net charge on surface functional group.*

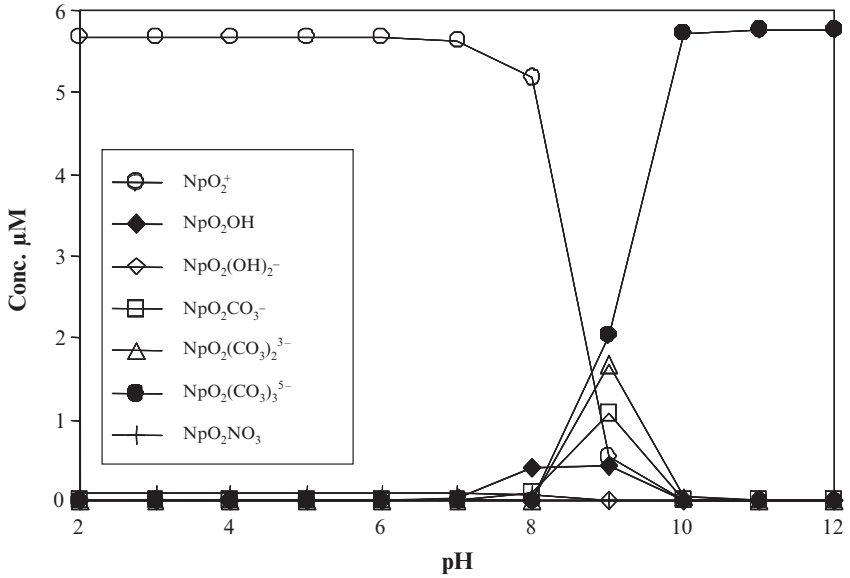

Fig. 33.12 *Np(V) speciation as a function of pH in* 0.1 *M* $NaNO_3$ *electrolyte, in equilibrium with carbonate in the atmosphere, and with* 5.76 μ*M initial Np(V) concentration.*

towards $NpO_2(CO_3)^-$, $NpO_2(CO_3)_2^{3-}$, and $NpO_2(CO_3)_3^{5-}$, such that the amine site ($pK_a > 10$) is the dominant complexant of Np(V)-carbonate species. Above pH 10, the amine site starts to deprotonate so that Np(V) complexation on *S. alga* is reduced.

33.3.4 Bioprecipitation of actinides

Unlike biological treatment of organic contaminants, which often can be mineralized to harmless products, such treatment does not work on metals and radionuclides because they cannot be "destroyed" to harmless products (Rittmann *et al.*, 2002a; Lins *et al.*, 2008). For these contaminants, immobilization strategies, such as bioprecipitation, can be envisioned to render them immobile and less toxic (Dodge and Francis, 1994; Yong and Macaskie, 1995; Phillips *et al.*, 1995; Thomas and Macaskie, 1996; Mackasie *et al.*, 1997; NRC, 2000a).

Bioprecipitation is the process of biologically mediated precipitation of metals and radionuclides into an insoluble form. While reduced forms of actinides are generally less soluble than oxidized forms, and supersaturated cationic and anionic species will have a higher tendency of precipitation, other parameters/factors may also contribute towards spontaneous precipitation: for example, presence of nucleation sites, where all of these potentially can be mediated by microbial activity. Before we highlight microbial contribution to precipitation, we first discuss the mechanisms of precipitation.

Solid precipitation can occur via two distinct mechanisms, homogeneous and heterogeneous. In a homogeneous system, no solid nucleation sites are present. Due to the energy cost of forming the initial solid surfaces, precipitation only occurs when the solution is significantly supersaturated with the participating cationic and anionic species, and the energy required for initial nucleation of the solid is less than the energy released during precipitation (Morel and Hering, 1993; Stumm and Morgan, 1996).

Contrary to homogeneous system, precipitation in heterogeneous system is facilitated by the presence of nucleation sites, which lower the free energy barrier of precipitation, thereby decreasing the amount of super-saturation required to initiate precipitation. This reduction in the nucleation free-energy barrier is related to the structural similarity between the precipitate and the nuclei; precipitates similar to the nuclei have lower energy barrier to precipitation (Raymond *et al*., 1989).

Next, we discuss how microbial activity facilitates bioprecipitation. Banaszak *et al*. (1999a) provided a comprehensive review of how microorganisms directly or indirectly affect the actinide precipitation. We provide a succinct summary here.

First, microorganisms can directly cause actinide precipitation by using them as electron acceptors, because the reduced form in most cases is less soluble and more prone to precipitate than does the oxidized form. Second, bacteria can accelerate precipitation of super-saturated solids by acting as nucleation sites, reducing the free energy barrier to the point where precipitation becomes possible. The large areas of lipid bilayers are interspersed with membrane-bound proteins that can act as specific metal binding sites. Not only can these surface sites be viewed as general heterogeneous nucleation sites (McLean *et al*., 1996; Schultze-Lam *et al*., 1996), but their overall negative charge can attract positively charged metal species as counter-ions. Third, after precipitate initiation at the nucleation sites, microorganisms may further accelerate precipitation by changing the local chemical gradient, which may support crystal growth.

The rate of crystal growth depends on how much the solution has supersaturated, transfer of mass of chemical species to the solid surface, and the surface area of solid where accumulation will occur. This can be described as (Morel and Hering, 1993; Stumm and Morgan, 1996; Rittmann *et al*., 2002b):

$$\frac{dSp_{solid}}{dt} = ka\left[\frac{1 - K_{sp}}{Q}\right][Sp] \tag{33.20}$$

where Q is the ion activity product of precipitating species in solution, K_{sp} is the solubility product, k represents precipitation or dissolution of mass-transfer rate constant, a is the surface area of the solid surface, and $[Sp]$ is the rate-controlling concentration of the aqueous species.

It is easier to understand the change in local chemical gradient if the bacterium produces an anionic species that is a precipitation anion with the metal or actinide. A typical example is the generation of carbonate/bicarbonate, where

the local carbonate gradient (i.e., carbonate concentration immediately adjacent to the cell) is governed by the rate of carbonate mass transfer away from the bacteria. Thus, if the rate of carbonate production exceeds the rate at which it is removed from its immediate surrounding, precipitation will be accelerated because the elevated/accumulated carbonate exceeds the solubility of metal/actinide carbonate species.

Similar to carbonates, microbial activity can facilitate bioprecipitation by altering the local concentrations of other precipitating anions, such as S^{2-}, SO_3^{2-}, NO_3^{-}, and PO_4^{3-}. Additionally, microbial activity may influence bioprecipitation by manipulating the geochemical environment through biodegradation products that change the pH (VanBriesen and Rittmann, 1999) or that complex with actinides. Examples of such products are acidic hydrogen (Rittmann *et al.*, 2002a) and microbe-produced extra-cellular polymeric substances (Songkasiri, 2003).

33.4 MATHEMATICAL MODELING OF THE INTERACTION OF THE ACTINIDES WITH MICROORGANISMS

Subsurface biological and chemical processes interact with actinide species in complicated but clearly identifiable ways. While externally imposed geochemical conditions of pH, ionic strength, and redox potential may establish the initial speciation of actinides in the environment, microbiological processes can change the local geochemical conditions in ways that directly or indirectly influence speciation and fate of the actinides. The microorganisms change their environment as they carry out their normal metabolic reactions, which begin with oxidations and reductions of electron-donor and acceptor substrates, respectively. Not only do these reactions alter the redox state of their environment, but they also produce or consume materials that are acids, bases, and complexing ligands. Furthermore, the microorganisms themselves present complexing ligands that can sorb actinides (Banaszak *et al.*, 1999a). Interactions of this complexity can only be understood and controlled by using mathematical modeling that comprehensively couples all the relevant biogeochemical processes.

One such mathematical model was developed specifically to achieve the goal of comprehensively coupling the biological and chemical reactions. Called CCBATCH (Rittmann and VanBriesen, 1996; Banaszak *et al.*, 1998a; VanBriesen and Rittmann, 2000; Rittmann *et al.*, 2002b; Schwarz and Rittmann, 2007a, b), it explicitly couples biological electron-donor and -acceptor consumption to simultaneously occurring geochemical reactions, namely complexation, acid/base, and precipitation/dissolution reactions. CCBATCH also includes transport processes when relevant (Schwarz and Rittmann, 2007b). CCBATCH provides the framework for understanding the biogeochemical reactions and how they interact with each other to control the fate of actinides.

While CCBATCH provides a comprehensive framework and one that is directly suited to understanding the fate of actinides, several other models related to biotransformation of actinides are available in batch or transport systems. Batch systems include simple models that simulate bacterial reduction of uranium (U(VI)) without coupling to bacterial growth (Truex *et al.*, 1997; Spear *et al.*, 1999, 2000; Lall and Mitchell, 2007; Nyman *et al.*, 2007) and coupled models (that provide links among bacterial growth, substrate utilization, and chemical speciation) in the presence of neptunium(V), NpO_2^+ (Banaszak *et al.*, 1998a; Rittmann *et al.*, 2002a). Coupled models in transport systems have been reported that simulate bioimmobilization of U(VI) (Burgos *et al.*, 2003; Wang *et al.*, 2003; Roden and Scheibe, 2005; Scheibe *et al.*, 2006; Luo *et al.*, 2007a; Yabusaki *et al.*, 2007).

The fundamental principle behind modeling interaction of actinides with microorganisms is to link quantitatively and mechanistically the microbial-growth and substrate-utilization reactions to the chemical species and reactions that affect actinide speciation. These reactions apply a combination of thermodynamics and kinetics. In this section, we show how these thermodynamically and kinetically controlled reactions are coupled.

33.4.1 Coupling microbial synthesis, substrate utilization, and geochemical reactions

Like all living things, microorganisms need to consume food from their environment to grow and sustain themselves. More specifically, food means a source of available electrons that the cells use a source of energy to drive their metabolic processes. Thus, the microorganisms' food is called its electron-donor substrate. All microorganisms need a bioavailable electron donor if they are to grow and survive.

Like all organisms, microorganisms generate useful energy by transferring electrons from their electron-donor substrate to an oxidized material that is bioavailable. That material is called the electron acceptor, and the energy-producing transfer of electrons to the acceptor is called respiration. Common electron acceptors include oxygen (O_2), nitrate (NO_3^-), sulfate (SO_4^{2-}), and carbon dioxide (CO_2). For some microorganisms, oxidized forms of actinides can be electron acceptors: e.g., NpO_2^+, PuO_2^+, and UO_2^{2+}. We can represent the respiration reaction by combining the half reactions of electron donor and acceptor, as shown by the following example:

Electron donor substrate (lactate being partially oxidized to acetate and inorganic C):

$$0.25CH_3CH(OH)COO^- + 0.5H_2O \rightarrow 0.25CH_3COO^- + 0.25H_2CO_3 + H^+ + e^- \quad (33.21)$$

Electron acceptor substrate (oxygen being reduced to water):

$$0.25O_2 + H^+ + e^- \rightarrow 0.5H_2O \tag{33.22}$$

Overall respiration reaction:

$$0.25CH_3CH(OH)COO^- + 0.25O_2 \rightarrow 0.25CH_3COO^- + 0.25H_2CO_3 \tag{33.23}$$

Similarly, actinides can be represented in the respiration reaction, since they normally are in an oxidized form and can accept electrons. We show this using NpO_2^+ as an example:

$$NpO_2^+ + 4H^+ + e^- \rightarrow Np^{4+} + 2H_2O \tag{33.24}$$

$$0.25CH_3CH(OH)COO^- + NpO_2^+ + 3H^+ \rightarrow 0.25CH_3COO^- + Np^{4+} + 0.25\,H_2CO_3 + 1.5H_2O \tag{33.25}$$

The energy gained in respiration is invested, at least in part, to synthesize new biomass. This also means that some of the electrons in the donor substrate must be invested in the newly formed biomass. Furthermore, nutrients must be taken up to form biomass, which can be represented as $C_5H_7O_2N$. A simple synthesis half reaction is:

$$0.05NH_4^+ + 0.25H_2CO_3 + 0.95H^+ + e^- \rightarrow 0.05C_5H_7O_2N + 0.65H_2O \tag{33.26}$$

In order to combine the synthesis reaction with the energy-generating respiration reaction, we need to account for the fraction of electrons invested in respiration (f_e°) and synthesis (f_s°) (Rittmann and McCarty, 2001) The overall synthesis reaction (R_{overall}) combines the half-reactions of acceptor (R_{acceptor}), donor (R_{donor}), and synthesis ($R_{\text{synthesis}}$) in the following manner:

$$R_{\text{overall}} = R_{\text{donor}} - f_s^{\circ} R_{\text{synthesis}} - f_e^{\circ} R_{\text{acceptor}} \tag{33.27}$$

where all the half-reactions are written in their oxidized form. Thus, the overall synthesis reaction in the above example using oxygen as the electron acceptor and $f_s^\circ = 0.59$ is:

$$0.25CH_3CH(OH)COO^- + 0.1025O_2 + 0.0295NH_4^+ \rightarrow 0.0295C_5H_7O_2N + 0.25CH_3COO^- + 0.1025H_2CO_3 + 0.0295H^+ + 0.0885H_2O \tag{33.28}$$

Developing complete stoichiometry for the overall reaction is crucial for accurately defining/calculating the change in chemicals that are consumed and produced during microbial reactions. In the example above, four electrons are released per mole of lactate oxidized, of which 59% is used in cell synthesis (f_s°) and 41% is used in respiration (f_e°) that generates energy for metabolism. Similarly, overall reactions for other combinations of electron-donor and -acceptor substrates can be developed that link substrate utilization to microbial growth.

When substrate and nutrients are consumed, a range of products is produced, including inorganic carbon and acidic hydrogen, both of which directly or indirectly affect actinide speciation; these were discussed in Section 33.3.

As in all biological processes, microorganisms use enzymes to catalyze these kinetically controlled energy- and electron-generating reactions, as long as the reactions are thermodynamically feasible. Thus, the basic microbiological reactions need to be represented through kinetic expressions, such as the dual-limitation Monod expression (Bae and Rittmann, 1996) that describes the utilization rate of primary electron donor substrate (r_d):

$$r_d = -q_m X \left(\frac{S}{S + K_s}\right)\left(\frac{A}{A + K_A}\right) \tag{33.29}$$

where S is the concentration of primary electron donor substrate ($M_s L^{-3}$), A is the concentration of electron acceptor substrate ($M_a L^{-3}$), K_S is the half-maximum-rate concentration for the electron donor substrate ($M_s L^{-3}$), K_A is the half-maximum-rate concentration for the electron acceptor substrate ($M_a L^{-3}$), X is the concentration of active biomass ($M_x L^{-3}$) and q_m is the maximum specific rate of donor substrate utilization ($M_s M_x^{-1} t^{-1}$).

The Monod model has wide application in modeling biodegradation reactions. It reduces to single Monod formulation or zero order when one or both the substrates are in excess, respectively. In a case in which the substrate is in a very low concentration, the model reduces to first-order dependence with respect to that substrate.

The half-maximum-rate concentration, $K_{(A \text{ or } S)}$, measures the microorganism's affinity towards a particular substrate: High K value denotes low affinity for the substrate, and vice versa. The maximum specific rate of substrate utilization, q_m, represents how fast the microorganisms can consume the substrates when they can work at their maximum rate, which occurs with $S >> K$. $K_{(A \text{ or } S)}$ and q_m have important implications for predicting the dominant population in subsurface environment. With high substrate availability, microorganisms having a high q_m will be dominant. On the other hand, substrate utilization in substrate-limited environment is controlled by K, and microorganisms with a small value of K can dominate even if they have a smaller q_m (Bae and Rittmann, 1996).

Additionally, the substrate-utilization rate depends on the concentration of active biomass, X. Because the substrate utilization (r_d) is directly proportional to the biomass concentration, a high substrate-utilization rate requires that the active biomass concentration be large. A goal of bioremediation often is to stimulate biomass growth so that X is large.

Once we have established the utilization rate of primary electron donor substrate (r_d), we can calculate the rate of consumption or production of other species involved in the microbial growth reaction by taking advantage of the stoichiometry of the overall reaction. For the example above, rate of

biomass synthesis is linked to rate of electron donor substrate utilization, lactate, as:

$$r_{synthesis} = \frac{0.0295}{0.25} r_d \tag{33.30}$$

Besides new growth, biomass is lost to cell death and endogenous respiration. Collectively called cell decay, the rate of biomass loss is represented by:

$$r_{decay} = -bX \tag{33.31}$$

where r_{decay} is the rate of cell decay, b is the biomass decay coefficient (T^{-1}), and the negative sign indicates loss of biomass. Combining the two rate expressions, the net rate of biomass growth is:

$$r_{net} = r_{synthesis} + r_{decay} \tag{33.32}$$

Often, kinetic parameters of the Monod expression are determined in "non-growth" conditions. In such a case, the net rate of cell synthesis is kept to a minimum, thereby avoiding the complication of effect of changing biomass concentration on rate of substrate utilization.

Although the basic metabolic reactions are kinetically controlled, most of the reactions involving the products are rapid geochemical reactions that immediately come to equilibrium. Therefore, we must couple the kinetically controlled microbial reactions with thermodynamically controlled geochemical reactions (Rittmann and VanBriesen, 1996; VanBriesen and Rittmann, 1999).

In brief, coupled biogeochemical codes employ the same strategy as commonly used chemical speciation models (e.g., MINTEQA, PHREEQE, and The Geochemist's Workbench): all the species of the medium are divided into components, such that the components can be combined to form all possible species, but cannot be further divided into other components (Morel and Hering, 1993). Components often can combine with more than one other component to form multiple species, such as different complexes. A mass balance is written for each component and contains all species that contain the component.

In most cases, the combination of components to form complexes is nearly instantaneous and can be described by equilibrium expressions. This is true for all acid/base complexes and almost all metal–ligand complexes. It also may be true for complexes that precipitate as solids.

The mass-balance equations and all equilibrium expressions must be solved simultaneously for equilibrium. Typically, a Newton–Raphson iterative solution method that simultaneously solves these multiple non-linear algebraic equations is used (Press *et al.*, 1992). The output is the speciation (or species distribution) of all components at equilibrium.

The geochemical speciation is affected by the microbial reactions because the components can be produced or consumed in the coupled microbial-growth and substrate-utilization reactions. Each component's total mass is updated

continuously by the consumptions and productions from the microbiological reactions. Likewise, the model needs to continuously update the equilibrium chemical speciation as the total mass of the components changes.

So far, we have treated bacteria as suspended biomass. However, in reality bacteria in subsurface environments usually are attached to a solid surface in the form of a biofilm (Rittmann, 1993). Depending on the thickness of the biofilm, substrate utilization can be limited by its concentration gradient within the biofilm; bacteria attached near the inner surface of the biofilm have lower substrate availability compared to bacteria residing away from the attached surface (Rittmann and McCarty, 1981). Taking this into account, a substrate gradient demands that mass-transport equations be added to the coupled microbial-growth and substrate-utilization expressions. However, if the accumulation of biomass per unit surface area in subsurface is very small, substrate limitation due to concentration gradient within biofilm can be neglected during modeling in subsurface environment (Odencranz, 1992); this avoids a significant addition of complexity and computing demand. On the other hand, substrate gradients are normal along the flow path of the water, due to the slow water flow velocities (Bedient *et al*., 1994; Rittmann and VanBriesen, 1996). Thus, it is important to distinguish between concentration gradient along the water flow path and within the biofilm; the former is normal, while the latter often can be ignored.

We have outlined the basic framework for biogeochemical modeling that links equilibrium chemical speciation reactions to coupled microbial growth and substrate utilization reactions. Next, we highlight how some of the direct and indirect microbial influences are handled in codes like CCBATCH, which was specially designed to handle the coupling of chemical speciation and microbial reactions.

(a) Acid/base reactions and proton condition

Unlike typically used equilibrium-controlled models, coupled equilibrium-kinetic models consist of kinetically controlled consumption or production of acidic hydrogen that requires modification of the standard mass balance on acidic hydrogen. Acidic hydrogen is handled with the proton condition, a special mass balance on acid and base equivalents that is defined by using reference species (Stumm and Morgan, 1996). In a proton condition, species with more protons than the reference level are placed on the left side of the equation, and those with fewer protons than reference level are placed on the right side of the equation. The units for all terms in the proton condition are proton equivalents per liter. In this way, the fundamental principle of acid/base chemistry is quantified: formation of every acid equivalent generates a conjugate-base equivalent. All the terms in proton condition are the mole per liter concentration multiplied by the difference in protons from the reference level.

In order to clearly explain proton condition, we use the following stoichiometry for aerobic biodegradation of citrate in the presence of Fe^{3+} as an example

(VanBriesen and Rittmann, 1999). The reaction of oxidation of citrate and synthesis of biomass is:

$$\begin{aligned} C_6H_5O_7^{3-} + 0.64NH_4^+ + 1.26O_2 + 2.36H^+ \\ + 0.071H_2O \rightarrow 2.77H_2CO_3 + 0.64C_5H_7O_2N \end{aligned} \tag{33.33}$$

Fe^{3+} is not involved in the synthesis reaction, but forms several complexes with citrate. The components are $C_6H_5O_7^{3-}$ – Cit^{3-} (citrate ion), Fe^{3+}, H_2CO_3, NH_4^+, H_2O, O_2, and cells – $C_5H_7O_2N$). The forms listed here are chosen as the reference levels for acid/base reactions. In this case, the proton condition is written as:

$$\begin{aligned} 3[H_3Cit] + 2[H_2Cit^-] + [HCit^{2-}] + [H^+] = [FeOHCit^-] \\ + 2[Fe(OH)_2Cit^{2+}] + [FeOH^{2+}] + 2[Fe(OH)_2^+] \\ + 3[Fe(OH)_3] + 2[Fe_2(OH)_2^{3+}] + [OH^-] + 2[CO_3^{2-}] \\ + [HCO_3^-] + [NH_3] + ACID \end{aligned} \tag{33.34}$$

where the square brackets indicate moles per liter concentration and the coefficients indicate the number of acid or base equivalents difference from the reference level. For example, H_3Cit has a coefficient of 3, because it has three more protons for its reference species, Cit^{3-}. $HCit^{2-}$ has a coefficient of 1; it holds just one proton compared to Cit^{3-}. On the right side, CO_3^{2-} has a coefficient of 2; it has two fewer H^+ equivalents than its reference species, H_2CO_3. $Fe(OH)_2Cit^{2+}$ has a coefficient of 2, because it was added to OH^- groups to the reference levels of Fe^{3+} and Cit^{3-}.

A very important term in the proton condition of CCBATCH is ACID, which is used to capture the changes in the net acid equivalents brought about through the kinetically controlled biological reactions. The microbial reaction written just above for citrate biodegradation shows that 2.34 H^+ equivalents are added for each mole of $C_6H_5O_7^{3-}$ consumed. In this case, ACID is a positive number that is proportional to the consumption of citrate by biodegradation. If the biodegradation reaction consumes H^+, ACID takes a negative value.

The proton condition is the special feature in CCBATCH that allows it to accurately calculate the effects of biologically produced acids and bases on pH and component speciation. CCBATCH also has provisions for handling a well-buffered system in which the pH and acidic hydrogen concentration are fixed.

In summary, the effects of kinetic reactions on chemical speciation are handled by changing the total component concentration and capturing the consumption and production of acidic hydrogen through the ACID term in the proton condition. Then, the new component mass balances (including the proton condition) are solved simultaneously with the equilibrium equations for the complexes to determine the new pH and concentrations of all complexes.

(b) Precipitation and dissolution reactions

Given the significance of immobilizing contaminants in the biogeochemical arena, it is essential to include precipitation and dissolution reactions in the biogeochemical mathematical model. CCBATCH has this feature in two options, kinetic and equilibrium. The equilibrium feature calculates precipitation/dissolution by comparing the product of metal–cation and ligand–anion concentrations against the solubility product (K_{sp}) of the relevant solid. It allows precipitation or dissolution to occur until the actual concentrations are consistent with the solubility product. Precipitation removes component mass from solution, while dissolution increases the component concentration in solution.

Calculation with the kinetic feature represents the rate of precipitation using the following expression:

$$R_p = k'a\left(\frac{1 - {}^cK_{sp}}{{}^cQ}\right)[Me] \tag{33.35}$$

in which ${}^cQ = [Me][L]$ ($[Me]$ refers to metal–cation concentration, and $[L]$ refers to ligand anion concentration), R_p is the rate of precipitation ($ML^{-3}t^{-1}$), ${}^cK_{sp}$ is the concentration-based solubility product of metal cation and ligand anion, $k' = k_p[L]$ (k_p refers to the second-order precipitation rate coefficient $[L_4M^{-1}t^{-1}]$, and a is the solid surface area of the nucleation site).

The rate expression combines thermodynamic and kinetic controls, both of which are important to precipitation kinetics. The thermodynamic part is represented by ${}^cK_{sp}/{}^cQ$. When ${}^cK_{sp}/{}^cQ = 1$, the system is at equilibrium, representing no net reaction. If ${}^cK_{sp}/{}^cQ < 1$, the system is supersaturated, and R_p is positive, meaning that precipitation is thermodynamically favorable. If ${}^cK_{sp}/{}^cQ > 1$, dissolution is thermodynamically favorable, and R_p is negative. The kinetic part is represented by $k'a$ and $[Me]$. Fast intrinsic kinetics (k'), a high surface area for solids accumulation or dissolution (a), and a high concentration of the rate-limiting species ($[Me]$) make the rate larger.

Similar to biodegradation reactions, precipitation changes the total mass of a component. Precipitation makes the component concentration lower. In many cases, precipitation/dissolution reactions add or remove acidic hydrogen and affect ACID. Changes to component mass and ACID from precipitation/dissolution reactions are added to changes from microbial reactions in CCBATCH so that all effects are evaluated together, since all the reactions occur simultaneously.

33.4.2 "Special features" required for modeling biodegradation with actinides

(a) Actinide toxicity and strong complexation with electron donor substrate

We have discussed the effects of biodegradation of organic chelating agents on actinide speciation (Section 33.3.3). In this section, we use CCBATCH to

illustrate an investigation of the effects of aerobic biodegradation of NTA on neptunium and plutonium speciation (Banaszak *et al.*, 1998a).

Aerobic biodegradation of NTA ($C_6H_9O_6N$) is described by the following stoichiometry:

$$0.055C_6H_9O_6N + 0.0875O_2 + 0.023H^+ + 0.069H_2O \rightarrow 0.032C_5H_7O_2N + 0.17H_2CO_3 + 0.023NH_4^+ \quad (33.36)$$

Experimental results showed that, in the presence of Pu(IV), biodegradation of NTA was retarded, most likely due to formation of a very stable complex of $PuNTA^+$ in a 1:1 ratio. The observed residual NTA was properly simulated when ligand-to-metal ratio of greater than 1:1 was assumed. As shown in Fig. 33.13, model calculation accurately computed the long-term (>20 h) concentration of NTA remaining in solution, while radiotoxicity was suggested for slowed NTA degradation at times <10 h.

On the other hand, in the presence of Np(V), NTA was completely mineralized, although the biodegradation rate slowed. Unlike the Pu system, equilibrium speciation revealed that most of the Np(V) in the presence of NTA should remain uncomplexed, so that the slowed biodegradation rate should be unaffected by limited substrate availability. Because toxicity of Np(V) on aerobic NTA degradation by *Chelatobacter heintzii* had been reported (Banaszak *et al.*,

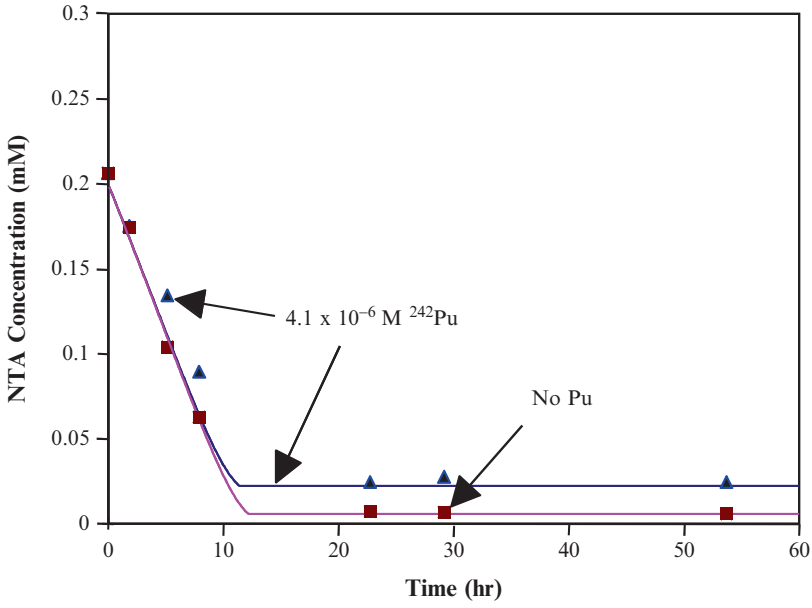

Fig. 33.13 *Comparison of model calculated to experimental NTA degradation in the presence of* 0 *and* 4.1×10^{-6} *M* ^{242}Pu *when Pu(IV)-NTA complexes with ligand-to-metal ratios greater than* 1:1 *are considered* (*adapted from Banaszak et al.*, 1998a).

1998b), toxicity mechanisms were involved to account for the slowed biodegradation of NTA.

A chemical-toxicity approach that adjusts the maximum rate of substrate utilization is given by the following:

$$\frac{K_{Me}}{K_{Me} + [Me]} \tag{33.37}$$

where K_{Me} is the threshold concentration and $[Me]$ is the concentration of the toxic metal. Thus, as the concentration of toxic metal exceeds the estimated threshold concentration, microbial growth and substrate utilization is slowed, eventually leading to complete inhibition.

Figure 33.14 shows the application of the toxicity term, which accurately describes the reduced rate of NTA degradation. Furthermore, unlike the fixed pH case, where all Np remained in solution, Np resulted in precipitation when the solution was unbuffered. Inspection of the overall NTA-degradation reaction shows that, for every mole of NTA consumed, 2.42 mol of acidic hydrogen and 3.4 mol of carbonic acid are produced. Because the reaction was open to the atmosphere, the pH increase was significant and led to the precipitation of NpO_2^+ in the presence of Ca^{2+} and PO_4^{3-} as NpO_2CaPO_4.

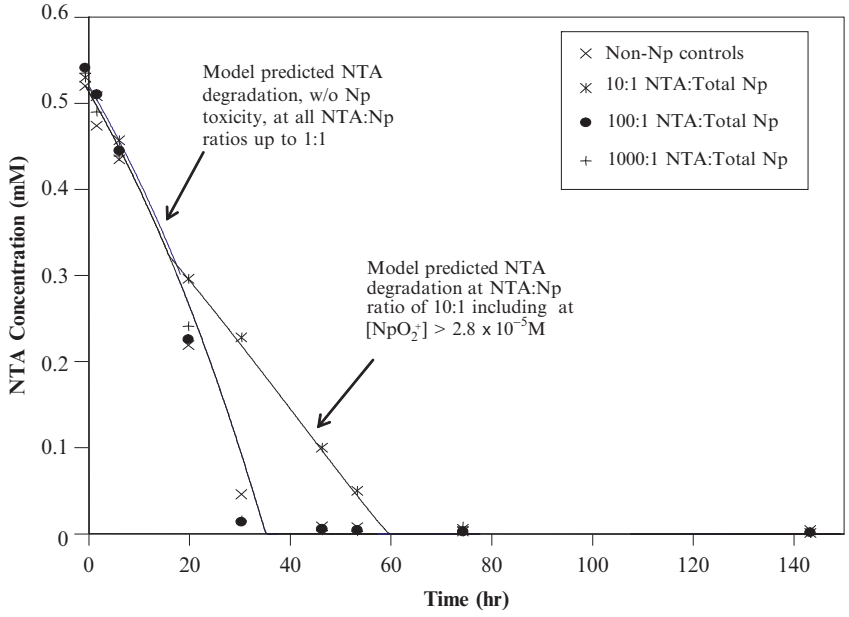

Fig. 33.14 *Comparison of actual to model-calculated NTA degradation by Chelatobacter heintzii in fixed-pH* 6.1 *NTA growth medium, in equilibrium with atmospheric carbon dioxide, and in the presence of increasing total neptunium concentrations* (*adapted from Banaszak et al.*, 1998a).

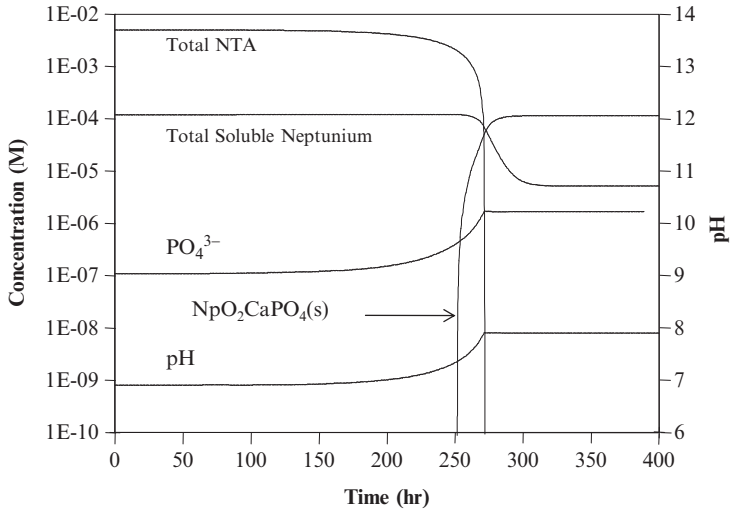

Fig. 33.15 *Calculated equilibrium speciation of* 1.2×10^{-4} *M Np during the degradation of NTA by Chelatobacter heintzii. The solution contains a* 5 *mM NTA growth medium and* 12 *mM phosphate buffer and is in equilibrium with atmospheric carbon dioxide. Calculated results are based on precipitation of the assumed solid phase* NpO_2CaPO_4. (*adapted from Banaszak et al.*, 1998a).

The formation of NpO_2CaPO_4 solid was captured as shown in Fig. 33.15. The Np(V) precipitation was most likely due to an increase in pH that led to an increase in NpO_2^+ and PO_4^{3-} until the solubility of the solid phase was exceeded.

(b) Actinide complexation with strong complexing intermediates

In a study to investigate the fate of Np(V) when it could be reduced by a sulfate-reducing consortium, Np(IV) precipitation was observed only when H_2 was the electron donor (Rittmann *et al.*, 2002a). Increasing the concentration of pyruvate, either in the presence or absence of H_2, delayed precipitation of Np(IV). The authors hypothesized that a fermentation product of pyruvate, most probably succinate, was responsible for keeping Np(IV) soluble. This was tested using the following overall reaction, which involves pyruvate (CH_3COCOO^-) fermentation to acetate (CH_3COO^-) and succinate ($(CH_2COO^-)_2$) coupled to biomass synthesis:

$$\begin{aligned} CH_3COCOO^- + 1.2305H_2O + 0.0635NH_4^+ &\rightarrow 0.0635C_5H_7O_2N \\ &+ 0.0655(CH_2COO^-)_2 + 0.855CH_3COO^- + 0.7105H_2CO_3 \\ &+ 0.471H_2 + 0.0495H^+ \end{aligned} \tag{33.38}$$

The following assumptions were implemented:

- Only 10% of the cells utilize succinate.
- Succinate degradation does not support growth.

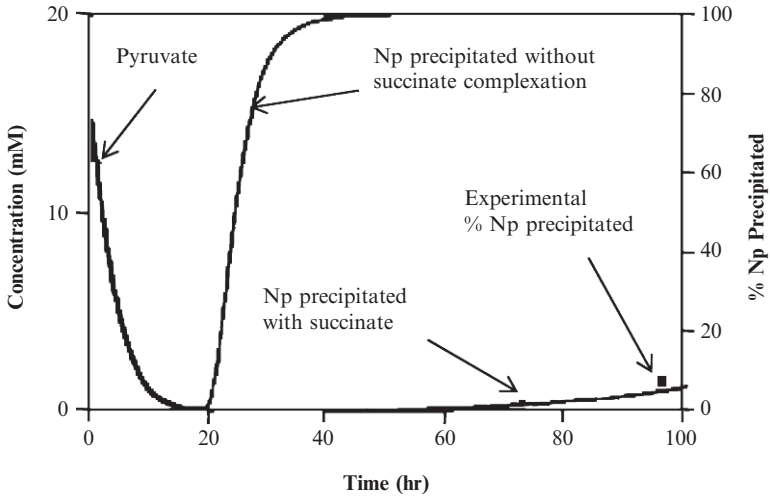

Fig. 33.16 *Model-calculated Np(IV) precipitation with and without Np(IV) complexation by succinate. Slow degradation of the intermediate succinate could account for the delayed Np (IV) precipitation observed when pyruvate was added as a supplemental electron donor (adapted from Rittmann et al.*, 2002a).

- The maximum specific rate of succinate utilization is 5% of the pyruvate-utilization rate.
- The Np(IV)-$(succinate)_2$ formation constant is $10^{20..9}$.

Modeling results show that strong complexation of Np(IV) with succinate, based on the assumptions, could retard Np(IV) precipitation. As shown in Fig. 33.16, Np(IV) precipitation is prevented in the presence of succinate for about 50 h. However, in the absence of succinate complexation to Np(IV), complete precipitation occurs within 50 h.

The features included in model like CCBATCH are essential for understanding systems in which multiple biological reactions are anticipated, especially if they generate strong organic complexants. Although the Np-succinate example illustrates the trends, details of the results should not be extrapolated directly to other situations, due to lack of experimental evidence on the assumptions implemented in the model.

Despite its clear value and documented successes, modeling the fate of actinides in complex subsurface environment is faced with challenges. For example, it is essential to have accurate formation constants for actinide complexation with organic ligands; small errors in complexation constants can result in large differences in calculated speciation (Banaszak *et al.*, 1999a). Additionally, actinide reduction due to indirect (chemical) mechanisms needs to be included in the model (see Section 33.3.1 (a)). Species involved in indirect

reduction are inorganic reduced end products (e.g., ferrous iron or sulfide) (Behrends and Cappellen, 2005; Tabak *et al.*, 2005; Reed *et al.*, 2007) or organic compounds (e.g., dicarboxylic acids, citrate, EDTA) (Choppin and Rao, 1992; Reed *et al.*, 1997).

33.5 ACTINIDE BIOREMEDIATION AND NATURAL ATTENUATION IN THE SUBSURFACE

The interest in microbiological effects on actinide speciation in the subsurface is primarily twofold: (1) understanding the key interactions between actinides and microorganisms and how they affect their subsurface mobility, and (2) using this understanding to engineer a bioremediation solution for existing and future actinide-contaminated sites. The prior sections of this chapter show that the speciation of actinides in the subsurface is greatly influenced by microorganisms in biologically active systems. Likewise, actinides can affect the growth and viability of the microorganisms themselves. However, many questions remain about the mechanisms involved and how to represent them quantitatively. Whether or not the interactions between microorganisms and actinides can be used to implement a remediation or containment strategy for a contaminated site is not yet resolved and may be answered differently for the various actinides and sites.

Current remediation strategies are not centered on removal and cleanup, but on in situ immobilization and containment of actinides in the subsurface. This relatively recent shift in emphasis away from complete site cleanup is in recognition of the intractability of the contamination problems at many sites. There is also the concern that a remediation process that removes actinides from the subsurface may lead to greater effective dose-to-man exposure than the simple management of the subsurface problem (e.g., a subsurface immobilization and containment strategy). The utility of this approach depends on natural attenuation and our ability to predict, with confidence, the long-term fate of actinide contaminants. Prediction depends on having a sound understanding and validation in the field.

Relatively few real-system bioremediation studies of actinides have been carried out, in one part due to the sensitivity of these studies to the owners of the contamination problem and in another part due to the inherent difficulty in measuring all the factors that contribute to real-system results. However, quite a significant amount of data to monitor and track contaminant plumes is generated at most contaminated sites and, typically, annual site-specific reports are published. Additionally, naturally occurring uranium has been extensively investigated, and much is known about its geochemistry.

In the United States, there are a number of efforts to perform in situ experiments to bioremediate uranium subsurface contamination. These are focused on nutrient augmentation to select for biogeochemical microcosms that will reduce, and therefore immobilize, the uranium(VI) groundwater plumes. Three

well-studied subsurface systems are the Rifle site in Western Colorado, a uranium plume in the Hanford 300 area, and a uranium plume at Oak Ridge National Laboratory.

An UMTRA (Uranium Mill Tailings Remedial Action) uranium site in Western Colorado is being studied extensively (Long *et al*., 2006; Yabusaki *et al*., 2007; Fang *et al*., 2009) for prolonged immobilization of U(VI) in a plume that persists from past uranium mining operations. In these experiments acetate additions stimulated iron-reducing bacteria (primarily *Geobacter* sp.) leading to the reduction of U(VI) and subsequent precipitation. This reduction could not be completely sustained after acetate depletion and the onset of sulfate reduction may have caused some re-oxidation of the uranium. Research at this site continues with an emphasis on sustained removal of uranium from the aquifer and the coupling of abiotic reduced iron amendments with acetate additions.

The Oak Ridge site was a near-surface uranium nitrate plume where biostimulation to immobilize the uranium was investigated (Zhou and Gu, 2005; Phelps and Balkwill, 2006; Criddle *et al*., 2006; Luo *et al*., 2007a, b; Madden *et al*., 2007; Luo *et al*., 2009; Madden *et al*., 2009). In these experiments the predominant focus was on the effects of denitrifiers due to the presence of high concentrations of the nitrate co-contaminant. It was not possible to establish sustained bioreduction of uranium under these near-surface conditions but the reduction/precipitation of uranium was observed as the result of a variety of organic nutrient additions. The Hanford 300 studies (Zachara *et al*., 2005; Catalano *et al*., 2006; Gee *et al*., 2007; McKinley *et al*., 2007; Zachara *et al*., 2007) are vadose zone studies with a complex groundwater recharge scenario due to seasonal variations associated with the nearby Columbia river.

Although these in situ efforts have led to a significant increase in our understanding of subsurface processes and the associated complexities that come with real-system bioremediation, there is not yet a clear-cut demonstration of sustained bioreduction of uranium. In these near-surface systems, the effects of co-contaminants can be substantial (e.g., nitrate in the Oak Ridge U(VI) plume) and short-term immobilization and precipitation can be undone by longer-term processes that lead to re-oxidation.

Although real-system bioremediation studies of neptunium and plutonium do not exist, it is fairly rare to observe plutonium mobility under subsurface conditions even though it often coexists with uranium as a contaminant and often exists at or near the surface. Plutonium mobility is generally associated with colloidal transport (Kersting *et al*., 1999; Novikov *et al*., 2006) rather than transport as a dissolved aqueous species. This suggests that the biogeochemistry of plutonium is much more favorably affected by microbial interactions with respect to immobilization. At Hanford, vertical profiles of plutonium show the linkages between microbial activity as it varies with depth and plutonium speciation (Druteikiene and Luksiene, 2003). The potential for microbially mediated immobilization and mobilization of plutonium is the subject of current discussion (Macaskie *et al*., 1994; Gillow *et al*., 2000b; Santschi *et al*., 2002;

Neu *et al.*, 2005). The issue of the bioreduction of Pu(IV) to form Pu(III) species is relatively recent and the potential mobilization of plutonium through this process is not yet addressed in the literature. The potential contribution of plutonium biogeochemistry to the formation of transportable colloidal species is also not well understood.

Neptunium bioremediation, as is the case for plutonium, is less understood than that of uranium in part because neptunium is not routinely detected as a contaminant in the subsurface (Riley *et al.*, 1992). The bioreduction reported by several investigators under anoxic reducing conditions provides the most straightforward path for the reduction of Np(V), a highly mobile oxidation state that is difficult to reduce abiotically, under subsurface conditions. That neptunium is not routinely observed as a mobile subsurface species also argues that its subsurface biogeochemistry is favorable for immobilization and containment.

Natural attenuation of any of the actinides occurs through immobilization, mainly through formation of a precipitate. The most likely pathways towards precipitation incorporate bioreduction and raising the pH. In the case of bioreduction, an electron donor must be present in a high enough concentration that it reduces co-occurring acceptors, such as O_2, NO_3^-, and SO_4^{2-} and still remains to drive actinide reduction. The most likely ''naturally'' occurring donors with subsurface contamination by actinides are organic chelating agents. Raising the pH is most readily accomplished when an acidic electron donor is oxidized. Citric acid is the example shown above, but other organic complexing ligands will have the same effect. Thus, the success of natural attenuation at actinide-contaminated sites is likely to be predicated on the co-contamination of organic chelating agents that are biodegradable under site conditions. Relying on bioreduction and biodegradation of chelates to drive precipitation puts a premium on an increased understanding of biogeochemical processes and biogeochemical modeling that links the multiple biological, chemical, and transport processes.

LIST OF ABBREVIATIONS

An	generic for actinide
CCBATCH	co-contaminants in a *BATCH* reactor
CFU	colony-forming units
DOE	Department of Energy
DFB	desferrioxamine B
DFE	desferrioxamine E
DTPA	diethylentriaminepentaacetic acid
EDTA	ethylenediaminetetraacetic acid
EPS	extracellular polymeric substances
EXAFS	extended X-ray absorption fine-structure spectroscopy

HPW	high-purity water
IDA	iminodiacetic acid
LANL	Los Alamos National Laboratory
LET	linear energy transfer
Lig	ligand
LPS	lipopolysaccharides
Me	metal
NAD	nicotinamide-adenine dinucleotide
NADH	nicotinamide-adenine dinucleotide hydrogen
NRC	National Research Council
NTA	nitrilotriacetic acid
PIPES	piperazine-N′,N′-bis[2-ethanesulfonic acid])
SRB	sulfate-reducing bacteria
TRU	transuranic actinide
UMTRA	Uranium Mill Tailings Remedial Action
XAFS	X-ray absorption fine-structure spectroscopy
XANES	X-ray absorption near-edge spectroscopy

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