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Role of Microbes as Biocolloids in the Transport of Actinides From a Deep Underground Radioactive Waste Repository

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Abstract

We investigated the interaction of dissolved actinides ^{232}Th, ^{238}U, ^{237}Np ^{239}Pu, and ^{243}Am, with a pure and a mixed culture of halophilic bacteria isolated from the Waste Isolation Pilot Plant repository under anaerobic conditions to evaluate their potential transport as biocolloids from the waste site. The sizes of the bacterial cells studied ranged from 0.54 x 0.48 μm to 7.7 x 0.67 μm. Using sequential microfiltration, we determined the association of actinides with free-living (mobile) bacterial cells suspended in a fluid medium containing NaCl or $MgCl_2$ brine, at various phases of their growth cycles. The number of suspended bacteria ranged from 10^6 to 10^9 cells ml^{-1}. The amount of actinide associated with the suspended cell fraction (calculated as mol $cell^{-1}$) was very low: Th, 10^{-12} ; U, 10^{-15} - 10^{-18} ; Np, 10^{-15} - 10^{-19} ; Pu, 10^{-18} - 10^{-21} ; and Am, 10^{-18} - 10^{-19} ; and it varied with the bacterial culture studied. The differences in the association are attributed to the extent of bioaccumulation and biosorption by the bacteria, pH, the composition of the brine, and the speciation and bioavailability of the actinides.

Introduction

The transuranic (TRU) waste slated for disposal at the Waste Isolation Pilot Plant (WIPP), New Mexico, consists of clothing, tools, rags, and other organic and inorganic

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constituents contaminated with actinides. Colloidal particles are likely to be generated in the repository environment from the microbial degradation of cellulose and other organic compounds, and by other processes such as corrosion of steel-waste containers. Therefore there is the potential for radionuclides to migrate away from the WIPP repository due to the transport of actinides as colloids, including those bound to microbes (biocolloids). Microorganisms fall within the colloidal size range and can be transported with bulk liquid flow in the subsurface strata. The size of bacteria varies considerably, depending on the species and nutrients available. Growing bacteria are several micrometers long with volumes of several cubic microns, while non-growing bacteria under oligotrophic conditions may be as small as 0.2-0.3 μm with a volume not less than 0.05 μm^3; the minimum size is approximately 0.2 μm with a volume of 0.05 μm^3 [1].

Significant numbers of bacteria exist in all subterranean environments including extreme environments, such as deep-sea hydrothermal vents, hot springs, and deep aquifers. At the WIPP site, concentrations of naturally occurring microbes in the hypersaline environments are about 10^4 to 10^7 cells ml^{-1} [2]. The numbers of bacteria are likely to increase after the TRU waste, which is rich in cellulose, is biodegraded. Free-living bacteria constitute mobile suspended particles that may have a radionuclide-sorbing capacity. In addition, biofilms develop in all ecosystems that harbor microbes, and the biomass accumulated on surfaces can release cells via detachment. The microbial cell surfaces are negatively charged due to the presence of various anionic structures, including phosphate and carboxylate moieties [3], giving them considerable ability to bind metal cations. In many cases, the bacteria can accumulate metals in amounts greater than their own weight.

We evaluated the potential impact of microbes on actinide sorption and transport in support of the WIPP Colloid Research Program [4]. In this paper, we discuss the abundance, size, and growth characteristics of bacteria isolated from the WIPP's surficial and subsurface environment, and the effect of ^{232}Th, ^{238}U, ^{237}Np, ^{239}Pu, and ^{243}Am, on the

growth of a pure culture and mixed culture of halophilic bacteria. In addition, we assessed the potential for the association of actinides with suspended bacteria, which then could be transported as biocolloids.

Materials and Methods

Enumeration of bacteria in WIPP samples. The number of bacteria present in the (i) WIPP salt, (ii) brine from the repository, (iii) surficial samples around the repository, and (iv) groundwater from the Culebra Dolomite Member of the Rustler Formation (Culebra) were enumerated by direct microscopy [5]. The Culebra is the potential far-field release path at the WIPP [1].

Pure culture. A pure culture of bacteria (WIPP1A) was isolated from a sediment slurry sample at the WIPP surficial environment [2]. This bacterium is a gram-positive rod capable of rapid growth aerobically, or anaerobically using nitrate as electron acceptor. It does not reduce iron or uranium. The bacterium was grown in medium containing sodium succinate, 5.0 g; KNO_3, 1.0 g; K_2HPO_4, 0.25 g; yeast extract, 0.5 g; WIPP salt (primarily halite), 200 g; deionized water, 1000 ml, pH 6.3. The NaCl concentration in the medium was 18% w/v. The brine composition of this medium simulates brine from the Castile Formation below the repository's horizon.

Mixed Culture. A mixed culture, consisting of short and long rods of 5 different cell morphologies, all gram-negative, was isolated from mixed inoculum prepared for the WIPP-Gas Generation Study at Brookhaven National Laboratory (BNL) [2]. The culture was grown in Brine A, a synthetic brine simulating that found in the Salado Formation at the repository horizon. Brine A growth medium consisted of sodium succinate, 5.0 g; KNO_3, 1.0 g; K_2HPO_4, 0.25 g; yeast extract; 0.5 g; NaCl, 100.1 g; Na_2SO_4, 6.2 g; $Na_2B_4O_7.10H_2O$, 1.95 g; $NaHCO_3$, 0.96 g; NaBr, 0.52 g; KCl, 57.2 g; $MgCl_2.6H_2O$, 292.1 g; $CaCl_2$, 1.66 g, deionized H_2O, 1000 ml and pH 6.3. This mixed culture, designated as BAB, grows very slowly aerobically and anaerobically using nitrate as an

electron acceptor in Brine A medium only. Like the pure culture (WIPP1A), the mixed culture (BAB) does not reduce iron nor uranium anaerobically.

The media were pre–reduced by purging with filtered ultra–high–purity (UHP) nitrogen gas and then filter-sterilized through 0.2 μm sterile filters. They were transferred to an anaerobic (N_2) glovebox, and 40 ml were dispensed into sterile (acid-washed and autoclaved) 60 ml glass serum bottles. The bottles were sealed with sterile butyl rubber stoppers. The media were inoculated with 1 ml of an early log–phase culture of WIPP1A (24-hrs. old) or BAB (5-days old) and incubated in the dark at 30 ± 2°C without shaking. Aliquots from the top fluid column were withdrawn periodically from a set of samples well-mixed (shaken) before sampling, or from another dedicated set of undisturbed samples, and analyzed for cell number and optical density (600 nm) using a Spectronic 20D spectrophotometer. The operational pH (uncorrected for ionic strength effects) was measured with a Beckman ϕ-11 pH meter and AgCl combination electrode.

The total number of bacteria was determined by direct microscopy using the DAPI staining technique [5]. A Zeiss Axioskop microscope equipped with an Optronics 3-chip CCD camera interfaced to a PC was used to acquire images for cell size analysis. Images (1850x magnification) were captured and analyzed using Sigma Scan (Jandel Scientific) software. A 10 μm stage micrometer (WILD #310345) traceable to the U.S. National Institute of Standards and Technology (NIST) through Klarmann Rulings, Inc. (Manchester, NH) provided a pixel to micron conversion. In addition, NIST-traceable Bangs Laboratories (Carmel, IN) uniform microspheres (0.538 μm and 1.90 μm) were analyzed to check the precision of measurement.

Effect of Actinides on Growth of Bacteria. The effect of ^{232}Th, ^{238}U, ^{237}Np, ^{239}Pu, and ^{243}Am on the growth of the pure and mixed cultures was determined by adding a known concentration of the actinide to the growth medium and inoculating with the respective bacteria, as described before. Uranium nitrate (BDH Chemicals Ltd., England) and thorium nitrate (J.T. Baker, NJ) and citrate or EDTA complexes were prepared from

the nitrate salts. Equimolar EDTA complexes of Pu, Np, and Am (Los Alamos National Laboratory, NM) were prepared by combining the stock solution of the inorganic salt. When uranyl nitrate or thorium nitrate was added, the composition of the nutrient solution was adjusted to give the appropriate final concentration of nitrate in the medium. Uninoculated samples served as abiotic controls. All treatments were prepared in triplicate. Samples were withdrawn at appropriate intervals to determine optical density, pH, concentration of actinide, and cell number. Actinide in solution was determined by filtering an aliquot through a 0.22 μm syringe filter (Whatman Puradisc®) into a scintillation vial and acidifying with 0.1 ml concentrated HCl. Uranium or Th were analyzed by ICP-AES; samples containing Np, Pu or Am were filtered through a 0.22 μm filter (Gelman Acrodisc®) into pre-weighed scintillation vials for analysis by liquid scintillation counting (LSC) using an Ultima-Gold AB® liquid scintillation cocktail.

Actinide Association with Suspended Bacteria. At appropriate intervals, aliquots were removed from the fluid column of the undisturbed bottles for counting the suspended bacteria, determining total actinide concentration, and for sequential microfiltration using track-etched PETE Poretics® membrane filters. Fifteen milliliters of the sample was removed, 3-5 ml aliquots transferred into each of 3-15 ml capacity Amicon® stirred cells, and sequentially filtered through 10 μm, 0.4 μm and 0.03 μm filters. Nitrogen overpressure was applied to the cells while stirring them, and the filtrate was collected in a scintillation vial. The filtrate and filters were analyzed for the actinides. The filters were digested by acidification with 5 ml of 1M HCl or 1 M HNO_3 and analyzed by ICP-AES for U and Th, and LSC for Pu, Am, or Np. Data for actinide in solution and on the filter were calculated as the mean ± SEM of triplicate filtrations from a single sample.

Results

Bacterial Population in WIPP Samples. Table 1 shows the populations and size distribution of the bacteria present in samples collected from the WIPP surficial and

subsurface environments. The populations generally ranged from 10^5 to 10^8 cells ml^{-1} depending on the presence of nutrients, and sizes of the cells ranged from 0.54 x 0.48 μm to 7.73 x 0.67 μm (l x w). The natural bacterial populations in the groundwater sample collected from the 8-m thick Culebra Dolomite Member of the Rustler Formation had 10^5 cells ml^{-1} with an average size of 0.82 x 0.66 μm; this number and size is representative of typical populations found in oligotrophic (low-nutrient) environments. Adding carbon and nitrogen to the groundwater samples increased the total population with a concomitant increase in cell size (data not shown).

Growth Characteristics of the Pure and the Mixed Culture. The growth characteristics of the pure and mixed culture of bacteria isolated from the WIPP site were very different (Figure 1a-c and d-f). The pure culture followed the typical bacterial growth pattern, and the number of suspended bacteria in the liquid phase decreased at the onset of the stationary growth phase of the culture (Figure 1a). The cell size of the culture (reported as the calculated surface area), increased to a maximum of 4.5 μm^2 during the logarithmic growth and started to decrease to ~1.5 μm^2 during the stationary phase with little difference between the total and suspended populations (Figure 1c). The pH of the culture medium increased from 7.0 to 8.3 due to the consumption of succinic acid. In contrast, the number of cells in the mixed culture consisting of five different size ranges (Table 1) continually increased with time and there was no settling of the cells during the entire incubation period; both the total and the suspended cell numbers remained the same. However, the size of the suspended cells was much smaller than that of the total population. The pH of the medium showed a slight increase (from 6.5 to 6.8) and there was no significant change between the uninoculated control and the inoculated samples. The pure culture did not grow in medium containing $MgCl_2$, nor did the mixed culture in NaCl.

Effect of Actinides on the Growth of Bacteria. The effect of actinides on the growth rate of the pure and mixed culture is given in Table 2. The growth rate constant, (k) is the generation time, or number of doublings $hour^{-1}$ (the time required for the

population to double is the reciprocal, 1/k) and was determined at the mid-log growth phase. Thorium added as either its nitrate or as an equimolar EDTA complex had no effect on the mixed culture at concentration up to $4.2x10^{-3}$M, but it retarded the growth of the pure culture at concentrations $>4.2x10^{-4}$M. Uranium completely inhibited the growth of the pure culture at concentrations $>2.1x10^{-3}$M. Neptunium, added as the EDTA complex to maintain its solubility, caused the growth of the pure culture to decline at concentrations $>5.0x10^{-5}$M but it had no effect on the mixed culture even at concentration up to $5.0x10^{-4}$M. Plutonium, on the other hand, decreased the growth rate of the mixed culture at concentrations $>1.0x10^{-6}$M with no effect on the pure culture. Americium at a concentration $>5.0x10^{-7}$M, inhibited both the pure and mixed culture, with a more pronounced effect on the latter. These results are not adequate to differentiate between whether the observed toxicity is due to radiolytic or heavy-metal interactions. However, the actinides tested altered the growth rate of the bacteria to varying degrees with the overall effect depending upon the bacterial species, the composition of the growth medium, actinide concentration, speciation, and bioavailability.

Actinides Associated with the Bacterial Fraction. Table 3 shows the association of actinides with the suspended bacterial-cell fraction, analyzed by microfiltration generally at 11 or 13 days for the pure culture, and 21 days for the slower growing mixed culture. Actinide detected on the 0.4 μm filter represents the amount of cell-associated actinide for the equilibrium population of suspended cells. Actinide retained on the 10 and 0.03μm filters was also determined (data not shown). About 74% of the total Th present in the fluid column was associated with the pure culture cell fraction (0.4 μm filter), whereas none was detected with the cells of the mixed culture. Both the pure and mixed culture showed <1% of the total U detected in solution (unfiltered) associated with the cell fraction. Neptunium, however, was detected in the cell fraction of the pure culture ($2.3x10^{-6}$M); this amount was more than that detected in the mixed culture fraction ($2.7x10^{-8}$M). The reasons for this variation between the pure and mixed culture are currently being investigated. Very

low levels of Pu was detected with the cell fraction of the pure and mixed culture. The amount detected with the pure culture ($9.2x10^{-10}$ M) was similar to that of the mixed culture ($1.2x10^{-9}$ M). The association of Am with the pure and mixed culture was similar. The amount of actinide per cell was determined by dividing the amount of actinide detected at the 0.4 μm filter fraction by the number of suspended bacterial cells $liter^{-1}$ (Table 3).

Effect of Pu on Growth and Association with Bacterial Fraction. Pu ($1x10^{-6}$M) had no effect on the growth of the pure culture initially, but, at late logarithmic growth phase, it had an effect (Fig. 2a). The pH of the medium increased from 6.5 to 8.5 (Fig. 2b) and the concentration of Pu in solution decreased (Fig. 2c). The mixed culture was not affected by $1x10^{-6}$M Pu (Fig. 2d); the pH of the medium increased slightly in both the uninoculated (control) and the inoculated medium (Fig. 2e). As the growth of the culture increaseed, the concentration of Pu in solution declined (Fig. 2f). Microfiltration of the fluid column of the pure culture sample showed that Pu was associated with the cell fraction (0.4μm fraction) and it decreased with a decrease in the number of suspended cells (Fig. 3a). Analysis of the amount of Pu associated with the cell fraction of the mixed culture showed a small increase with an increase in the number of suspended cells (Fig. 3b). The exact nature of association of Pu in the cell fraction is unknown; studies are underway to clarify this.

Discussion

Microorganisms are important sources of colloids as are their metabolic by-products and exocellular polymers. Microbes bioaccumulate and biosorb actinides intracellularly or extracellularly. Bioaccumulation, which is an active process, usually occurs by an energy-dependent transport system in viable or growing cells where the metals are taken up into cells and sequestered intracellularly by complexing with specific metal-binding cell components or through precipitation reactions. In contrast, biosorption binds metal ions by ligand interactions or by ion exchange mechanisms at the cell surface. Both living and dead microorganisms possess an abundance of functional groups, such as carboxyl, hydroxyl,

and phosphate, on their cell surface able to bind metal ions. Desorption and recovery of the biosorbed radionuclides is easily achieved. Biosorption/bioaccumulation of Th, U, Np, Pu and Am by microorganisms has been reported [6-9]. For example, the uptake of Pu and Am by *Aeromonas hydrophila*, a fresh-water bacterium, was observed in laboratory cultures [6, 7]. Bacterial uptake of soluble Am in aqueous media appears to be primarily due to adsorption to the cell surface. Adsorption is reversible and depends upon the nutrients present, the physiological state of the cell, the pH, and release of bacterial exometabolites [8]. Uptake of Np in excess of 10 mg/g dry weight of cells was observed in *Pseudomonas aeruginosa, Streptomyces viridochromogenes, Scenedesmus obliquus* and *Micrococcus luteus*, when these cultures were exposed to solutions containing 36 mg/L of Np [9].

Bacterial growth, attachment, and detachment of cells together with the hydrogeological conditions affect the transport of actinides [10]. Transport from the waste site can be facilitated by free-living suspended bacteria through bioaccumulation and biosorption. The chemical form of the actinide i.e., ionic, organic, and inorganic complexes and its solubility affect its bioavailability to bacteria. The overall association of the actinides, especially Pu and Am, with the cell fraction was quite low. These results suggest that the extent of actinide association with suspended bacteria, under WIPP-relevant conditions, will be minimal suggesting that the other mechanisms may play a major role in regulating the availability of actinides and that the transport of actinides as microbial colloids may be insignificant. These results support the conclusions of other studies [10, 11]. An assessment of the influence of bacteria on the migration of radionuclides from a deep spent-fuel repository, based on total number of bacteria found there, showed the released radionuclides bound to unattached bacteria is negligible [11]. Microbial processes, such as bioreduction and bioprecipitation [12], which are effective especially in anaerobic environments can limit the solubility of the actinides. The speciation of actinides in solution can affect their bioavailability to the suspended bacteria. These processes need to

taken into consideration in evaluating the potential role of microbes involved in colloidal transport of actinides.

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Figure Legends

Figure 1. Growth characteristics (number of cells, pH, and cell area) for the pure culture (a-c) and mixed bacterial culture (d-f).

Figure 2. Effect of $1x10^{-6}$ M Pu on the growth of the pure culture (a-c) and mixed bacterial culture (d-f).

Figure 3. Association of Pu with the bacterial cell fraction: ☐ total Pu in the unfiltered fluid column; ▒ Pu associated with the cell fraction (0.4 μm filter fraction); ● number of suspended cells for pure culture (a) and mixed culture (b).

Table 1. Enumeration of bacterial populations in WIPP repository-relevant samples.

Source of Sample	Number of Bacteria ml^{-1}	Size (μm)	
		length	width
WIPP Environs			
Culebra groundwater	$1.02 \pm 0.49 \times 10^5$	0.82 ± 0.14	0.66 ± 0.14
G-Seep brine	$1.24 \pm 0.13 \times 10^5$	n/a	n/a
Mixed Inoculum	$3.89 \pm 0.08 \times 10^6$	n/a	n/a
Isolates from WIPP Environs			
Monoculture[a]	$4.30 \pm 0.20 \times 10^8$	1.53 ± 0.09	0.79 ± 0.03
Mixed culture[b] (Mean size)	$3.57 \pm 0.22 \times 10^8$	1.46 ± 0.45	0.66 ± 0.07
(Classified by size)			
Group a		0.54 ± 0.03	0.48 ± 0.03
Group b		0.66 ± 0.01	0.62 ± 0.02
Group c		1.47 ± 0.14	0.76 ± 0.04
Group d		2.66 ± 0.29	0.92 ± 0.06
Group e		7.73 ± 0.96	0.67 ± 0.04
Gas Generation Experiments[c]			
No Nutrient Addition	$1.59 \pm 0.15 \times 10^7$	n/a	n/a
Nutrient Addition	$1.62 \pm 0.07 \times 10^8$	n/a	n/a
Nutrient Addition + Nitrate	$2.24 \pm 0.24 \times 10^8$	n/a	n/a

[a] Population at 175 hrs. at 30°C
[b] Population at 241 hrs. at 30°C
[c] Reference [2]
n/a = not analyzed

Table 2. Effect of actinides on the growth of pure and mixed cultures of bacteria.

Actinide	Form	Concentration (M)	Activity (μCi/L)	Growth Rate (k)	
				Pure Culture	Mixed Culture
None	--	--	--	0.129	0.014
^{232}Th(IV)	nitrate	4.20×10^{-4}	0.01	0.131	0.013
		1.05×10^{-3}	0.03	0.093	0.014
		2.10×10^{-3}	0.05	0.017	0.014
		4.20×10^{-3}	0.10	0.009	0.013
	1:1 EDTA	4.20×10^{-4}	0.01	0.066	0.012
		1.05×10^{-3}	0.03	0.046	0.011
		2.10×10^{-3}	0.05	0.025	0.014
		4.20×10^{-3}	0.10	0.011	0.014
^{238}U(VI)	nitrate	4.20×10^{-4}	0.03	0.133	0.010
		1.05×10^{-3}	0.08	0.130	0.011
		2.10×10^{-3}	0.15	0.004	0.011
		4.20×10^{-3}	0.34	0.000	0.013
	1:1 citrate	4.20×10^{-4}	0.03	0.141	0.012
		1.05×10^{-3}	0.08	0.098	0.013
		2.10×10^{-3}	0.15	0.000	0.012
		4.20×10^{-3}	0.34	0.000	0.011
^{237}Np(V)	1:1 EDTA	5.0×10^{-6}	0.83	0.132	0.016
		5.0×10^{-5}	8.3	0.096	0.012
		5.0×10^{-4}	84	0.087	0.014
^{239}Pu(V)	1:1 EDTA	1.0×10^{-7}	1.5	0.143	0.014
		1.0×10^{-6}	15	0.131	0.013
		1.0×10^{-5}	150	0.130	0.005
^{243}Am(III)	1:1 EDTA	5.0×10^{-8}	2.4	0.125	0.012
		5.0×10^{-7}	24	0.122	0.012
		5.0×10^{-6}	240	0.098	0.003

n/a = not analyzed

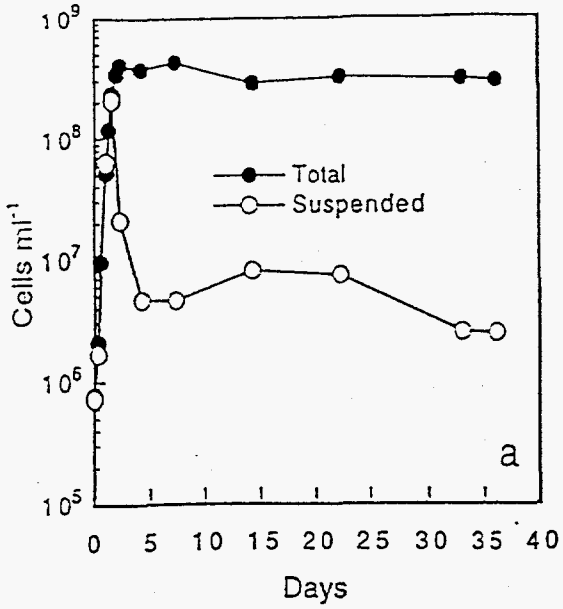
Table 3. Actinide (An) association with the suspended equilibrium bacterial population in the fluid column.

Actinide	Chemical Form	Bacterial (M)	Bacterial Cells ml^{-1}	Actinide Concentration (M)[b] Total in Fluid Column (Unfiltered)	Associated with Cells (<10.0 µm, >0.40 µm)	Soluble (<0.03 µm)	mol An $Cell^{-1}$
^{232}Th	EDTA	Pure (13)	3.7×10^{5}	9.40×10^{-4}	$6.95 \pm 0.76 \times 10^{-4}$ [74]	$1.55 \pm 0.06 \times 10^{-4}$ [16]	2×10^{-12}
	EDTA	Mixed (28)	3.2×10^{8}	2.70×10^{-4}	n/d	$2.50 \pm 0.06 \times 10^{-4}$ [93]	n/d
^{235}U	nitrate	Pure (13)	3.0×10^{6}	8.34×10^{-4}	$2.11 \pm 0.26 \times 10^{-6}$ [<1]	$9.83 \pm 0.17 \times 10^{-4}$ [118]	7×10^{-16}
		Mixed (13)	2.3×10^{8}	1.17×10^{-3}	$2.22 \pm 0.19 \times 10^{-6}$ [<1]	$1.16 \pm 0.00 \times 10^{-3}$ [99]	1×10^{-18}
	citrate	Pure (13)	2.4×10^{6}	9.67×10^{-4}	$3.78 \pm 0.42 \times 10^{-6}$ [<1]	$9.83 \pm 0.17 \times 10^{-4}$ [101]	2×10^{-15}
		Mixed (13)	1.0×10^{8}	1.22×10^{-3}	$4.20 \pm 0.42 \times 10^{-6}$ [<1]	$1.24 \pm 0.04 \times 10^{-3}$ [102]	4×10^{-17}
^{237}Np	EDTA	Pure (7)	2.3×10^{6}	$1.95 \pm 0.03 \times 10^{-5}$	$2.33 \pm 1.24 \times 10^{-6}$ [12]	$1.98 \pm 0.09 \times 10^{-7}$ [1]	1×10^{-15}
	EDTA	Mixed (20)	1.8×10^{8}	$7.41 \pm 0.03 \times 10^{-8}$	$2.74 \pm 0.06 \times 10^{-8}$ [53]	$1.12 \pm 0.01 \times 10^{-8}$ [15]	2×10^{-19}
^{239}Pu	EDTA	Pure (11)	8.1×10^{5}	1.02×10^{-8}	$9.24 \pm 1.70 \times 10^{-10}$ [9]	$6.33 \pm 0.32 \times 10^{-9}$ [62]	1×10^{-18}
	EDTA	Mixed (21)	3.0×10^{8}	4.81×10^{-8}	$1.22 \pm 0.20 \times 10^{-9}$ [2.5]	5.46 ± 1.11 x 10-8 [113]	4×10^{-21}
^{243}Am	EDTA	Pure (11)	2.5×10^{6}	1.28×10^{-8}	$1.22 \pm 0.05 \times 10^{-8}$ [95]	$1.59 \pm 0.30 \times 10^{-9}$ [12]	5×10^{-18}
		Mixed (21)	3.0×10^{8}	$1.69 \pm 0.01 \times 10^{-7}$	$4.72 \pm 0.10 \times 10^{-8}$ [28]	$5.73 \pm 0.43 \times 10^{-8}$ [33]	2×10^{-19}

[a] Nos. in parentheses are the incubation time at 30 ± 2°C (days).

[b] Nos. in brackets are the percent (of the total in the fluid column) of An associated with the particular fraction (cell-bound or soluble).

n/d = not detected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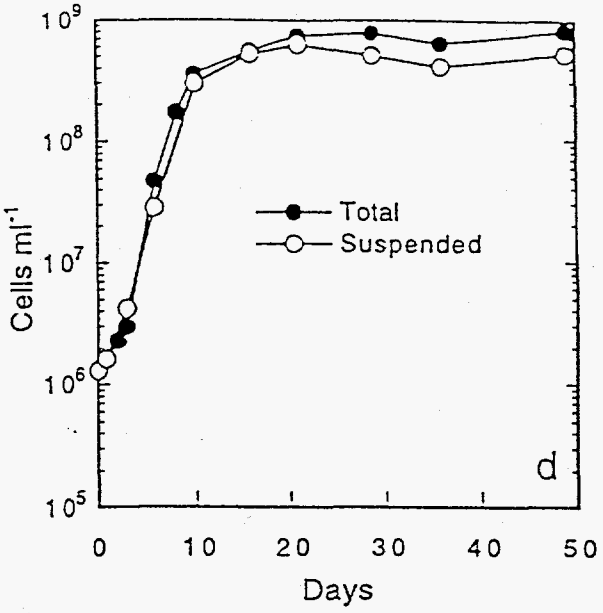
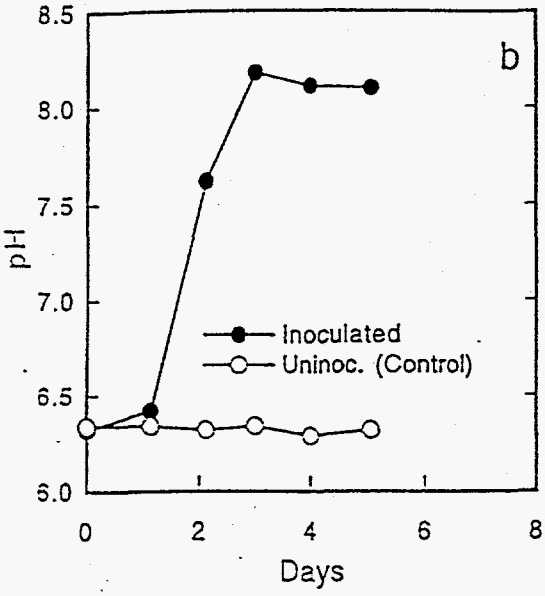
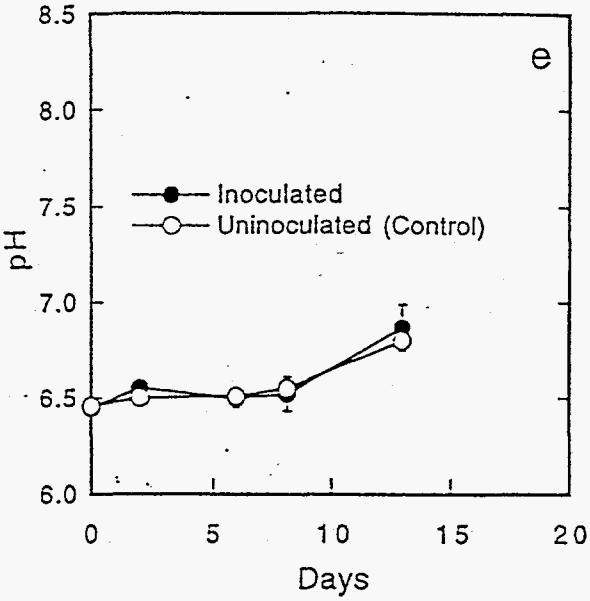
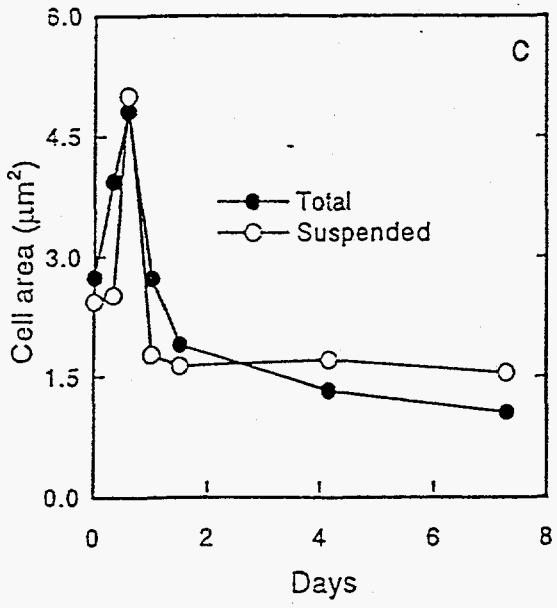
a
Days


Cells ml-1
Total
Suspended
d
Days


pH
b
Inoculated
Uninoc. (Control)
Days


pH
e
Inoculated
Uninoculated (Control)
Days


Cell area (µm2)
c
Total
Suspended
Days

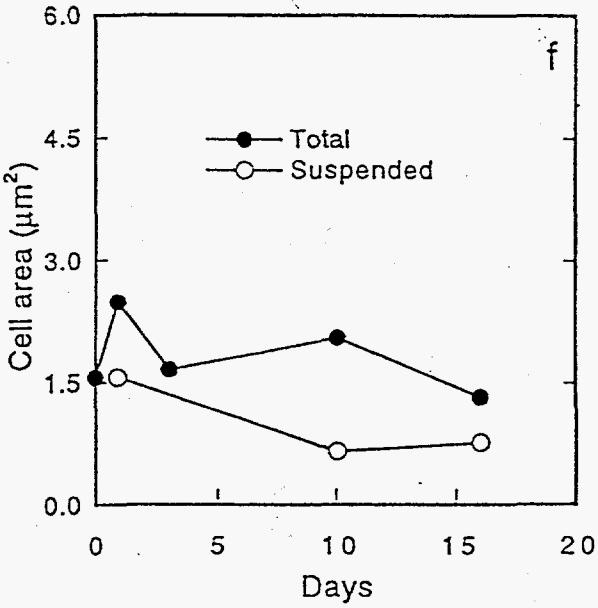
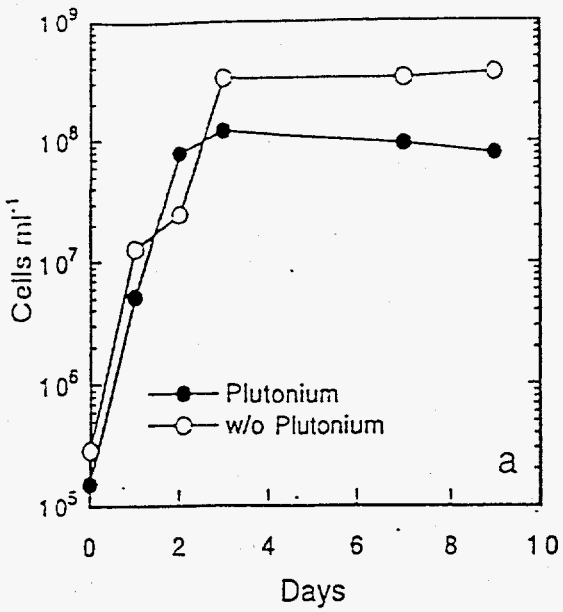
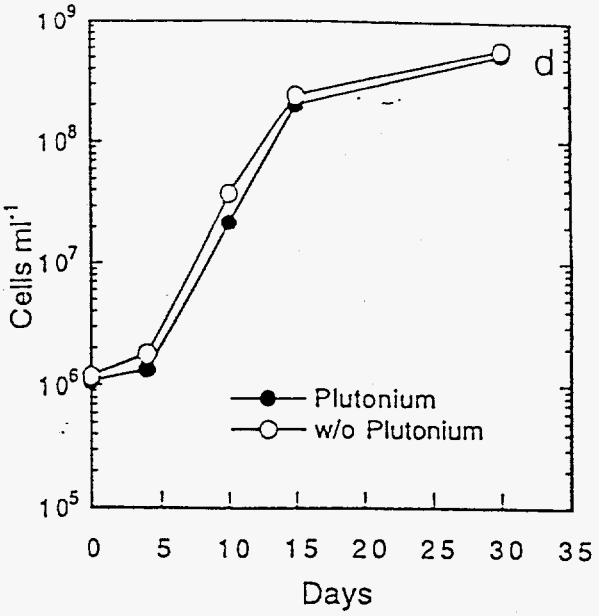
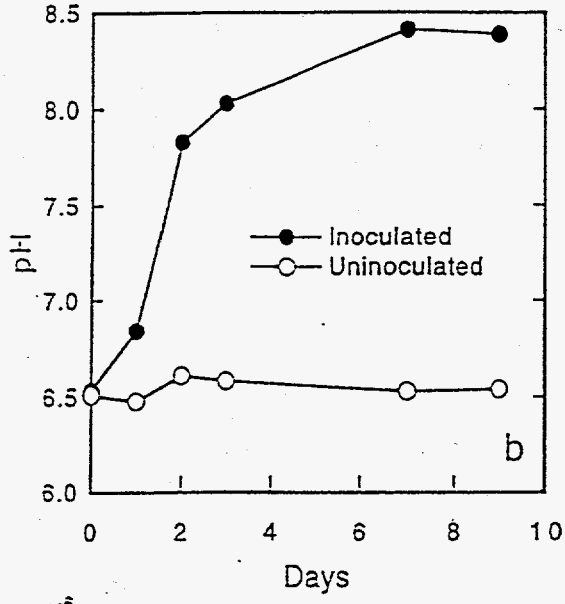
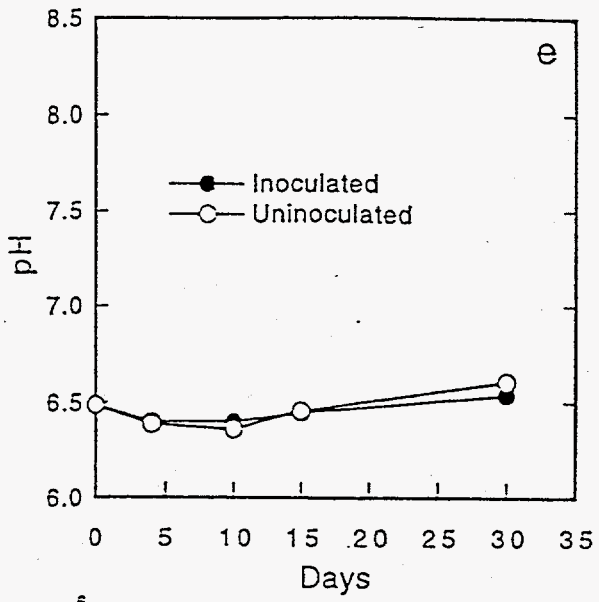

Cell area (µm2)
f
Total
Suspended
Days

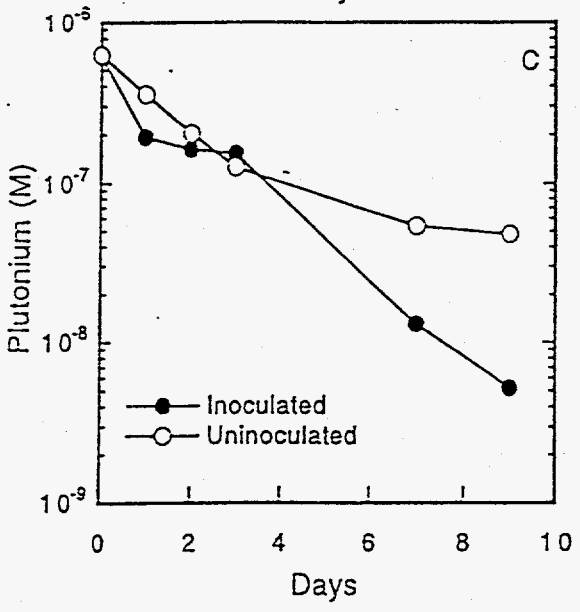
Figure 1


Cells ml-1
Plutonium
w/o Plutonium
a
Days


Cells ml-1
Plutonium
w/o Plutonium
d
Days


pH
Inoculated
Uninoculated
b
Days


pH
Inoculated
Uninoculated
e
Days


Plutonium (M)
Inoculated
Uninoculated
c
Days

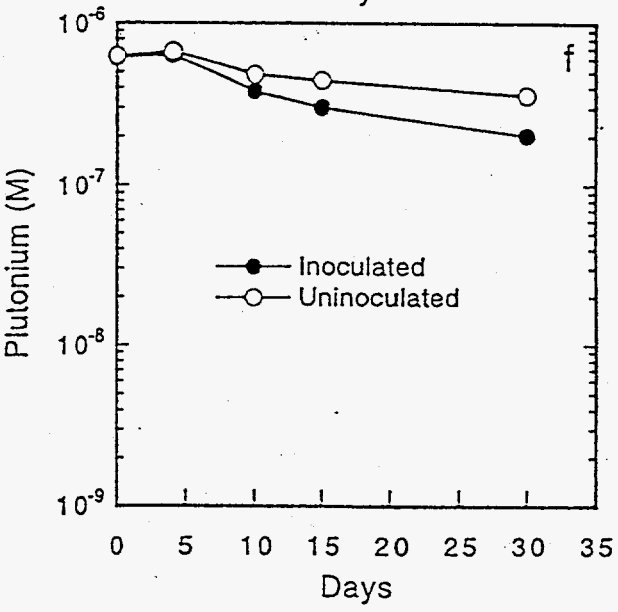
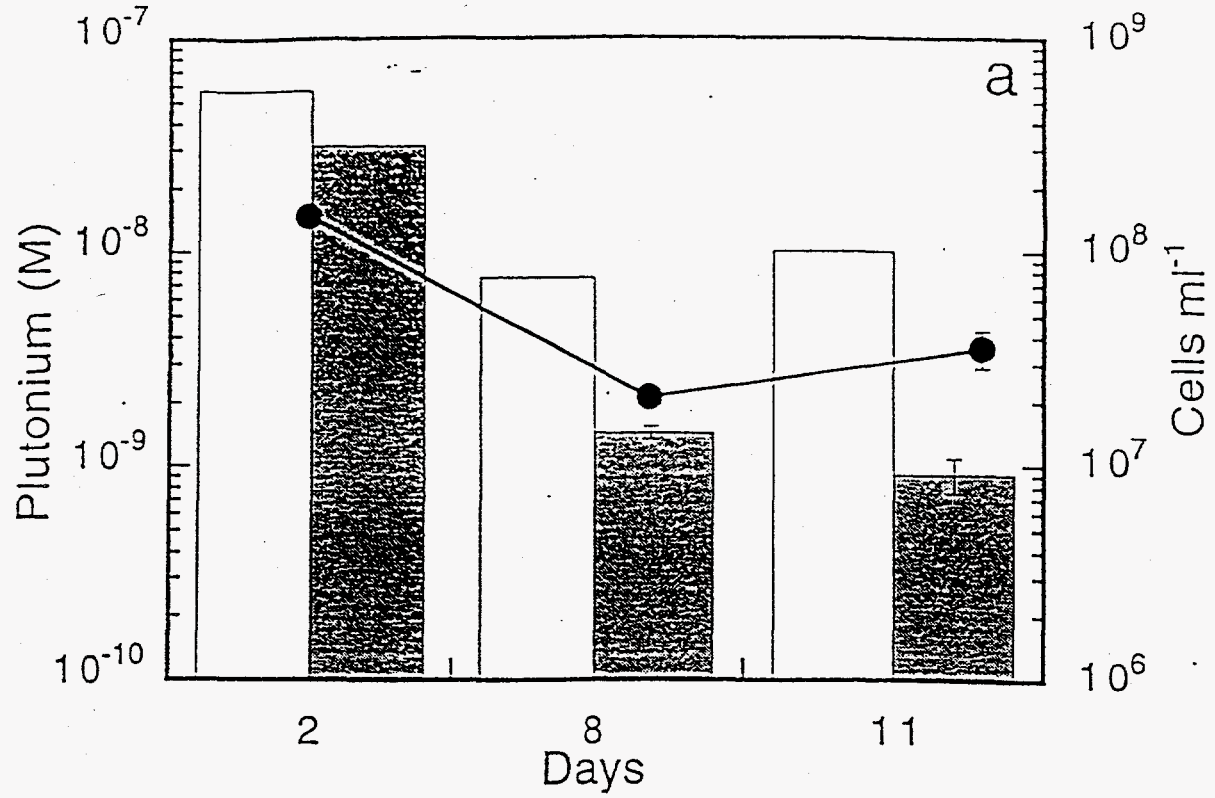

Plutonium (M)
Inoculated
Uninoculated
f
Days

Figure 2


a
Plutonium (M)
10^{-7}
10^{-8}
10^{-9}
10^{-10}
10^{9}
10^{8}
10^{7}
10^{6}
Cells ml^{-1}
2
8
11
Days

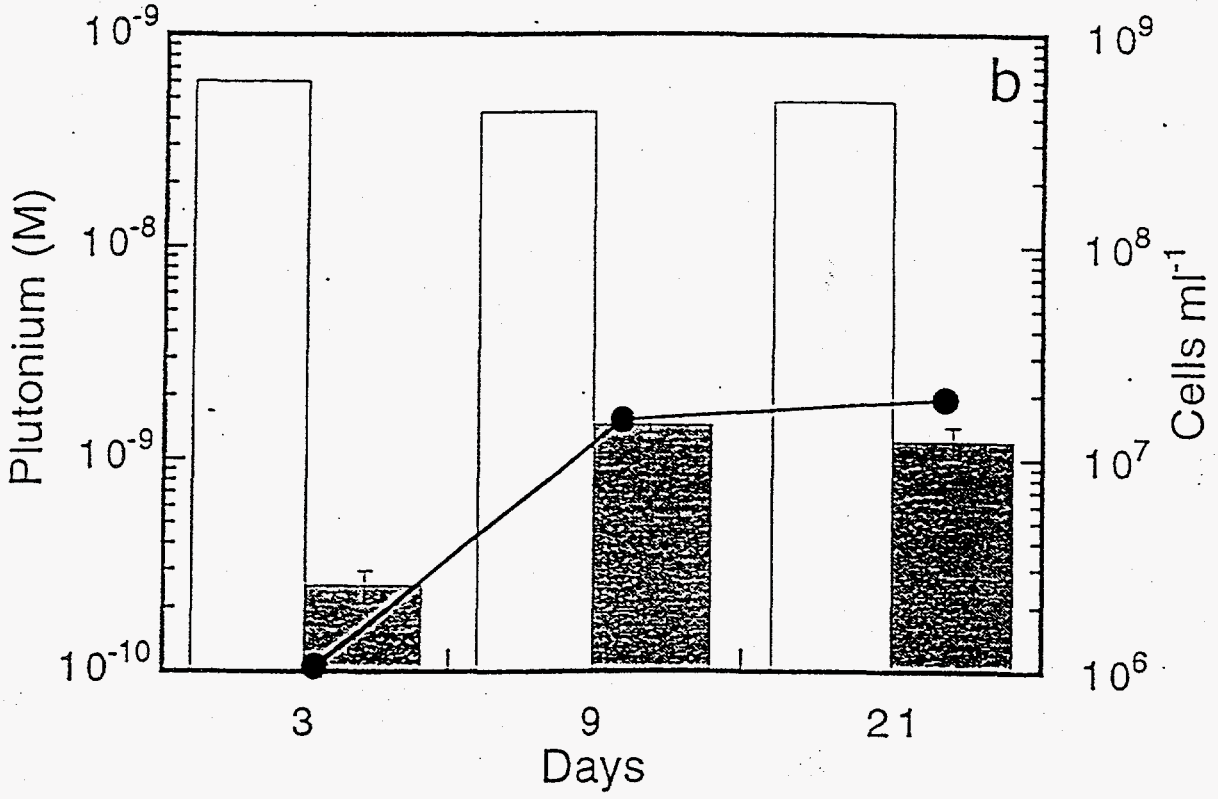

b
Plutonium (M)
10^{-9}
10^{-8}
10^{-9}
10^{-10}
10^{9}
10^{8}
10^{7}
10^{6}
Cells ml^{-1}
3
9
21
Days

Figure 3