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RADIOCHEMICAL METHOD FOR ASSESSMENT OF PLUTONIUM AFTER RADIONUCLIDE INTERACTION WITH MICROORGANISMS

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Abstract. In this study, referring to the co-precipitation method (Lovett and Nelson 1981), separation of oxidized and reduced plutonium fractions was achieved. Based on differences in property to form (Pu - IV) and not to form (Pu - III) anionic complexes in high molar (8 mol/L) HNO_3 solution, the anion exchange method for separation and determination of Pu (V, VI), Pu (III) and Pu (IV) was developed. The developed radiochemical method was examined using real samples. Fungi *Absidia spinosa* and *Paecilomyces lilacinus* as well as Gram-positive bacteria *Bacillus mycoides* and *Rhodococcus luteus* and one morphotype of Gram-negative bacterium isolated from the samples in the radioactive waste repository territory were tested for their ability to participate in Pu(IV) redox reactions. To determine Pu in its different oxidation states the method of Pu co-precipitation with neodymium fluoride and the modified anion exchange method were applied. Alpha spectrometric counting following radiochemical analysis showed that out of tested microorganisms the highest ability to change Pu oxidation state should be attributed to bacteria *Bacillus mycoides* and Gram–negative bacterium. Pu (IV) after its interaction with above-mentioned bacteria for 24 hours in brine at pH 2.7-2.9 under aerobic conditions was reduced by up to 15 % to valence (III) plutonium.

Keywords: radiochemical analysis, bacteria, fungi, plutonium oxidation states.

1. Introduction

Plutonium is one of the most important anthropogenic radionuclides related to nuclear weapon production and testing, reprocessing of spent nuclear power plant fuel, radioactive waste treatment and reactor accidents. Two plutonium isotopes, ^{239}Pu (half-life 24110 yr) and ^{240}Pu (half-life 6584 yr) are the most abundant isotopes in the environment. In recent years, studies on the distribution of Pu isotopes and their application in the environmental science have been increasing considerably. For instance, Pu isotopes were widely used for the source identification of radioactive contaminants, for sediment dating, for the study of particle scavenging process and for tracing soil erosion (Zheng *et al.,* 2008). In the environment, Pu mostly exists as low soluble Pu(IV) oxides and hydroxides. The geochemical and physical characteristics of the environment (mineralogy, pH, chelating agents, redox conditions, temperature and humidity) as well as microbial activity play an important role in plutonium behavior in environmental systems. Microorganisms interact with their surroundings and in some cases they greatly modify the characteristics of their environment. Several such interactions may have a significant influence on the behaviour of radionuclides possibly escaping from underground radioactive waste repositories. Microbes can mobilize trace elements. Unattached microbes may act as large colloids, transporting radionuclides on their cell surfaces with the groundwater flow. Many microbes produce ligands that can mobilize trace elements from solid phases and that can inhibit trace element sorption to solid phases (Pedersen 2005). Under appropriate conditions the activity of microorganisms could affect the chemical nature of plutonium due to changing redox conditions and biotransformation of its oxidation states. Interaction of microorganisms with radionuclides affects radionuclide geochemical processes, in particular radionuclide migration (Dighton *et al.* 2008; Ehrlich 2006; Fomina and Gadd 2007). Microorganisms show a multiplicity of interactions with metals in soil and can contribute to metal mobility or immobilization. Interaction of microorganisms with radionuclides also affects radionuclide geochemical processes, in particular radionuclide migration (Keith-Roach 2002; Pedersen 2005; Ehrlich 2006). Preliminary investigation of ^{242}Pu accumulation by fungal biomass showed that all the metal-tolerant fungi were able to sorb ^{242}Pu in their biomass and the most effective radionuclide sorption was demon-

strated by fungi *Eupenicillium sp., Penicillium oxalicum and Aspergillus niger* (Levinskaitė *et al.* 2009).

Some microbes are able to reduce Pu(IV) oxyhydroxides, especially under anaerobic conditions, and reductively solubilize Pu(IV) to Pu(III). Bacterium *Bacillus subtilis* solubilized about 90 % of solid PuO_2 under anaerobic conditions in the presence of the chelating agent nitrilotriacetic acid but only 40% of Pu (IV) was reductively dissolved without this chelating agent (Rusin *et al.* 1994). Organic acids as bacteria metabolic products can increase Pu solubility and mobility (Neu *et al.* 2005).

Different instrumental techniques for the determination of Pu oxidation states were tested in research centers. The spectrophotometric methods for a quantitative determination of oxidation states of aqueous Pu are generally effective only at relatively high concentrations (Schramke *et al.* 1989; Lee *et al.* 2008)**.** X-ray absorption spectroscopy (XAS) is an established technique for determination of oxidation states and speciation in transition metals and is coming into use to study actinides. A simplified model for data collection and evaluation was presented for X-ray Absorption Near Edge structure (XANES) that has been recently used for the determination of Pu oxidation states at the cross section of a Pu-containing "hot" particle coming from a nuclear weapon test site (Bielewski *et al.* 2009). For the speciation of the plutonium oxidation states in aqueous solutions, the online coupling of capillary electrophoresis (CE) with ICP-MS has been developed, and improvement of the sensitivity of the CE method due to the offline coupling of CE to resonance ionization mass spectrometry (RIMS) has been explored (Bürger *et al.* 2007). Consequently, it should be recognized that some of physical-instrumental methods are applicable to solids and liquids, they require no sample preparation, however their employment in each institution is limited. Among the speciation technique methods listed (Ervin and Conradson 2002; Chopin 2005) the radiochemical trace analysis and Electron Spin Resonance (ESR) are the most advantageous methods to determine plutonium oxidation states because of their sensitivity (10^{-8} – 10^{-12} M) and (10^{-5} – 10^{-12} M), respectively. There are numerous analytical methods to determine the concentration of plutonium in environmental and other samples. Many radiochemical separation techniques to separate plutonium from the matrix and interferring radionuclides are based on anion exchange chromatography, solvent extraction chromatograhy and extraction chromatography.

The aim of this study was to investigate capability of aerobic bacteria and fungi to change the Pu (IV) oxidation state applying the developed modified radiochemical method of anion-exchange chromatography.

2. Materials and methods

To modify the anion exchange method, that is used for bulk plutonium separation, and to apply it to the Pu oxidation state determination, model aqueous samples were used. The acidic solution was spiked with appropriate quantities of both reduced and oxidized ^{239}Pu. Oxidized plutonium (V, VI) fraction retained in solution, while plutonium (III, IV) fraction was co-precipitated with neodymium fluoride (Lovett and Nelson 1981). Solution with oxidized plutonium and precipitate with reduced plutonium were treated according to the radiochemical procedure, the final solution being 8 M in HNO_3. Oxidized plutonium fraction was separated using the reported method for the bulk plutonium (Talvite 1971; Lukšienė *et al.* 2006). The separation of Pu (IV) from Pu (III) was based on the Pu (III) property not to form anionic complexes in highly acidic HNO_3 solution. Loading of the solution containing both Pu(IV) and Pu(III) onto the anion exchange resin column without valence adjustment, sorption of only oxidation state (IV) plutonium is available. Pu (III) in the eluate is converted to Pu (IV) due to application of the oxidation-reduction cycle. This solution is then loaded onto another anion exchange resin column. The plutonium amount in each fraction after radiochemical analysis was estimated by the α-spectrometric measurement.

Fungi *Absidia spinosa* and *Paecilomyces lilacinus* as well as Gram-positive bacteria *Bacillus mycoides* and *Rhodococcus luteus* and one morphotype of Gram-negative bacterium isolated from samples from the low-level radioactive waste repository site at the Ignalina NPP were selected for the investigation of their ability to transform the oxidation states of plutonium under aerobic conditions. Bacteria were isolated and characterized using culture identification following a morphological identification, using Gram staining reaction and other biochemical tests (Holt and Bergey 1994). All fungal morphotypes were transferred on MEA, PDA, corn meal agar (CMA) and Czapek's agar (CA), grown for up to 21 days and identified. Standard procedures based on colony, spore and structural morphology were followed for identification to a species level (Gilman 1966; Barnet 1967; Barron 1968; Ellis 1971; Domsch *et al.* 1980; Kiffer and Morelet 1999; Watanabe 2000).

Microorganism cells were separated from growing media by centrifugation at 3000 rpm for 5 min (for bacteria) or by filtration through filter paper (for actionomycetes and fungi), washed thoroughly with deionized water (conductivity 0.055 µS/cm, TOC < 10 ppb) and then with 0.08 M NaCl. The cell biomass (0.03 g L^{-1} dry weight basis) was suspended in 20 mL of 0.08 M NaCl solution, and 9.2 x 10^{-12} M of Pu (IV) was added. A set of the experiment included 24-hour exposure of the cells to Pu (IV) under static conditions. After 24-hour exposure of microorganisms to Pu(IV), microorganism cells were separated from supernatant by centrifugation at 3000 rpm for 5 min. The percentage distribution of ^{239}Pu oxidation states in both supernatant and biomass was analyzed. The supernatant and biomass were spiked with appropriate quantities of both reduced and oxidized ^{242}Pu to monitor chemical yield. By using acid leaching, Pu from the biomass was extracted into solution. In the supernatant and biomass extract, using appropriate procedures neodymium fluoride precipitate was formed. Oxidized plutonium fraction Pu (V, VI) remained in solution and reduced fraction Pu (III, IV) was co-precipitated onto neodymium fluoride. The separation of Pu (IV) from Pu (III) was carried out after specific chemical procedures for neodymium fluoride which allowed getting Pu (III, IV) fraction into nitric acid solution. Final separation of Pu (III) from

Pu (IV) was based on the differences in sorption of these Pu oxidation states onto the anion exchange resin. Eluates from the anion exchange resin columns are conditioned for plutonium separation. Thin plutonium samples for alpha spectrometry were obtained by electrodeposition for 1 hour under direct 0.6 A/cm^2 electric current onto a stainless steel disc in sulphate medium at pH 2.2-2.4. The electrodeposited Pu samples were measured using the alpha-spectrometer Octete Plus (Ortec) with the detector of 450 mm^2. Alpha-counting efficiency was 25 % and resolution - 24 keV (Lukšienė *et al.* 2006; Druteikienė and Lukšienė 2008).

3. Results and discussion

To assess plutonium of different oxidation state distribution in samples with plutonium-microorganism suspensions, a run of procedures according to developed method is presented in Figs 1, 2.

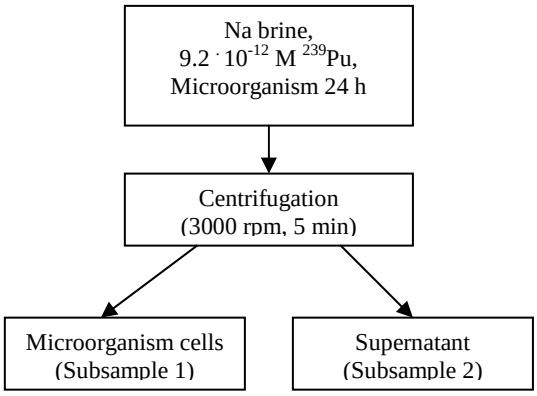

Fig 1. Initial sample preparation after microorganism exposure to ^{239}Pu (IV)

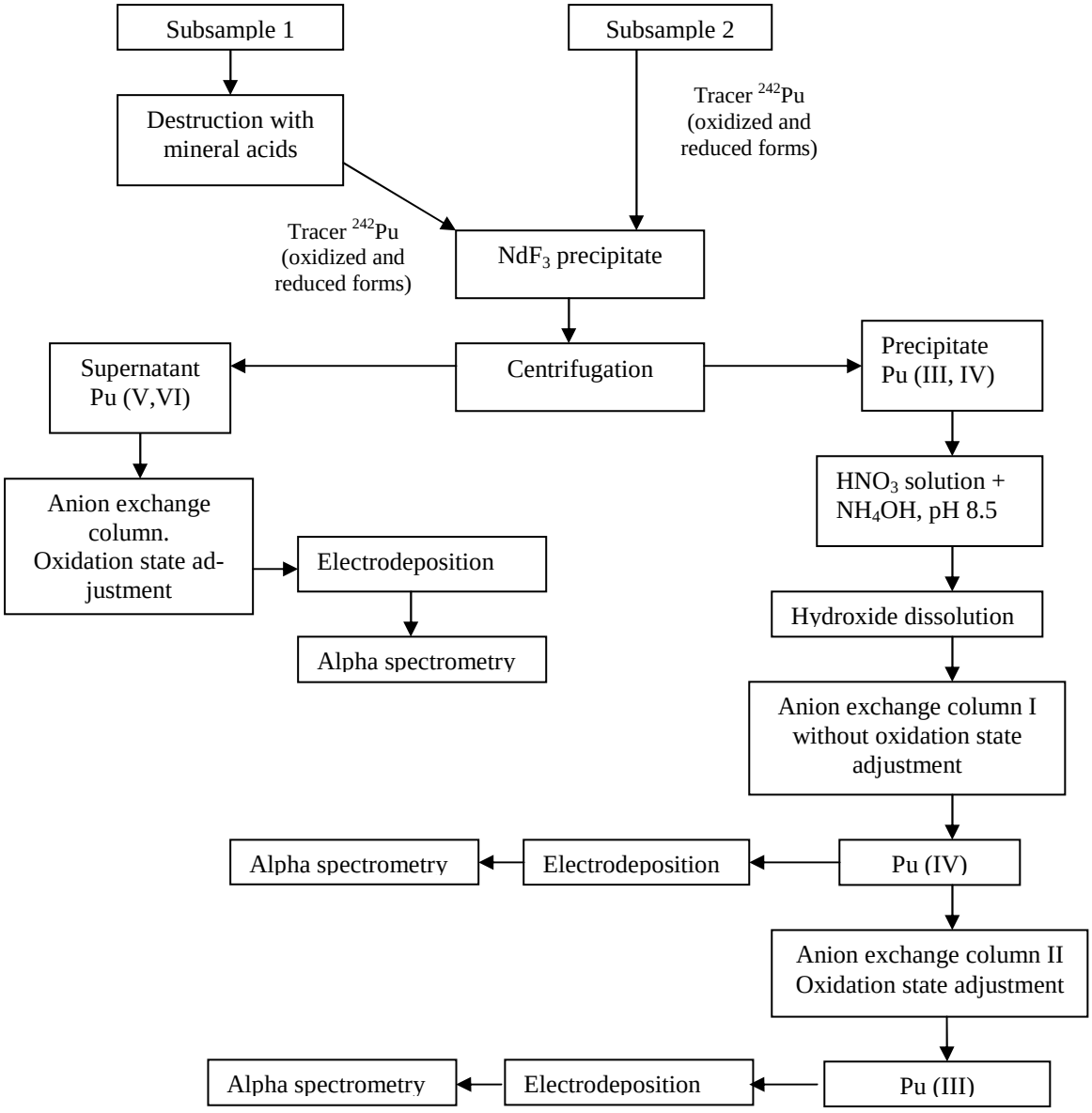

Fig 2. Flow chart of different Pu oxidation state separation and determination

After 24-hour exposure of each microorganism (Bacteria *Bacillus mycoides, Rhodococcus luteus,* Gram$^-$ and fungi *Paecillomyces lilacinus, Absidia spinosa*) to 9.2 x 10^{-12} M ^{239}Pu(IV) at pH 2.7-2.9 under aerobic ambient conditions, centrifugation at 3000 rpm for 5 min. of suspensions allowed dividing samples into two subsamples: microorganism cells and supernatant. This initial stage of the procedure is presented in Fig. 1.
According to the developed modified anion exchange method identification of Pu of oxidation state (III), formed by microbial activity, was carried out.

Percentage distribution of Pu(III) calculated according to the results of alpha spectrometric measurement following radiochemical analysis is presented in Table 1. Procedures of Pu radiochemical analysis applying the modified anion exchange method are presented in Figs. 2,3. The developed modified anion exchange method allowed separating Pu oxidation state (III), Pu oxidation state (IV) and Pu (V, VI) fraction. Percentage distribution of Pu (III) calculated according to the results of alpha spectrometric measurement following radiochemical analysis is presented in Table 1.

Table 1. Percentage amount of reductively solubilized plutonium in tested medium of Na brine at low pH after 24-hour interaction under aerobic conditions

Tested microorganism	^{239}Pu (IV) added	Pu (III), % Brine/ biomass	Brine/ biomass, S.d., ±
Bacterium *Bacillus mycoides*	9.2 x 10^{-12} M	14.4/1.1	2.6/0.22
Bacterium *Rhodococcus lutesus*	9.2 x 10^{-12} M	0.7/0.5	0.15/0.12
Bacterium Gram$^-$	9.2 x 10^{-12} M	15.0/0.3	2.4/0.06
F. *Paecillomyces lilacinus*	9.2 x 10^{-12} M	0.6/1.0	0.14/0.23
F. *Absidia* sp.	9.2 x 10^{-12} M	0.2/0.3	0.05/0.06

Results obtained in our investigations indicate that Pu (IV) is more sensitive to bacteria activity compared to that of fungi (Table 1). After 24-hour interaction, we determined that tested bacteria converted from (0.7±0.15) % to (15.0.±2.4) % of Pu(IV) to Pu(III) in brine and from (0.3±0.06) % to (1.1±0.22) % in biomass. Tested fungi *Paecillomyces lilacinus and Absidia spinosa* had insignificant influence on the Pu(IV) oxidation state change under studied aerobic conditions. The fraction of Pu (V, VI) under studied conditions was not observed. The highest amount of Pu (IV) was not changed by microorganisms and Pu of this oxidation state was immobilized by microorganism biomass.

By generalizing results of our investigations we can state the following. Application of the radiochemical method that included co-precipitation and modified anion exchange chromatography was very useful in the Pu oxidation state study. The method showed rather good Pu recoveries (60-70 %), however procedures are very time consumable. The capability of microorganisms to convert Pu(IV) into Pu(III) to some extent under aerobic conditions indicates that the presence of such microorganisms under reducing conditions, for example, in geochemical systems of nuclear waste repositories, is a potential tool of Pu being changed into its more mobile state. The immobilization of Pu (IV) by micoorganism biomass indicates that studied microorganisms are effective radionuclide-accumulators and their application in removal of radionuclides from polluted substrata could be investigated (Parekh *et al* 2008; Sar *et al.* 2004). On the other hand the presence of these microorganisms in soil creates biotic barrier zone that prevents radionuclide downward migration.

4. Conclusions

1. Results of investigations have demonstrated that the developed modified anion exchange method is a rather advantageous and sensitive (10^{-12}-10^{-13}M) for routine plutonium oxidation state quantification.
2. A higher amount of reductively solubilized Pu because of activity of tested bacteria (*Bacillus mycoides, Rhodococcus luteus*, Gram-negative) was found in brine (0.7-15 %), while in biomass it ranged between 0.3-1.1 %. The activity of tested fungi (*Paecillomyces lilacinus, Absidia spinosa*) conditioned a higher amount of Pu(III) in biomass (0.6 % and 0.2 %, respectively) compared to that in brine (1.0 % and 0.3 %).
3. The largest part of Pu (IV) was not changed by microorganisms and Pu of this oxidation state was immobilized by microorganism biomass.

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