

The effect from the number of cells, pH and lanthanide concentration on the sorption of promethium on gramnegative bacterium (Shewanella Putrefaciens)

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TEL 08-665 28 00 TELEX 13108 SKB S TELEFAX 08-661 57 19 THE EFFECT FROM THE NUMBER OF CELLS, pH AND LAN-THANIDE CONCENTRATION ON THE SORPTION OF PROMETHIUM ON THE GRAMNEGATIVE BACTERIUM (Shewanella Putrefaciens)

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This report concerns a study which was conducted for SKB. The conclusions and viewpoints presented in the report are those of the author(s) and do not necessarily coincide with those of the client.

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The aim of this work was to investigate the sorption of the lanthanide promethium on bacteria and the distribution ratio K_d , of the lanthanide between bacteria and water at different cell numbers, pH and lanthanide concentrations. There was a negative linear relationship between the number of cells and the amount of the Pm sorbed, and also between the number of cells and the K_d. The sorption decreased from a stable level at 90% as pH was raised above 7. There was a linear quantitative relation between the Pm concentration and the amount of Pm sorbed on the cells while the the relation to K_d was relatively constant. The results indicate the sorption of trivalent actinides and lanthanides on bacteria to be a reversible surface adsorption.

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SUMMARY

The disposal of high level radioactive waste in deep geologic formations has been proposed. The Swedish concept, presented in the KBS-3 study, is to isolate the waste in copper canisters embedded in bentonite in an excavated granitic rock repository at 500 m depth. Dissolution and transport by the ground water is then the most important dispersion mechanism for the trace elements eventually released from the waste.

The possibly enhanced transport of trace elements with bacteria would preferentially be with elements with a high sorption capacity eg. Cs, lanthanides and trivalent and tetravalent actinides. In this investigation we have used promethium, a trivalent lanthanide which has a chemistry very similar to the trivalent actinides, especially americium. The aim of this work was to investigate the sorption of the lanthanide promethium on bacteria and the distribution ratio K_d , of the lanthanide between bacteria and water at different cell numbers, pH and lanthanide concentrations. The influence of the cell number, the pH and the Pm concentration on the sorption of Pm to *Shewanella putrefaciens* isolated from ground water (463 m) was studied.

Cell suspensions were portioned in 10 ml aliquots in 14 ml polycarbonate centrifuge tubes with caps. Pm was added after any eventual pH adjustment. The tubes were left for 17 h at 15 °C. Aliquotes of 1 ml of the cell-Pm suspensions were mixed with 10 ml scintillation cocktail and counted for 10 min in a liquid scintillation counter. The cells were then centrifuged down at 9000 g during 30 min and 1 ml aliquotes of the supernatant were measured according to above. Then the pH was measured in the tubes. The difference between the measured radioactivity in the water phase in the cell-Pm suspension and in the supernatant was assumed to show the amount of Pm sorbed on the cells. Control measurements for precipitation of the Pm, sorption to the tube wall, etc, were prepared in the same way as above but without cells. The distribution ratio, K_d (1 g⁻¹), is defined as the ratio between the amount of Pm sorbed per g cells and the amount Pm left nonsorbed per l cell-Pm suspension.

There was a negative linear relationship between the number of cells and the amount of Pm sorbed, and also between the number of cells and the K_d. This result demonstrate the importance of having a good control of the number of cells used in sorption experiments - else, unnecessary variance of the results will appear. The average sorption was 50 nmol Pm g⁻¹ when the pH was lower than 7. At higher pH there was a decrease in the sorption down to 20 nmol Pm g^{-1} at pH 10.5 with a corresponding drop in K_d from 100 to 2 l g⁻¹. This may be explained by the increasing hydrolysis of Pm as the pH was raised, resulting in neutral Pm aggregates out of reach for the charged complexing or chelating groups on the cell surface. There was a linear quantitative relation between the Pm concentration and the amount of Pm sorbed on the cells while the relation to K_d was relatively constant. This can possibly be explained by a precipitation of the highly hydrolysed Pm onto the few particles available at low colloid or cell concentration, giving high sorption and K_d values.

Although this work indicate the sorption of trivalent actinides and lanthanides on bacteria to be a reversible surface adsorption, one must consider that other processes beside surface adsorption could occur. These processes include cation transport systems into the cell, irreversible bounding to specific metabolic components of the cell and the production of complexing agents that can affect speciation and thus mobility of a trace element.

1. INTRODUCTION

1.1 Background

The disposal of high level radioactive waste in deep geologic formations has been proposed. The Swedish concept, presented in the KBS-3 study [1], is to isolate the waste in copper canisters embedded in bentonite in an excavated granitic rock repository at 500 m depth. Dissolution and transport by the ground water is then the most important dispersion mechanism for the trace elements eventually released from the waste.

Pedersen [2] investigated the distribution of bacteria in ground water from 16 different levels in 5 boreholes in granite bed-rock down to 860 m. There was between 6.8.107 to 1.7.109 l-1 bacteria. The chemical environment in the ground water was reducing with a pH around 8, an E_h between -112 to -383 mV, a conductivity range between 232 and 3532 mS m⁻¹ and a temperature range of 10.2 to 20.5 °C, depending on the depth. Plate counts showed that there were facultative anaerobic, gram-negative, non-fermenting heterotrophs in the ground water. Enrichment cultures indicated the presence of anaerobic bacteria capable of growth on organic one carbon compounds and hydrogen, presumably methanogenic bacteria. Most probable number assays with sulphate and lactate revealed up to 5.6.107 l⁻¹ viable sulphate reducing bacteria. Biofilm development experiments have indicated active attached microbial populations of up to 2.2.10¹¹ m⁻² bacteria on surfaces exposed to flowing ground water (0.2 mm s⁻¹) during 8 weeks.

1.2 Purpose and scope of the investigation

The presence of microorganisms can influence the transport of trace elements from a repository in different ways. Three principal mechanisms can be identified.

 The microorganism constitutes a mobile suspended particle which may have a trace element sorbing capacity. The trace element is sorbed on the outside of the cell or accumulated inside the cell. [3, 4].

2. The trace element is sorbed in a microbial biofilm on the rock

surface [5].

3. The microorganism may produce complexing agents that can affect speciation and thus mobility of trace elements [6, 7].

The possibly enhanced transport of trace elements with bacteria would preferentially be with elements with a high sorption capacity eg. Cs, lanthanides and trivalent and tetravalent actinides. In this investigation we have used promethium, a trivalent lanthanide which has a chemistry very similar to the trivalent actinides, especially americium.

The aim of this work was to investigate the sorption of the lanthanide promethium on bacteria and the distribution ratio K_d , of the lanthanide between bacteria and water at different cell numbers, pH and lanthanide concentrations. The influence of the cell number, the pH and the Pm concentration on the sorption of Pm to Shewanella putrefaciens isolated from ground water (463 m) was studied.

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2. EXPERIMENTAL PROCEDURES

2.1 Preparation of cell suspensions

The bacterium Shewanella putrefaciens (CCUG-22948, Culture Collections University of Göteborg) isolated from 463 m deep ground water [8] was used for the experiments. It is a facultative anaerobic, gram-negative, non-fermenting, rod-shaped heterotroph. The cells were grown over night in Nutrient Broth (Difco) to approximately 10¹³ l⁻¹ cells. They were washed by centrifugation at 2500 g during 10 min and resuspended in 0.1 M NaCl twice. The cells were subsequently diluted to the appropriate cell number for each experiment in 0.1 M NaCl and left for 72 h at 5 °C in dark. The number of cells was counted again after this period. The material and medium used for cultivation of the cells was sterilized in an autoclave at 121 °C and 1.1 atmosphere overpressure. All solutions used were first centrifuged at 16000 g during 15 min to remove any solids or colloids that else might have interfered with the measurements.

2.2 Determination of the total number of cells

Acridine orange stained direct count (AODC) [9] was used to determine the total number of cells. Nuclepore filters (0.2 μ m pore size, 13-mm diameter) were pre-stained with a Sudan-black solution which was prepared by dissolving 25 mg Sudan-black in 75 ml 99% ethanol and then diluted with 75 ml de-ionized water. The filters were thoroughly rinsed with de-ionized water before use. An acridine orange (AO) solution was prepared by dissolving 10 mg AO in 1 1 of 6.6 mM sodium potassium phosphate buffer, pH 6.7. The phosphate buffer was a mixture of 2.45 mM KH₂PO₄ and 4.15 mM Na₂HPO₄ in de-ionized water. The AO solution was stored as 10 ml aliquots. All solutions and the water were autoclaved and filter sterilized (0.2 μ m). A portion of the sample was filtered onto a pre-stained nuclepore filter at 20 KPa underpressure and stained for 7 minutes with AO. The number of cells was counted using blue light (390-490 nm) in a epi-fluorescence microscope (Olympus BH-2) at 1250 enlargement. Between 500 and 600 cells or a minimum of fifteen microscopic fields (1 field = 80 x 80 μ m =

 0.0064 mm^2), were counted on each filter.

2.3 Trace element

The almost pure β emitter ¹⁴⁷Pm (t_{1/2}=2.6 y, E_{β}=0.2 MeV) was used in this investigation. The carrierfree ¹⁴⁷Pm was obtained from Amersham, England. To make up the different Pm concentrations, inactive neodyn was used as a carrier.

2.4 Measurement of sorption

The cell suspensions were portioned in 10 ml aliquots in 14 ml polycarbonate centrifuge tubes with caps. Pm was added after any eventual pH adjustment. The tubes were left for 17 h at 15 °C. Aliquotes of 1 ml of the cell-Pm suspensions were mixed with 10 ml scintillation cocktail (Emulsifier safe, Packard, USA) and counted for 10 min in a liquid scintillation counter (Intertechnique, SL30, France). The cells were then centrifuged down at 9000 g during 30 min and 1 ml aliquotes of the supernatant were measured according to above. Then the pH was measured in the tubes. The difference between the measured radioactivity in the water phase in the cell-Pm suspension and in the supernatant was assumed to show the amount of Pm sorbed on the cells. Control measurements for precipitation of the Pm, sorption to the tube wall, etc, were prepared in the same way as above but without cells. The distribution ratio, K_d (1 g⁻¹), is defined as the ratio between the amount of Pm sorbed per g cells and the amount Pm left nonsorbed per l cell-Pm suspension.

2.4.1 Determination of the dry weight per cell

A total of 10^{13} cells were centrifuged down at 2500 g to a pellet, which was dried in an exicator with dehydrated copper sulphate under vaccum for 96 h and subsequently weighed (A&D company, Japan d=0.0001g). The dry weight of the cell after 72 h in 0.1 M NaCl was determined to be $4 \cdot 10^{-13}$ g. All sorption and K_d data presented here are based on this dry weight.

2.4.2 Determination of the relation between the number of cells and sorption

Three dilution series of 7.6.108, 7.4.109, 8.1.1010 and 5.6.1011 l-1

cells were prepared and supplemented with Pm to a concentration of 0.14 nM. The pH was adjusted to 6.5 with NaOH.

2.4.3 The effect from pH

The pH was adjusted with HCl or NaOH to between 4 and 10.5 in suspensions with $5 \cdot 10^{11} \ 1^{-1}$ cells and supplemented with Pm to a concentration of 11 nM. Two separate experimental runs were made.

2.4.4 The effect from the Pm concentration

The sorption was studied at different concentrations of Pm in 4 separate experimental runs in n parallels at the following Pm concentrations and cell numbers: 0.14 nM, $5.8 \cdot 10^{11} \, 1^{-1}$ cells, n=3; 1.2 nM, $2.2 \cdot 10^{11} \, 1^{-1}$ cells, n=14; 1.7 nM, $1 \cdot 10^{11} \, 1^{-1}$ cells, n=6; 2.8 nM, $1 \cdot 10^{11} \, 1^{-1}$ cells, n=5; 10 nM, $1 \cdot 10^{11} \, 1^{-1}$ cells, n=6; 11 nM, $4.9 \cdot 10^{11} \, 1^{-1}$ cells, n=55; 100 nM, $1 \cdot 10^{11} \, 1^{-1}$ cells, n=3. The pH was adjusted to between 4 and 7.

2.5 Measurement of desorption

Cell suspensions of $2.1 \cdot 10^{10}$ and $2.3 \cdot 10^{12}$ l⁻¹ cells were portioned in 10 ml aliquots in 14 ml polycarbonate centrifuge tubes with caps. Pm was added to a concentration of 0.6 nM. The pH was approximately 5.5. The tubes were left for 17 h at 15 °C whereafter the radioactivity in the water phase before and after centrifugation at 9000 g for 30 min was measured. The supernatant was decanted and the cell pellet resuspended in 9 ml 0.1 M NaCl and left for 17 h at 15 °C, whereafter the activity in the water phase before and after centrifugation was measured again. Pm present in the supernatant after the second centrifugation was assumed to have been desorbed from the cells.

3. **RESULTS**

Adjusting the pH to desired values turned out to be difficult. The cells had a tendency to buffer the pH of the cell-Pm suspension to around 6, indicating functional groups with pKa values around this value.

3.1 Determination of the effect from the number of cells on sorption

Fig. 1 shows a negative linear relationship between the number of cells and the amount of Pm sorbed. There was 220-fold decrease from 110 to 0.5 nmol Pm g⁻¹, as the number of cells were increased 760 fold, from $7.6 \cdot 10^8$ to $5.8 \cdot 10^{11}$ l⁻¹. The percentage sorption of Pm in the samples was: $7.6 \cdot 10^8$ l⁻¹ cells, 24%; $7.4 \cdot 10^9$ l⁻¹ cells, 40%; $8.1 \cdot 10^{10}$ l⁻¹ cells, 62%; $5.6 \cdot 10^{11}$ l⁻¹ cells, 83%. The corresponding K_d decreased 50-fold from 1000 to 20 l g⁻¹. The standard deviation (SD) for the means in Fig. 1 ranged between 50% for the lowest and 1.6 % for the highest cell number. The background in tubes without cells was 20 % (n=4, SD=6.8%) in this experiment.

3.2 The effect from pH on the sorption

The sorption was approximately 53 nmol Pm g⁻¹ when the pH was lower than 7 (Fig 3). This corresponds to a 90% sorption of Pm to the cells. At higher pH there was a decrease in the sorption down to approximately 20 nmol Pm g⁻¹ (30% sorbed) at pH 10.5. There was a drop in the corresponding K_d from 100 to 3 l g⁻¹ as the pH was increased from 4 to 10.5 (Fig.3). The number of samples was 87. The background in tubes without cells was 5.5%, (n= 10, SD=9.9%).

3.3 The effect from the Pm concentration

The sorption of Pm at different concentrations in the pH interval between 4.0 and 7 is shown in Fig. 4. There was a linear quantitative relation between the Pm concentration and the amount of Pm sorbed on the cells. The variation of the means around the regression line was due to the variation in the cell numbers used. There was not any major change in the K_d as the concentration of Pm was varied. Fig. 5 shows that the K_d ranged between 20 and 90 l g⁻¹ as the Pm concentration was varied between 0.14 and 100 nM. The background in tubes without cells was 23.0% (n=23, SD=18.1%).

3.4 Measurement of desorption

Table 1 shows that Pm was sorbed reversibly to the cells. Pm was desorbed with a new K_d 1.5 to 2-fold higher than the first one after resuspension of the cells in 0.1 M NaCl. The background in tubes without cells was 0.8% (n=6, SD=1.2%).

Table 1 The sorption after a first centrifugation and the desorption after resuspension in Pm free 0.1 M NaCl and a second centrifugation of the cells.

Number of cells 1	Sorption			Desorption		
	%	nmol Pm g ⁻¹	K _d l g ⁻¹	⁰∕₀	mol Pm g ⁻¹	K _d lg ⁻
2.1.10 ¹⁰ l ⁻¹ 2.3.10 ¹² l ⁻¹	64.5 81.8	71 0.57	206 4.8	26.8 11.2	19 0.06	311 8.4



Figure 1. The sorption of Pm on Shewanella putrefaciens (\bigcirc), and the distribution ratio, K_d, for Pm between Shewanella putrefaciens and the water phase (\blacksquare), at a Pm concentration of 0.14 nM in suspensions with different cell numbers and a pH of 6.5.



Figure 2. The sorption of Pm on Shewanella putrefaciens at different pH. The concentration of Pm was 11 nM and the cell number was $5 \cdot 10^{11} 1^{-1}$.



Figure 3. The distribution ratio, K_d (l g⁻¹), for Pm between Shewanella putrefaciens and the water pshase at different pH. The concentration of Pm was 11 nM and the cell number was $5 \cdot 10^{11} l^{-1}$.



Figure 4. The sorption of Pm on *Shewanella putrefaciens* at different concentrations of Pm. The cell numbers were between $1 \cdot 10^{11}$ and $5.8 \cdot 10^{11}$ l⁻¹ and the pH was between 4 and 7.



Figure 5. The distribution ratio, K_d (l g⁻¹), for Pm between Shewanella putrefaciens and the water phase at different concentrations of Pm. The cell numbers were between 1.10¹¹ and 5.8.10¹¹ 1⁻¹ and the pH was between 4 and 7.

4. DISCUSSION

4.1 The cell surface

The envelope that bounds a gram-negative cell is composed of an inner membrane, a peptidoglucan layer and an outer membrane. The outer membrane is built up as the unit cell membrane, but many of the phospholipids are replaced by a lipopolysaccharide (LPS) unique to the outer membrane. The lipid moiety of the LPS forms the hydrophobic portion of the leaflet. The core polysaccharide with it's attached side chains projects outwards and the fatty acid residue projects towards the center of the cell. The outer membrane also contains a number of different transport proteins in contact with the medium surrounding the cell. They make the membrane to act as a molecular sieve with control of not all but many of the molecules that must pass in and out of the cell. It is the outer membrane of the cell that first comes in contact with eventual trace elements.

Organic molecules can form complex with metal ions. The bounding of the metal ion can be specific and irreversible on fixed sites, eg. the chelating of some metal ions in nitrogen or sulphur containing prosthetic groups of enzymes. The bounding can also be nonspecific and reversible to charged ion exchange sites as the carboxylic acids and the amino acids of the outer membrane LPS and transport proteins. Adjacent complexing groups may form metal chelate complexes if their distance suit the size of the metal ion.

4.2 The effect from the number of cells on sorption

There was a negative linear relationship between the number of cells and the amount of Pm sorbed, and also between the number of cells and the K_d (Fig. 1). This result demonstrate the importance of having a good control of the number of cells used in sorption experiments - else, unnecessary variance of the results will appear. The AODC method for the total number of cells was appropriate for this purpose. We have used a number of cells at or higher than $1 \cdot 10^{11} 1^{-1}$ cells, thereby assuring sorption data corresponding to 70-80% sorption of Pm at a concentration as low as 0.14 nM and with

a low variance.

4.3 The effect from pH on sorption

The average sorption was 53 nmol Pm g^{-1} when the pH was lower than 7 (Fig 3). At higher pH there was a decrease in the sorption down to 20 nmol Pm g^{-1} at pH 10.5 with a corresponding drop in K_d from 100 to 2 l g^{-1} . This may be explained by the increasing hydrolysis of Pm as the pH was raised, resulting in neutral Pm aggregates out of reach for the charged complexing or chelating groups on the cell surface.

4.4 The effect from the concentration of Pm on sorption

There was a linear quantitative relation between the Pm concentration and the amount of Pm sorbed on the cells (Fig 5) while the relation to K_d was relatively constant (Fig. 5). MacCordick et. al. [10] achieved similar results with 1 to 500 μ M concentrations of Europium, but observed a sorption limit at the highest concentrations.

4.5 Sorption mechanism

The sorption and the K_d values were very dependent on the number of cells (Fig. 1). This phenomenon has been found more or less in exactly the same manner for Pm when using silica colloids (diameter around 200 nm) as sorbent [11]. It can possibly be explained by a precipitation of the highly hydrolysed Pm onto the few particles available at low colloid or cell concentration, giving high sorption and K_d values. At higher number of cells, more surface was available for sorption and less or no precipitation should then be observed. In the case of Cs, which is uncomplexed in water, the concentration of colloides had no effect on the sorption [11].

The phenomenon of precipitation of the metal ions on bacteria has been visualized by transmission electron microscopy (TEM) as needlelike (La) or clumped (Ag) deposits on the outside of *Pseudomonas aerunginosa* and *Bacillus subtilis* respectively [12]. They used metal ion concentrations in the 1 to 100000 μ M region and the number of cells was 10¹³ to 10¹⁴ l⁻¹. The sorption measured was 1-1000 μ mol La or Ag g⁻¹ cells⁻¹. MacCordick et. al. [10] used 1 to 500 μ M Eu, 10¹³ to 10¹⁴ l⁻¹ cells and measured a sorption between 0.1 to 16 μ mol Eu g⁻¹. This can be compared to our measurements with a Pm concentration in the 0.1 to 100 nM region with sorption of 0.5 to 2000 nmol Pm g⁻¹. It seems likely that the presumed precipitation effect ranges from at least nM to mM concentrations of metal ions.

4.6 Irreversible contrary reversible sorption

A crucial issue is if the trace elements attach themselves irreversibly or reversibly on bacteria. For most irreversible cases it can be assumed that the whole rock is short circuited as the retardation of the trace elements due to matrix diffusion in the rock [1] will be arrested. All the trace elements released from the near field and irreversible attached on bacteria will follow the ground water. For the reversible case there will be an influence from bacteria on the trace element migration if the product of the distribution ratio, K_d (l g⁻¹), and the mass of cells present, (g l⁻¹), is larger then 1 (Note by Ivars Neretnieks). The data presented here, indicate the sorption of Pm to *Shewanella putrefaciens* to be reversible (Table 1) and the product (K_d)*(mass of cells) to be smaller than 1.

Although this work indicate the sorption of trivalent actinides and lanthanides on bacteria to be a reversible surface adsorption, one must consider that other processes beside surface adsorption could occur. These processes include cation transport systems into the cell, irreversible bounding to specific metabolic components of the cell and the production of complexing agents that can affect speciation and thus mobility of a trace element.

4.7 Acknowledgements

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5. **REFERENCES**

1	KBS (1983) Final storage of spent nuclear fuel - KBS-3, Part I-IV, Report by Swedish Nuclear Fuel Supply Co, SKBF/KBS, Stockholm. Available from SKB, Box 5864, 102 48 Stockholm, Sweden, tel. 46-8-665 28 00
2	Pedersen K Ekendahl S. (1990) Distribution and activity of bacteria in deep granitic ground waters in SE Sweden Microbial Ecology 19: - in press
3	Beveridge TJ, Fyfe WS (1985) Metal fixation by bacterial cell walls. Can Jour Earth Sci 22:1893-1898
4	Strandberg GW, Starling E, Shumate II, Parrott JR (1981) Microbial cells as biosorbents for heavy metals: Accumulation of uranium by Saccharomyces cerevisiae and Pseudomonas aerunginosa. Appl Environ Microbiol 41:237-245
5	Ferris FG, Starling E, Shumate II, Parrott JR, (1989) Metal interactions with microbial biofilms in acidic and neutral pH environments Appl Environ Microbiol 55:1249-1257
6	Jardim WF, Pearson HW (1984) A study of the copper-complexing compounds released by some species of cyanobacteria. Wat Res 18:985-989
7	Rudd T, Sterritt RM, Lester JN (1984) Formation and conditional stability constants of complexes formed between heavy metals and bacterial extracellular polymers. Wat Res 18:379-384
8	Pedersen K (1990) Potential effects of bacteria on radionuclide transport from a Swedish high level nuclear waste repository. SKB technical report 90-05 Stockholm. Available from SKB, Box 5864, 102 48 Stockholm, Sweden, tel. 46-8-665 28 00
9	Hobbie JE, Daley RJ, Jasper S (1977). Use of nuclepore filters for counting bacteria by fluorescence microscopy.

Appl Environ Microbiol 33:1225-1228
MacCordick HJ, Hubert JC Schleiffer JJ (1989) Selective adsorption of europium/III/ and americium/III/ in non-proleferative myxobacterial suspensions J Radioanal Nucl Chem Letters 349-357
Sätmark B & Albinsson Y (1990) Sorption of radionuclides on silica and granite colloids and stablity of different colloids in natural sys- tems. SKB technical report 90-xx Stockholm. Available from SKB, Box 5864, 102,48 Stockholm, Sweden, tel, 46-8-665, 28,00
5004, 102 48 Stockholm, Sweden, 161. 40-5-005 20 00
Mullen MD, Wolf DC, Ferris FG, Beveridge TJ, Flemming CA, Baily GW (1989) Bacterial sorption of heavy metals. Appl. Environ. Microbiol. 55:3143-3149

List of SKB reports

Annual Reports

1977–78 TR 121 **KBS Technical Reports 1 – 120.** Summaries. Stockholm, May 1979.

1979

TR 79-28 The KBS Annual Report 1979. KBS Technical Reports 79-01 – 79-27.

Summaries. Stockholm, March 1980.

1980

TR 80-26

The KBS Annual Report 1980. KBS Technical Reports 80-01 – 80-25. Summaries. Stockholm, March 1981.

1981

TR 81–17 The KBS Annual Report 1981. KBS Technical Reports 81-01 – 81-16. Summaries. Stockholm, April 1982.

1982

TR 82–28

The KBS Annual Report 1982. KBS Technical Reports 82-01 – 82-27. Summaries. Stockholm, July 1983.

1983

TR 83-77

The KBS Annual Report 1983.

KBS Technical Reports 83-01 – 83-76 Summaries, Stockholm, June 1984.

1984

TR 85-01

Annual Research and Development Report 1984

Including Summaries of Technical Reports Issued during 1984. (Technical Reports 84-01–84-19) Stockholm June 1985.

1985

TR 85-20

Annual Research and Development Report 1985

Including Summaries of Technical Reports Issued during 1985. (Technical Reports 85-01-85-19) Stockholm May 1986.

1986

TR 86-31

SKB Annual Report 1986

Including Summaries of Technical Reports Issued during 1986 Stockholm, May 1987 1987

TR 87-33

SKB Annual Report 1987

Including Summaries of Technical Reports Issued during 1987 Stockholm, May 1988 1988

TR 88-32 SKB Annual Report 1988

Including Summaries of Technical Reports Issued during 1988 Stockholm, May 1989

Technical Reports

List of SKB Technical Reports 1990

TR 90-01 FARF31 – A far field radionuclide migration code for use with the PROPER package Sven Norman¹, Nils Kjellbert²

¹ Starprog AB ² SKB AB January 1990

TR 90-02

Source terms, isolation and radiological consequences of carbon-14 waste in the Swedish SFR repository

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TR 90-03

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Lars Lovius¹, Sven Norman¹, Nils Kjellbert² ¹ Starprog AB ² SKB AB February 1990

TR 90-04

Examination of the surface deposit on an irradiated PWR fuel specimen subjected to corrosion in deionized water

R.S. Forsyth, U-B. Eklund, O. Mattsson, D. Schrire Studsvik Nuclear March 1990

TR 90-05

Potential effects of bacteria on radionuclide transport from a Swedish high level nuclear waste repository

Karsten Pedersen University of Gothenburg, Department of General and Marine Microbiology, Gothenburg January 1990

TR 90-06

Transport of actinides and Tc through a bentonite backfill containing small quantities of iron, copper or minerals in inert atmosphere

Yngve Albinsson, Birgit Sätmark, Ingemar Engkvist, W. Johansson Department of Nuclear Chemistry, Chalmers University of Technology, Gothenburg April 1990

TR 90-07

Examination of reaction products on the surface of UO_2 fuel exposed to reactor coolant water during power operation

R S Forsyth, T J Jonsson, O Mattsson Studsvik Nuclear March 1990

TR 90-08

Radiolytically induced oxidative dissolution of spent nuclear fuel

Lars Werme¹, Patrik Sellin¹, Roy Forsyth² ¹ Swedish Nuclear Fuel and waste Management Co (SKB) ² Studsvik Nuclear May 1990

TR 90-09

Individual radiation doses from unit releases of long lived radionuclides

Ulla Bergström, Sture Nordlinder Studsvik Nuclear April 1990

TR 90-10

Outline of regional geology, mineralogy and geochemistry, Poços de Caldas, Minas Gerais, Brazil

H D Schorscher¹, M E Shea² ¹ University of Sao Paulo ² Battelle, Chicago December 1990

TR 90-11

Mineralogy, petrology and geochemistry of the Poços de Caldas analogue study sites, Minas Gerais, Brazil.

I: Osamu Utsumi uranium mine

N Waber¹, H D Schorscher², A B MacKenzie³, T Peters¹

- University of Bern
- ² University of Sao Paulo
- ³ Scottish Universities Research & Reactor Centre (SURRC), Glasgow December 1990

TR 90-12

Mineralogy, petrology and geochemistry of the Poços de Caldas analogue study sites, Minas Gerais, Brazil. II: Morro do Ferro

N Waber University of Bern

December 1990

TR 90-13

Isotopic geochemical characterisation of selected nepheline syenites and phonolites from the Poços de Caldas alkaline complex, Minas Gerais, Brazil M E Shea

Battelle, Chicago December 1990

TR 90-14

Geomorphological and hydrogeological features of the Poços de Caldas caldera, and the Osamu Utsumi mine and Morro do Ferro analogue study sites, Brazil

D C Holmes¹, A E Pitty², R Noy¹ ¹ British Geological Survey, Keyworth ² INTERRA/ECL, Leicestershire, UK December 1990

TR 90-15

Chemical and isotopic composition of groundwaters and their seasonal variability at the Osamu Utsumi and Morro do Ferro analogue study sites, Poços de Caldas, Brazil

- D K Nordstrom¹, J A T Smellie², M Wolf³
- ¹ US Geological Survey, Menio Park ² Contorro AB, Uppealo
- ² Conterra AB, Uppsala
- ³ Gesellschaft für Strahlen- und Umweltforschung (GSF), Munich December 1990

TR 90-16

Natural radionuclide and stable element studies of rock samples from the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil

A B MacKenzie¹, P Linsalata², N Miekeley³,

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² New York Medical Centre

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⁴ Florida State University

5 Los Alamos National Laboratory

December 1990

TR 90-17

Natural series nuclide and rare earth element geochemistry of waters from the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil

N Miekeley¹, O Coutinho de Jesus¹,

- C-L Porto da Silveira¹, P Linsalata², J N Andrews³, J K Osmond⁴
- Catholic University of Rio de Janeiro (PUC)
- ² New York Medical Centre
- ³ University of Bath

⁴ Florida State University December 1990

TR 90-18

Chemical and physical characterisation of suspended particles and colloids in waters from the Osamu Utsumi mine and Morro do Ferro analogue study sites, Poços de Caldas, Brazil

N Miekeley¹, O Coutinho de Jesus¹, C-L Porto da Silveira¹, C Degueldre² Catholic University of Rio de Janeiro (PUC) ² PSI, Villingen, Switzerland December 1990

TR 90-19

Microbiological analysis at the Osamu Utsumi mine and Morro do Ferro analogue study sites, Pocos de Caldas, Brazil

J West¹, A Vialta², I G McKinley³

- British Geological Survey, Keyworth
- ² Uranio do Brasil, Poços de Caldas

³ NAGRA, Baden, Sitzerland December 1990

TR 90-20

Testing of geochemical models in the Poços de Caldas analogue study

J Bruno¹, J E Cross², J Eikenberg³, I G McKinley⁴,

- D Read⁵, A Sandino¹, P Sellin⁶
- Royal Institute of Technology (KTH), Stockholm AERE, Harwell, UK
- ³ PSI, Villingen, Switzerland
- 4 NAGRA, Baden, Switzerland
- 5 Atkins ES, Epsom, UK
- 6 Swedish Nuclear and Waste Management Co (SKB), Stockholm

December 1990

TR 90-21

Testing models of redox front migration and geochemistry at the Osamu Utsumi mine and Morro do Ferro analogue sites, Poços de Caldas, Brazil

J Cross¹, A Haworth¹, P C Lichtner²,

- A B MacKenzi³, L Moreno⁴, I Neretnieks⁴, D K Nordstrom⁵, D Read⁶, L Romero⁴,
- S M Sharland¹, C J Tweed¹
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December 1990

TR 90-22

Near-field high temperature transport: Evidence from the genesis of the Osamu Utsumi uranium mine analogue site, Poços de Caldas, Brazil

L M Cathles¹, M E Shea² University of Cornell, New York ² Battelle, Chicago December 1990

TR 90-23

Geochemical modelling of water-rock interactions at the Osamu Utsumi mine and Morro do Ferro analogue sites, Poços de Caldas, Brazil

D K Nordstrom¹, I Puigdomenech², R H McNutt³ US Geological Survey, Menlo Park

² Studsvik Nuclear, Sweden

³ McMaster University, Ontario, Canada December 1990

TR 90-24

The Poços de Caldas Project: Summary and implications for radioactive waste management

N A Chapman¹, I G McKinley², M E Shea³,

- J A T Smellie⁴
- ¹ INTERRA/ECL, Leicestershire, UK ² NAGRA, Baden, Switzerland
- ³ Battelle, Chicago
- ⁴ Conterra AB, Uppsala

TR 90-25

Kinetics of UO₂(s) dissolution reducing conditions:

numerical modelling

- I Puigdomenech¹, I Cass², J Bruno³
 ¹ Studsvik AB, Nyköping, Sweden
 ² Department of Chemical Engineering, E.T.S.E.I.B.
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May 1990