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Äspö Hard Rock Laboratory

Prototype repository

Analyses of microorganisms, gases, and water chemistry in buffer and backfill, 2010

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June 2011

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Summary

The prototype repository (hereafter, “Prototype”) is an international project to build and study a full-scale model of the planned Swedish final repository for spent nuclear fuel. However, the Prototype differs from a real storage in that it is drained, which makes the swelling pressure lower in the Prototype than in a real storage facility. The heat from the radioactive decay is simulated by electrical heaters. The project is being conducted at the Äspö Hard Rock Laboratory (HRL) in crystalline rock at a depth of approximately 450 m. A monitoring programme is investigating the evolution of the water chemistry, gas, and microbial activity at the site, and a specific aim is to monitor the microbial consumption of oxygen *in situ* in the Prototype. This document describes the results of the analyses of microbes, gases, and chemistry inside the Prototype in 2010.

Hydrogen, helium, nitrogen, oxygen, carbon monoxide, carbon dioxide, methane, ethane, and ethene were analysed at the following sampling points in the Prototype: KBU10001, KBU10002, KBU10004, KBU10008, and KFA04. Where the sampling points in the Prototype delivered pore water, the water was analysed for amount of ATP (i.e. the biovolume), culturable heterotrophic aerobic bacteria (CHAB), sulphate-reducing bacteria (SRB), methane-oxidizing bacteria (MOB), and iron-reducing bacteria (IRB). The pore water collected from the Prototype was subject to as many chemical analyses as the amount of water allowed. Chemical analyses were also performed on pore water from two additional sampling points, KBU10005 and KBU10006. Chemical data from a previous investigation of the groundwater outside the Prototype were compared with the pore water chemistry.

The improved sampling and analysis protocols introduced in 2007 worked very well. The International Progress Report (IPR) 08-01 (Eriksson 2008) revealed that many of the hydrochemical sampling points differ greatly from each other. The 16 sampling points were therefore divided into seven sampling groups, each with similar properties. The properties of one sampling group (i.e. KBU10002 + KBU10008) resembled those of the groundwater, while others (i.e. KBU10004 + KBU10006, KBU10005, and KFA01–KFA04) differed, for example, in microbial composition, salinity, sulphate content, and the concentrations of calcium, potassium, magnesium, sodium, and many dissolved metals, actinides, and lanthanides. One sampling group comprised sampling points that seemed to be in contact with tunnel air (KBU10003 + KBU10007). Another sampling group comprised sampling points, near the canisters in the buffer (KB513–614), with very little pore water with high pH and a high salt content. One sampling point in the backfill, which had not been reached by the groundwater as of May 2007 (KBU10001), now yielded pore water with properties resembling those of groundwater.

The gas composition in the sampling groups was uniform in that the proportion of nitrogen in the extracted gas was increasing while the oxygen content was decreasing with time. ATP analyses demonstrated that the biomass in the Prototype was higher than in the surrounding groundwater. The microbiological results indicated that aerobic microbes, such as methane-oxidizing bacteria and culturable heterotrophic bacteria, thrived in the aerobic Prototype environment. The chemical data indicated differences between the sampling groups: concentrations of sodium and potassium were higher in the Prototype pore water than in the groundwater outside it, while calcium was lower than in the groundwater. Obviously, cation exchange occurs in the montmorillonite interlayers. At sampling points containing active microbes, copper, rubidium, vanadium, and uranium were enriched up to 225 times the groundwater levels; microbes are possibly responsible for dissolving these substances by excreting compound-specific ligands.

Overall, the observations presented here strongly support our hypothesis that oxygen will be consumed by bacteria within a short period (i.e. weeks to years), as opposed to the long period associated with abiotic processes (i.e. many years). The gas data generally indicate that oxygen was disappearing and that methane-oxidizing bacteria were responsible for at least some of this oxygen decrease. The microbes also affected the chemistry in the Prototype, both indirectly (by being active and changing the redox and pH) and possibly directly (via compound-specific ligands).

Sammanfattning

Prototypförvaret är ett internationellt projekt bestående av en fullskalemodell av det slutförvar som planeras byggas för Sveriges använda kärnbränsle. Till skillnad från ett riktigt förvar är emellertid Prototypförvaret dränerat och värmen från det radioaktiva sönderfallet simuleras med hjälp av elvärme. Detta medför till exempel att svälltrycket blir högre i det riktiga förvaret. Prototypförvarsprojektet genomförs på Äspölaboratoriet, i kristallin berggrund på 450 m djup. Ett övervakningsprogram undersöker förändringar av kemi, gas och mikrobiell aktivitet. Ett av de specifika målen är att utreda mikrobiell reduktion av syremängden i förvaret. Denna rapport beskriver resultaten av de analyser som genomförts angående mikrober, gaser och vattenkemi inuti Prototypförvaret under 2010.

Analyser av vätgas, helium, kvävgas, syrgas, kolmonoxid, koldioxid, metan, etan och eten utfördes på följande provtagningspunkter i Prototypförvaret: KBU10001, KBU10002, KBU10008 och KFA04. Dessa provtagningspunkter analyserades även med avseende på ATP innehåll (dvs biovolymen), odlingsbara heterotrofa aeroba bakterier (CHAB), sulfatreducerande bakterier (SRB), metanoxiderande bakterier (MOB) och järnreducerande bakterier (IRB). Det uppsamlade porvattnet från Prototypförvaret skickades efter gas och mikrobanalyser för kemisk analys av så många ämnen som vattnet räckte till. Utöver detta utfördes kemisk analys på porvatten från ytterligare två provtagningspunkter i Prototypförvaret, KBU10005 och KBU10006. Kemidata från en tidigare undersökning av grundvattnet runt Prototypförvaret användes för jämförelser mellan porvattnet och omgivningen.

De under 2007 förbättrade provtagnings- och analysprotokollen fungerade mycket bra även denna provtagning. I tidgare undersökningar visade det sig att de 16 olika provtagningspunkterna skiljde sig relativt markant från varandra. Dessa 16 punkter i Prototypförvaret har därför delats upp i sju provgrupper med likartade egenskaper. En provgrupp (KBU10002 + 8) liknade grundvatten medan andra (KBA10004 + 6, KBU10005, KFA01–04) skiljde sig när det tex gäller mikrobiell sammansättning och salinitet, sulfatinnehåll, koncentrationer av kalcium, kalium, magnesium, natrium och många lösta metaller, aktinider och lantanider. En provgrupp innehöll provtagningspunkter som såg ut att ha kontakt med tunneln (KBU10003 + 7). En provgrupp innehöll provtagningspunkter nära kapslarna i bufferten (KB513-614) med mycket lite porvatten med högt pH och hög salinitet. En provgrupp i återfyllnaden var torrlagd 2007 (KBU10001), men innehöll denna gång porvatten med grundvattenliknande egenskaper.

Gassammansättningen i de olika provgrupperna var enhetlig när det gäller kvävgashalten som ökade och syrgashalten som minskade över tiden. ATP analyserna visade att biomassan i Prototypförvaret är hög jämfört med det omgivande grundvattnet. De mikrobiologiska resultaten visade att aeroba bakterier såsom metanoxiderande bakterier (MOB) och heterotrofa aeroba bakterier frodades i det aeroba Prototypförvaret. Kemidata visade ännu en gång skillnader mellan provgrupperna. Koncentrationerna av natrium och kalium var högre i porvattnet än i grundvattnet utanför. Koncentrationerna av kalcium däremot var lägre än i grundvattnet. Detta tyder på att katjonbyte i montmorillonitens mellanlager förekommit. I provtagningspunkter som innehöll aktiva mikrober anrikades koppar, rubidium, vanadin och uran upp till 225 gånger jämfört med grundvattnet. Det är möjligt att mikrober var ansvariga för denna anrikning genom exkretion av ämnesspecifika ligander.

Övergripande visar de observationer som presenteras här att syre kommer att konsumeras av bakterier under ett relativt kort tidspann (dvs veckor till år) i motsats till det långa tidsspann som förväntas genom abiotiska processer (många år). Gasdata visade att syre försvinner och att MOB var ansvariga för åtminstone en del av denna syreminskning. Mikroberna påverkade också kemin i Prototypförvaret, både indirekt (genom att vara aktiva och förändra redox och pH) och möjligen också direkt (genom specifika ligander).

Contents

1 Introduction

The prototype repository (hereafter, “Prototype”) is an international project to build and study a full-scale model of the planned deep repository for Swedish spent nuclear fuel. The Prototype differs from a real storage facility in that it is drained. For example, this makes the swelling pressure lower in the Prototype than in a real storage facility. The heat from the radioactive decay is simulated by electrical heaters. The project is being conducted at the Äspö Hard Rock Laboratory (HRL) in crystalline rock at a depth of approximately 450 m.

The evolution of chemistry, gas, redox, and oxygen reduction in various parts of the Prototype is being monitored. One specific aim is to monitor the microbial consumption of oxygen. Because oxygen has previously been found to be consumed within 2 weeks in granitic media at Äspö HRL (Puigdomenech et al. 2001), it is hypothesized that oxygen will also be consumed by bacteria in the buffer and backfill within a short period (i.e. weeks to years), as opposed to the long period associated with abiotic processes (i.e. many years).

Gases and microorganisms are regularly sampled and analysed to monitor the biogeochemical processes taking place in the Prototype. A method for sampling and analysing the gases present in buffer and backfill has been tested *in situ*. The results and evaluation of the first *in situ* measurements were presented in an IPR (Pedersen et al. 2004). Analyses were subsequently performed in fall 2004, 2005, 2006, 2007, and 2009. The results and interpretations of these measurements were reported previously (Eriksson 2007, Lydmark 2010).

1.1 Design of the prototype repository

The Prototype has six full-scale deposition holes distributed in two sections, as shown in Figure 1-1. The inner section farthest from the tunnel, section 1, contains four deposition holes while the outer section nearest the tunnel, section 2, contains two. A full-size, electrically heated canister surrounded by bentonite has been placed in each deposition hole.

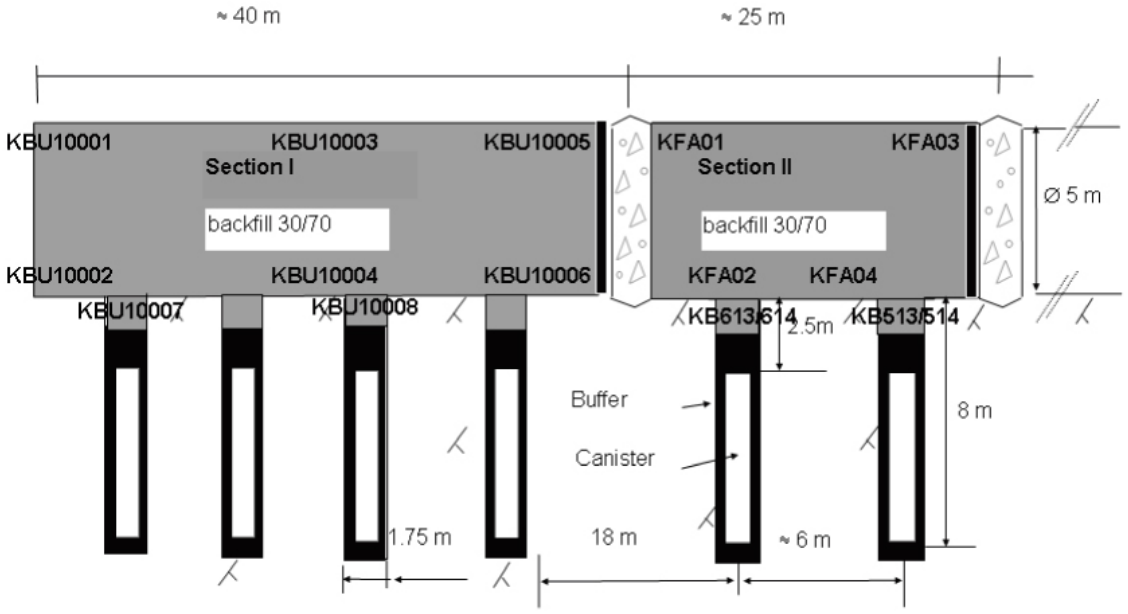

Figure 1-1. *Schematic of the prototype repository (adapted from IPR 99-34).*

1.2 Sampling points and sample collectors

The instrumented deposition holes in section 1 (the inner section farthest from the tunnel), DA3587G01 and DA3575G01, are labelled hole numbers 1 and 3, respectively, in Figure 1-2. Eight sample collectors have been installed for continuous hydrochemical sampling in section 1 (inner section) (Table 1-1), six in the backfill (Figure 1-3), one at the top of deposition hole DA3587G01, and one at the top of deposition hole DA3575G01. The instrumented deposition holes in section 2 (the outer section nearest the tunnel), DA3545G01 and DA3551G01, are labelled hole numbers 5 and 6, respectively, in Figure 1-2.

In section 2 (outer section) in Table 1-2, four sample collectors were placed in the backfill (Figure 1-3), two in the rock/bentonite interface at the top of deposition hole DA3545G01, and two in the rock/bentonite interface at the top of deposition hole DA3551G01.

Each sample collector consists of a titanium cup with a titanium filter mounted on top and polyether-etherketone (PEEK) tubes connected to the bottom. The length of each tube is at most approximately 79 m and the inner diameter is 2 mm, for a maximal sample tube volume of approximately 250 mL.

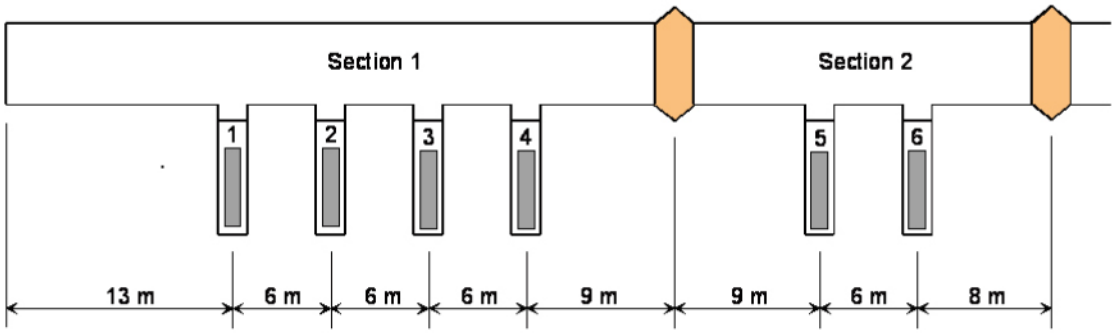

Figure 1-2. *The deposition holes in the prototype repository.*

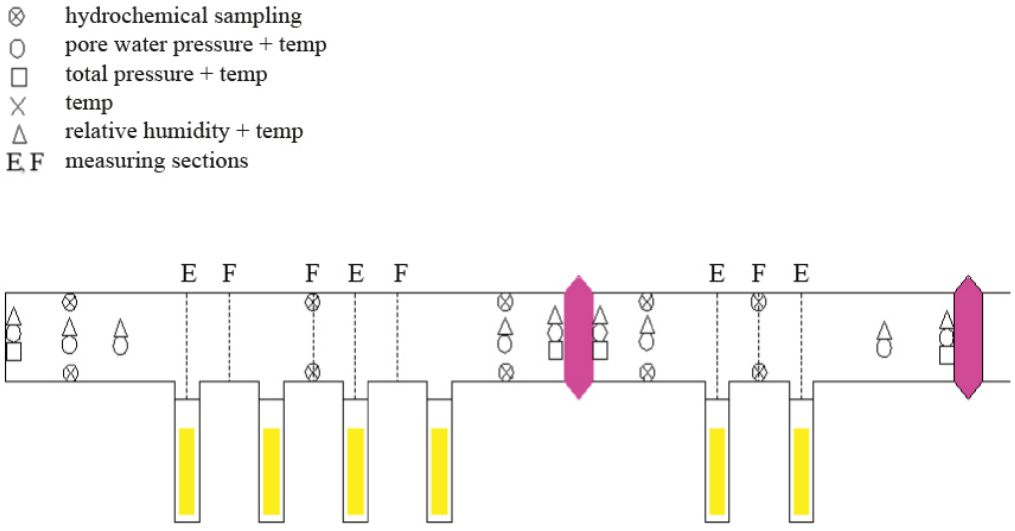

Figure 1-3. *Figure showing the positions of the titanium cups in the backfill in sections 1 and 2.*

Table 1-1. Sample collectors in section 1, the inner section farthest from the tunnel.

ID code	Deposition hole/backfill	Labell	Block/section
PXPKBU101	Backfill	KBU10001	Inner part
PXPKBU102	Backfill	KBU10002	Inner part
PXPKBU103	Backfill	KBU10003	Between dep. holes 2 and 3
PXPKBU104	Backfill	KBU10004	Between dep. holes 2 and 3
PXPKBU105	Backfill	KBU10005	In front of plug
PXPKBU106	Backfill	KBU10006	In front of plug
PXPKBU107	DA3587G01	KBU10007	C4 (hole 1)
PXPKBU108	Da3575G01	KBU10008	C4 (hole 3)

Table 1-2. Sample collectors in section 2, the outer section nearest the tunnel.

ID code	Deposition hole/backfill	Mark	Block/section
PXP0KFA01	Backfill	KFA01	Inner part
PXP0KFA02	Backfill	KFA02	Inner part
PXP0KFA03	Backfill	KFA03	Between dep. holes 5 and 6
PXP0KFA04	Backfill	KFA04	Between dep. holes 5 and 6
PXP0KB513	DA3551G01	KB513	C4 (hole 6)
PXP0KB514	DA3551G01	KB514	C4 (hole 6)
PXP0KB613	DA3545G01	KB613	C4 (hole 5)
PXP0KB614	DA3545G01	KB614	C4 (hole 5)

1.3 Microorganisms, gases, and chemistry in buffer and backfill

The Prototype lets us study various processes that will take place in a storage facility of the KBS-3 type, such as:

- Water uptake in buffer and backfill.
- Temperature distribution in canisters, buffer, backfill, and rock.
- Displacement of canisters.
- Swelling pressure and displacement in buffer and backfill.
- Stress and displacement in near-field rock.
- Water pressure build-up and pressure distribution in rock.
- Gas pressure in buffer and backfill.
- Chemical processes in rock, buffer, and backfill.
- Bacterial growth and migration in buffer and backfill.

This P-report deals with the three last processes in this list: gas pressure in buffer and backfill; chemical processes in rock, buffer, and backfill; and bacterial growth and migration in buffer and backfill. The report presents compiled results from 2010 and comparisons with earlier findings. The microbes active in the deep subsurface and currently considered important to the near-field KBS-3 repository may be responsible for the following processes:

- **Methanotrophy** – an aerobic microbial life strategy that includes the reduction of oxygen (which is corrosive to copper canisters) as well as the oxidation of methane and the resulting production of carbon dioxide.
- **Sulphate reduction** – an anaerobic microbial life strategy that includes the reduction of sulphur in sulphate and the oxidation of organic carbon as well as the production of sulphide, which is corrosive to copper canisters.
- **Iron reduction** – an anaerobic microbial life strategy that includes the reduction of ferric iron with organic carbon to ferrous iron. Such microorganisms have been proposed to catalyse the itillization of montmorillonite in bentonite under good growth conditions (Dinh et al. 2004).

2 Aims

One main question to be answered in this project was whether microbial activity could reduce the oxygen levels *in situ* in the Prototype. It was also important to examine whether microbial activity (i.e. sulphate reduction ending in sulphide production or iron reduction possibly enhancing illitization) could in any manner compromise the integrity of the copper canisters.

3 Methods

3.1 Experimental approach

Water samples from the Prototype were analysed to determine the numbers of the following types of microbes: culturable heterotrophic aerobic bacteria (CHAB), methane-oxidizing (aerobic) bacteria (MOB), sulphate-reducing bacteria (SRB), and iron-reducing bacteria (IRB).

According to the above, the differences in the gas composition of a water sample over a given period could be affected by microbial activity in the water. In addition to the microbial analyses, the gas composition (i.e. proportions of particular gases) was analysed in the gas or gas/water phases at the various sampling points in the Prototype; the analysed gases were nitrogen, oxygen, helium, methane, ethane and ethene, carbon dioxide, carbon monoxide, and hydrogen.

The pore water was sent for partial Class 5 analysis, since microbial activity can affect the chemistry of pore water from the buffer and the backfill in several ways:

- Microbial activity can lead to the **dissolution of various minerals** in the bentonite (i.e. gypsum, $CaSO_4 \times 2\ H_2O$), which contains sulphate that SRB need for their respiration.
- The effect can be **direct** if microbes excrete certain compounds, such as bioligands, that can complex specifically with various metals they need for their metabolism.
- The effect can also be **indirect**, depending on the extent to which the microbial activity increases the pH, which in an unspecific manner can change the solubility of all tested elements.

3.2 Sampling

3.2.1 Sampling and analyses inside Prototype

In November 2010, samples for gas, microbe, and chemical analyses were taken from four sampling points inside the repository, KBU10001, KBU10002, KBU10008, and KFA04 (Tables 1-1 and 1-2), were performed. In addition, samples for chemical analyses were taken from two sampling points, i.e. KBU10005 and KBU10006. These samples were analysed as given in Table 3-1.

Positions KBU10001, KBU10002, KBU10008, and KFA04 were sampled on 2010-11-15 using special 45-mL high-vacuum, stainless steel pressure vessels (Mymeko, Göteborg, Sweden) (Figure 3-1). Before the pressure vessels were connected, the pressure at each sampling point inside the Prototype was

Figure 3-1. *Pressure vessels used for extracting pore water and gas from the prototype repository.*

Table 3-1. Analyses of samples from inside and outside the prototype repository, 2010.

Borehole	Sampling date	Gas analyses	Microbial analyses	Chemical analyses
KBU10001	2010-11-15	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN MOB, MPN IRB	Class 5
KBU10002	2010-11-15	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN MOB, MPN IRB	Class 5
KBU10003	2010-11-15	Pressure		
KBU10004	2010-11-15	Pressure		
KBU10005	2010-12-14	Pressure		Class 5
KBU10006	2010-12-14	Pressure		Class 5
KBU10007	2010-11-15	Pressure		
KBU10008	2010-11-15	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN MOB, MPN IRB	Class 5
KB513	2010-11-15	Pressure		
KB514	2010-11-15	Pressure		
KB613	2010-11-15	Pressure		
KB614	2010-11-15	Pressure		
KFA01	2010-11-15	Pressure		
KFA02	2010-11-15	Pressure		
KFA03	2010-11-15	Pressure		
KFA04	2010-11-15	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN MOB, MPN IRB	Class 5

registered using a 0–40 bar manometer (no. 7082534; WIKA Instruments, Klingenberg, Switzerland). After that, the tubing was flushed with nitrogen gas and the pressure vessel was connected to the four sampling points from which water was to be extracted. The valve on the pressure vessel was opened so that water could be extracted from the Prototype because of the vacuum inside the vessel. The pressure increase was measured in each pressure vessel and, when the pressure inside the vessel was the same as at the sampling point before the connection, the valve was closed and the pressure vessel transported to Microbial Analytics Sweden AB in Mölnlycke, Sweden. At the laboratory, samples for gas and microbial analyses were extracted directly from the high-vacuum, stainless steel pressure vessels. The remaining water was sent to the SKB chemistry laboratory for Class 5 analysis.

Additional samples were taken from two sampling points inside the repository, KBU10005 and KBU10006, for chemical analysis (Table 3-1).

3.3 Gas analyses

The sampling was performed as previously described, except that the vessels were left until 2010-12-14 due to the slow filling time, using special 45-mL high-vacuum, stainless steel pressure vessels. In the laboratory, the samples were transferred to a vacuum container and any gas in the water was boiled off under vacuum (i.e. at water vapour pressure) at room temperature; the transfer time was approximately 20–30 min. After extraction, the gas was compressed and transferred to a 10-mL syringe (SGE Analytical Science, Victoria, Australia) and the volumes of extracted gas and water were measured. The captured gas was subsequently transferred to a 6.6-mL glass vial stoppered with a butyl rubber stopper and sealed with an aluminium crimp seal.The vial had previously been evacuated and flushed twice with nitrogen, in two cycles, and left at high vacuum (1 Pa). Dehydrant was added to adsorb any traces of water remaining in the gas (water causes troublesome baseline drifts in the gas chromatographs). Thereafter analysis was performed using gas chromatography.

Two different chromatographs were used and equipped as follows. Hydrogen and carbon monoxide were analysed on a KAPPA-5/E-002 analytical gas chromatograph (AMETEK/Trace Analytical, formerly Trace Analytical, Menlo Park, CA, USA) equipped with a 156×1/16-inch stainless steel HayeSep column in line with a 31×1/8-inch stainless steel Molecular Sieve 5A column, which was subsequently attached to a reduction gas detector. Helium, argon, and nitrogen were analysed on a Varian Star 3400CX gas chromatograph (Varian Analytical Instruments, Varian AB, Bromma, Sweden) using a thermal conductivity detector at an oven temperature of 65°C, a detector temperature of 120°C, and a filament temperature of 250°C. The gases were separated using a Porapak-Q column (2 m×1/8 inch diameter) followed by a Molecular Sieve 5A column 6 m×1/8 inch) with argon (for helium and nitrogen) and nitrogen (for argon) as the respective carrier gases. Methane and ethane were analysed on a Varian Star 3400CX gas chromatograph using a flame ionization detector (FID) at an oven temperature of 65°C and a detector temperature of 200°C. The gases were separated using a Porapak-Q column (2 m×1/8 inch diameter) and analysed on the FID with nitrogen as the carrier gas. Carbon dioxide was transformed to methane using a 10% Ni_2NO_3 "methanizer" fed with hydrogen gas (9.375×1/8 inch diameter, temperature 370°C) and analysed as methane on the FID with nitrogen as the carrier gas.

3.4 Microbial analyses

3.4.1 ATP analysis

The ATP Biomass Kit HS for determining total ATP in living cells was used (no. 266-311; BioThema, Handen, Sweden). This analysis kit was developed based on the results of Lundin et al. (1986) and Lundin (2000). Sterile and "PCR clean" epTIPS with filters (GTF, Göteborg, Sweden) were used in transferring all solutions and samples to prevent ATP contamination of pipettes and solutions. Light may cause delayed fluorescence of materials and solutions, so all procedures described below were performed in a dark room and all plastic material, solutions, and pipettes were stored in the dark. A new 4.0-mL, 12-mm-diameter polypropylene tube (no. 68.752; Sarstedt, Landskrona, Sweden) was filled with 400 µL of the ATP Biomass Kit HS reagent (BioThema, Handen, Sweden) and inserted into an FB12 tube luminometer (Sirius Berthold, Pforzheim, Germany). The quick measurement FB12/Sirius software, version 1.4 (Berthold Detection Systems, Pforzheim, Germany), was used to calculate light emission as relative light units per second (RLU s^{-1}). Light emission was measured for three 5-s intervals with a 5-s delay before each interval, and the average of three readings was registered as a single measurement. The background light emission (I_{bkg}) from the HS reagent and the tube was monitored and allowed to decrease to below 50 RLU s^{-1} before registering a measurement. ATP was extracted from 100-µL aliquots of sample within 1 h of collection, by mixing for 5 s with 100 µL of B/S extractant from the ATP kit in a separate 4.0-mL polypropylene tube. Immediately after mixing, 100 µL of the obtained ATP extract mixture was added to the HS reagent tube in the FB12 tube luminometer, and the sample light emission (I_{smp}) was measured. Subsequently, 10 µL of an internal ATP standard was added to the reactant tube, and the standard light emission (I_{std}) was measured. The concentration of the ATP standard was 10^{-7} M. Samples with ATP concentrations approaching or higher than that of the ATP standard were diluted with B/S extractant to a concentration of approximately 1/10 that of the ATP standard. Mixtures of HS reagent and B/S extractant were measured at regular intervals to control for possible ATP contamination. Values of 1,600 ± 500 amol ATP mL^{-1} (n = 10) were obtained with clean solutions, while solutions displaying values above 1,600 amol ATP mL^{-1} were disposed of. The ATP concentration of the analysed samples was calculated as follows:

$$\text{amol ATP mL}^{-1} = (I_{smp} - I_{bkg}) / ((I_{smp+std} - I_{bkg}) - (I_{smp} - I_{bkg})) \times 10^6 / \text{sample volume},$$

where I represents the light intensity measured as RLU s^{-1}, smp represents sample, bkg represents the background value of the reagent HS, and std represents the standard (referring to a 10^{-7} M ATP standard).

This ATP biomass method has been evaluated for use with Fennoscandian groundwater, including Olkiluoto groundwater (Eydal and Pedersen 2007).

3.4.2 Determining culturable aerobic bacteria

Petri dishes containing agar with nutrients were prepared for determining the numbers of culturable heterotrophic aerobic bacteria (CHAB) in groundwater samples. This agar contained 0.5 g L^{-1} of peptone (Merck, VWR, Stockholm, Sweden), 0.5 g L^{-1} of yeast extract (Merck), 0.25 g L^{-1} of sodium acetate (Merck), 0.25 g L^{-1} of soluble starch (Merck), 0.1 g L^{-1} of K_2HPO_4 (Merck), 0.2 g L^{-1} of $CaCl_2$ (Merck), 10 g L^{-1} of NaCl (Merck), 1 mL L^{-1} of trace element solution, and 15 g L^{-1} of agar (Merck) (Pedersen and Ekendahl 1990). The medium was sterilized in 1-L batches by autoclaving at 121°C for 20 min, cooled to approximately 50°C in a water bath, and finally distributed in 15-mL portions in 9-cm-diameter plastic Petri dishes (GTF, Göteborg, Sweden). Ten-times dilution series of culture samples were made in sterile analytical-grade water (AGW) containing 0.9 g L^{-1} of NaCl; 0.1-mL portions of each dilution were spread on the plates in triplicate using a sterile glass rod. The plates were incubated for 7–9 d at 20°C, after which the number of colony forming units (CFU) was counted; plates with 10–300 colonies were counted.

3.4.3 Preparing media for most probable numbers of culturable anaerobic microorganisms

Media for determining the most probable number of microorganisms (MPN) in groundwater were formulated based on previously measured granitic groundwater chemistry data. This allowed the formulation of artificial media that most closely mimicked *in situ* groundwater chemistry for optimal microbial cultivation (Haveman and Pedersen 2002). Media for the iron-reducing bacteria (IRB), sulphate-reducing bacteria (SRB), and autotrophic acetogens (AA) were autoclaved and anaerobically dispensed, according to the formulations outlined in Hallbeck and Pedersen (2008), into 27-mL, sealable anaerobic glass tubes (no. 2048-00150; Bellco Glass, Vineland, NJ, USA) stoppered with butyl rubber stoppers (no. 2048-117800; Bellco Glass) and sealed with aluminium crimp seals (no. 2048-11020; Bellco Glass).

3.4.4 Inoculations and analysis of anaerobic microorganisms

Inoculations for IRB, SRB, and AA were performed in the laboratory as soon as possible after sampling. After inoculating, the headspaces of only the AA cultures were filled with H_2 to an overpressure of 2 bar; all MPN tubes were incubated in the dark at 20°C for 8–13 w. After incubation, the MPN tubes were analysed by testing for metabolic products. The production of ferrous iron by IRB was determined using the 1.10-phenanthroline method (method no. 8146; HACH LANGE, Sköndal, Sweden). SRB were detected by measuring sulphide production using the $CuSO_4$ method according to Widdel and Bak (1992) on a UV–visible spectrophotometer (Genesys10UV, VWR, Stockholm, Sweden). Product formation at a concentration twice or above that of the uninoculated control tubes was taken as positive for all MPN analyses.

The MPN procedures resulted in protocols for tubes that scored positive or negative for growth. The results of the analyses were rated positive or negative relative to control levels. Three dilutions (five replicate tubes each) were used to calculate the MPN of each microbial group, according to the calculations found in Greenberg et al. (1992).

3.4.5 Inoculations and analysis of aerobic methane-oxidizing bacteria

Sets of MPN tubes were prepared in a nitrate mineral salts (NMS) medium (Whittenbury et al. 1970), as follows: 1.0 g L^{-1} of KNO_3, 1 g L^{-1} of $MgSO_4 \times 7\ H_2O$, 0.2 g L^{-1} of $CaCl_2 \times 2\ H_2O$, 1 mg L^{-1} of $CuCl_2 \times 2H_2O$, 7 g L^{-1} of NaCl, 1 mg L^{-1} of copper chloride dehydrate, 1 mL L^{-1} of an iron solution made of 0.5 g of ferric (III) chloride in 1,000 mL AGW, 1 mL L^{-1} of a trace element solution, and 2 mL L^{-1} of a phosphate buffer solution made of 3.6 g of Na_2HPO_4 and 1.4 g of NaH_2PO_4 in 100 mL of AGW. The pH was adjusted to 6.8–7.0.

MPN inoculations were completed as soon as possible after sampling. Five replicate tubes were made for each dilution. All transfers were performed aseptically using new sterile syringes and needles. After each transfer, the tubes were vortexed to achieve homogeneity. Control tubes contained nitrate minimal salt medium and 1 mL of filtered groundwater. After inoculation, methane filter-sterilized through .2-µm-pore-size Sartorius Minisart CA syringe filters (GTF, Göteborg, Sweden) was injected into the headspace of each tube to 1-bar overpressure. The tubes were then incubated horizontally in the dark at 20°C. Growth of cells was detected after 2–4 w, as judged by the turbidity compared with that of negative controls and by the concomitant production of CO_2 by methane oxidation in turbid tubes. MPN calculations were done using a combination of positive tubes in a three-tube dilution series (15 tubes) according to Greenberg et al. (1992); the detection limit was < 0.2 cells mL^{-1}.

3.5 Chemical analyses

3.5.1 Sampling and analysis of groundwater inside the prototype repository

Samples for the chemical analysis of the pore water inside the Prototype were taken by collecting the water phase after the gas was extracted from the pressure vessels. The samples were sent to SKB, and the chemical analyses were performed by the SKB chemistry laboratory at Äspö HRL according to their standard protocols, or were subcontracted to external laboratories.

3.5.2 Evaluation of the chemistry

The chemistry data from the pore water in the sample groups were compared with the groundwater chemistry reported in IPR-08-01. The results were calculated by dividing the mean amount of a specific compound in the groundwater by the mean amount (when available) in the various sample groups, and were presented as enrichment factors.

4 Results

4.1 Sample groups

The Prototype is a field experiment located in the Äspö HRL, where processes inside a KBS-3-type nuclear waste repository are studied under near-authentic conditions. This report provides an overview of the data from 2010, the interaction between gas composition, chemistry, and microbial life, and the influence of the groundwater surrounding the Prototype.

As described by Eriksson (2007), the sampling points inside the Prototype have been divided into seven sample groups based on the gas composition, water pressure development, pore water content, and pore water chemistry. These sample groups are also applied, when data are available, in presenting the results from 2010 regarding the sampling of Prototype pore water and surrounding groundwater from five of the groups:

- **KBU10001:** This sampling point from the backfill in section 1 (inner section farthest from the tunnel) increases in pressure with time. In November 2010, the pressure at this sampling point was 4 bars and water was extractable within 24 h (Table 4-1).
- **KBU10002 and KBU10008:** These two sampling points, one in the backfill and one at the top of deposition hole 3 in section 1 (inner section), produced extractable water within 24 h as of November 2010. The pressure at these sampling points increases with time, and was 4 bars as of November 2010 (Table 4-1).
- **KBU10004 and KBU10006:** Gas could not be extracted from the pore water in the KBU10004+6 group and the filling time was too long for proper microbial analysis. Enough water for pore water chemistry could be obtained from KBU1006.
- **KBU10005:** Gas could not be extracted from the pore water in the KBU10005 group and the filling time was too long for proper microbial analysis. Enough water for pore water chemistry could be obtained from KBU1005.
- **KFA01, KFA02, KFA03, and KFA 04:** Sufficient water could be extracted from KFA04 within 24 h. The pressure at this sampling point increases with time and was 12 bar as of November 2010 (Table 4-1).

4.2 Dissolved gas composition

4.2.1 Group KBU10001

Water was extractable from the KBU10001 sampling point (Table 4-1). The oxygen level had increased to approximately 12% versus 2% in 2009. The nitrogen level had decreased from almost 100% in 2009 to approximately 89% in 2010. This change in the ratio between oxygen and nitrogen could be due to leakage of oxygen somewhere in the sampling tube or to conditions on the particular sampling occasion. The amounts of carbon dioxide, methane, and hydrogen varied but remained below 1%. Helium was not detected at the KBU10001 sampling point.

4.2.2 Group KBU10002 + KBU10008

Water could easily be extracted from the KBU10002 + KBU10008 sampling points (Table 4-1). The gas composition in pore water in the group generally lacked distinctive trends, although the oxygen levels in these pore waters were generally lower in 2009 and in this sampling in 2010 than in previous sampling performed in 2005–2007 (i.e. 0.9–5% versus 7–8% in 2007). The amounts of methane and hydrogen varied but remained below 1% in 2010. However, the carbon dioxide content in KBU10002 had increased to approximately 4%. Helium was not detected in the KBU10002 + KBU10008 sampling points.

4.2.3 Group KFA01–KFA04

Water could be extracted from the KFA04 sampling point (Table 4-1). The gas composition at this point generally follows the trends described previously (Lydmark 2010), with low oxygen (2% in 2010) and high nitrogen contents (96% in 2010). The concentrations of methane, carbon dioxide, and helium reached 0.2–0.5%.

4.3 Microbial composition

4.3.1 Group KBU10001

On 2010-11-15, the numbers of CHAB, SRB, IRB, and MOB in as well as the ATP content of pore water from KBU10001 were determined (Table 4-2). In this pore water, the ATP content was approximately 14,000 amol mL^{-1} and the numbers of CHAB and MOB were 43, and 0.2 mL^{-1}, respectively. SRB and IRB were not detected.

4.3.2 Group KBU10002 + KBU10008

On 2010-11-15, the numbers of CHAB, SRB, AA, and MOB in as well as the ATP content of pore water from the KBU10002 + KBU10008 group were determined (Table 4-2). In these pore waters, the ATP contents ranged from approximately 40,000–50,000 amol mL^{-1}, and the numbers of CHAB, SRB, IRB, and MOB were 13–47, 2, 0.2–8, and 8,000–17,000 mL^{-1}, respectively. Obviously, MOB were the most abundant type of bacteria in this group. IRB were the most abundant anaerobic bacteria, reaching 8 mL^{-1} at the most.

4.3.3 Group KFA01–KFA04

On 2010-11-15, the numbers of CHAB, SRB, AA, and MOB in as well as the ATP content of pore water from KFA04 were determined (Table 4-2). In this pore water, the ATP content was approximately 10,000 amol mL^{-1}, and the numbers of CHAB, SRB, IRB, and MOB were 3, 0.2, < 0.2, and 0.8, respectively.

4.4 Chemical composition

4.4.1 Group KBU10001

The pore water in the KBU10001 group was similar to the groundwater in salinity, and in concentrations of sulphate, potassium, magnesium, sodium, calcium and silica (Tables 4-3 and 4-4). Many of the metals examined were present in concentrations of 0.1–100 µg L^{-1}. However, there was one exception; the concentration of copper was 913 µg L^{-1}. The concentrations of vanadium and of the actinide uranium were approximately 3 times higher and 8 times higher, 0.46 and 1.3 µg L^{-1}, than in the groundwater outside the repository.

4.4.2 Group KBU10002 + KBU10008

The pore water in the KBU10002 + KBU10008 group was fairly similar to the groundwater from KBU10001 in salinity and in concentrations of potassium, magnesium, sodium, calcium, and silica (Tables 4-3 and 4-4). However, the mean amount of sulphate was approximately 11 times higher (145–2,330 mg L^{-1}) in the KBU10002 + KBU10008 group than in the groundwater. Many of the metals examined were present in high concentrations relative to groundwater levels; copper, for example, reached 10,600 µg L^{-1}. The concentrations of vanadium and of the actinide uranium were approximately 11 and 10 times higher, respectively, than in the groundwater outside the repository.

4.4.3 Group KBU10004 + KBU10006

The pore water in the KBU10004 + KBU10006 group was different from that of the KBU10001 and KBU10002 + KBU10008 groups (Tables 4-3 and 4-4). The pore water had sodium and potassium levels 7 and 21 times higher and calcium levels 3 times lower, respectively, than in the groundwater outside the Prototype. Sulphate was present in levels 17 times that of the surrounding groundwater. The silica level was 7 times that of the groundwater. The concentrations of metals were higher in the pore water than in the groundwater outside the Prototype. The metals examined were mostly present in concentrations of 0.1–100 µg L^{-1}, except for copper, molybdenum, and rubidium, which were more abundant, being present in concentrations of 947, 1,430, and 279 µg L^{-1}, respectively. The concentration of vanadium was 4 times higher (0.7 µg L^{-1}) and the concentration of the actinide uranium was approximately 24 times higher (3.8 µg L^{-1}) than in the groundwater outside the repository.

Table 4-1. The gas content at the sampling points inside the prototype repository on the 2010-11-15 sampling occasion. The gas is presented in ppm or ppt of the total amount of extracted gas.

Sampling point	SKB no.	Date	P[a] (bar)	H_2 (ppm)	CO (ppm)	CH_4 (ppm)	CO_2 (ppm)	C_2H_6 (ppm)	C_2H_4 (ppm)	He (ppt)	O_2 (ppt)	N_2 (ppt)	Gas/water (mL/mL)
KBU10001	20502	2010-11-15	4.4	22.7	7.15	54.5	7,790	bd	bd	bd	116	887	2.6/45
KBU10002	20503	2010-11-15	4.4	19.5	11.0	1440	39,600	bd	2.23	bd	55.5	883	3.7/45
KBU10003		2010-11-15	1.1										
KBU10004		2010-11-15	1.7										
KBU10005		2010-11-15	1.0										
KBU10006		2010-11-15	1.1										
KBU10007		2010-11-15	1.1										
KBU10008	20507	2010-11-15	4.4	186	6.10	914	5,730	bd	bd	bd	41.0	966	6.0/44
KB513		2010-11-15	1.1										
KB514		2010-11-15	1.2										
KB613		2010-11-15	1.2										
KB614		2010-11-15	1.8										
KFA01		2010-11-15	1.9										
KFA02		2010-11-15	1.2										
KFA03		2010-11-15	1.7										
KFA04	20509	2010-11-15	12.1	17.9	4.49	2,180	5,410	2.25	bd	1.51	18.9	964	8.3/45

Table 4-2. The microbial composition at the sampling points inside the prototype repository on the 2010-11-15 sampling occasion.

Sampling point	SKB no	Date	TNC (mL^{-1})	Stdev TNC	ATP (amol mL^{-1})	Stdev ATP	CHAB (mL^{-1})	Stdev CHAB	MPN SRB (mL^{-1})	MPN IRB (mL^{-1})	MPN MOB (mL^{-1})
KBU10001	20502	2010-11-15			13,800	3,190	43	38	bd	bd	0.2 (0.1–1)
KBU10002	20503	2010-11-15	50,000		39,900	1,520	13	15	2.3 (0.9–8.6)	0.2 (0.1–1.1)	17,000 (7,000–48,000)
KBU10008	20507	2010-11-15	170,000	45,000	47,700	2,090	47	25	2.2 (0.9–5.6)	8 (3–25)	8,000 (3,000–25,000)
KFA04	20509	2010-11-15	45,000		10,200	700	3	6	0.2 (0.1–1.1)	bd	0.8 (0.3–2.4)

4.4.4 Group KBU10005

The pore water in the KBU10005 group differed from those of the KBU10001 and KBU10002 + KBU10008 groups (Tables 4-3 and 4-4). The pore water had sodium and potassium levels 3 and 8 times higher and calcium levels 3 times lower, respectively, than those in the groundwater outside the Prototype. Sulphate was present in levels 3 times that of the surrounding groundwater. The silica level was 3 times that of the groundwater. The concentrations of metals were higher in the pore water than in the groundwater outside the Prototype. The metals examined were mostly present in concentrations of 0.1–100 μg L^{-1}, except for copper and rubidium, which were more abundant, being present in concentrations of 444 and 188 μg L^{-1}, respectively. The concentration of vanadium was 68 times higher than in the groundwater (11 μg L^{-1}), and the concentration of the actinide uranium was approximately 206 times higher (34 μg L^{-1}) than in the groundwater outside the repository.

4.4.5 Group KFA01–KFA04

The pore water in the KFA01–KFA04 group differed from those of the KBU10001 and KBU10002 + KBU10008 groups, resembling those of the KBU10004 + KBU10006 and the KBU10005 groups (Tables 4-3 and 4-4). This pore water had sodium and potassium levels 2 and 5 times higher and calcium levels 10 times lower, respectively, than those of the groundwater outside the Prototype. Sulphate was present in levels 3 times that of the surrounding groundwater. The silica level was 2 times that of the groundwater. The concentrations of metals were higher in the pore water than in the groundwater outside the Prototype. The metals examined were mostly present in concentrations of 0.1–100 μg L^{-1}, except for nickel and rubidium, which were more abundant, being present in concentrations of 150 and 105 μg L^{-1}, respectively. The concentration of vanadium was 63 times higher than in groundwater (10 μg L^{-1}), and the concentration of the actinide uranium was approximately 226 times higher (37 μg L^{-1}) than in the groundwater.

4.5 Conclusions

4.5.1 Microbial decrease of oxygen and its consequences

The ATP content of the pore water inside the Prototype remained high relative to the ATP content of the groundwater outside. In IPR-08-01, the mean ATP content of the groundwater in 12 sections near the Prototype was calculated to be 5,000 amoles mL^{-1}. Inside the Prototype, the pore water contained 10,000–50,000 amoles mL^{-1} of ATP, which is 2–5 times higher (Table 4-2). Thus, the pore water must be regarded as a biologically active environment.

The main purpose of the microbiological investigations of the Prototype was to evaluate whether the oxygen level in a newly built storage facility would decrease faster due to microbial activity than it would in an abiotic environment. Oxygen levels were generally observed to decrease with time in the Prototype, even though the most recent sampling in 2010 found generally higher oxygen levels (2–12%) than in 2009 (0.6–4%) (Lydmark 2010). There could be several reasons for this uncharacteristically high reading; for example, the sampling tubes could have become damaged since the 2009 sampling, creating oxygen leakage. However, MOB, with their ability to reduce the oxygen content, continued to thrive inside the Prototype sampling points (reaching 17,000 mL^{-1} at the most). This could be beneficial for the long-term storage of spent nuclear fuel, because they eliminate corrosive oxygen. As the oxygen level decreases, however, another possibly problematic microbial group, the anaerobic SRB, will emerge. These microbes are not active in aerobic environments. SRB produce sulphide, another compound corrosive to copper. Anaerobic conditions appear to prevail at some sampling points.

The number of SRB in the backfill and buffer remained fairly low in the pore water, i.e. approximately 2 SRB mL^{-1}, there have occasionally been higher readings, such as 130 mL^{-1} in 2009 (Lydmark 2010). A source of SRB is the bentonite itself, as they have been reported to exist and survive in the MX-80 bentonite used in the Prototype (Masurat et al. 2010). The Prototype decommissioning will indicate how well SRB have coped in recent years in the oxygenated environment and whether they can survive in a water-saturated repository, or whether they will migrate into the backfill and buffer after the oxygen is depleted in the Prototype. The abundance of SRB should thus be investigated in detail during Prototype decommissioning.

Table 4-3. The chemical composition at the sampling points inside the prototype repository on the 2010-11-15 sampling occasion.

Sample point	Sampling occasion	SKB no.	Na (mg L^{-1})	K (mg L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	Cl (mg L^{-1})	SO_4^{2-} (mg L^{-1})	F (mg L^{-1})	Br (mg L^{-1})	Si (mg L^{-1})	Fe (mg L^{-1})	Mn (mg L^{-1})	Li (mg L^{-1})	Sr (mg L^{-1})
KBU10001	2010-11-15	20502	2,060	18.0	656	114		132			9.03	0.30	0.30	0.41	12.8
KBU10002	2010-11-15	20503	1,900	16.1	578	106		2,330			10.1	14.8	1.08	0.38	11.5
KBU10008	2010-11-15	20507	1,620	14.7	613	98.9		145			8.26	0.92	0.66	0.34	11.2
KFA04	2010-11-15	20509	3,510	53.3	51.5	48.9		296			16.5	0.17	0.06	0.15	0.97
KBU10005	2010-11-15	20565	5,950	81.4	241	180		328			23.0	0.006	0.01	0.53	5.93
KBU10006	2010-11-15	20562	11,700	231	213	122		1,960			50.1	0.03	0.25	1.60	8.15

Sample point	Sampling occasion	SKB no.	S (mg L^{-1})	Al (µg L^{-1})	Ba (µg L^{-1})	Cd (µg L^{-1})	Co (µg L^{-1})	Cr (µg L^{-1})	Cu (µg L^{-1})	Hg (µg L^{-1})	Mo (µg L^{-1})	Ni (µg L^{-1})	P (µg L^{-1})	Pb (µg L^{-1})	V (µg L^{-1})
KBU10001	2010-11-15	20502		51.4	70.7	0.10	5.18	46.5	913	0.04	68.8	302	15.9	0.11	0.46
KBU10002	2010-11-15	20503		1,360	121	0	42.4	721	10,600	0.04	460	2,930	53.2	6.17	2.79
KBU10008	2010-11-15	20507		41.9	62.7	0.40	2.27	12.6	165	0.005	33.5	74.8	41.1	2.03	0.74
KFA04	2010-11-15	20509		33.4	44.1	0	2.00	1.27	10.2	0.005	241	150	245	0	10.3
KBU10005	2010-11-15	20565		6.34	151	0.15	0.69	0.67	444	0.02	73.6	15.6	0	0.57	11.1
KBU10006	2010-11-15	20562		25.3	83.4	0	2.03	3.14	947	0.17	1,430	82.2	1,490	0.30	0.67

Sample point	Sampling occasion	SKB no.	Zn (µg L^{-1})	La (µg L^{-1})	Ce (µg L^{-1})	Pr (µg L^{-1})	Nd (µg L^{-1})	Sm (µg L^{-1})	Eu (µg L^{-1})	Gd (µg L^{-1})	Tb (µg L^{-1})	Dy (µg L^{-1})	Ho (µg L^{-1})	Er (µg L^{-1})	Tm (µg L^{-1})
KBU10001	2010-11-15	20502	82.5	0.05	0.04	0	0	0	0	0	0	0	0	0	0
KBU10002	2010-11-15	20503	111	1.75	2.65	0.30	1.27	0.21	0	0.22	0	0.16	0	0.08	0
KBU10008	2010-11-15	20507	104	0.25	0.18	0.04	0.22	0.04	0	0.04	0	0.03	0	0.02	0
KFA04	2010-11-15	20509	23.6	0	0	0	0	0	0	0	0	0	0	0	0
KBU10005	2010-11-15	20565	82.0	0	0	0	0	0	0	0	0	0	0	0	0
KBU10006	2010-11-15	20562	6.95	0	0	0	0	0	0	0	0	0	0	0	0

Sample point	Sampling occasion	SKB no.	Yb (µg L^{-1})	Lu (µg L^{-1})	Sc (µg L^{-1})	Rb (µg L^{-1})	Y (µg L^{-1})	Zr (µg L^{-1})	Sb (µg L^{-1})	Cs (µg L^{-1})	Hf (µg L^{-1})	Tl (µg L^{-1})	U (µg L^{-1})	Th (µg L^{-1})
KBU10001	2010-11-15	20502	0	0	0	41.1	0.22	0.14	0.62	3.77	0	0.10	1.30	0
KBU10002	2010-11-15	20503	0.06	0	0	31.4	1.75	0.51	1.42	0.60	0	0	2.42	0
KBU10008	2010-11-15	20507	0	0	0	31.3	0.87	0.11	0.20	2.88	0	0	0.67	0
KFA04	2010-11-15	20509	0	0	0	105	3.40	0	1.18	9.42	0	1.19	36.8	0
KBU10005	2010-11-15	20565	0	0	0	188	0	0.51	2.30	9.74	0	2.18	33.6	0
KBU10006	2010-11-15	20562	0	0	0	279	0	0.59	0.28	7.82	0	0	3.83	0

Table 4-4. The chemical enrichment factors in the prototype repository sample groups as of 2010-11-15 compared with those of Äspö groundwater.

Sample group	Na	K	Ca	Mg	SO_4^{2-}	Si	Fe	Mn	Li	Sr	Ba	Cd	Hg
KBU10001	1.2	1.7	0.9	1.6	1.1	1.3	1	0.6	0.9	1.1	1.4	1.9	155
KBU10002 + 8	0.7	1.4	0.8	1.4	10.7	1.3	26.2	1.7	0.8	0.9	1.8	4	86.8
KBU10004 + 6	6.7	21.4	0.3	1.7	17.0	7	0.1	0.5	3.4	0.7	1.6	–	654
KBU10005	3.4	7.5	0.3	2.5	2.5	3.2	–	–	1.1	0.5	2.9	2.9	95.1
KFA01–04	2.0	4.9	0.1	0.7	2.6	2.3	0.6	0.1	0.3	0.1	0.8	–	20.6
	Sm	Gd	Dy	Er	Ce	Nd	Rb	Y	Zr	Cs	U	V	
KBU10001	–	–	–	–	0.3	–	–	1.1	14.6	1.3	8.0	2.8	
KBU10002 + 8	427	116	287	89.6	9.4	26.3	14.1	6.4	33.6	0.6	9.5	10.8	
KBU10004 + 6	–	–	–	–	–	–	–	–	63.3	2.6	23.5	4.1	
KBU10005	–	–	–	–	–	–	–	–	55.1	3.2	206	68.1	
KFA01–04	–	–	–	–	–	–	–	16.6	–	3.1	226	63.2	

4.5.2 Mineral dissolution and bacterial activity affects the pore water chemistry

Prototype tunnel backfill was prepared from 70% crushed rock and 30% sodium-exchanged bentonite material from Milos in Greece (Gunnarsson 2002). It has been suggested that cation exchange and interactions with, for example, calcite, gypsum, and cristobalite could affect the pore water chemistry (Karnland 2007). Enriched amounts of ions and dissolved solids could be found in the KBU10004 + KBU10006, KBU10005, and KFA01–KFA04 groups (Tables 4-3 and 4-4). The high sodium and potassium and lower calcium concentrations in pore water could be due to cation exchange; this is possible, because the univalent sodium and potassium from the bentonite can readily be replaced with the divalent magnesium and calcium in the montmorillonite interlayers, resulting in higher sodium and potassium concentrations and lower magnesium and calcium concentrations in the pore water. This ion exchange process is noticed also in other experiments such as the ABM experiment. Calcium concentrations in the pore water could be lowered still further by calcite precipitation; Table 4-1 shows that this scenario likely occurred inside the Prototype.

Gypsum dissolution increases the amount of sulphate in the pore water, and this has happened in the KBU10002 + KBU10008, KBU10004 + KBU10006, KBU10005, and KFA01–KFA04 sampling groups in the Prototype (Table 4-1). Cristobalite dissolution could have caused the small rise in silica concentration in the pore water in the KBU10004 + KBU10006, KBU10005, and KFA01–KFA04 sampling groups.

The pore water chemistry data suggest that dissolution of the minerals cristobalite and gypsum occurs in the Prototype. The data also suggest that cation exchange occurs from the sodium and potassium to calcium in the interlayers of the montmorillonite. All these exchanges, in particular the increased sulphate concentration in the pore water, would affect the microbial activity, since sufficient sulphate is one prerequisite for sulphate reduction to occur.

Microbes are experts at both adapting to and exploiting their environment. Microbes can thus both be affected by and affect the chemical composition of the pore water in the Prototype. Many microbes can produce siderophores, which are chelating agents used for iron uptake in deficient environments, i.e. aerobic environments. It is well-known that, using these chelating agents, microbes can also mobilize also other trace elements (Pedersen 2002) and inhibit trace element sorption to solid phases (Kalinowski et al. 2004, 2006). Some microorganisms produce very powerful bioligands, usually denoted pyoverdins, which have a very strong binding affinity for many radionuclides (Johnsson et al. 2006, Essén et al. 2007, Moll et al. 2008b). Chelating agents have been reported for many of the compounds enriched in the Prototype pore water (Table 4-1), i.e. vanadium (Baysse et al. 2000), uranium (Moll et al. 2008a), and cesium (Wendling et al. 2005). Such compounds may have influenced the dissolution of uranium, vanadium, and cesium in the Prototype pore water, since these elements – in particular, uranium – were found to be enriched up to 225 times. Uranium dissolution in the near- and far-field should of course be investigated in this environment, because of the nature of the nuclear waste. The analysis of pore water also revealed very high copper concentrations, up to 10,600 μg L^{-1}, at several sampling points (groups KBU10004 + KBU10006, KBU10005, and KFA01–KFA04). This could indicate that the canisters or, due to short circuit, the cables to the heaters might have released copper and possibly other metals during the incubation underground.

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