International
Progress Report

IPR-10-04

Äspö Hard Rock Laboratory

Prototype Repository

Analyses of microorganisms, gases and water chemistry in buffer and backfill, 2009

Sara Lydmark
Microbial Analytics Sweden AB

Svensk Kärnbränslehantering AB

September 2010

Svensk Kärnbränslehantering AB
Swedish Nuclear Fuel
and Waste Management Co

Box 250, SE-101 24 Stockholm
Phone +46 8 459 84 00

Äspö Hard Rock
Laboratory

Report no.
IPR-10-04
No.
KBP1001
Author
Sara Lydmark
Date
October 2010
Checked by
Daniel Svensson
Date
April 2011
Approved
Mats Ohlsson
Date
September 2011

Äspö Hard Rock Laboratory

Prototype Repository

Analyses of microorganisms, gases and water chemistry in buffer and backfill, 2009

Sara Lydmark

Microbial Analytics Sweden AB

October 2010

Keywords: Fractured rock, Transport of solutes, Performance assessment, Site characterisation

Abbreviations

Explanations to some of the abbreviations used in the text.

Abbrivation	Meaning	Short description
AA	Autotrophic Acetogens	Microbes able to produce acetate from carbon dioxide and hydrogen
ATP	Adenosine Tri Phosphate	Energy carrier in a living organism
cDNA	Complementary DeoxyriboNucleic Acid	RNA that has been altered to DNA by enzymatic teqnuiqes
CFU	Colony Forming Unit	A cell which has divided repetedly, e.g. on an agar plate, forming a dense colony of a large number of identical cells
CHAB	Culturable Heterotrophic Aerobic Bacteria	Microbes able to live on oxygen and organic carbon and that grow in the laboratory
DNA	DeoxyriboNucleic Acid	The genetic code, which builds the genome unique for each organism
FID	Flame Ionization Detector	Detector for flammable gases
GC	Gas Chromatograph	Separates different gases in specific columns
IRB	Iron-Reducing Bacteria	Microbes able to reduce iron in their respiration
MOB	Methane-Oxidizing Bacteria	Oxygen-dependent microbes able to use methane as a carbon and energy source
MPN	Most Probable Number	Method for enumeration of microbes
PCR	Polymerase Chain Reaction	Technique applied to exponentially increase DNA or RNA above detection limit
PEEK	PolyEtherEtherKetone	Material resistant to most chemicals and with low permeability to gases
RGD	Reduction Gas Detector	Detector for reduced gases
RNA	RiboNucleic Acid	Part of the ribosome, which constructs all the proteins in an organism.
SRB	Sulphate-Reducing Bacteria	Microbes able to reduce sulphate in their respiration
TCD	Thermal Conductivity Detector	Detector able to detect all gases other than the carrier gas.
qPCR	Quantitative Polymerase Chain Reaction	Technique applied to exponentially increase DNA or cDNA and quantitate the number of genes by comparison to a known standard

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 Prototypförvaret dränerat. 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 och utanför Prototypförvaret under 2009.

Analyser av vätgas, helium, kvävgas, syrgas, kolmonoxid, koldioxid, metan, etan och eten utfördes på följande provtagningspunkter i Prototypförvaret: KBU10001, KBU10002, KBU10004, KBU10006, KBU10008, KFA01 och KFA04. De provtagningspunkter i Prototypförvaret som innehöll porvatten analyserades med avseende på ATP innehåll (dvs biovolymen), odlingsbara heterotrofa aeroba bakterier (CHAB), sulfatreducerande bakterier (SRB), metanoxiderande bakterier (MOB) autotrofa acetogener (AA) och i vissa fall järnreducerande bakterier (IRB). Odlingsmetoderna jämfördes även med molekylära qPCR metoder för att utvärdera dessa inför brytningen av förvaret 2011. Det uppsamlade porvattnet från Prototypförvaret skickades efter gas och mikrobanalyserna till kemisk analys för så många ämnen som vattnet räckte till. Utöver detta analyserades grundvatten från två borrhållssektioner i den omgivande bergmatrisen med avseende på gas, mikrobiologi och redox. 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 de molekylära metoderna som testades för första gången på prover från Prototyp förvaret visade stor potential för användning under brytningen 2011. I IPR 08-01 framkom att de olika provtagningspunkterna skiljer sig relativt markant från varandra. De 16 punkterna har därför delats därför 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, pH 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 backfillen nåddes ännu inte av grundvattnet år 2007 (KBU10001), men innehöll nu porvatten med grundvattenliknanade egenskaper.

Gassammansättningen i de olika provgrupperna var enhetlig när det gäller kvävgashalten som ökade och syrgashalten som minskade över tiden. Från maj 2007 har syrgashalten i porvattnet minskat från 3-7 % till 0.6-4 %, med undantag för ett porvatten som innehöll 15 % syrgas. Detta kan även jämföras med syreandelen i gasfasen 2005 som var 10–18 %. Koncentrationerna av vätgas, metan, helium and koldioxid varierade, speciellt i de provgrupper där det fanns extraherbart porvatten. ATP analyserna visade att biomassan i Prototypförvaret är hög jämfört med det omgivande grundvattnet. De

mikrobiologiska resultaten visade att aeroba bakterier som MOB och CHAB frodades i det aeroba Prototypförvaret. Kemidata visade ännu en gång skillnader mellan provgrupperna. pH och koncentrationerna av natrium och kalium var högre i porvattnet än i grundvattnet utanför. Koncentrationerna av kalcium och stundtals magnesium däremot var lägre än i grundvattnet. Detta tyder på att katjonbyte i montmorillonitens mellanlager förekommit. I provtagningspunkter som innehöll aktiva mikrober anrikades rubidium, cesium, vanadin och uran från två till 200 gånger jämfört med grundvattnet. Det är möjligt att mikrober var ansvariga genom exkretion av ämnesspecifika ligander.

Övergripande visade observationerna som presenteras här att vår hypotes håller om att syre kommer att konsumeras av bakterier i ett relativt kort tidspann (dvs veckor till år) i motsats till det långa tidsspann som förväntas av 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).

Abstract

The Prototype repository is an international project to build and study a full-scale model of the planned Swedish final repository for spent nuclear fuel. The Prototype repository differs from a real storage in that it is drained. For example, this makes the swelling pressure lower in the Prototype repository compared with a real storage. 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 one of the specific aims is to monitor the microbial consumption of oxygen *in situ* in the Prototype repository. This document describes the results of the analyses of microbes, gases, and chemistry inside and outside the Prototype in 2009.

Hydrogen, helium, nitrogen, oxygen, carbon monoxide, carbon dioxide, methane, ethane, and ethene were analysed in the following sampling points in the Prototype repository: KBU10001, KBU10002, KBU10004, KBU10006, KBU10008, KFA01 and KFA04. Where the sampling points in the Prototype delivered pore water, the water was analysed for amount of ATP (i.e., the biovolume), cultivable heterotrophic aerobic bacteria (CHAB), sulphate-reducing bacteria (SRB), methane-oxidizing bacteria (MOB), autotrophic acetogens (AA) and in some cases iron-reducing bacteria (IRB). Cultivation methods were also compared with qPCR molecular techniques to evaluate these before next year´s decommission of the Prototype repository. The collected pore water from the Prototype repository was subject to chemistry analysis (as many analyses were conducted as the amount of water allowed). In addition, groundwater from two borehole sections in the rock surrounding the Prototype was analysed regarding its gas composition, microbiology and redox. Chemistry data from a previous investigation of the groundwater outside the Prototype repository were compared with the pore water chemistry.

The in 2007 improved sampling and analysis protocols worked very well. Also, the molecular methods that were tested for the first time in the Prototype showed promising potential. IPR 08-01 revealed that many of the hydrochemical sampling points differ quite remarkably from each other. The 16 sampling points were therefore divided into seven sampling groups with similar properties. The properties of one sampling group (i.e., KBU10002+8) resembled those of the groundwater, while others (i.e., KBU10004+6, KBU10005, and KFA01-04) differed, for example, in microbial composition, salinity, sulphate content, pH, and the concentrations of calcium, potassium, magnesium, sodium, and many dissolved metals, actinides, and lanthanides. One sampling group contained sampling points that seemed to be in contact with tunnel air (KBU10003+7). Another sampling group contained 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 consisted of 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 and the oxygen content decreasing with time. In most sampling groups, the oxygen content in the pore water had decreased from 3-7% as of May 2007 to 0.6-4% in 2009. This can also be compared with the proportion

of oxygen in the gas phase in 2005, which was 10-18%. Hydrogen, methane, helium, and carbon dioxide concentrations varied, especially in the sampling groups with extractable pore water. ATP analyses demonstrated that the biomass in the Prototype repository is high compared to the surrounding groundwater. The microbiological results indicated that aerobic microbes, such as MOB and CHAB, thrived in the aerobic Prototype environment. The chemistry data indicated differences between the sampling groups: pH and concentrations of Na and K were higher in the Prototype pore water than in the groundwater outside, while Ca and sometimes Mg concentrations were lower than in the groundwater. Obviously, cation exchange in the montmorillonite interlayers occurs. At sampling points containing active microbes, however, Rb, Cs, V, and U were enriched from two to almost 200 times the groundwater levels; microbes are possibly responsible for the dissolution of these substances by the excretion of compound-specific ligands.

Overall, the observations presented here strongly supported 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 indicated that oxygen is disappearing and that MOB were responsible for at least some of the oxygen decrease. The microbes also affected the chemistry in the Prototype repository, both indirectly (by being active and changing redox and pH) and possibly directly (via compound-specific ligands).

Contents

1 Introduction

The Prototype repository is an international project to build and study a full-scale model of the planned deep repository for Swedish nuclear fuel. The Prototype repository differs from a real storage in that it is drained. For example, this makes the swelling pressure lower in the Prototype repository compared with a real storage. 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 different parts of the Prototype repository is being monitored. One specific aim is to monitor the microbial consumption of oxygen. Because oxygen previously has been shown to be consumed within two weeks in granitic media at Äspö (TR 01-05), oxygen is hypothesized to be consumed by bacteria also 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 gases in buffer and backfill has been tested *in situ* (AP TD F63P1-04-012). The results and evaluation of the first *in situ* measurements were presented in an international progress report (IPR), IPR-04-26 (2004). Analyses were subsequently performed in fall 2004, 2005, and 2006, and in fall/summer 2007. The results and interpretations of these measurements are reported in IPR-08-01 (2008)

1.1 Design of the Prototype repository

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

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 2. Eight sample collectors have been installed (AP TD F63-01-054) for continuous hydrochemical sampling in section 1 (inner section) (Table 1), six in the backfill (Figure 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 closest to the tunnel), DA3545G01 and DA3551G01, are labelled hole numbers 5 and 6, respectively, in Figure 2.

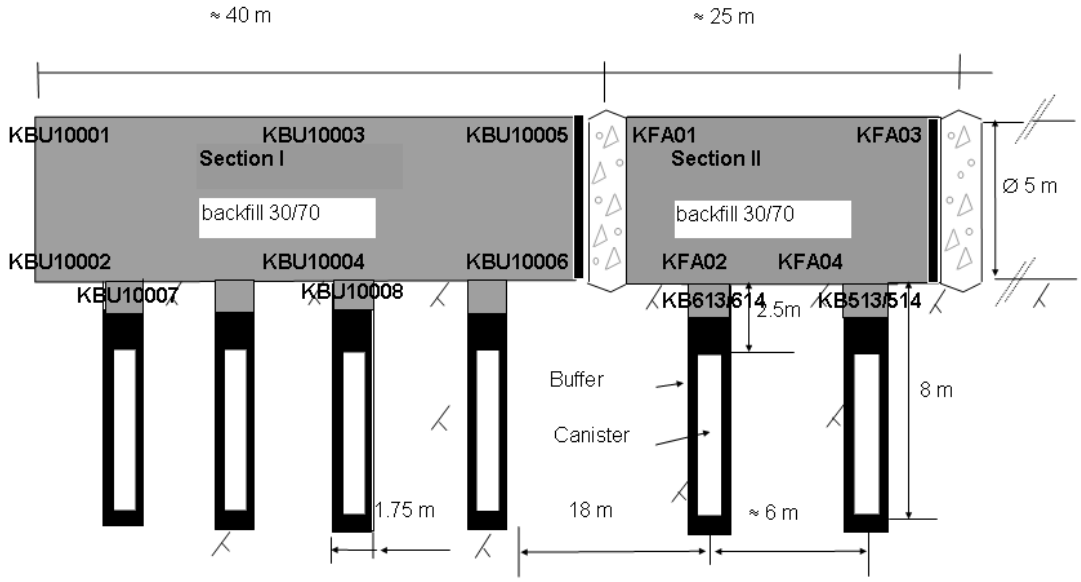

Figure 1. *Schematic of the Prototype repository (adapted from IPR 99-34)*

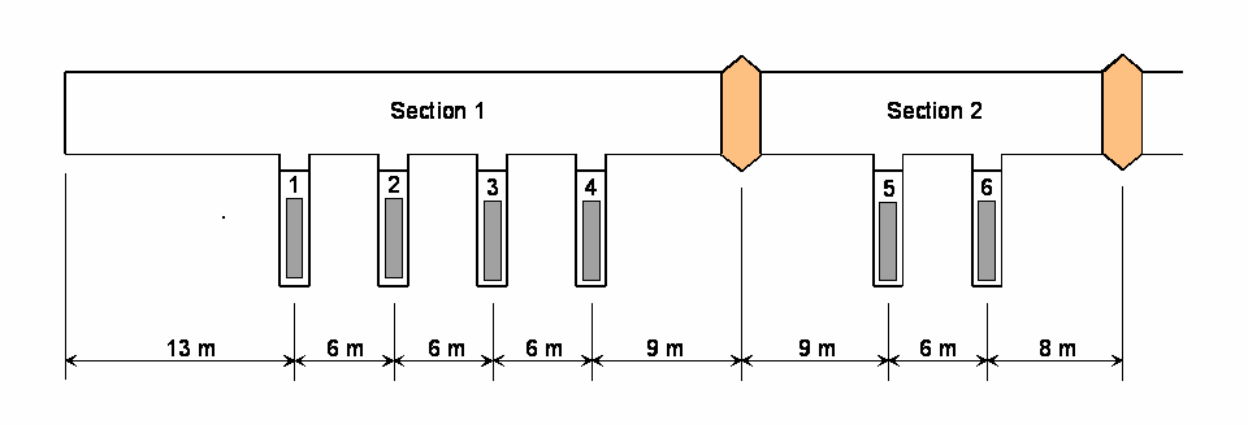

Figure 2. *The deposition holes in the Prototype repository (from AP TD F63.1-04-018)*

In section 2 (outer section) (AP TD-F63-02-040) (Table 2), four sample collectors were placed in the backfill (Figure 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. The exact positions (coordinates) of the titanium cups in the buffer and backfill are given in AP TD F63-01-054 and AP TD-F63-02-040.

Table 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)

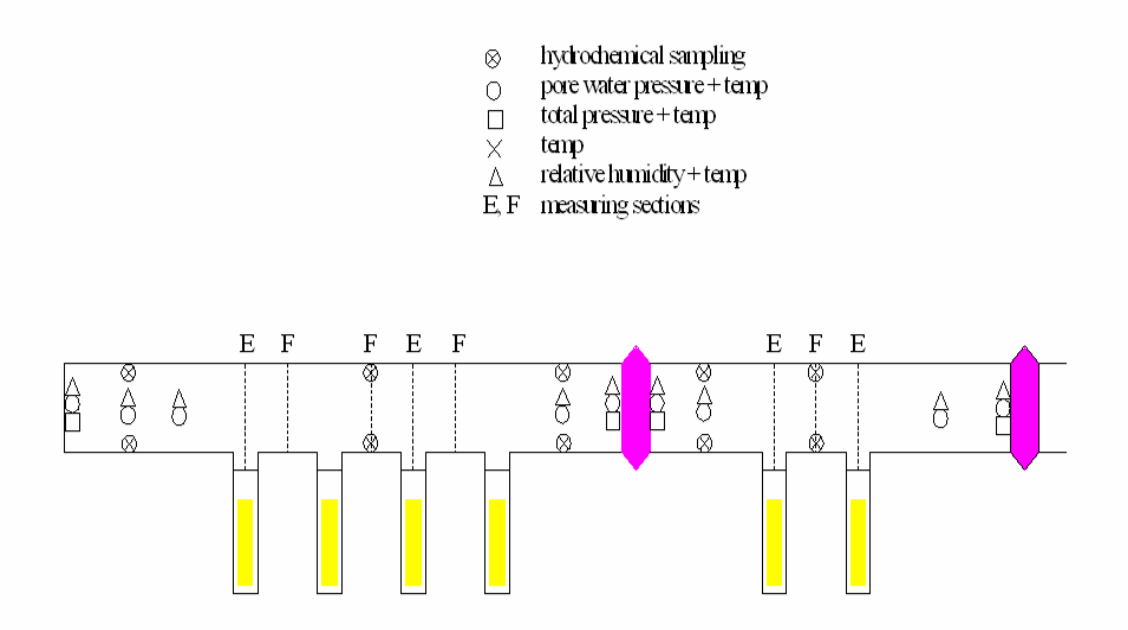

Figure 3. *Figure showing the positions of the titanium cups in the backfill in sections 1 and 2 (from AP TD F63-02-040)*

The sample collectors consist of a titanium cup with a titanium filter mounted on top and polyetheretherketone (PEEK) tubes connected to the bottom. The length of each tube is at most approximately 79 m and the inner diameter is 2 mm. This gives a maximal sample tube volume of approximately 250 mL.

Table 2. Sample collectors in section 2, the outer section closest to 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 canisters in the deposition holes contain heaters to simulate the heat emitted by the nuclear waste during actual storage. The Prototype repository will let us study many different 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
- Stresses and displacements 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 international progress report (IPR) 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 contains compiled results from 2009 and comparisons to 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** – a microbial life strategy that includes consumption of oxygen and methane and production of carbon dioxide
- **Acetogenesis** – a microbial life strategy that produces organic carbon in the form of acetate using carbon dioxide and hydrogen gas.

- **Heterotrophy** – a microbial life strategy that includes consumption of oxygen, ferric iron and sulphate (and other compounds not discussed in this context) and organic carbon and production of biomass and carbon dioxide

- **Autotrophy** – a microbial life strategy that includes consumption of sulphate, carbon dioxide, and hydrogen gas (autotrophy also includes other compounds than sulphate and hydrogen gas, but these are not discussed in this context) and production of biomass or organic carbon.

One of the main questions to be answered in this project is whether microbial activity can decrease the oxygen levels *in situ* in the Prototype repository. It is also important to examine whether microbial activity (i.e., sulphate reduction ending in sulphide production, acetogenisis producing organic carbon or iron reduction enhancing illitisation) could compromise the stability of the copper canisters.

Water samples from the Prototype repository and outside 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), autotrophic acetogens (AA) and iron-reducing bacteria (IRB). In addition, molecular qPCR methods were also used to examine the activity and the numbers of MOB and SRB in the water.

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

In the study, we succeeded in extracting pore water from several sampling collectors in the Prototype repository. The pore water was sent for partial class 5 analysis. Microbial activity can affect the chemistry in the pore water from the buffer and the backfill in several ways:

- Microbial activity can lead to **dissolution of various minerals** in the bentonite (i.e., gypsum, $CaSO_4 \times 2\ H_2O$), which contains sulphate that SRB need for their metabolism.

- The effect can be **direct** if microbes excrete certain compounds, such as bioligands, that can complex specifically with various metals they need in their metabolism.

The effect can also be **indirect**, depending on the extent to which the microbial activity increases the pH, which in a unspecific manner can change the solubility constants of all the tested elements.

2 Material and methods

2.1 Sampling

2.1.1 Sampling and analyses inside Prototype

In June 2009, gas, chemistry and microbe samplings from seven sampling points inside the repository, KBU10001, KBU10002, KBU10004, KBU10006, KBU10008, KFA01 and KFA04 (Table 1, Table 2), were performed. These samples were analysed as given in Table 3. Sampling was performed using special 45-mL high-vacuum, stainless steel pressure vessels (Mymeko, Göteborg, Sweden) (Figure 4). Before the pressure vessels were connected, the pressure at each sampling point inside the Prototype repository was registered using a 0-40 bar WIKA manometer (order no. 7082534, Klingenberg, Switzerland). After that, the tubing was flushed with nitrogen gas and the pressure vessel was connected. The handle on the pressure vessel was opened so that water could be extracted from the Prototype repository because of the vacuum inside the vessel. The pressure increase was measured at each pressure vessel and, when the pressure inside the vessel was the same as it was in the sampling point before the connection, the handle was closed and the pressure vessel transported to Microbial Analytics Sweden AB in Mölnlycke. At the laboratory, samples for gas and microbial analyses were extracted directly from the high-vacuum, stainless steel pressure vessels. The remaining water was send to SKB chemistry laboratory for Class 5 analysis.

In October 2009, additional molecular samplings from two sampling points inside the repository, KBU10008 and KFA04, were performed (Table 3). The sampling was performed as described aboved using special 45-mL high-vacuum, stainless steel pressure vessels with the exception the the vessels were pre-filled with 33 mL RNA Later (no. AM7021; Ambion, Stockholm, Sweden) to conserve the sampled water.

Figure 4. *Pressure vessels used for extracting pore water and gas from the Prototype repository.*

2.1.2 Sampling and analyses outside Prototype

In October 2009, gas, chemistry, microbial and molecular samplings from two sections with groundwater outside the repository were performed. The sampled groundwater came from KA3542G01:3 and KA3600F:2 (Figure 5). Water standing in the tube to the section was rinsed out before sampling. Gas samplings were performed using a PVB sampler filled with neon at a pressure of 3 bar in the lower compartment. The PVB was connected to the groundwater section and opened. Before filling, the upper compartment was flushed with 100 mL groundwater. For microbiological analyses, samples were collected in sterile anaerobic tubes (27 mL) stopped with butyl rubber stoppers. These were evacuated and flushed twice with N_2/CO_2 (80/20%) gas and left filled with this gas mixture at atmospheric pressure. Each tube was attached to its borehole via a short sterile tube connector and a needle. After sampling, the tube was transported to the MICROBE field laboratory, approximately 50 m from the sampling site. Inoculation and sample preparation were undertaken there, immediately after sampling. Samples for molecular analyses were taken in 50-mL Falcon tubes prefilled with 33 ml RNA Later. The SKB chemistry laboratory performed a Class 3 analysis on the water.

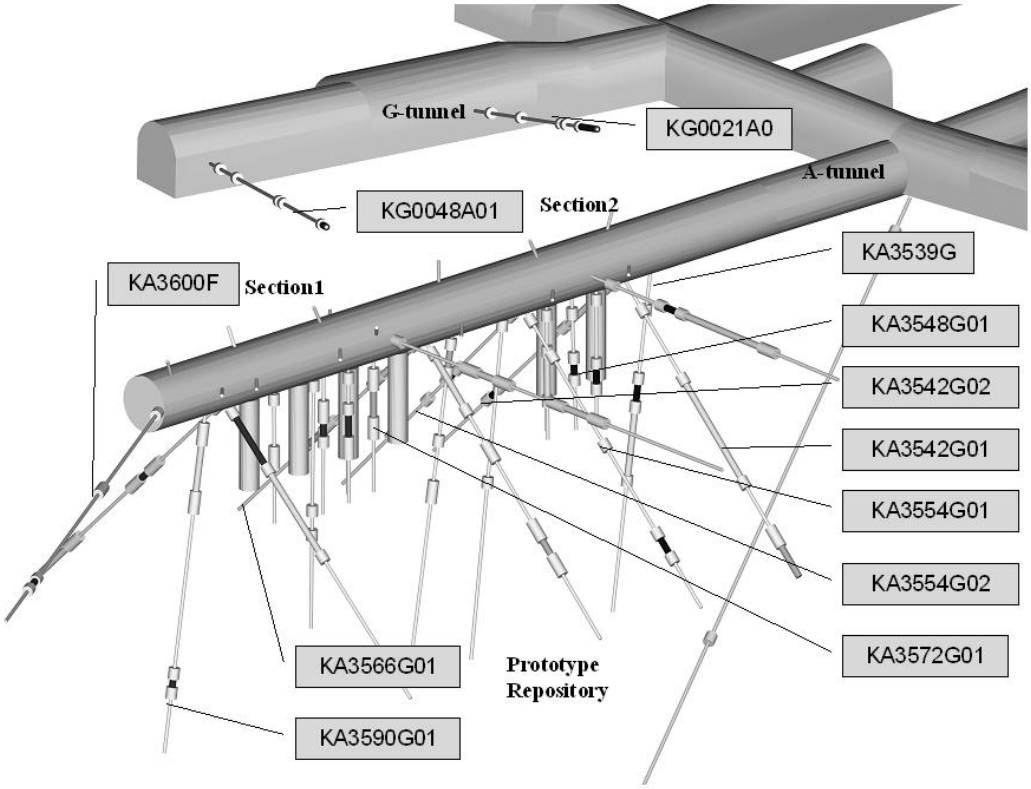

Figure 5. *Location of groundwater boreholes outside the Prototype repository.*

Table 3. Analyses of samples from inside and outside the Prototype repository, 2009

Borehole	Sampling date	Gas analyses	Microbial analyses	Molecular analyses	Chemical analyses
KBU10001	2009-06-01	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN AA, MPN MOB		Class 5
KBU10002	2009-06-01	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN AA, MPN MOB		Class 5
KBU10003	2009-06-01	Pressure			
KBU10004	2009-06-01	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2			
KBU10005	2009-06-01	Pressure			
KBU10006	2009-06-01	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN AA, MPN MOB		Class 5
KBU10007	2009-06-01	Pressure			
KBU10008	2009-06-01	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN AA, MPN MOB, MPN IRB		Class 5
KB513	2009-06-01	Pressure			
KB514	2009-06-01	Pressure			
KB613	2009-06-01	Pressure			

Borehole	Sampling date	Gas analyses	Microbial analyses	Molecular analyses	Chemical analyses
KB614	2009-06-01	Pressure			
KFA01	2009-06-01	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN AA, MPN MOB		Class 5
KFA02	2009-06-01	Pressure			
KFA03	2009-06-01	Pressure			
KFA04	2009-06-01	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, CHAB, MPN SRB, MPN AA, MPN MOB, MPN IRB		Class 5
KA3542G01:3	2009-10-22	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	MPN SRB	qPCR ApsA, MxaF, PmoA (numbers and activity)	SKB Class 3
KA3600F:2	2009-10-22	Pressure, H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	MPN SRB	qPCR ApsA, MxaF, PmoA (numbers and activity)	SKB Class 3
KBU10008	2009-10-22			qPCR ApsA, MxaF, PmoA (numbers and activity)	
KFA04	2009-10-22			qPCR ApsA, MxaF, PmoA (numbers and activity)	

2.2 Gas analyses

2.2.1 Extraction of gas

The gas was extracted from the pressure vessels and PVB samplers and the volume of the extracted gas was measured using equipment specially built and designed for analysing gas in groundwater.

The sample was transferred to a vacuum container, and the gas in the sampled pore water was boiled off under vacuum (i.e., water vapor pressure) at room temperature. After this extraction, the gas was compressed and transferred to a 10-mL syringe (order no. 10MDR-VLLMA-GT, 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 with a butyl rubber stopper sealed with an aluminum crimp seal. The vial was evacuated and flushed twice with nitrogen and left at high vacuum (10^{-4} bar). Copper sulphate (dehydrant) was added to adsorb any traces of water in the gas, because water causes the gas chromatographs to experience troublesome baseline drifts.

2.2.2 Calibration and reproducibility

The chromatographs were calibrated and tested using four gas mixtures, as follows:

Special gas 1 (Linde specialgas, AGA, certificate no.: 28810-3)

He	25,700	ppm
H_2	964	ppm
O_2	10,900	ppm
Nitrogen		962,436 ppm

Special gas 2 (Linde specialgas, AGA, certificate no.: 28757-1)

Ar	1000	ppm
CH_4	2740	ppm
CO_2	1040	ppm
CO	9.75	ppm
Nitrogen		995,210 ppm

Special gas 3 (Linde specialgas, AGA, certificate no.: 28749-1)

C_2H_6	253	ppm
C_2H_4	257	ppm
C_2H_2	248	ppm
C_3H_8	252	ppm
C_3H_6	238	ppm
Nitrogen		998,752 ppm

Special gas 4 (Linde specialgas, AGA, certificate no.: 30008-1)

H_2	24.6	ppm
CO	24.9	ppm
Nitrogen		999,950 ppm

Multiple calibration points were used for the Varian Star 3400CX gas chromatograph (Varian, Solna , Sweden). The KAPPA-5 chromatograph (Trace Analytical, Menlo Park, CA, USA) used single-point calibrations. Calibration gases were analysed

immediately before analysis of samples and the calibration results were used in calculating the concentrations of all gases in the samples.

Volumes of 1–1000 µL were injected into each gas chromatograph. The injection volume used was adjusted according to the sensitivity range of each instrument and detector. Several injections were commonly needed to determine the proper injection volume for each gas. Based on previous analyses of gas from the Äspö area, the following was concluded:

- The precision of the extractions was approximately ± 6%
- The uncertainty of the instruments and of the repeated injections was low, typically 0–4%.
- The calibration gases used had a maximum accepted mixing uncertainty of ± 2%.
- In total, the analytical uncertainty was no higher than ± 12%.

2.2.3 Gas analysis

Low concentrations of hydrogen (<20 ppm) were analysed on a KAPPA-5/E-002 analyser gas chromatograph (Trace Analytical) equipped with a 156 × 1/16-inch stainless steel HayeSep column (Scantec Lab, Partille, Sweden) in line with a 31 × 1/8-inch stainless steel Molecular Sieve 5A column (Sigma-Aldrich, Stockholm Sweden), which was subsequently attached to a reduction gas detector (RGD). Nitrogen was used as the carrier gas. The sample was injected into a 1000-µL injection loop. The sample usually had to be diluted to reach the detection range of the instrument, as it has the most sensitive hydrogen detector on the market. Calibration gas 4 was used. The detection limit of the instrument with a 0.1-mL injection loop is 10^{-12} L (1 ppb).

High concentrations of hydrogen (>20 ppm) were analysed on a Varian Star 3400CX gas chromatograph (Varian) using a thermal conductivity detector (TCD) with an oven temperature of 65°C, a detector temperature of 120°C, and a filament temperature of 250°C. The hydrogen gas was separated using a Porapak-Q column (2 m × 1/8 inch diameter; Agilent Technologies, Santa Clara, CA, USA) followed by a 6-m × 1/8-inch molecular sieve 5A column (Scantec Lab) with argon as the carrier gas. Calibration gases 1 and 2 were used. The detection limit of the instrument with a 250-µL injection loop is 5×10^{-9} L (20 ppm).

Carbon monoxide was analysed on a KAPPA-5/E-002 analyser gas chromatograph (Trace Analytical) equipped with a 156 × 1/16-inch stainless steel HayeSep column (Scantech Lab) in line with a 31 × 1/8-inch stainless steel molecular sieve 5A column (Sigma-Aldrich) , which was subsequently attached to a reductive gas detector (RGD, Trace Analytical). Nitrogen was used as the carrier gas. The sample was injected into a 1000-µL injection loop. The sample usually had to be diluted to reach the detection range of the instrument, which has the most sensitive carbon monoxide detector on the market. These results were compared with those obtained using the Varian Star 3800CX analyser and reported when they agreed. The detection limit of the instrument with a 0.1-mL injection loop is 10^{-12} L (1 ppb).

Methane was analysed on a Varian Star 3400CX gas chromatograph (Varian) using a flame ionization detector (FID) with an oven temperature of 65°C and a detector temperature of 200°C. The methane gas was separated using a Porapak-Q column (2 m × 1/8 inch diameter; Agilent Technologies) and analysed on the FID with nitrogen as

the carrier gas. This configuration used a 156 × 1/16-inch stainless steel HayeSep column and a FID detector.

High concentrations of methane, above 1%, required very small injection volumes, with nitrogen as the carrier gas, on the FID. The use of a small injection volume increased the uncertainty of the results. Therefore, the sensitivity of the analysis was reduced as required by analysing methane with helium as the carrier gas and using the TCD. The results obtained using an FID were compared with those obtained using a TCD and reported when they agreed. The detection limit of the instrument with a 250-µL injection loop is 0.1×10^{-9} L (0.4 ppm).

Carbon dioxide was analysed on a Varian Star 3400CX gas chromatograph using a flame ionization detector (FID) with an oven temperature of 65°C and a detector temperature of 200°C. The carbon dioxide gas was separated using a Porapak-Q column (2 m × 1/8 inch diameter) and transformed to methane using a 10% Ni_2NO_3 "methanizer" fed with hydrogen gas (9.375 × 1/8 inch diameter, temperature 370°C). Carbon dioxide was finally analysed as methane on the FID with nitrogen as the carrier gas. This configuration used a 156 × 1/16-inch stainless steel HayeSep column (Scantec Lab) and an FID detector. The detection limit of the instrument with a 250-µL injection loop is 0.1×10^{-9} L (0.4 ppm).

Ethane, and ethane + ethylene were analysed on a Varian Star 3400CX gas chromatograph using a flame ionization detector (FID) with an oven temperature of 65°C and a detector temperature of 200°C. The ethane, and ethane + ethylene 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. This configuration used a 156 × 1/16-inch stainless steel HayeSep column and a FID detector. Ethene and ethylene cannot be separated using the present configuration (Porapack Q). The detection limit of the instrument when using a 250-µL injection is 0.1×10^{-9} L (0.4 ppm).

Helium was analysed on a Varian Star 3400CX gas chromatograph using a thermal conductivity detector (TCD) with an oven temperature of 65°C, a detector temperature of 120°C, and a filament temperature of 250°C. The helium gas was 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 as the carrier gas. The detection limit of the instrument with a 250-µL injection loop is 5×10^{-9} L (20 ppm).

Oxygen was analysed on a Varian Star 3400CX gas chromatograph using a thermal conductivity detector (TCD) with an oven temperature of 65°C, a detector temperature of 120°C, and a filament temperature of 250°C. The oxygen gas was 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 as the carrier gas. The detection limit of the instrument with a 250-µL injection loop is 250×10^{-9} L (100 ppm).

Nitrogen was analysed on a Varian Star 3400CX gas chromatograph using a thermal conductivity detector (TCD) with an oven temperature of 65°C, a detector temperature of 120°C, and a filament temperature of 250°C. The nitrogen gas was separated using a Porapak-Q column (2 m × 1/8 inch diameter) followed by a molecular sieve 5A column (6 m × 1/8 inch). Argon or helium can be used as the carrier gas. The results obtained using argon were compared with those obtained using helium and reported when they agreed. The detection limit of the instrument with a 250-µL injection loop is 25×10^{-9} L (100 ppm).

2.3 Microbial analyses

2.3.1 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 kit reagent HS (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 reagent HS and the tube was monitored and allowed to decrease to below 50 RLU s^{-1} prior to 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 reagent HS 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 reagent HS and B/S extractant were measured at regular intervals to control for possible ATP contamination. Values of 1600 ± 500 amol ATP mL^{-1} (n = 10) were obtained with clean solutions, while solutions displaying values above 1600 amol ATP mL^{-1} were disposed of.

The ATP concentration of the analysed samples was calculated as follows:

$$\text{amol ATP mL}^{-1} = (I_{smp} - I_{bkg}) / ((I_{smp + std} - I_{bkg}) - (I_{smp} - I_{bkg})) \times 10^9 / \text{sample volume}$$

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, and the results were recently published (Eydal and Pedersen, 2007).

2.3.2 Determining cultivable aerobic bacteria

Petri dishes containing agar with nutrients were prepared for determining the numbers of cultivable 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 AGW containing 0.9 g L^{-1} of NaCl; 0.1-mL portions of each dilution were spread with a sterile glass rod on the plates in triplicate. The plates were incubated for between 7 and 9 days at 20°C, after which the number of colony forming units (CFU) was counted; plates with between 10 and 300 colonies were counted.

2.3.3 Preparing media for most probable numbers of cultivable anaerobic microorganisms

Media for determining the most probable number of microorganisms (MPN) in groundwater were formulated based on previously measured chemical data regarding granitic groundwater. 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) sealed with butyl rubber stoppers (no. 2048-117800; Bellco Glass) and sealed with aluminium crimp seals (no. 2048-11020; Bellco Glass).

2.3.4 Inoculations and analysis for anaerobic microorganisms

Inoculations for IRB, SRB, and AA were performed in the laboratory as soon as possible. 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). AA were detected by means of acetate production using an enzymatic UV method (enzymatic bioanalysis kit no. 10 139 084 035; Boehringer Mannheim/R-Biopharm, Food diagnostics, Göteborg, Sweden) with a UV–visible spectrophotometer (per SRB detection). Product formation at a concentration twice or above that of the un-inoculated 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 compared with 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).

2.3.5 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 1000 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. 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 between 2 and 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}.

2.4 Molecular analyses

2.4.1 DNA extraction

DNA were extracted from the water/RNA Later mixture. First, the samples were centrifuged at 8000 rpm (5800 g), for 15 min in a Heraus Multifuge 3SRplus centrifuge (Thermo Fischer Scientific, Waltham, MA, USA). The supernatant was discarded, and the DNA was extracted from the pellet using the DNeasy Blood&Tissue DNA extraction kit (no. 69504; QIAGEN, Solna, Sweden) according to the manufacturer's protocol for Gram positive bacteria. The DNA extractions were stored at –20°C.

2.4.2 RNA extraction

To extract RNA from the water/RNA Later mixture, the samples were centrifuged at 8000 rpm (5800 g), for 10 min in a Heraus Multifuge 3SRplus centrifuge (Thermo Fischer Scientific, Waltham, MA, USA). The supernatant was discarded, and the RNA was extracted from the pellet using the RNeasy mini kit according to the manufacturer's protocol (Qiagen inc.) The RNA extractions were stored at –80°C.

cDNA synthesis

Complementary DNA (cDNA) was produced with the RNA using the QuantiTect Reverse Transcription kit according to the manufacturer's protocol (Qiagen inc.).

Quantitative PCR

DNA or cDNA was serially diluted six times in ¼ increments, 16 ng per reaction being the most concentrated standard sample. The qPCR reactions were run in duplicate. Primers for the genes ApsA (designed for the experiment), MxaF (Horz et al. 2001) and PmoA (Horz et al. 2001) were used. The gene ApsA is present in SRB, MxaF in MOB able to degrade methanol and PmoA in MOB able to degrade methane. Each PCR mixture contained 1.0 µL of the primer (10 pmol μl^{-1}), 16 ng of DNA, 12.5 µL Stratagene Brilliant SYBR II qPCR Mastermix 2X (AH Diagnostics AB, Skärholmen,

Sweden) and sterile water to a final reaction volume of 25 µL. Amplification was carried out on a Startagene Mx35005P qPCR thermal cycler (AH Diagnostics AB). The primers were temperature optimized and the products with the standard samples were checked on agarose gels to verify the size of the fragments. The dissociation curves (melting curves) were also checked to evaluate the specificity of the primers. The optimal program for the 16S rRNA gene consisted of initial denaturation for 10 min at 95°C, and then 45 cycles were performed, each cycle consisting of 30 s at 95°C, 1 min at 58°C, and 40 s at 72°C; a final extension step was carried out for 7 min at 72°C. The results are given as units (i.e. number of gene copies) mL^{-1}, which is a relative measurement of the numbers and activity of different bacteria and not the absolute numbers.

2.5 Chemical analyses

2.5.1 Sampling and analyses of the groundwater inside the Prototype repository

Samples for chemical analysis of the pore water inside the Prototype repository 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.

2.5.2 Sampling and analyses of the groundwater outside the Prototype repository

The chemical sampling and analyses of the groundwater surrounding the Prototype repository were performed by the SKB chemistry laboratory at Äspö HRL according to their standard protocols.

2.5.3 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 different sample groups, and were presented as an enrichment factor.

2.6 Statistical analyses

Statistical analyses and graphics were performed using STATISTICA software, version 8.0 (Statsoft, Tulsa, OK, USA).

3 RESULTS

3.1 Sample groups

As described in IPR08-01, the sampling points inside the Prototyp Repository 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 is available, in this presentation of the results from 2009 regarding the sampling of Prototype pore water and groundwater around the Prototype repository:

- **KB513, KB514, KB613, and KB614:** These four sampling points on the top of the deposition holes 5 and 6 in section 2 (outer section closest to the tunnel) gave no or very little water as of May 2007. After 5 weeks, only a small amount of water could be extracted from KB513 and KB613. In May 2007, there was an under-pressure of approximately 0.5 bar in the KB513-614 sampling group. In June 2009, the pressure in this group had increased to 1-2 bar (Table 4).

- **KBU10001:** This sampling point from the backfill in section 1 (inner section farthest from the tunnel) delivered no water but had increasing pressure, approximately 3 bar as of May 2007. In June 2009, the pressure in this group had increased to 5 bar and water was extractable within an hour (Table 4).

- **KBU10003 and KBU10007:** These two sampling points, one from the backfill and one from on the top of deposition hole 1 in section 1 (inner section), delivered only gas as of May 2007. In May 2007, the pressure in these sampling points was the same as the atmospheric pressure in the tunnel. The situation was the same in June 2009 (Table 4).

- **KBU10005:** In May 2007, this sampling point from the backfill in section 1 (inner section) produced extractable water after 5 weeks using a pressure vessel. The pressure in the sampling point was decreasing with time, and was 0.5 bar as of May 2007. In June 2009, the pressure in this group had increased to 1 bar (Table 4).

- **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 15 h as of May 2007. The pressure in these sampling points increased with time, and was 3 bar as of May 2007. In June 2009, the pressure in these sample group was 5.2 bar (Table 4).

- **KBU10004 and KBU10006:** In 2007, these two sampling points in the backfill in section 1 (inner section) produced extractable water within 15 h. The pressure in these sampling points increased with time, and was 3 bar as of May 2007. As of June 2009, the pressure was 1–3 bar in this sample group (Table 4).

- **KFA01, KFA02, KFA03, and KFA 04:** These four sampling points in the backfill in section 2 (outer section) produced fairly easily extractable water. Sufficient water could be extracted from KFA01 and KFA04 within 24 h, from KFA02 within 72 h, and from KFA03 within 3 weeks. The pressure in these sampling points increased with time, and was 3–8 bar as of May 2007. As of

June 2009, the pressure was 9–12 bar in sampling points KFA01 and KFA04, and 1.2 in sampling points KFA02 and KFA03 (Table 4).

3.2 Dissolved gas composition

3.2.1 Groundwater outside the Prototype Repository

Two groundwater boreholes sections in the close vicinity of the Prototype Repository, KA3600F:2 and KA3542G01:3, were examined regarding gas composition (Table 4). The gas compositions were relatively similar between the two, with O_2 below detection limit, a CH_4 content of about 1 %, He between 3-5%, and H_2 below 0.01% . The only big differences between the two waters were the CO_2 and N_2 contents. In section KA3600F:2, the N_2 content was higher and the CO_2 was lower (97% and around 1%) compared to section KA3542G01:3 (86% and around 10%).

3.2.2 Group KBU10001

For the first time, water was extractable from the KBU10001 sampling point (Table 4). The gas composition in pore water in KBU10001 nevertheless followed the trend of the borehole described in IPR 08-01, with a O_2 level decreasing from 15% in 2005 to about 2% as of 2009 and an increasing N_2 level from 80% to almost 100% in the same period of time. The amounts of CO_2, CH_4, and H_2 varied but remained below 1% throughout 2005-2009, except CO_2 in 2009 (1.1%). He was not detected in the KBU10001 sampling point.

3.2.3 Group KBU10002 and KBU10008

Water can easily be extracted from the KBU10002+KBU10008 sampling points (Table 4). The gas composition in pore water in the group generally lack distinctive trends, although the O_2 levels in these pore waters generally were lower in 2009 compared to previous sampling performed in 2005-2007 (0.9-4% compared to 7-8% as of 2007). The amounts of CO_2, CH_4, and H_2 varied but remained below 1% in 2009. Small amount of He was detected in the KBU10002 sampling point.

3.2.4 Group KBU10004 and KBU10006

Water could be extracted from the KBU10006 sampling point, and a small amount of gas (3.3 mL) from the KBU10004 sample point (Table 4). The gas compositions in these two sampling points differs from each other in the sampling 2009. In KBU10004, the gas contains 3% O_2 and 98% N_2, and KBU10006 contains 15% O_2 and 83% N_2. The other gases, i.e. CO_2, CH_4, and H_2 varied but remained below 1% in 2009. No He was detected in the KBU10004 and KBU10006 sampling group.

3.2.5 Group KFA01-KFA04

Water could be extracted from the KFA01 and KFA04 sampling points (Table 4). The gas composition in these two sampling points generally follows the trends described in IPR 08-01, with decreasing O_2 levels (0.6-0.7% in 2009 compared to 5% in 2007) and increasing N_2 contents (97-98% in 2009 compared to 90% in 2007). The amounts of CH_4, CO_2, He and occasionally even H_2 reached concentrations of 0.2-0.5%.

Table 4. The gas content in the sampling points inside and outside the Prototype Repository at the sampling occasions 2009-06-01 and 2009-10-22. The gas is given 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	14892	2009-06-01	5.2	15.3	26.0	655	11100	bd	bd	bd	19.8	986	7.3/45
KBU10002	14891	2009-06-01	5.2	10.2	16.2	768	8970	bd	bd	1.5	9.46	1020	6.5/44
KBU10003		2009-06-01	1.1										
KBU10004		2009-06-01	2.4	18.5	38.0	157	5660	bd	bd	bd	33.9	980	3.3/0
KBU10005		2009-06-01	1.0										
KBU10006	14886	2009-06-01	1.1	5.1	21.2	15.1	1360	bd	0.54	bd	148	830	33/20
KBU10007		2009-06-01	1.1										
KBU10008	14890	2009-06-01	5.2	15.8	25.0	209	7250	bd	bd	bd	44.3	930	9.3/44
KB513		2009-06-01	1.3										
KB514		2009-06-01	1.7										
KB613		2009-06-01	1.2										
KB614		2009-06-01	0.8										
KFA01	14970	2009-06-01	9	1600	5.59	2460	3420	1.15	bd	3.35	6.84	970	14/42
KFA02		2009-06-01	1.2										
KFA03		2009-06-01	1.2										
KFA04	14893	2009-06-01	11.8	11.3	14.2	2130	2700	1.95	bd	5.08	5.66	981	22/43
KA3600F:2	20018	2009-10-22		92	8.82	8690	7450	0.75	bd	25	bd	965	15/192
KA3542G01:3	20041	2009-10-22		40	3.93	9480	90600	bd	bd	44.8	bd	862	10/195

3.3 Microbial composition

3.3.1 Groundwater outside the Prototype Repository

In 2009-10-22, samples for determination of the numbers of SRB as well as MOB outside the Prototype repository were taken from the borehole sections KA3600F:2 and KA3542G01:3. The numbers of SRB were determined both with MPN and qPCR (Table 3,). The numbers of SRB in KA3600F:2 were determined to 80 mL^{-1} by the MPN method and below 100 by qPCR on DNA with the ApsA primer (Table 5, Table 6). The active SRB determined by qPCR on RNA with the ApsA primer were lower than 100 as well. The number of MOB capable of methanol degradation was determined to below 10 as were the active MOB of the same kind. The number of MOB capable of methane degradation was determined to below 10 and the active MOB of the same was determined to approximately 3000 units mL^{-1}. The numbers of SRB in KA3542G01:3 were determined to 30000 mL^{-1} by the MPN method and about 20000 units mL^{-1} by qPCR on DNA with the ApsA primer (Table 5, Table 6). The active SRB determined by qPCR on RNA with the ApsA primer were determined to about 20000 units mL^{-1} as well. The number of MOB capable of methanol degradation was determined to below 10 as were the active MOB of the same kind. The number of MOB capable of methane degradation was determined to below 10 and the active MOB of the same was determined to approximately 3000 units mL^{-1}.

3.3.2 Group KBU10001

In 2009-06-01, the numbers of CHAB, SRB, AA and MOB as well as the ATP content in the pore water of KBU10001 were determined (Table 5). In this pore water, the ATP content was 243000 amol mL^{-1}, and the numbers of CHAB, SRB, AA and MOB were 15000, 17, 1 and 2400 mL^{-1}, respectively. Obviously MOB and CHAB were the most abundant types of bacteria in KBU10001. Of the anaerobic bacteria, SRB were most abundant, the AA did not appear to be very active in the pore water.

3.3.3 Group KBU10002 and KBU10008

In 2009-06-01, the numbers of CHAB, SRB, AA and MOB as well as the ATP content in the pore water of KBU10002+KBU10008 group were determined (Table 5). Two samples were extracted from KBU10008. In this pore waters, the ATP contents ranged from 10700-118000 amol mL^{-1}, and the numbers of CHAB, SRB, AA and MOB ranged from <10-5900, 2-130, <0.2-0.2 and 3000-30000 mL^{-1}, respectively. The number of IRB was determined to 5 mL^{-1} in KBU10008. Obviously MOB and CHAB were the most abundant types of bacteria in this group as well as in KBU10001. Of the anaerobic bacteria, SRB were most abundant reaching 130 mL^{-1} at the most, the AA did not appear to be very active in the pore water.

In 2009-10-22, the numbers of SRB and MOB in KBU10008 were analysed by means of qPCR. The number of SRB and the active SRB were determined to below 100 (Table 6). The number of MOB capable of methanol degradation was determined to approximately 200 units mL^{-1}, but all these were not found to be very active as the MxaF RNA qPCR were below 100. The number of MOB capable of methane degradation was determined to below 10 but the active MOB of the same was determined to approximately 300 units mL^{-1}.

3.3.4 Group KBU10004 and KBU10006

In 2009-06-01, the numbers of CHAB, SRB, AA and MOB as well as the ATP content in the pore water of KBU10006 were determined (Table 5). In this pore water, the ATP content was 301000, and the numbers of CHAB, SRB, AA and MOB were 68000, <0.2, <0.2 and >16000 mL^{-1}, respectively. Obviously MOB and CHAB were the completely dominating types of bacteria in KBU10006. Anaerobic bacteria were missing all together.

3.3.5 Group KFA01-KFA04

In 2009-06-01, the numbers of CHAB, SRB, AA and MOB as well as the ATP content in the pore water of KFA01-04 group were determined. In this pore waters, the ATP contents were about 120000 amol mL^{-1}, and the numbers of CHAB, SRB, AA and MOB ranged from 400-1400, <0.2-0.4, <0.2-0.2 and 170-800, respectively. The number of IRB was determined to less than 0.2 mL^{-1} in KFA04. The microbial composition was also determined in KFA01 in 2009-06-15. At this point, the ATP content and CHAB were considerable higher (30 and 1000 times higher, at least). However, this sample had been left standing for 15 days before inoculation. Also, the pressure in this sample was lower.

In 2009-10-22, the numbers of SRB and MOB in KFA were analysed by means of qPCR. The number of SRB and the active SRB were determined to below 100. The number of MOB capable of methanol degradation was determined to approximately 50 units mL^{-1}, accordingly the active MOB of the same type, determined by MxaF RNA qPCR, were below 100. The number of MOB capable of methane degradation was determined to below 10 but the active MOB of the same was determined to approximately 500 units mL^{-1}.

Table 5. The microbial composition in the sampling points inside and outside the Prototype Repository at the sampling occasions 2009-06-01 and 2009-10-22.

Sampling point	SKB no	Date	ATP (amol mL^{-1})	Stdev ATP	CHAB (mL^{-1})	Stdev CHAB	MPN SRB (mL^{-1})	MPN AA (mL^{-1})	MPN IRB (mL^{-1})	MPN MOB (mL^{-1})
KBU10001	14892	2009-06-01	243000	17000	15000	4400	17 (8-41)	1 (0.5-4)	nd	2400 (1000-9400)
KBU10002	14891	2009-06-01	19200	1320	<10		130 (50-390)	0.2 (0.1-1)	nd	9000 (3000-29000)
KBU10006	14886	2009-06-01	301000	15300	68000	30000	<0.2	<0.2	nd	>16000
KBU10008	14890	2009-06-01	118000	10400	5900	210	7 (2-9)	<0.2	nd	30000 (1000-130000)
KBU10008	14890	2009-06-01	10700	500	<10		2 (1-9)	<0.2	5 (2-17)	3000 (1000-13000)
KFA01	14883	2009-06-01	117000	9260	1400	210	<0.2	<0.2	nd	800 (300-2500)
KFA04	14893	2009-06-01	124000	21000	400	110	0.4 (0.1-2)	0.2 (0.1-1)	<0.2	170 (80-410)
KFA01	14970	2009-06-15	2930000	158000	>2000000		1 (0.5-4)	0.6 (0.2-2)	nd	dm
KA3600F:2	20018	2009-10-22	nd		nd		80 (30-250)	nd	nd	nd
KA3542G01:3	20041	2009-10-22	nd		nd		30000 (10000-120000)	nd	nd	nd

Table 6. The microbial composition, as determined by molecular methods, in the sampling points inside and outside the Prototype Repository at the sampling occasion 2009-10-22.

Sampling point	SKB no	Date	qPCR DNA MxaF (± stdev) (units mL^{-1})	qPCR RNA mxaF (± stdev) (units mL^{-1})	qPCR DNA PmoA (± stdev) (units mL^{-1})	qPCR RNA pmoA (± stdev) (units mL^{-1})	qPCR DNA ApsA (± stdev) (units mL^{-1})	qPCR RNA apsA (± stdev) (units mL^{-1})
KBU10008		2009-10-22	163 (± 19.2)	<100	<10	307	<100	<100
KFA04		2009-10-22	49.9 (± 12.0)	<100	<10	463	<100	<100
KA3600F:2	20018	2009-10-22	<10	<100	<10	2970	<100	<100
KA3542G01:3	20041	2009-10-22	<10	<100	<10	2740 (± 2860)	17400 (± 141)	16700 (± 12900)

3.4 Chemical composition

3.4.1 Groundwater outside the Prototype Repository

In Table 7 the chemical compositions of the groundwater sampled outside the Prototype repository are shown. The groundwater in the borehole sections KA3600F:2 and KA3542G01:3 were strikingly similar to the mean chemical composition of Äspö groundwater reported in IPR 08-01, showing them representative for the environment outside the Prototype repository.

3.4.2 Group KBU10001

The pore water in the KBU10001 group was similar to the groundwater in salinity, pH, and in concentrations of sulphate, potassium, magnesium, sodium, and calcium, but was depleted in iron (Table 7, Table 8). Many of the metals examined were present in concentrations of 0.1–100 µg L^{-1}. In most cases, the concentrations of metals were about the same in the KBU10001 group and in the groundwater outside the Prototype repository. However the concentrations of mercurium and vanadium were about 20 and 4 times higher, 0.005 µg L^{-1} and 0.7 µg L^{-1}. The concentration of the actinide uranium was approximately 9 times higher, 1.5 µg L^{-1}, than in the groundwater.

3.4.3 Group KBU10002 and KBU10008

The pore water in the KBU10002+10008 group was similar to the groundwater and group KBU10001 in salinity, pH, and in concentrations of sulphate, potassium, magnesium, sodium, and calcium, but was depleted in iron (Table 7, Table 8). Many of the metals examined were present in concentrations of 0.1–100 µg L^{-1} (Table 7). In most cases, the concentrations of metals were about the same in the KBU10002+8 group, KBU10001 group and in the groundwater outside the Prototype repository. However the concentrations of mercurium and vanadium were about 15 and 5 times higher, 0.004 µg L^{-1} and 0.8 µg L^{-1} than in the surrounding groundwater. The concentration of the actinide uranium was approximately 9 times higher, 1.5 µg L^{-1}, than in the groundwater.

3.4.4 Group KBU10004 and KBU10006

The pore water in the KBU10004+6 group was different from that of the KBU10001 and KBU10002+8 groups (Table 7, Table 8). The pore water had sodium and potassium levels 4 and 16 times higher and calcium levels 5 times lower than that of the groundwater outside the Prototype repository. Sulphate was present in levels 5 times the surrounding groundwater. The silica level was 4 times the groundwater and the pore water was depleted in both iron and manganese. 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 molybdenum and rubidium, which were more abundant, being present in concentrations of 781 and 193 µg L^{-1}, respectively. The concentration of vanadium was 6 times higher than in groundwater (0.9 µg L^{-1}), and the concentration of the actinide uranium was approximately 11 times higher (1.8 µg L^{-1}) than in the groundwater.

3.4.5 Group KFA01-KFA04

The pore water in the KFA01-04 group was different from that of the KBU10001 and KBU10002+8 groups and resembled the KBU10004+6 group (Table 7, Table 8). The

pore water had sodium and potassium levels 2 and 6 times higher and calcium levels 10 times lower than that of the groundwater outside the Prototype repository. Sulphate was present in levels twice the surrounding groundwater. The silica level was 2 times the groundwater and the pore water was depleted in both iron and manganese. 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 molybdenum, which were more abundant, being present in concentrations of 200 µg L^{-1}, respectively. The concentration of vanadium was 64 times higher than in groundwater (10 µg L^{-1}), and the concentration of the actinide uranium was approximately 200 times higher (32 µg L^{-1}) than in the groundwater.

Table 7. The chemical composition in the sampling points inside and outside the Prototype Repository at the sampling occasions 2009-06-01 and 2009-10-22.

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	2009-06-01	14892	1620	14.9	571	90		339			7.19	0.0038	0.615	0.392	10.4
KBU10002	2009-06-01	14891	1600	13.1	633	96.1		309			6.65	0.0044	0.649	0.385	10.2
KBU10006	2009-06-01	14886	6860	136	165	68.1		2960			31.6	0.0035	0.059	0.857	5.12
KBU10008	2009-06-01	14890	1470	13.3	752	102		358			7.52	0.0038	0.865	0.378	8.35
KFA01	2009-06-01	14970	2950	80.6	44	30.5		763			7.45	0.0025	0.075	0.092	0.903
KFA04	2009-06-01	14893	3410	57.4	43.6	42		779			15.8	0	0.086	0.164	0.792
KA3600F:2	2009-10-22	20018	1880	11.6	1000	66.3	4351	340	2.3	19.3	8	0.3			
KA3542:G01:3	2009-10-22	20041	1570	9.03	690	63	3531	291	2.5	15.7	9.1	4			

	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	2009-06-01	14892		2.04	61.3	0	1.56	0.454	225	0.0045	121	61.6	16.2	0	0.688
KBU10002	2009-06-01	14891		2.36	123	0	3.27	0.681	20.1	0.0026	63.4	174	13.4	0	0.983
KBU10006	2009-06-01	14886		1.28	75.8	0	0.478	0.635	105	0.041	781	18.5	233	0.148	0.893
KBU10008	2009-06-01	14890		1.96	57.3	0.0345	1.69	0.429	41.8	0.0048	47.4	75.4	47.8	0	0.629
KFA01	2009-06-01	14970		2.98	31	0	0.671	0.734	0.998	0	163	12	16.9	0	9.25
KFA04	2009-06-01	14893		0	53	0	1.93	0.558	516	0.0083	233	122	281	0	11.6
KA3600F:2	2009-10-22	20018													
KA3542:G01:3	2009-10-22	20041													

Sample point	Sampling occasion	SKB	Zn	La	Ce	Pr	Nd	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm

		no	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})	(µg L^{-1})
KBU10001	2009-06-01	14892	52	0.0283	0.0341	0	0	0	0	0	0	0	0	0	0
KBU10002	2009-06-01	14891	52.6	0.109	0.101	0	0.0488	0	0	0	0	0	0	0	0
KBU10006	2009-06-01	14886	34.8	0.0072	0.0074	0	0.0071	0	0	0	0	0	0	0	0
KBU10008	2009-06-01	14890	103	0.0293	0	0	0.024	0	0	0	0	0	0	0	0
KFA01	2009-06-01	14970	18.6	0	0	0	0	0	0	0	0	0	0	0	0
KFA04	2009-06-01	14893	22.9	0	0	0	0	0	0	0	0	0	0	0	0
KA3600F:2	2009-10-22	20018													
KA3542:G01:3	2009-10-22	20041													

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})	**pH**
KBU10001	2009-06-01	14892	0	0	0	35.3	0.187	0	0.224	2.57	0	0.183	1.49	0	7.29
KBU10002	2009-06-01	14891	0	0	0	29.8	0.229	0	0.145	0.299	0	0.235	2	0	7.52
KBU10006	2009-06-01	14886	0	0.0326	0	193	0.0824	0.0596	0.331	5.64	0	0.0354	1.81	0	7.76
KBU10008	2009-06-01	14890	0	0	0	30.3	0.276	0	0.141	2.46	0	0	0.905	0	7.59
KFA01	2009-06-01	14970	0	0	0	91.1	0.051	0	1.51	3.24	0	0.579	18.3	0	7.33
KFA04	2009-06-01	14893	0	0	0	102	0.0912	0	1.25	9.53	0	1.39	45.6	0	8.26
KA3600F:2	2009-10-22	20018													
KA3542:G01:3	2009-10-22	20041													

Table 8. The chemical enrichment factors in the Prototype Repository sample groups compared to Äspö groundwater.

Sample group	Na	K	Ca	Mg	SO_4^{2-}	Si	Fe	Mn	Li	Sr	Ba	Cd	Hg
KBU10001	0.9	1.4	0.8	1.3	1.0	1.0	0.013	1.2	0.8	0.9	1.2	0	18
KBU10002+8	0.9	1.2	1.0	1.4	1.0	1.0	0.014	1.5	0.8	0.8	1.7	0.3	15
KBU10004+6	3.9	13	0.2	0.9	8.6	4.4	0.012	0.1	1.8	0.4	1.5	0	160
KFA01-04	1.8	6.4	0.1	0.5	2.2	1.6	0.004	0.2	0.3	0.1	0.8	0	16

	Nd	Rb	Y	Zr	Cs	U	V
KBU10001	0	1.1	0.9	0	0.9	9.1	4.2
KBU10002+8	0.7	0.9	1.2	0	0.5	8.9	4.9
KBU10004+6	0.1	6	0.4	6.4	1.9	11	5.5
KFA01-04	0	3	0.3	0	2.1	200	64

4 Discussion

The Prototype repository 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 overviews the data from 2009 and the interplays between gas composition, chemistry, and microbial life, and the influence of the surrounding groundwater in the Prototype repository. In addition, molecular methodology was evaluated on Prototype samples for the up-coming decommission of the Prototype repository. The pore water chemistry, gas content, pressure, and prerequisites for microbial life at the sampling points of the Prototype repository are not uniform and the 16 sampling points in the Prototype are divided into seven sampling groups, as described in detail in IPR 08-01. These are: the KB513-614 group, the KBU10001 group, the KBU10003+10007 group, the KBU10002+8 group, the KBU10004+6 group, the KBU10005 group, and the KFA01-04 group.

4.1 Microbial decrease of oxygen and its consequences

The ATP content in the pore water inside the Prototype repository continues to be very high compared to the ATP content in the groundwater outside. In IPR 08-01, the mean ATP content in 12 groundwater sections close to the Prototype repository were calculated to be 5000 amoles mL^{-1}. Inside the Prototype Repository, the pore water contained 11000- 2930000 amoles mL^{-1}, which is 2-600 times more (Table 5). Thus, the pore water has to be regarded as a highly biologically active environment.

The main purpose of the microbiological investigations of the Prototype is to evaluate whether the oxygen level in a newly built storage facility decreases faster due to microbial activity than it would in an abiotic environment.

In Figure 6, we see that the oxygen level generally decreased over time in the Prototype repository (exemplified by the sample point KBU10001, where the oxygen decreased exponentially). Typically, the oxygen levels have decreased to >4 %.

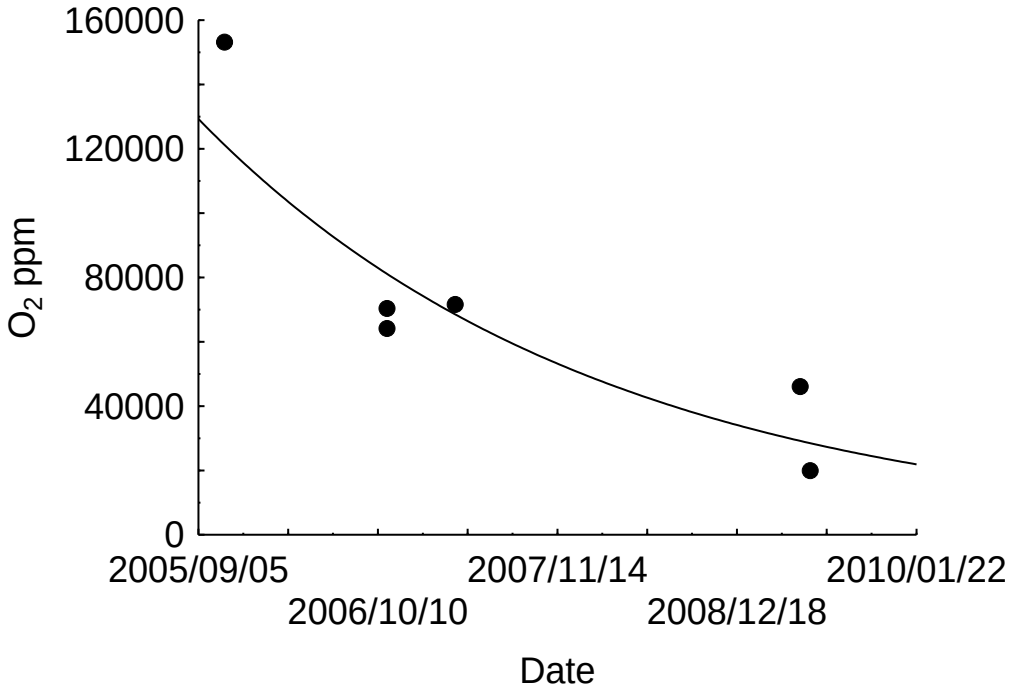

Figure 6. *The oxygen content in ppm of the total gas in sampling point KBU10001 during five years of monitoring (2005-2009).*

MOB with their ability to decrease the oxygen content, can 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. The number of SRB in the backfill and buffer have so far usually been found to be quite low in the pore water, approximately 1 SRB mL^{-1} but they are repeatedly occasionally higher, e.g this sampling showed a SRB number of 130 mL^{-1}. 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 repository (Masurat et al., 2010). The decommision will show 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 molecular methods detect microbes in the same order of magnitude as the culture dependent methods (i.e. CHAB and MPN), showing them useful in future studies. Theoretically, these methods can give us quantitative data within 24 h from sampling, which can be compared to 8 weeks for culture dependent methods. The numbers of different microbes was detected by measuring how much DNA specific for the examined microbes that was present in the Prototype samples. In addition, we also detected to what extent this microbial population was active. This is done by measuring the amount of RNA specific for the examined microbes. The RNA is only present if the microbes perform the specific process in situ. For example, presence and activity of SRB are detected by looking for ApsA, either in its DNA form for total numbers or its RNA form for active numbers. The total numbers as well as the active numbers of SRB

correlate with the culture dependent MPN numbers of SRB in the examined waters. Shown in Table 5 and Table 6, the numbers of SRB were found to be below detection in three of four analysed waters (KBU10008, KFA04 and KA3600:2) using the three different methods (MPN, ApsA and apsA). In KA3542G01:3, the numbers of total as well as active number of SRB were determined to approximately 20000 mL^{-1} and by MPN to approximately 30000 mL^{-1}.

4.2 Mineral dissolution and bacterial activity affects the pore water chemistry

Prototype repository tunnel backfill was prepared from 70% crushed rock and 30% Na-exchanged bentonite material from Greece (Karnland, 2007). 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+6 and KFA01-04 groups (Table 7, Table 8). The high sodium and potassium and lower calcium and magnesium concentrations in pore water could be due to cation exchange (Luukkonen, 2007); this is because the univalent sodium and potassium from the bentonite can be readily 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. Calcium concentrations could be further lowered in the pore water by calcite precipitation (Luukkonen, 2007); Table 8 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 KBU10004+6 and KFA01-04 sampling groups in the Prototype repository (Table 8). Cristobalite dissolution can result in the small rise in Si concentration in the pore water in the KBU10004+6 and KFA01-04 sampling groups (Luukkonen, 2007).

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 repository. Many microbes can produce siderophores, chelating agents used for iron uptake in deficient environments, i.e., aerobic environments. It is well-known that, with 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 8); i.e., V (Baysse et al., 2000), U (Moll et al., 2008a), and Cs (Wendling et al., 2005).

5 Concluding remarks

- Methodologies for sampling and analysing pore water from the Prototype repository worked very well. We thus have good background data for support when the Prototype Repository is being decommissioned in 2011. The molecular methods used in 2009, especially the SRB qPCR, were proved to be a good methodology for the up-coming decommission of the Prototype repository, because they are quick methods compared to traditional culture-dependant methods.

- The oxygen gas content in the pore water has decreased drastically in the last 2 years. MOB were proven to be abundant as well as active in pore water. When MOB have used the oxygen, the repository will enter anaerobic mode, giving the SRB a playground. It appears that anaerobic conditions prevail in some of the sampling points. The abundance of SRB should thus be investigated extremely careful in future studies.

- The chemical data for the pore water suggest that dissolution of the minerals cristobalite and gypsum occurs in the Prototype repository. The data also suggest that cation exchange occurs from the sodium and potassium to the magnesium and 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.

- In iron-deficient environments, such as the Prototype repository pore water, microbes are known to use bioligands called siderophores specifically to acquire iron and transfer it inside the cell. Siderophores can sometimes unspecifically bind other metals as well. As well, other element-specific bioligands can dissolve various metals. 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 almost 200 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.

6 References

Baysse C, De Vos D, Naudet Y, Vandermonde A, Ochsner U, Meyer J-M, Budzikiewicz H, Schäfer M, Fuchs R, Cornelis P, 2000. Vanadium interferes with siderophore-mediated iron uptake in *Pseudomonas aeruginosa*. Microbiology **146**, 2425–2434.

Essén S A, Johnsson A, Bylund D, Pedersen K, Lundström U S, 2007. Siderophore production by P*seudomonas stutzeri* under aerobic and anaerobic conditions. Appl. Environ. Microbiol. **73**, 5857–5864.

Eydal H S C, Pedersen P, 2007. Use of an ATP assay to determine viable microbial biomass in Fennoscandian Shield groundwater from depths of 3–1000 m. J. Microbiol. Methods **70**, 363–373.

Greenberg A E , Clesceri L S, Eaton A D, 1992. Estimation of Bacterial Density Standard Methods for the Examination of Water and Wastewater, 18th ed. American Public Health Association, Washington. pp 9–49.

Hallbeck L, Pedersen K, 2008. Characterization of microbial processes in deep aquifers of the Fennoscandian Shield. Appl. Geochem. **23**, 1796–1819.

Haveman S A, Pedersen K, 2002. Distribution of culturable anaerobic microorganisms in Fennoscandian Shield groundwater. FEMS Microbiol. Ecol. **39**, 129–137.

Herz H-P, Yimga M T, Liesack W, 2001. Detection of Methanotroph Diversity on Roots of Submerged Rice Plantsby Molecular Retrieval of *pmoA*, *mmoX*, *mxaF*, and 16S rRNA and Ribosomal DNA, Including *pmoA*-Based Terminal Restriction Fragment Length Polymorphism Profiling. Appl. Environ. Microbiol. **67**, 4177–4185.

Johnsson A, Arlinger J, Pedersen K, Ödegaard-Jensen A, Albinsson Y, 2006. Solid–aqueous phase partitioning of radionuclides by complexing compounds excreted by subsurface bacteria. Geomicrobiol. J. **23**, 621–630.

Kalinowski B E, Johnsson A, Arlinger J, Pedersen K, Ödegaard-Jensen A, Edberg F, 2006. Microbial mobilization of uranium from shale mine waste. Geomicrobiol. J. **23**, 157–164.

Kalinowski B E, Oskarsson A, Albinsson Y, Arlinger J, Ödegaard-Jensen A, Andlid T, Pedersen K, 2004. Microbial leaching of uranium and other trace elements from shale mine tailings at Ranstad. Geoderma **122**, 177–194.

Karnland O, 2007. PM concerning: Mineralogical composition of the bentonite used for the tunnel backfilling material in the Prototype repository at Äspö HRL. Clay Technology AB, personal communication

Lundin A, 2000. Use of firefly luciferase in ATP-related assays of biomass, enzymes, and metabolites. Methods Enzymol. **305**, 346–370.

Lundin A, Hasenson M, Persson J, Pousette Å, 1986. Estimation of biomass in growing cell lines by adenosine triphosphate assay. Methods Enzymol. **133**, 27–42.

Luukkonen A, 2007. Memorandum: A survey of porewater compositions detected from the prototype repository tunnel backfill, Äspö, Sweden. VTT, Finland, personal communication

Masurat P, Eriksson S, Pedersen K, 2010. Evidence for indigenous sulphate-reducing bacteria in commercial MX-80 Wyoming bentonite. Appl. Clay Sci. **47**, 51–57.

Moll H, Glorius M, Bernhard G, Johnsson A, Pedersen K, Schäfer M, Budzikiewicz H, 2008a. Uranium(VI) complexation with pyoverdins secreted by a subsurface strain of P*seudomonas fluorescens*. Geomicrobiol. J. **25**, 157–166.

Moll H, Johnsson A, Schäfer M, Pedersen K, Budzikiewicz K, Bernhard G, 2008b. Curium(III) complexation with pyoverdins secreted by a groundwater strain of P*seudomonas fluorescens*. Biometals **21**, 219–228.

Pedersen K, 2002. Microbial processes in the disposal of high-level radioactive waste 500 m underground in Fennoscandian Shield rocks. In: Interactions of Microorganisms with Radionuclides. Edited by Keith-Roach M J, Livens F R. Elsevier, Amsterdam. pp 279–311.

Pedersen K, Ekendahl S, 1990. Distribution and activity of bacteria in deep granitic groundwaters of southeastern Sweden. Microb. Ecol. **20**, 37–52.

Puigdomenech I, Ambrosi JP, Eisenlohr L, Lartigue JE, Banwart SA, Bateman K, Milodowski AE, West JM, Griffault L, Gustafsson E, Hama K, Yoshida H, Kotelnikova S, Pedersen K, Michaud V, Trotignon L, Rivas Perez J, Tullborg EL, 2001. O_2 depletion in granitic media. The REX project. SKB-TR-01-05. Swedish Nuclear Fuel Waste Management Co,Stockholm.

Wendling L A, Harsh J B, Ward T E, Palmer C D, Hamilton M A, Boyle J S, Flury M, 2005. Cesium desorption from illite as affected by exudates from rhizosphere bacteria. Environ. Sci. Technol. **39**, 4505–4512.

Whittenbury R, Philips K C, Wilkinson J F, 1970. Enrichment, culturing, isolation and some properties of methane-utilizing bacteria. J. Gen. Microbiol. **61**, 205–218.

Widdel F, Bak F, 1992. Gram-negative, mesophilic sulphate-reducing bacteria. In: The Prokaryotes. Edited by Balows A, Truper H G, Dworkin M, Harder W, Schleifer K-Z. Springer-Verlag, New York. pp 3352–3378.