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Äspö Hard Rock Laboratory

Prototype Repository

Analysis of microorganisms, gases, and water chemistry in buffer and backfill, 2004 – 2007

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December 2007

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Abstract

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. 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 the Prototype, 2004 to 2007.

Hydrogen, helium, nitrogen, oxygen, carbon monoxide, carbon dioxide, methane, ethane, and ethene were analysed in samples from 16 hydrochemical sampling points in the Prototype Repository. Where the sampling points in the Prototype delivered pore water, the water was analysed for total number of cells (TNC), amount of ATP (i.e., the biovolume), cultivable heterotrophic aerobic bacteria (CHAB), sulphate-reducing bacteria (SRB), methane-oxidizing bacteria (MOB), and autotrophic acetogens (AA). 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 boreholes in the rock surrounding the Prototype was analysed regarding its gas composition and microbiology. Chemistry data from a previous investigation of the groundwater outside the Prototype Repository were compared with the pore water chemistry.

The sampling and analysis protocols – already working properly – were improved in 2007, when stainless steel pressure vessels began to be used to extract pore water from the Prototype Repository. This method allowed us to extract water from nine, rather than the previous six, of the 16 sampling points. Several years of examination of the gas composition, microbial composition, and chemistry of the pore water in the Prototype Repository has 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 had not been reached by the groundwater (KBU10001) as of May 2007.

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 of the gas phase was approximately 3–7% in May 2007, markedly lower than the oxygen content in 2005, which was 18–10%. Hydrogen, methane, helium, and carbon dioxide concentrations varied,

especially in the sampling groups with extractable pore water. The microbes found in the pore water correlated with some of the gases found: high numbers of MOB correlated with high oxygen content ($r^2 = 0.997$, $p = 0.002$), high numbers of CHAB with high carbon dioxide content ($r^2 = 0.81$, $p = 0.1$), and high numbers of AA with high hydrogen content and low carbon dioxide content ($r^2 = 0.94$, $p = 0.03$, $r^2 = 0.89$, $p = 0.06$). As well, hydrogen seemed to stimulate the growth of SRB. ATP analyses demonstrated that the biomass in the Prototype Repository increased over time. The microbiological results indicated that aerobic microbes, such as MOB and CHAB, thrived in the aerobic Prototype environment, where 120 and 2300 times greater numbers, respectively, were found than in surrounding groundwater. As well, anaerobic SRB increased in abundance in the Prototype, occasionally exceeding the number of SRB outside by a factor of 12. Autotrophic acetogens were found in numbers 200 times greater than in the surrounding groundwater. 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. Occasionally, high concentrations of Al, Ni, Zn, and Cu were found in the Prototype Repository pore water; corrosion of the heavy instrumentation could account for this. At sampling points containing active microbes, however, Rb, Cs, V, and U were enriched from two to almost 500 times the groundwater levels; microbes are possibly responsible for the dissolution of these substances by the excretion of compound-specific ligands.

The Prototype Repository gave a unique opportunity to explore microbe–gas–chemistry interactions in bentonite buffer and backfill of the KBS-3 type, even if it in difference to a real storage is drained which in turn can affect the survival of microbes and how gases and other compounds dissolve in the pore water. 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). Further sampling is necessary in order to determine exactly to what extent microbes affect the chemistry in a repository over the long term, and to clarify whether this could in any way compromise a spent nuclear fuel repository of the KBS-3 type.

Sammanfattning

Prototypförvaret är ett internationellt projekt bestående av en fullskalemodell av det djupförvar som planeras byggas för Sveriges utbrända kärnbränsle. Prototypförvarsprojektet genomförs på Äspö Hard Rock Laboratory, 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 Prototyp under 2004–2007.

Analyser av vätgas, helium, kvävgas, syrgas, kolmonoxid, koldioxid, metan, etan och eten utfördes på prov från de 16 hydrokemiska provtagningspunkterna i Prototypförvaret. De provtagningspunkter i Prototypförvaret som innehöll porvatten analyserades med avseende på totalantal av celler (TNC), ATP innehåll (dvs biovolymen), odlingsbara heterotrofa aeroba bakterier (CHAB), sulfatreducerande bakterier (SRB), metanoxiderande bakterier (MOB) och autotrofa acetogener (AA). 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 borrhåll i den omgivande bergmatrisen med avseende på mikrobiologi och gas. Kemidata från en tidigare undersökning av grundvattnet runt Prototypförvaret användes för jämförelser mellan porvattnet och omgivningen.

Provtagnings- och analysprotokollen fungerade mycket bra och förbättrades under 2007, när tryckkärl i rostfritt stål introducerades för att extrahera porvatten från Prototypförvaret. Med dessa kärl var det möjligt att extrahera vatten från nio av de 16 provtagningspunkterna, till skillnad från tidigare endast sex punkter. Under de år när Prototypförvaret undersökts med avseende på gasinnehåll, mikrobinnehåll och kemi framkom det att många av de hydrokemiska provtagningspunkterna skiljde sig relativt markant från varandra. De 16 punkterna delades 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 lanthanider. 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 (KBU10001).

Gassammansättningen i de olika provgrupperna var enhetlig när det gäller kvävgashalten som ökade och syrgashalten som minskade över tiden. I de flesta provgrupper var syrgashalten 3–7% i maj 2007, vilket kan jämföras med syreandelen i gasfasen 2005 som var 18–10%. Koncentrationerna av vätgas, metan, helium and koldioxid varierade, speciellt i de provgrupper där det fanns extraherbart porvatten. Vilka mikrober som hittades i porvattnet korrelerade med vissa av gaserna. Höga antal av MOB korrelerade med högt syrgasinnehåll ($r^2 = 0.997$, $p = 0.002$). Höga antal av CHAB korrelerade med högt koldioxid innehåll ($r^2 = 0.81$, $p = 0.1$) och höga antal av AA korrelerade med högt vätgasinnehåll och lågt koldioxid innehåll ($r^2 = 0.94$, $p =$

0.03, $r^2 = 0.89$, $p = 0.06$). Vätgas tycktes också stimulera SRB. ATP analyserna visade att biomassan i Prototypförvaret ökade över tid. De mikrobiologiska resultaten visade att aeroba bakterier som MOB och CHAB frodades i det aeroba Prototypförvaret, där 120–2300 gånger fler mikrober av respektive sort kunde hittas jämfört med det omgivande grundvattnet. Också de anaeroba SRB ökade i antal inne i Prototypförvaret och överträffade stundtals de SRB som fanns i grundvattnet utanför 12 gånger. Autotrofa acetogener påträffades i antal 200 gånger fler än utanför. Kemidata visade 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 visar på att katjonbyte i montmorillonitens mellanlager förekommit. I vissa fall förekom sporadiska höga koncentrationer av aluminium, nickel, zink och koppar i Prototypförvarets porvatten. Korrosion av den tunga instrumenteringen kan tänkas ligga bakom detta. I provtagningspunkter som innehöll aktiva mikrober anrikades rubidium, cesium, vanadin och uran från två till nästan 500 gånger jämfört med grundvattnet. Det är möjligt att mikrober var ansvariga genom exkretion av ämnesspecifika ligander.

Prototypförvaret gav ett unikt tillfälle att undersöka mikrob-gas-kemi interaktioner i bentonit buffert och backfill av KBS-3 typ, även om det till skillnad från ett riktigt förvar är dränerat vilket till exempel kan påverka överlevnad av mikrober och hur gaser och andra ämnen löser sig i porvattnet. 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). Ytterligare provtagningar är absolut nödvändiga för att säkerställa hur mikrober långsiktigt påverkar kemin i ett förvar, och för att klarlägga om detta kan äventyra förvaringen av utbränt kärnbränsle enligt KBS-3 metoden på något sätt.

Contents

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
AGW	Analytical Grade Water	Purified destilled water
AM	Autotrophic Methanogens	Microbes able to produce methane from carbon dioxide and hydrogen
AODC	Acridine Orange Direct Count	Method based on nucleic acid staining for determination of cell numbers
ATP	Adenosine Tri Phosphate	Energy carrier in a living organism
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
HA	Heterotrophic Acetogens	Microbes able to produce acetate from organic carbon
HM	Heterotrophic Methanogens	Microorganisms able to produce methane from organic carbon
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.
TNC	Total Number of Cells	The number of cells in a water sample or on a solid phase, usually determined by microscopy using the AODC method

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 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. Oxygen is hypothesized to 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).

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.

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 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 1-2. Eight sample collectors have been installed (AP TD F63-01-054) 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 closest to the tunnel), DA3545G01 and DA3551G01, are labelled hole numbers 5 and 6, respectively, in Figure 1-2.

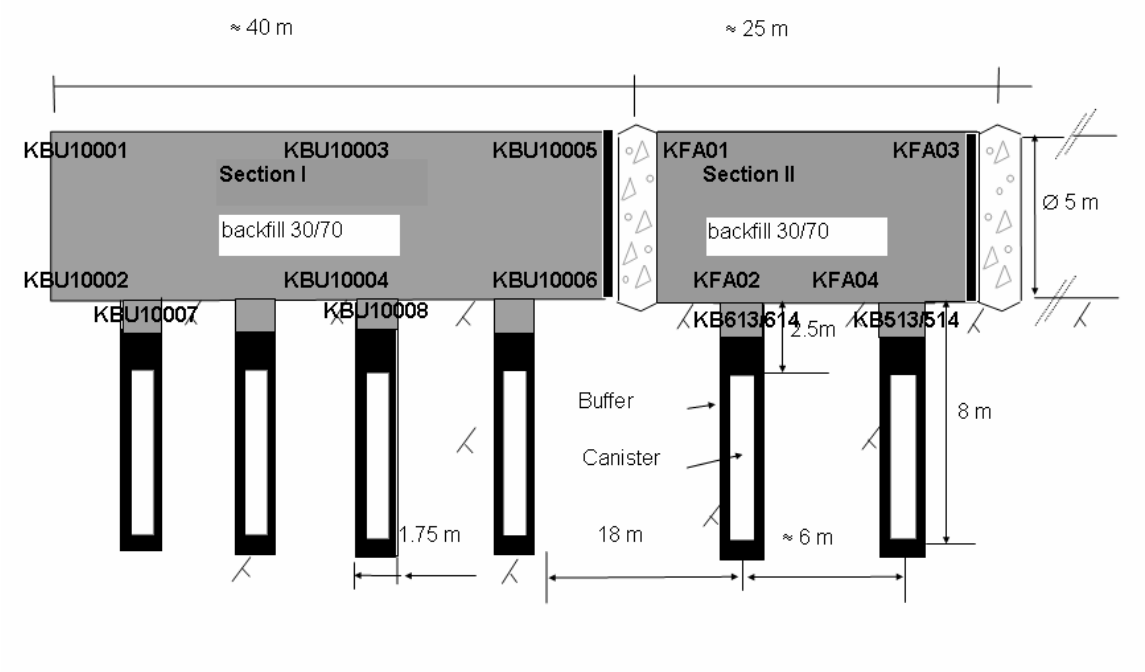


Figure 1-1. *Schematic of the Prototype Repository (adapted from IPR 99-34).*

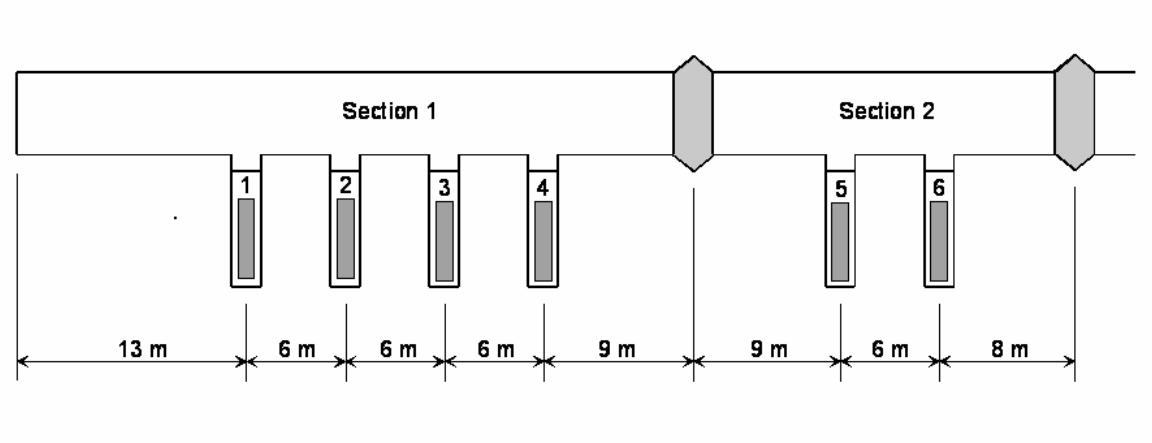


Figure 1-2. *The deposition holes in the Prototype Repository (from AP TD F63.1-04-018).*

In section 2 (outer section) (AP TD-F63-02-040) (Table 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. 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 1-1. 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)

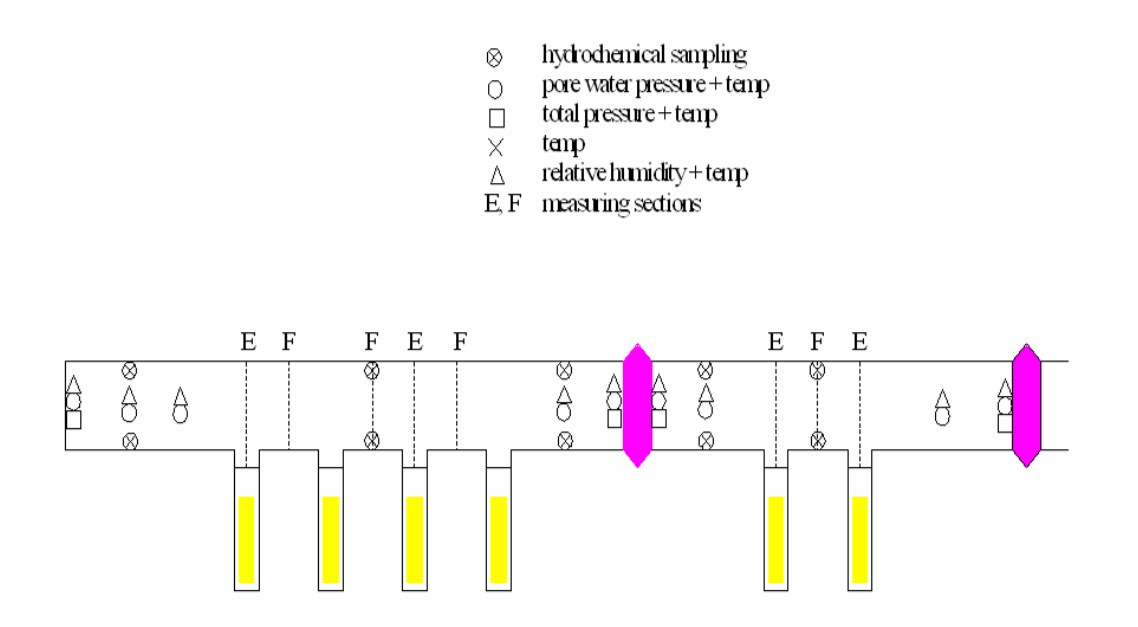


Figure 1-3. *Figure showing the positions of the titanium cups in the backfill in sections 1 and 2 (from AP TD F63-02-040).*

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 1-2. 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 1999 to the present, with a focus on the 2004–2007 period. The findings are discussed from a microbial point of view, which does not exclude that abiotic processes may also partly explain some phenomena.

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
- **Heterotrophy** – a microbial life strategy that includes consumption of oxygen or 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 not discussed in this context) and production of biomass or organic carbon.
- **Acetogenesis** – a microbial life strategy that produces organic carbon in the form of acetate using carbon dioxide and hydrogen gas.

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 or acetogenesis ending in acetate production) could compromise the stability of the copper canisters.

Water samples from the Prototype Repository 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 autotrophic acetogens (AA). In addition, the total number of bacteria (TNC) and the amount of ATP in the water were analysed. These parameters are determinants of the biomass in the sampled 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 various metals.
- The effect can also be **indirect**, depending on the extent to which the microbial activity increases the pH, which in turn can change the solubility constants of the tested elements.

1.4 Potential effects of the drainage in the Prototype Repository

The Prototype Repository differs from a real storage in that it is drained, through tubes in a pump hole in the middle of section 1 (situated farthest from the tunnel) and through tubes in the plug in section 2 (situated closest to the tunnel) (Johannesson, 2008). Water is constantly flowing through these draining tubes (Johannesson, 2008).

Draining makes the Prototype Repository different compared with a real storage. For example, the swelling pressure will be lower than in a repository and the inflow of groundwater to the Prototype is larger than to a real storage.

The drainage can affect the microbial processes inside the Prototype in several ways. Because of the drainage, water saturation of the backfill might not occur. In places were water reach but does not saturate the backfill, the number of microbes compared with a real storage can be exaggerated, as a consequence of the lower outer pressure on the cell walls. On the other hand, the drainage leaves dry places in the backfill, which in turn will give lower microbial numbers and activity in the Prototype compared with a real storage (since microbes need water).

The pore water chemistry would also potentially be affected. Because of the drainage, the Prototype Repository could act as a sink for components in the surrounding groundwater such as the indigenous groundwater gases (and possibly tunnel air). This would lead to inflow of gases and dissolved compounds that would otherwise be in equilibrium between the inside and the outside of the repository.

2 Material and methods

2.1 Sampling points and analyses performed

In 2001, the first section of the Prototype Repository was established (Figure 2-1). Both before and after this time, pore water and gas from sampling points outside (Table 2-1) and inside (Table 2-2) the repository were analysed on various occasions regarding gas content, microbes, and chemistry.

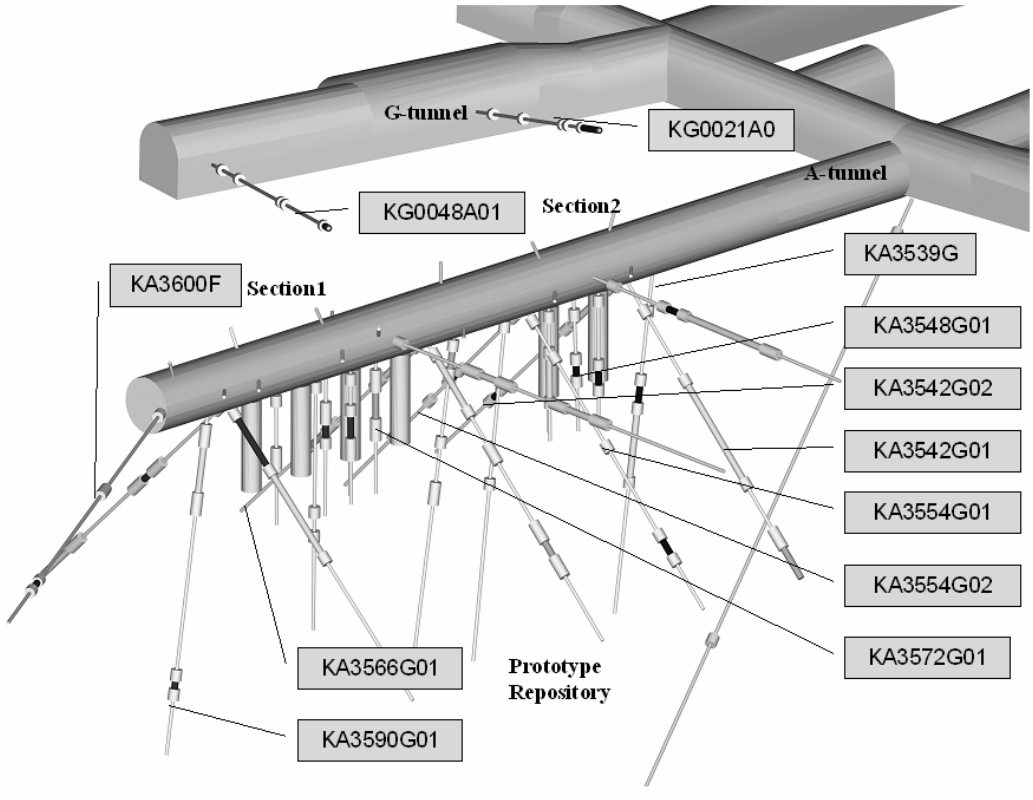


Figure 2-1. *Location of groundwater boreholes outside the Prototype Repository, except for boreholes KA3566G02:2 and KA3573A:1+2.*

The parameters analysed each year are listed in Table 2-3. The years given indicate when sampling vessels were installed at the sampling points.

Table 2-1. Analyses of groundwater samples from outside the Prototype Repository, 1999–2007.

Borehole	Gas analyses	Microbial analyses	Chemical analyses
KA3539G:2			2003, 2004
KA3542G01:2		2005, 2006	
KA3542G01:3		2005, 2006	2003, 2004
KA3542G02:5			2003, 2004
KA3548G01:3			2003, 2004
KA3554G01:1		2005, 2006	
KA3554G01:2		2005, 2006	2003, 2004
KA3554G02:4			2003, 2004
KA3566G01:2		1999	
KA3566G02:2		1999	2003, 2004
KA3572G01:2			2003, 2004
KA3573A:1		1999	
KA3573A:2		1999, 2006	
KA3590G01:2			2003, 2004
KA3600F:1		1999	
KA3600F:2		1999, 2006	2003, 2004
KG0021A01:2		2005, 2006	
KG0021A01:3		2005, 2006	2003, 2004
KG0048A01:3			2003, 2004
KJ0050F01	2006		
KJ0052F01	2006		
KJ0052F03	2006		

Table 2-2. Analyses of pore water samples and gas phases from inside the Prototype Repository, 1999–2007.

Sampling point	Gas analyses	Microbial analyses	Chemical analyses
KBU10001	2004, 2005, 2006, Apr 2007		
KBU10002	2004, 2006 , Apr 2007, May 2007	2005, 2006, Apr 2007, May 2007	Apr 2007, May 2007
KBU10003	2004, 2005, Apr 2007	2005	
KBU10004	2004, 2005, 2006 Apr 2007, May 2007	Apr 2007, May 2007	Apr 2007, May 2007
KBU10005	2004, 2005, Apr 2007	Apr 2007	Apr 2007
KBU10006	2004, 2005, 2006, Apr 2007, May 2007	Apr 2007	Apr 2007
KBU10007	2004, 2005, 2006, Apr 2007		
KBU10008	2004, 2005, 2006, Apr 2007, May 2007	2005, 2006, Apr 2007, May 2007	Apr 2007, May 2007
KB513	2004, 2005, Apr 2007	Apr 2007*	Apr 2007**
KB514	2004, 2005, Jan 2007, Apr 2007		
KB613	2004, 2005, Jan 2007, Apr 2007	Jan 2007*, Apr 2007	Apr 2007**
KB614	2004, 2005, Apr 2007	Apr 2007*	
KFA01	2004, 2006, Jan 2007, Apr 2007, May 2007	2005, Jan 2007, Apr 2007, May 2007	Jan 2007, Apr 2007, May 2007
KFA02	2004, 2005, Jan 2007, Apr 2007	Jan 2007*, Apr 2007	Jan 2007, Apr 2007
KFA03	2004, 2006, Jan 2007, Apr 2007	2005, Jan 2007*, Apr 2007	Jan 2007, Apr 2007
KFA04	2004, 2005, 2006, Jan 2007, Apr 2007, May 2007	2005, Jan 2007, Apr 2007, May 2007	Jan 2007, Apr 2007, May 2007

* Determination of ATP content was the only analysis performed. ** Cl^-, $SO4_2^-$, F^-, Br^-, and pH were analysed.

Table 2-3. Parameters analysed in samples from inside and outside the Prototype Repository from 1999 to 2007.

Year	Gas analyses	Microbial analyses	Chemical analyses
1999		TNC, MPN SRB, AA, IRB, HA, AM, and HM	
2003			Class 5
2004	H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2		Class 5
2005	H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	TNC, ATP, CHAB, MPN SRB, and MOB	
2006	H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	TNC, ATP, CHAB, MPN SRB, and MOB	
Jan 2007	H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, MPN SRB, and AA	Class 5
Apr 2007	H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP, MPN SRB, and AA	Class 5
May 2007	H_2, CO_2, CO, CH_4, C_2H_4, C_2H_6, O_2, He, N_2	ATP	Class 5

2.2 Gas analyses

2.2.1 Sampling of gas from groundwater outside the Prototype Repository

Groundwater outside the Prototype Repository was sampled using a standard PVB sampler, pressurized with 200 KPa nitrogen in the lower compartment. When installed, the sampler was filled and emptied three times, and then filled. After the last filling, flow through the sampler was continued at approximately 30 mL min^{-1} overnight. The sampler was subsequently closed, detached, and transported to Microbial Analytics Sweden AB in Göteborg.

2.2.2 Sampling of gas from the pore water and the gas phase inside the Prototype Repository

Before the first sampling at the hydrochemical sampling points in the Prototype Repository, a junction incorporating a valve and stopcock was mounted on the PEEK tube leading to each sample collector. Before sampling, the standing gas volume in the PEEK tube was evacuated with a vacuum pump for 15 min. This was done to ensure that the gas sample was representative of the interior of the Prototype Repository. The evacuation was done via a closed glass bottle to prevent water reaching the vacuum pump. When the evacuation cycle was completed, the stopcock was closed and the vacuum pump switched off. The stopcock remained closed, only being opened to permit sampling.

Before each ensuing sampling, a needle was mounted on the valve. The needle was inserted through the butyl rubber membrane of a 120-mL serum bottle. The stopcock was opened so that gas and/or water could pass. The sample bottle had been evacuated and flushed three times with N_2 and left fully evacuated. As the bottles were under vacuum, gas and/or water moved from the Prototype Repository into them. The bottles were left in contact with each collector for 20 min. Subsequently, the stopcock was closed and the needle removed from the bottle.

In December 2006, the sampling strategy for gas inside the Prototype Repository was changed. The sampling procedures used in former years worked well for some, but not all, sampling points, since difficulties were experienced extracting water from some points. There was also a risk the glass vessels could explode if high pore water or gas pressures were encountered. To overcome these problems, special high-vacuum, stainless steel pressure vessels (Mymeko, Göteborg, Sweden) began to be used to extract gas and/or water (Figure 2-2). Before the pressure vessels were connected, the pressure at each sampling point 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 Göteborg.

Figure 2-2. *Pressure vessels used for extracting pore water and gas from the Prototype Repository.*

2.2.3 Extraction of gas

The gas was extracted from the PVB samplers, bottles, and pressure vessels 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 vapour 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 aluminium 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.

2.2.4 Method development

The analytical procedures have undergone ongoing improvement and are now considered very reliable and reproducible. The data generated from the first sampling in 2004 have been treated as part of the method development process and have thus not been included in the graphs and calculations. The methodology and results of this sampling are discussed in TD-04-12.

2.2.5 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%.

2.2.6 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 Sampling of the groundwater outside the Prototype Repository

For microbiological analyses, samples were collected in sterile serum bottles (120 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 bottle was attached to its borehole via a short tube connector and a needle. The boreholes had been flushed before sampling. The connectors were sterilized with 70% ethanol. The connectors and needles were vigorously flushed with nitrogen gas prior to attachment, to remove the remaining ethanol. The valve was kept open until no more water entered the bottle; then the valve was closed and the bottle was transported to the MICROBE field laboratory, approximately 50 m from the sampling site. Inoculation and sample preparation were undertaken there, immediately after sampling.

2.3.2 Sampling of pore water inside the Prototype Repository

From 2005 to November 2006, samples for microbial analysis of the Prototype Repository pore water were taken in almost the same manner as the samples from the boreholes outside the Prototype. The only difference was that the sampling points inside the Prototype were not flushed, due to the restricted amount of water at these points. Starting in December 2006, the microbial samples were extracted from the pore water-filled pressure vessels (Figure 2-2) by adding 10 mL of sterile nitrogen to the pressure vessel while simultaneously extracting the same volume of water into sterile 27-mL anaerobic glass tubes (no. 2048-00150; Bellco Glass, Vineland, NJ, USA), which were sealed with butyl rubber stoppers (no. 2048-117800; Bellco Glass) and sealed with aluminium crimp seals (no. 2048-11020; Bellco Glass).

2.3.3 Sampling reproducibility of pore water inside the Prototype Repository

The effects of the lack of flushing were analysed by extracting three samples, one after each other, from sampling points KBU10008 and KFA04 in 2005 and KBU10002 and KBU10008 in November 2006. The microbial results of these samplings were compared and evaluated.

2.3.4 Determining total number of cells (TNC)

The total number of cells (TNC) was determined using the acridine orange direct count (AODC) method as devised by Hobbie et al. (1977) and modified by Pedersen and Ekendahl (1990). All solutions used were filtered through sterilized 32-mm-diameter, 0.2-µm-pore-size Sartorius Minisart CA syringe filters (GTF, Göteborg, Sweden). Stainless steel analytical filter holders, 13 mm in diameter (no. XX3001240; Millipore, Billerica, MA, USA), were rinsed with sterile, filtered, analytical grade water (AGW, Millipore Elix 3 purification system; Millipore). Samples of 1 mL were suction-filtered (–20 kPa) onto 0.22-µm-pore-size Sudan black-stained polycarbonate isopore filters, 13 mm in diameter (GTBP011300; Millipore). The filtered cells were stained for 5 min with 200 µL of an acridine orange (AO) solution (Sigma-Aldrich, St. Louis, MO, USA). The AO solution was prepared by dissolving 10 mg of AO in 1 L of a 6.6 mM sodium potassium phosphate buffer, pH 6.7 (Pedersen and Ekendahl, 1990). The filters were mounted between microscope slides and cover slips using fluorescence-free immersion oil (Olympus, Göteborg, Sweden). The number of cells was counted under blue light (390–490 nm) and, using a band-pass filter for orange light (530 nm), in an epifluorescence microscope (Nikon DIPHOT 300; Tekno-Optik, Göteborg, Sweden). Between 400 and 600 cells, or a minimum of 30 microscopic fields (1 field = 0.01 mm^2), were counted on each filter.

2.3.5 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).

2.3.6 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 (see Table 2-3D), 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.

2.3.7 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), autotrophic acetogen (AA), heterotrophic acetogen (HA), autotrophic methanogen (AM), and heterotrophic methanogen (HM) metabolic groups were autoclaved and anaerobically dispensed, according to the formulations outlined in Table 2-4, 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).

All culture tubes were flushed with 80/20% N_2/CO_2 gas and then filled with 9 mL of the appropriate medium. For IRB, 1 mL of hydrous ferric oxide (HFO), prepared from $FeCl_3$, was added to each culture tube. The final concentration of the iron solution was 0.44 M. The HM media also contained 20 mL L^{-1} of 100 g L^{-1} NaCOO, 3 mL L^{-1} of 6470 mM trimethylamine, 4 mL L^{-1} of methanol, and 20 mL L^{-1} of a 20 g L^{-1} solution of $NaCH_3COO$. The HA medium also contained 20 mL L^{-1} of 100 g L^{-1} NaCOO, 3 mL L^{-1} of 6470 mM trimethylamine, and 4 mL L^{-1} of methanol. The final pH was adjusted to between 6.5 and 7.5 with 1 M HCl or 1 M NaOH.

Table 2-4. A–G. Composition of anaerobic media used for MPN cultivation of different metabolic groups of anaerobic micro-organisms. All components were anoxic.

A) Ready medium	**Metabolic group**			
Component (mL L^{-1})	**IRB**	**SRB**	**AA & HA**	**AM & HM**
Basal medium (Table B)	940	860	860	890
Trace elements (Table C)	-	10	10	10
Trace elements (Table D)	1.0	-	-	-
Vitamins (Table E)	1.0	-	-	-
Vitamins (Table F)	-	10	10	10
Thiamine stock (Table G)	1.0	1.0	1.0	1.0
Vitamin B_{12} stock (Table G)	1.0	1.0	1.0	1.0
Fe stock (Table G)	-	5.0	5.0	5.0
Resazurin (Table G)	-	2.0	2.0	2.0
Cysteine hydrochloride (Table G)	-	10	10	10
$NaHCO_3$ (Table G)	30	60	60	60
Yeast extract (Table G)	1.0	10	10	10
$NaCH_3COO$ (Table G)	25	-	-	-
Lactate (Table G)	-	5.0	-	-
KNO_3 (Table G)	-	-	-	-
NaS × 9 H_2O (0.2 M) (Table G)	-	7.5	10	10

B) Basal medium	Metabolic group			
Component (g)	**IRB**	**SRB**	**AA & HA**	**AM & HM**
AGW	1000	1000	1000	1000
NaCl	7	7	7	7
$CaCl_2*2H_2O$	1.0	1.0	1.0	0.28
KCl	0.1	0.67	0.67	0.67
NH_4Cl	1.5	1.0	1.0	1.0
KH_2PO_4	0.2	0.15	0.15	0.15
$MgCl_2*6H_2O$	0.1	0.5	0.5	0.5
$MgSO_4*7H_2O$	0.1	3.0	-	-
$MnCl_2*4H_2O$	0.005	-	-	-
$Na_2MoO_4*2H_2O$	0.001	-	-	-

C) Trace element solution	
Component	**Amount**
AGW	1000 mL
Nitrilotriacetic acid	1500 mg
$Fe(NH_4)_2(SO_4)_2*6H_2O$	200 mg
Na_2SeO_3	200 mg
$CoCl_2*6H_2O$	100 mg
$MnCl_2*4H_2O$	100 mg
$Na_2MoO_4*2H_2O$	100 mg
$Na_2WO_4*2H_2O$	100 mg
$ZnSO_4*7H_2O$	100 mg
$AlCl_3$	40 mg
$NiCl_2*6H_2O$	25 mg
H_3BO_3	10 mg
$CuCl_2*2H_2O$	10 mg

D) Non-chelated trace elements	
Component	**Amount**
AGW	987 mL
HCl (25% = 7.7 M)	12.5 mL
$FeSO_4*7H_2O$	2.1 g
H_3BO_3	30 mg
$MnCl_2*4H_2O$	100 mg
$CoCl_2*6H_2O$	190 mg
$NiCl_2*6H_2O$	24 mg
$CuCl_2*2H_2O$	2 mg
$ZnSO_4*7H_2O$	144 mg
$Na_2MoO_4*2H_2O$	36 mg

E) Vitamin mixture for NRB, IRB, and MRB	
Component	**Amount**
Sodium phosphate buffer 10 mM, pH 7.1	100 mL
4-Aminobenzoic acid	4 mg
D(+)-biotin	1 mg
Nicotinic acid	10 mg
Pyridoxine dihydrochloride	15 mg
Calcium D(+) pantothenate	5 mg

F) Vitamin mixture for SRB, AA, HA, AM, and HM	
Component	**Amount**
Sodium phosphate buffer 10 mM pH 7.1	1000 mL
p-Aminobenzoic acid	10 mg
Nicotinic acid	10 mg
Calcium D(+) pantothenate	10 mg
Pyridoxine dihydrochloride	10 mg
Riboflavin	10 mg
D(+)-biotin	5 mg
Folic acid	5 mg
DL-6-8-thiotic acid	5 mg

G) Stock solutions	
Component	**Amount**
$NaHCO_3$	84 g L^{-1}
Thiamine chloride dihydrochloride in a 25 mM sodium phosphate buffer, pH 3.4	100 mg L^{-1}
Cyanocobalamin (B_{12})	50 mg L^{-1}
KNO_3	100 g L^{-1}
$NaCH_3COO$	100 g L^{-1}

G) Stock solutions, continued	
Component	**Amount**
Yeast extract	50 g L^{-1}
$Fe(NH_4)_2(SO_4)_2$*$6H_2O$, initially dissolved in 0.1 mL of concentrated HCl	2 g L^{-1}
Resazurin	500 mg L^{-1}
Cysteine-HCl	50 g L^{-1}
Sodium lactate solution	50%
NaS × 9 H_2O (0.2 M)	4.8 g L^{-1}

2.3.8 Inoculations and analysis for anaerobic microorganisms

Inoculations for IRB, SRB, AA, HA, AM, and HM were performed in the laboratory less that 2 h after sampling. After inoculating, the headspaces of only the AA and AM 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). Methanogens were detected by measuring the production of methane in the culture tube headspace. The methane was analysed using a Star 3400CX gas chromatograph (Varian) using a flame ionization detector (FID) at 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. Acetogens 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).

2.3.9 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 according to Table 2-4D 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 within 2 h of 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 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 Chemical analyses

2.4.1 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, or were subcontracted to external laboratories. The data used for evaluating the chemistry in the groundwater surrounding the Prototype were extracted from the primary SICADA database (SKB sample numbers 6039-6044, 6047-6049, 6051, 6184, 6186, 6199-6202, 6204, 6207, 6210-6211, 6263-668, 6274-6275, 6296, 6390, 6393-6394), and are traceable by borehole name and sampling occasion, as presented in Table 2-1.

2.4.2 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.

2.4.3 Evaluation of the chemistry

The chemistry data from the pore water in the sample groups were compared with the groundwater chemistry in those cases in which both types of data existed. The results were calculated by dividing the mean amount of a specific compound in the groundwater by the mean amount in the different sample groups, and were presented as an enrichment factor.

2.5 Statistical analyses

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

3 Results

3.1 Data

The water at different sampling points inside and outside the Prototype Repository was sampled at several occasions, from 1999 to May 2007 (Table 2-1, Table 2-2, and Table 2-3). All data generated from the pressure readings, microbial investigations, and gas analysis were quality checked and reported to SICADA. They are traceable by the sampling point name and sampling occasion. In Appendix A, all raw data can be found in Table 7-1, Table 7-2, and Table 7-3. All specific physical, chemical, and microbiological data in the following results section refer to these data.

3.2 Test of sampling reproducibility

We also tested for the reproducibility regarding samples extracted directly after each other from the same sampling point. Two or three approximately 100-mL samples were taken one after each other from each of sampling points KFA04 and KBU10008 (for location see Figure 1-1), respectively, and analysed in November 2005. Four additional samples from each of sampling points KBU10002 and KBU10008 were collected and analysed in November 2006. The results are shown in Table 3-1. The results varied between samples taken from the same sampling point. It was concluded that which of the sampled replicates is analysed may be significant. In pore water from sampling points KBU10002 and KBU10008, the number of SRB generally were lowest in the first sample extracted (Table 3-1), while this was not the case in water from sampling point KFA04. On the other hand, the MOB was highest in the first pore water extracted from sampling point KFA04, which was not the case in KBU10002 (Table 3-1). To eliminate any variation *within* the sampling points, the same replicate was used for each sampling point in further evaluating the results.

3.3 Prototype Repository sample groups

When examining the data collected from the sampling points in the Prototype, it was obvious that most of them differed from each other in a multitude of parameters, such as pressure, composition of the analysed gases, ATP content, and chemical composition. To illustrate this with an example, the pressure build-ups at the different sampling points over the lifetime of the Prototype have been plotted (Figure 3-1). Additionally, the sampling points differ in how much gas and pore water is available (Table 3-2). This extractable amount of pore water, and the gas content, changes with time in the different sampling points (Table 3-2).

Table 3-1. Sampling reproducibility test of microbes and composition of the extracted gas from the pore water inside the Prototype Repository. The gas content is given in ppm of the total amount of extracted gas from each sample.

Sample	Year	TNC (mL^{-1})	ATP (amol mL^{-1})	CHAB (mL^{-1})	SRB (mL^{-1})	MOB (mL^{-1})	H_2 (ppm)	CO (ppm)	CH_4 (ppm)	CO_2 (ppm)	C_2H_6 (ppm)	C_2H_4 (ppm)	He (ppm)	O_2 (ppm)	N_2 (ppm)
KBU10008:1	**2005**	370000	127000	bd		800									
KBU10008:2		360000		320000		240									
KBU10008:3		870000	217000	41000		24									
KFA04:1			81500	bd	2.7	800									
KFA04:2			11800	17	1.2	900									
KFA04:3				7	1.3	24									
KBU10002:1	**2006**	140000	45900	133	bd	1300	18.4	101	2140	38200	1.78	bd	bd	149000	817000
KBU10002:2		350000	74300	540	30	1100	18.9	83.6	688	25000	0.84	bd	bd	234000	742000
KBU10002:3		96000	41400	147	24	1700	22.3	64.1	105	90300	bd	bd	bd	156000	762000
KBU10002:4							21.1	36.2	54	101000	1.1	bd	5980	41300	862000
KBU10008:1		230000	124000		<0.2	70	14.6	65.5	49	30100	bd	bd	bd	26400	708000
KBU10008:2		240000	101000	9630	130	13	18.7	32.7	68	64600	bd	bd	17700	10100	876000
KBU10008:3		94000	24000	63	110	240	24.7	60.9	153	74500	0.50	bd	bd	bd	920000
KBU10008:4							45	44.2	7130	3950	0.50	bd	30500	13500	953000

Table 3-2. Pore water and gas extracted from the sampling points in the Prototype Repository, 2004–May 2007.

Sampling point	Sampling occasion	Pore water (mL)	n	Gas (mL)	n
KBU10001	2004	8	13	18	9
	2005	0	1	11	1
	2006	77	1	49	1
	Apr 2007	0	1	145	1
KBU10002	2004	25	4	5	4
	2006	90	1	2	1
	Apr 2007	38	1	11	1
	May 2007	36	1	4	1
KBU10003	2004	7	4	10	4
	2005	116	1	5	1
	Apr 2007	0	1	41	1
KBU10004	2004	0	4	29	4
	2005	0	1	11	1
	2006	60	1	8	1
	Apr 2007	34	1	13	1
	May 2007	35	1	2	1
KBU10005	2004	3	4	6	4
	2005	0	1	11	1
	Apr 2007	30	1	6	1
KBU10006	2004	0	13	26	7
	2005	0	1	12	1
	2006	29	1	36	1
	Apr 2007	38	1	12	1
KBU10007	2004	0	4	0	4
	2005	0	1	12	1
	2006	0	1	86	1
	Apr 2007	0	1	40	1
KBU10008	2004	24	4	6	4
	2005	115	1	8	1
	2006	86	1	3	1
	Apr 2007	38	1	10	1
	May 2007	36	1	4	1
KB513	2004	0	4	27	4
	2005	0	1	11	1
	Apr 2007	4	1	24	1

KB514	2004	0	4	27	4
	2005	0	1	11	1
	Jan 2007	0	1	33	1
	Apr 2007	0	1	38	1
KBU613	2004	0	4	6	4
	2005	0	1	11	1
	Jan 2007	0	1	15	1
	Apr 2007	5	1	16	1
KBU614	2004	0	4	27	4
	2005	0	1	11	1
	Apr 2007	0	1	26	1
KFA01	2004	1	6	10	6
	2006	47	1	6	1
	Jan 2007	37	1	2	1
	Apr 2007	38	1	8	1
	May 2007	33	1	6	1
KFA02	2004	2	8	9	8
	2005	30	1	12	1
	Jan 2007	32	1	3	1
	Apr 2007	33	1	7	1
KFA03	2004	1	4	17	4
	2006	28	1	6	1
	Jan 2007	37	1	9	1
	Apr 2007	35	1	10	1
KFA04	2004	9	3	8	3
	2005	107	1	12	1
	2006	45	1	8	1
	Jan 2007	40	1	2	1
	Apr 2007	38	1	8	1
	May 2007	38	1	7	1

Comparison of the results for each sampling point with those for the surrounding groundwater suggested that the individual sampling points could be divided into seven groups, each sharing similar properties. The sampling points were grouped together based similar gas composition, water pressure development, pore water content, and pore water chemistry. The following groups were identified:

- **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) produced no or very little water. 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.

- **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.

- **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. In May 2007, the pressure in these sampling points was the same as the atmospheric pressure in the tunnel.

- **KBU10005:** 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.

- **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. The pressure in these sampling points increased with time, and was 3 bar as of May 2007.

- **KBU10004 and KBU10006:** There 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.

- **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.

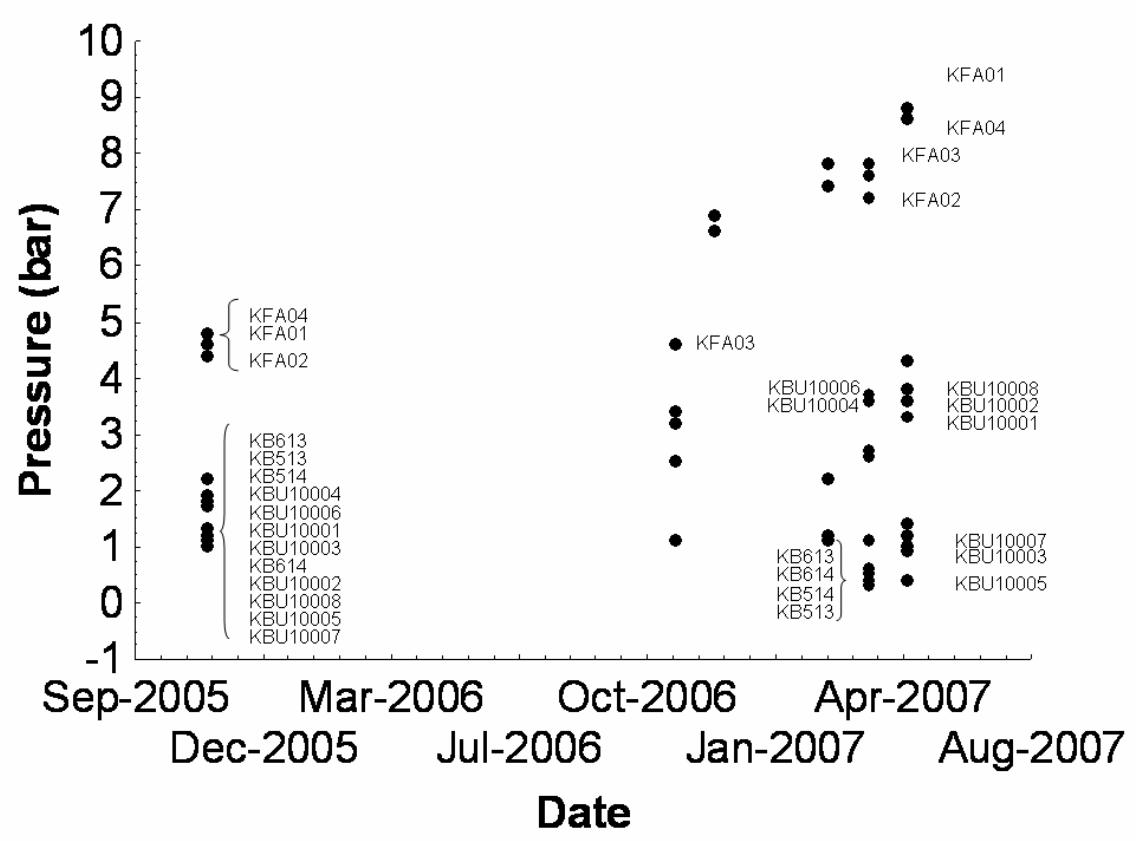


Figure 3-1. *Pressure readings in the Prototype Repository sampling points, 2005–2007.*

3.4 Composition of dissolved gases, microbes, and chemistry in the pore water in the sample groups in the Prototype Repository

The postulated sample groups were treated separately. The data for the different sample groups were also compared with the data for the Äspö groundwater surrounding the Prototype (Table 3-3, Table 3-4, and Table 3-5).

3.4.1 Dissolved gas composition:

The three boreholes KJ0050F01, KJ0052F01, and KJ0052F03 sampled at the MICROBE site near the Prototype were used for the comparison of groundwater gas composition to Prototype pore water gas composition (Table 2-1). On average, the gas in this groundwater contained no oxygen, 81% nitrogen, 14% helium, 1% methane, 0.3% carbon dioxide, and traces of carbon monoxide, hydrogen, and ethane (Table 3-3).

The following table describes the development of the gas composition in the Prototype sample groups in recent years. The values presented in the graphs are means of all the values for a group from one sampling occasion; these mean values are reported with the standard deviation in Table 3-3. In the appendices, all raw data from the gas analyses are reported.

Table 3-3. The mean gas composition in the sample groups inside the Prototype Repository at the different sampling occasions during 2005–2007. The gas content is given in ppm of the total amount of extracted gas.

Sample group	Sampling occasion	*n*	H_2 (ppm)	stddev	% stddev	CO (ppm)	stddev	%stddev	CH_4 (ppm)	stddev	%stddev	Comment
Äspö groundwater	2006	3	241	309	129	7.93	6.92	87	8340	2910	35	
KB513-614	2005	4	26.5	23.9	90	22.0	7.36	33	671	437	65	
	Jan 2007	2	99.1	43.8	44	18.3	5.59	31	1200	822	69	
	Apr 2007	4	32.2	33.4	104	143	217	151	3190	379	12	
KBU10001	2005	1	7.00			23.0			1440			
	2006	1	3.50			12.7			20.0			
	Apr 2007	1	1.80			4.90			10000			
KBU10003+7	2005	2	118	155	132	13.8	4.10	30	15000	20900	139	
	2006	1	2.60			26.4			117			
	Apr 2007	2	2.25	0.07	3	33.8	28.1	83	66	72.1	109	
KBU10005	2005	1	9.60			15.2			320			
	Apr 2007	1	7.90			154			39.0			
KBU10002+8	2005	1	17.9			15.1			7050			
	2006	2	23.5	1.70	7	62.5	2.26	4	129	33.9	26	
	Apr 2007	2	13100	18000	138	72.4	81.4	112	453	33.2	7	
	May 2007	2	9.45	2.33	25	200	24.0	12	235	177	76	
KBU10004+6	2005	2	8.60	1.84	21	13.4	0.21	2	252	107	42	
	2006	2	4.03	2.40	56	15.6	2.76	18	620	211	34	
	Apr 2007	2	91.7	88.2	96	28.1	34.7	124	46.0	35.4	77	
	May 2007	2	664	919	139	164	204	124	171	169	99	
KFA01-04	2005	2	11.1	0.21	2	25.8	13.6	53	2170	665	31	
	2006	3	14500	24000	165	24.7	17.8	72	743	906	122	*
	Jan 2007	4	59000	82000	139	78.5	58.0	74	537	161	30	
	Apr 2007	4	68.6	46.6	68	42.9	35.9	84	2130	1390	65	
	May 2007	2	79.4	85.8	108	291	393	135	1790	49.5	3	

Table 3-3, continued

Sample group	Sampling occasion	*n*	CO_2	stddev	% stddev	C_2H_6	stddev	%stddev	C_2H_4	stddev	%stddev	Comment
Äspö groundwater	2006	3	3330	1940	58	2.67	0.37	14	bd			
KB513-614	2005	4	699	826	118	0.63	0.60	95	0.08	0.15	200	
	Jan 2007	2	10900	7620	70	0.84	0.93	111	2.58	3.31	128	
	Apr 2007	4	6580	4250	65	2.25	3.70	164	0.76	0.72	94	
KBU10001:	2005	1	4930			2.10			2.10			
	2006	1	4040			0.33			bd			
	Apr 2007	1	259			bd			bd			
KBU10003+7	2005	2	199	134	68	1.60	0.57	35	0.20	0.28	141	
	2006	1	117			491			0.76			
	Apr 2007	2	762	42.4	6	0.49	0.69	141	0.13	0.18	141	
KBU10005	2005	1	25.3			bd			bd			
	Apr 2007	1	11300			bd			1.16			
KBU10002+8	2005	1	778			0.90			bd			
	2006	2	82400	11200	14	0.25	0.35	141	bd			
	Apr 2007	2	9200	771	8	0.15	0.21	141	bd			
	May 2007	2	11400	3970	35	bd			bd			
KBU10004+6	2005	2	1330	789	59	1.90	0.99	52	0.10	0.14	141	
	2006	2	8740	7020	80	1.59	0.06	4	0.05	0.07	141	
	Apr 2007	2	7110	672	9	0.33	0.47	141	bd			
	May 2007	2	25000	25300	101	bd			bd			
KFA01-04	2005	2	182	220	121	0.65	0.07	11	bd			
	2006	3	28300	14300	50	0.97	0.345	35	0.103	0.18	173	*
	Jan 2007	4	102000	111000	109	1.93	0.80	41	0.58	1.02	177	
	Apr 2007	4	9660	2900	30	0.85	0.98	116	0.22	0.44	200	
	May 2007	2	7740	6570	85	bd			bd			

Table 3-3, continued

Sample group	Sampling occasion	*n*	He (ppm)	stddev	% stddev	O_2 (ppm)	stddev	%stddev	N_2 (ppm)	stddev	%stddev	Comment
Äspö groundwater	2006	3	138000	40700	30	bd			810000	72500	9	
KB513-614	2005	4	5510	7000	127	144000	50200	35	813000	44400	5	
	Jan 2007	2	21700	21100	97	83500	24700	30	914000	18400	2	
	Apr 2007	4	34300	27000	79	72200	15100	21	892000	33700	4	
KBU10001:	2005	1	bd			153000			809000			
	2006	1	bd			64000			931000			
	Apr 2007	1	bd			71700			918000			
KBU10003+7	2005	2	bd			98100	119000	121	860000	124000	14	
	2006	1	bd			200000			790000			
	Apr 2007	2	bd			202000	1410	1	800000	1410	0.2	
KBU10005	2005	1	bd			178000			778000			
	Apr 2007	1	bd			30500			939000			
KBU10002+8	2005	1	bd			62200			901000			
	2006	2	bd			78000	110000	141	841000	112000	13	
	Apr 2007	2	bd			47500	23000	48	933000	42400	5	
	May 2007	2	bd			76700	9120	12	907000	3850	0.4	
KBU10004+6	2005	2	bd			121500	6360	5	829000	11300	1	
	2006	2	bd			69200	66200	96	921000	64300	7	
	Apr 2007	2	bd			12900	18200	141	969000	13400	1	
	May 2007	2	bd			52200	34300	66	913000	58700	6	
KFA01-04	2005	2	11500	5620	49	107000	61400	58	839000	62900	8	
	2006	3	1540	401	26	24200	26000	107	931000	81600	9	*
	Jan 2007	4	7570	11100	147	28100	33500	119	868000	62200	7	
	Apr 2007	4	9120	12200	134	370000	23400	63	937000	24800	3	
	May 2007	2	2010	1610	80	77300	59000	76	910000	53700	6	

*Different sampling procedures were applied.

KB513, KB514, KB613, and KB614: In the KB513-614 group, no or very little water was present, but the composition of gases could nevertheless be investigated. Figure 3-2 shows the contents of the major gases, i.e., nitrogen and oxygen, and the minor gases, i.e., helium, hydrogen, methane, ethane, ethene,and ethylene, carbon dioxide, and carbon monoxide, in the KB513-614 group over approximately a year and a half. The oxygen level decreased from 14% to approximately 6% over this period, while the nitrogen content increased from approximately 81% to 89% and the helium and carbon dioxide contents increased to 3% and 1%, respectively. The methane and hydrogen gas contents varied but remained below 1%.

KBU10001: Water could not be extracted from the KBU10001 group. Figure 3-3 shows the change in content of the gas phase in KBU10001 from November 2006 to April 2007. The oxygen level decreased from 15% to approximately 7% over this time, while the nitrogen content increased from approximately 81% to 92%. The amounts of carbon dioxide, methane, and hydrogen gas varied but remained below 1%. Helium was not detected at any time.

KBU10003 and KBU10007: The third sampling group in the Prototype Repository that produced gas, but no pore water, was the KBU10003+7 group. Despite the lack of water, this group displayed a gas profile different from those of the KB513-614 and KBU10001 groups (Table 3-3, Figure 3-4). Over the one and a half years from November 2005 to April 2007, the oxygen level increased from 10% to approximately 20%. At the same time, the nitrogen content decreased from approximately 86% to 79% and the methane content decreased from approximately 1% to below the detection limit. The amounts of carbon dioxide and hydrogen gas varied over the entire period, but remained below 1%. Helium was not detected at any time. The composition in April 2007 was thus the same as in the outside air, suggesting that either the sampling points were in contact with tunnel air, or the tubes connected to the sampling points were broken and in contact with the tunnel.

KBU10005: Gas could be extracted from the pore water in the KBU10005 group from the Prototype Repository on two occasions, in November 2005 and April 2007. The following trends were seen: The oxygen level decreased from 18% to approximately 3% over this time (Figure 3-5), while the nitrogen content increased from approximately 78% to 94% and the carbon dioxide content increased to approximately 1%. The amount of methane and hydrogen gas varied but remained below 1%. Helium was not detected at any time.

KBU10002 and KBU10008: Gas could easily be extracted from the pore water in the KBU10002+8 group. The gas composition for this sample group behaved differently from those of the other groups, in that it generally lacked distinctive trends (Figure 3-6). The oxygen content fluctuated between 4.5% and 8% and the nitrogen content between 84% and 92%. The carbon dioxide content reached 8% on one occasion and the hydrogen content reached 1%. Interestingly, the only obvious trend was a decrease in methane content from 0.7% to below 0.1%. No helium was detected at any time.

KBU10004 and KBU10006: Gas could be extracted from the pore water in the KBU10004+6 group. Figure 3-7 shows that, between November 2005 and May 2007, the oxygen level decreased from 12% to 5% while the nitrogen content increased from 83% to approximately 92%.The carbon dioxide content increased to 2.4% as of May 2007. The amount of methane and hydrogen gas varied, but remained below 1%. Helium was not detected at any time.

KFA01, KFA02, KFA03, and KFA04: Gas could be extracted from the pore water in the KFA01-04. The data were more scattered for this group than for the KBU10004+6 group, for example; in general, however, Figure 3-8 shows that the trends detected resembled those of the other groups. From November 2005 to May 2007 the oxygen level decreased from 11% to 5% while the nitrogen content increased from 84% to approximately 90%. The carbon dioxide and hydrogen contents were occasionally high and varied from below 1% to up to 10% and 6%, respectively. The amounts of methane and helium were generally decreasing and, when measurement began in 2005, their contents were approximately 2% and 1%, respectively.

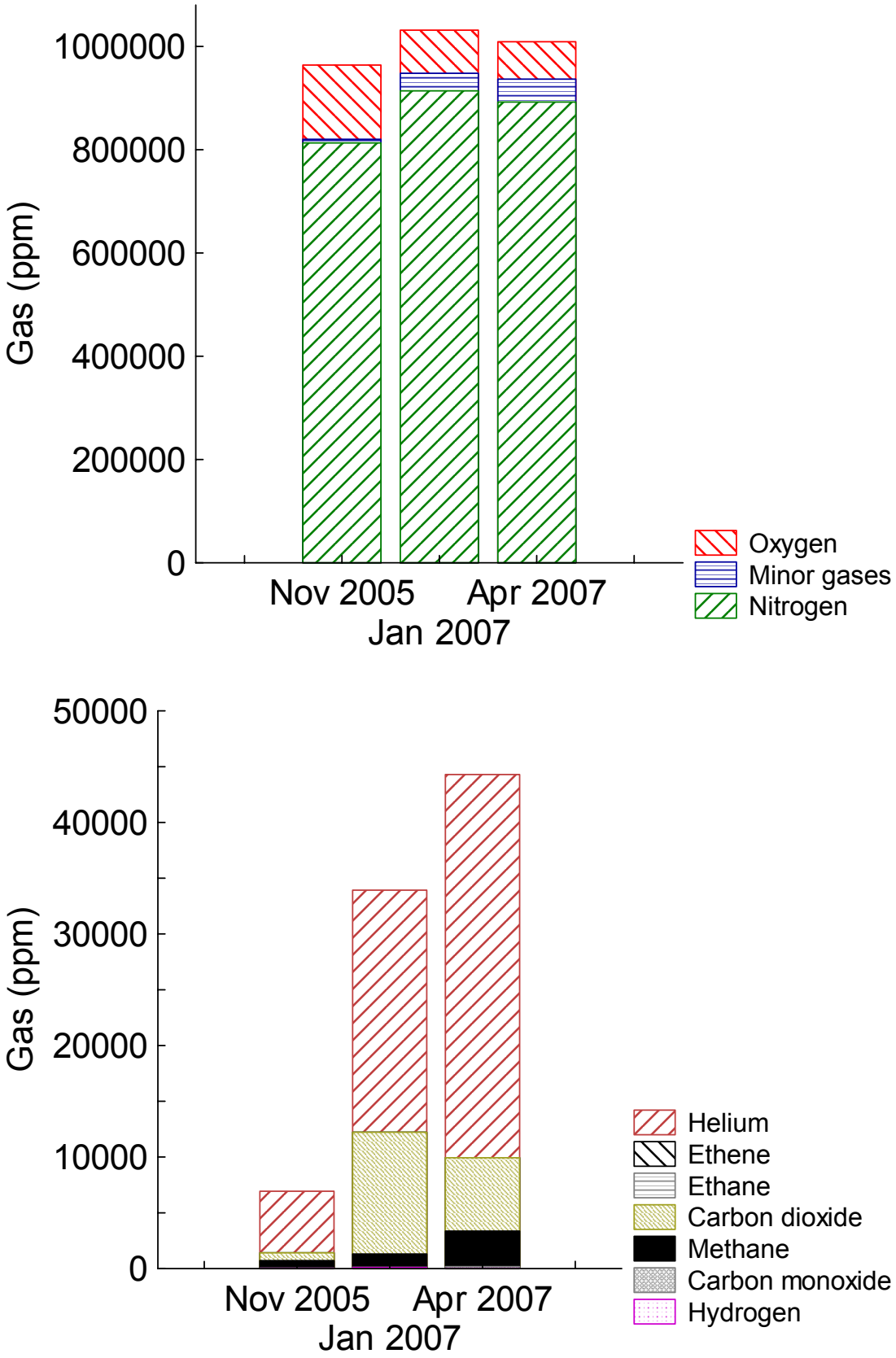


Figure 3-2. *The mean composition of major and minor gases in the KB513-614 group at the different sampling occasions during 2005–2007. The gas content is given in ppm of the total amount of extracted gas.*

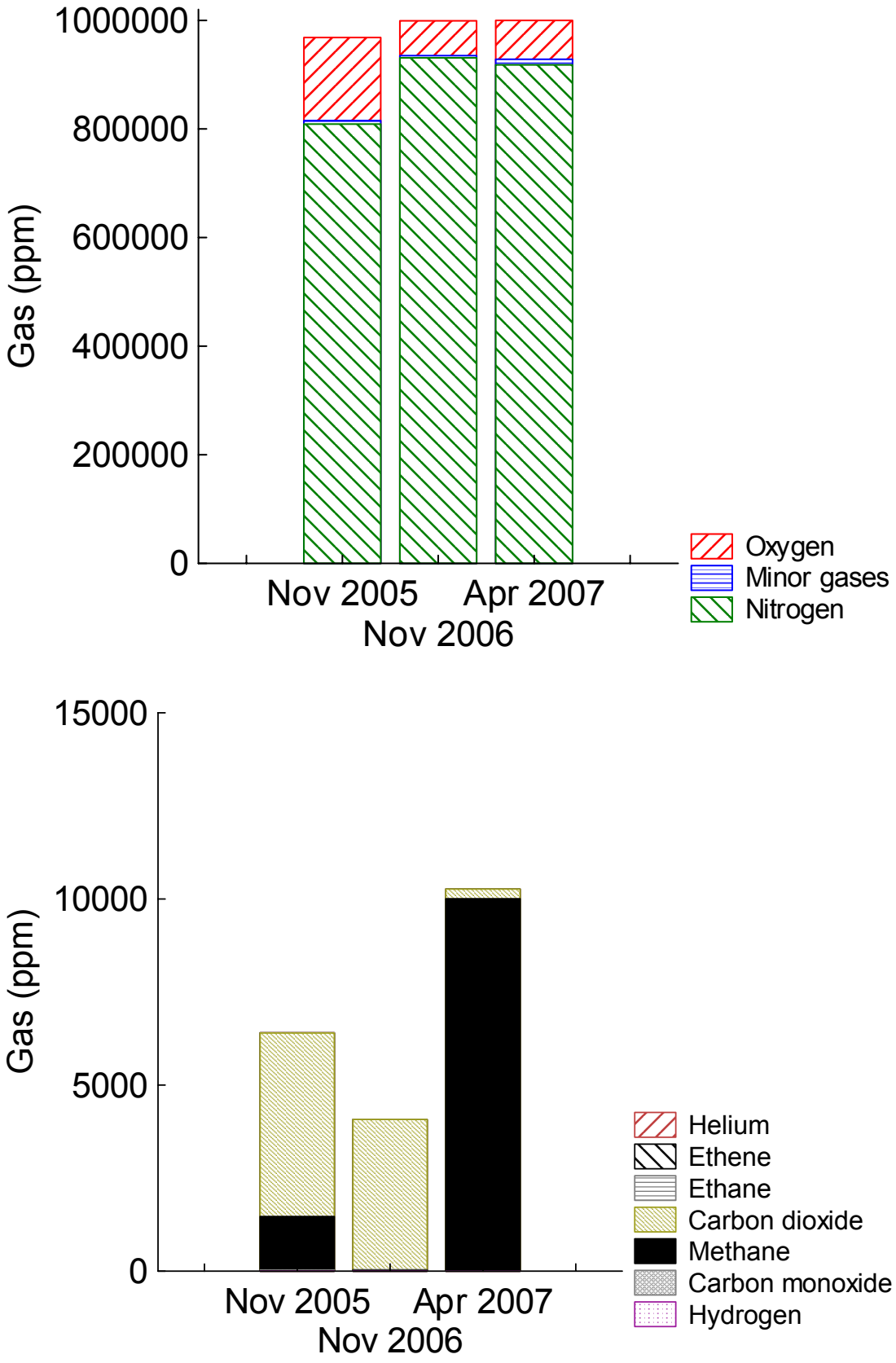


Figure 3-3. *The composition of major and minor gases in the KBU10001 group at the different sampling occasions during 2005–2007. The gas content is given in ppm of the total amount of extracted gas.*

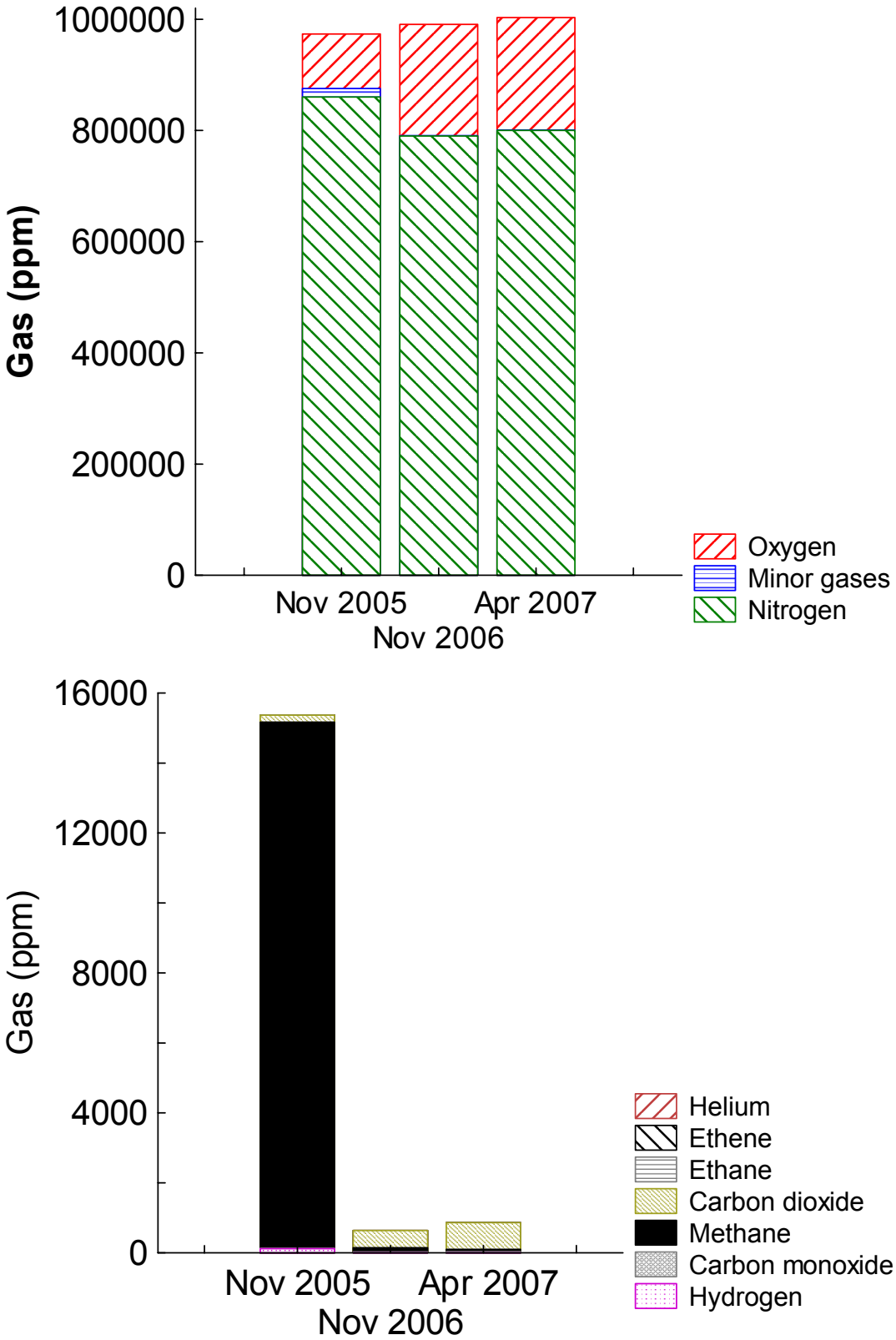


Figure 3-4. *The mean composition of major and minor gases in the KBU10003+7 group at the different sampling occasions during 2005–2007. The gas content is given in ppm of the total amount of extracted gas.*

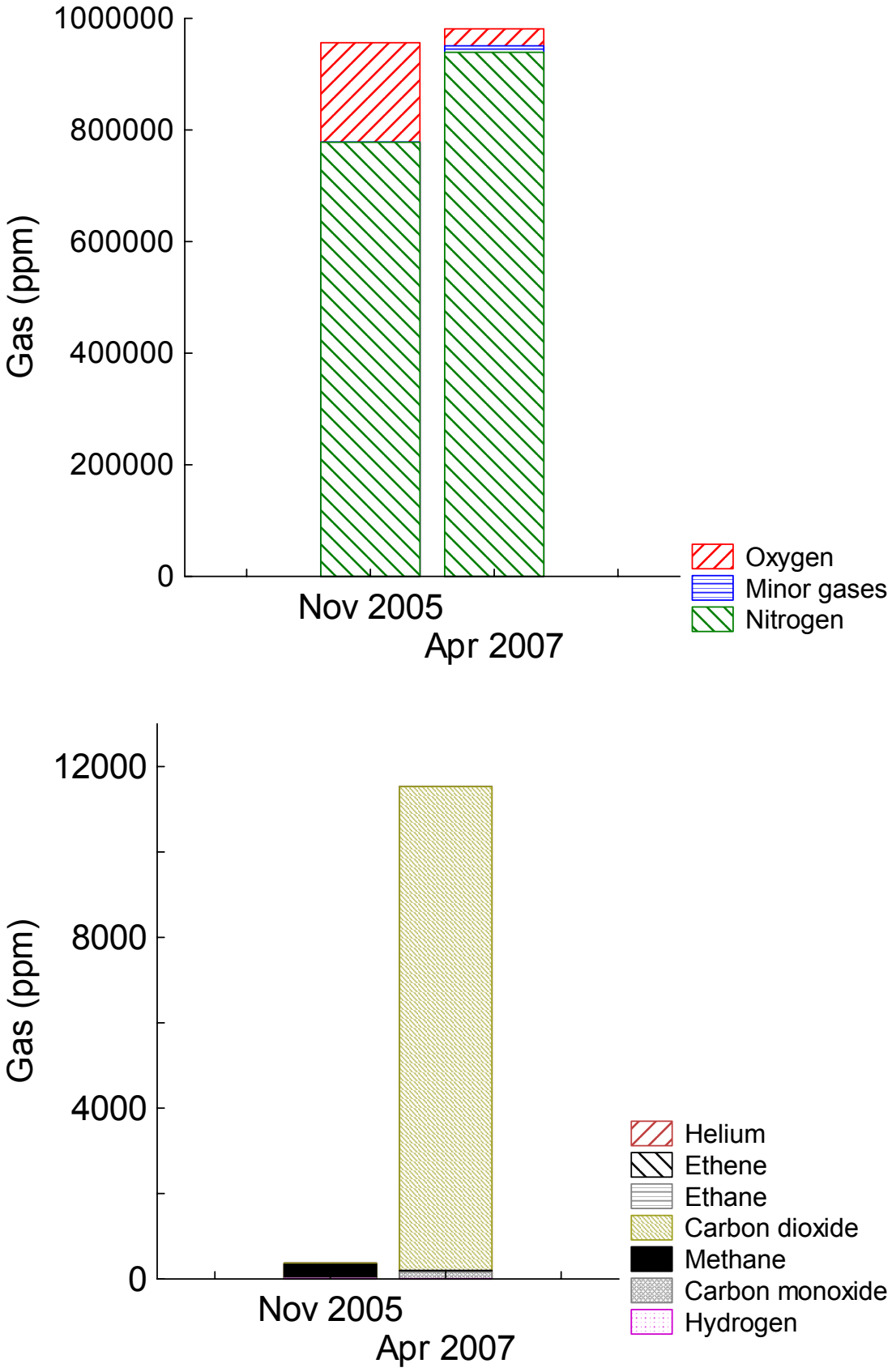


Figure 3-5. The *composition of major and minor gases in the* KBU10005 *group at the different sampling occasions during 2005–2007. The gas content is given in ppm of the total amount of extracted gas.*

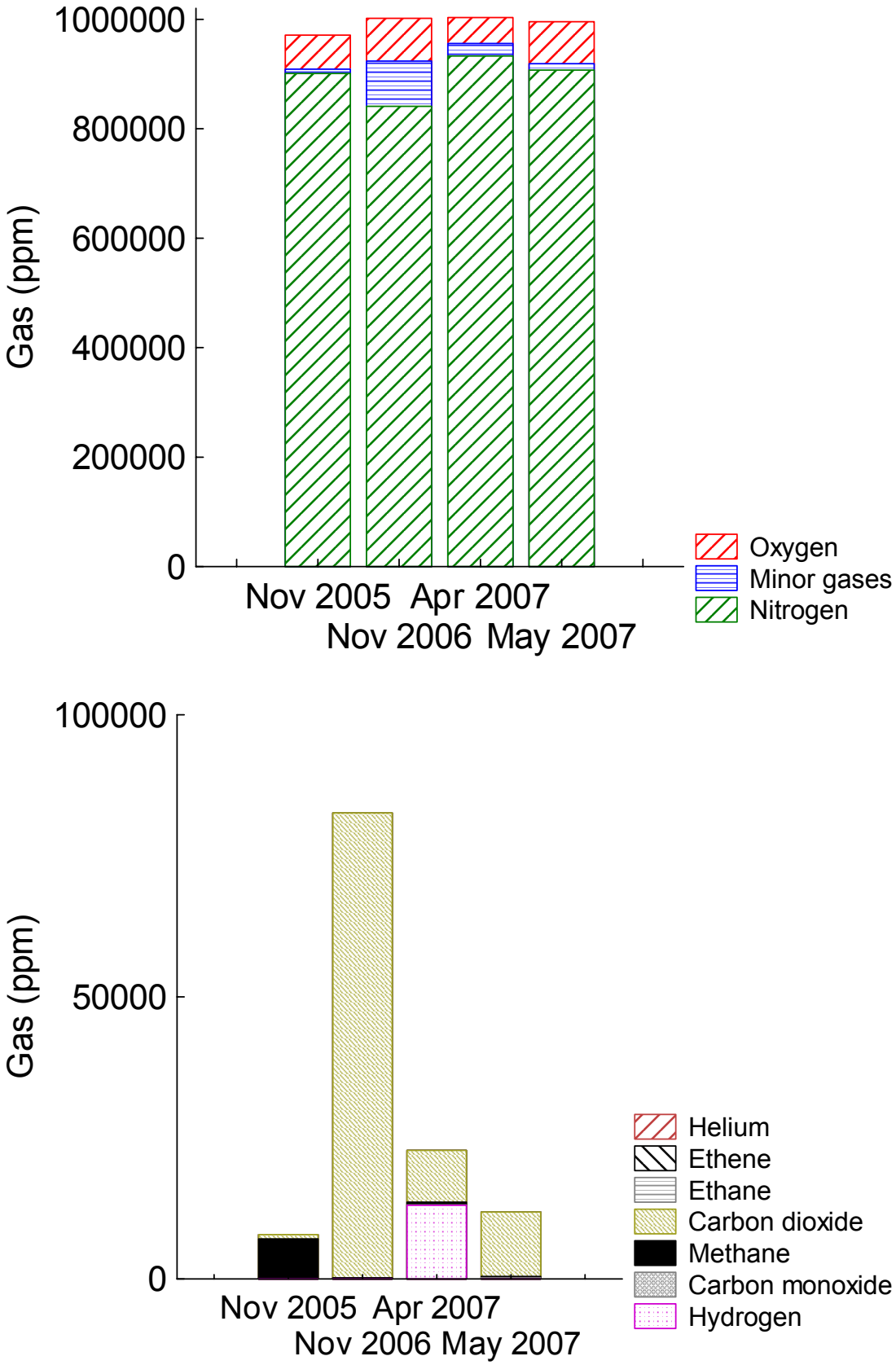


Figure 3-6. The *mean composition of major and minor gases in the* KBU10002+8 *group at the different sampling occasions during 2005–2007. The gas content is given in ppm of the total amount of extracted gas.*

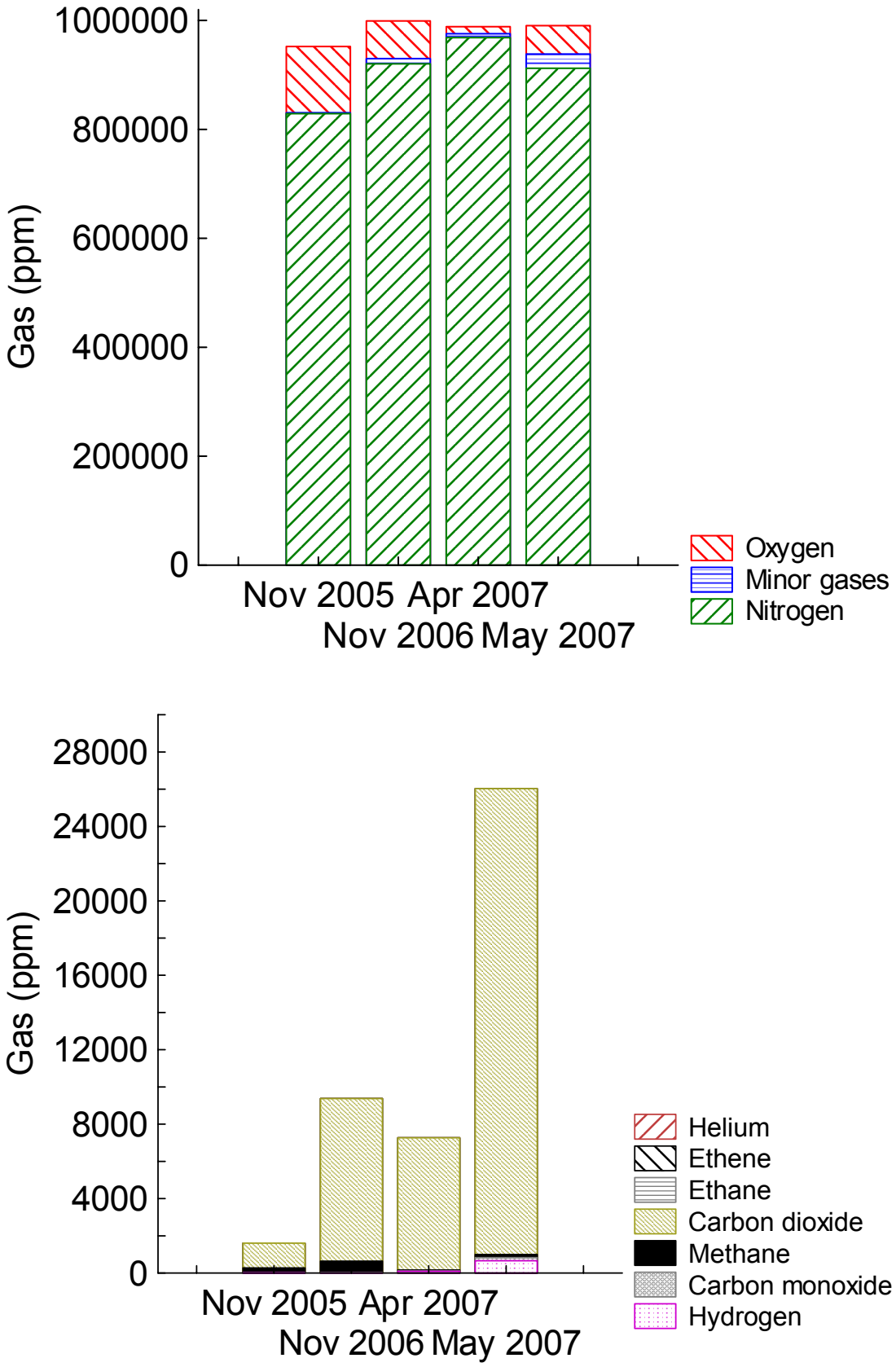


Figure 3-7. The *mean composition of major and minor gases in the* KBU10004+6 *group at the different sampling occasions during 2005–2007. The gas content is given in ppm of the total amount of extracted gas.*

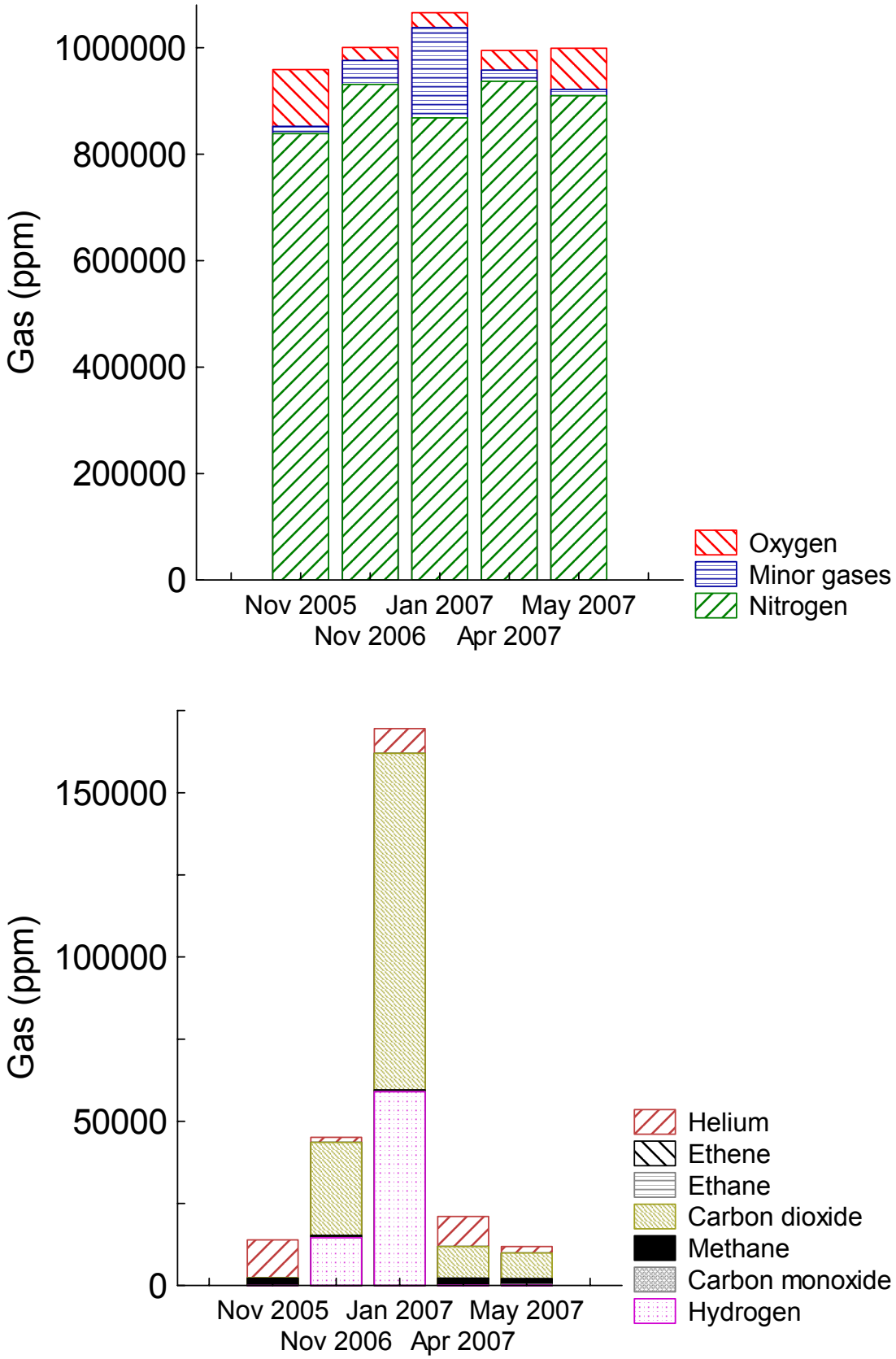


Figure 3-8. *The* mean composition of major and minor gases in the *KFA01-04* group at *the different sampling occasions during 2005–2007. The gas content is given in ppm of the total amount of extracted gas.*

3.4.2 Microbial composition

The microbial composition of the pore water inside the Prototype was compared with the mean results for the surrounding groundwater as sampled on 21 occasions from 1999 to 2006 (Table 3-4); this sampling was done from boreholes located near the Prototype (Table 2-1, Figure 2-1). In the groundwater from these boreholes, the numbers of SRB, MOB, CHAB, and AA were on average 96, 2, 8, and 0.13 mL^{-1}, respectively; the TNC was on average 28,600 and the ATP content 5000 amoles mL^{-1}. The mean data from the microbial analyses of the sample groups are shown in Table 3-4. In addition, the mean numbers of HA, IRB, HM, and AM (standard deviation within parentheses) were 647 (1410), 629 (1030), 4.15 (9.73), and below detection, respectively. The appendices present the raw microbial data (Table 7-2).

Table 3-4. The microbial composition of groundwater outside the Prototype Repository and of pore water inside the Prototype analysed in 1999-2007.

Sample group	Sampling occasion	TNC (mL^{-1})	*n*	stddev	% stddev	ATP (amol mL^{-1})	*n*	stddev	%stddev	CHAB (mL^{-1})	*n*	stddev	%stddev
Äspö groundwater	1999-2006	28600	21	30400	106	5000	12	2160	43	7.87	15	15.5	197
KB513-614	Jan 2007					74200000	1						
	Apr 2007					28900000	3	29400000	102				
KBU10003+7	2005	470000	1							1230	1		
KBU10005	Apr 2007					1680000	1						
KBU10002+8	2005	260000	1			77500	1			4600	1		
	2006	95000	2	1410	1	32700	2	12300	38	105	2	59.4	57
	Apr 2007					556000	2	719000	129				
	May 2007					1100000	2	186000	17				
KBU10004+6	Apr 2007					1450000	2	803000	55				
	May 2007					11000000	1						
KFA01-04	2005	410000	1			71400	3	14100	20	690	3	598	87
	Jan 2007					1930000	4	1190000	62				
	Apr 2007					3850000	4	6050000	157				
	May 2007					2860000	2	399000	14				

Table 3-4, continued

Sample group	Sampling occasion	SRB(mL^{-1})	*n*	stddev	% stddev	MOB (mL^{-1})	*n*	stddev	%stddev	AA (mL^{-1})	*n*	stddev	%stddev
Äspö groundwater	1999-2006	206	21	623	302	2.31	14	3.44	149	0.13	6	0.32	245
KB513-614	Jan 2007												
	Apr 2007	bd								1	2	1.41	141
KBU10003+7	2005	bd				23	1						
KBU10005	Apr 2007	bd								bd			
KBU10002+8	2005	9	1			24	1						
	2006	67	2	61	91	970	2	1030	106				
	Apr 2007	2540	2	3490	138					25.0	2	35.3	141
	May 2007												
KBU10004+6	Apr 2007	bd								bd			
	May 2007												
KFA01-04	2005	1.35	2	1.91	141	271	3	468	169				
	Jan 2007												
	Apr 2007	0.50	4	1.00	200					4.50	4	6.61	147
	May 2007												

KB513, KB514, KB613, and KB614: No or very little water was present in this group. Sufficient water could nevertheless be extracted on three sampling occasions in 2007 to allow investigation of the amount of ATP in the water (Table 3-4). These particular samples yielded the highest ATP values measured in the history of the Prototype Repository: 28,900,000–74,200,000 amoles mL^{-1}, approximately ten times the ATP content of other pore waters and 14,000 times that of the groundwater outside the Prototype (Table 3-4). On one sampling occasion, enough water was extracted to permit MPN analysis of AA and SRB as well.The MPN analysis made on this occasion revealed 2 AA mL^{-1} in the water. Appendix B includes a report on the molecular characterization of the microbes in KB613. It demonstrates that the microbial community largely consisted of a bacterium belonging to the genus *Microthrix* (99% similarity to the closest sequence according to 16S rRNA gene sequencing), able to grow in filaments in nutrient-rich environments.

KBU10001: Since water could not be extracted from this sampling group, microbial analyses were not performed.

KBU10003 and KBU10007: The microbial composition in the KBU10003+7 group was examined on one occasion in 2005 (Table 3-4). The TNC and CHAB in this water were 16 and 156 times higher, respectively, than in the surrounding groundwater. The number of MOB was 23 mL^{-1}, ten times higher than in Äspö groundwater. Sulphate-reducing bacteria were not detected.

KBU10005: In April 2007, the microbial composition of the pore water from sampling point KBU10005 was examined. The ATP value was 1,500,000 amoles mL^{-1} in this water, approximately 300 times higher than in the surrounding groundwater (Table 3-4). Cultivable AA or SRB could not, however, be detected.

KBU10002 and KBU10008: The KBU10002+8 group is well characterized. Seven sets of microbial analyses have been performed over the years. The ATP content at the sampling points increased from 30,000 to 1,000,000 amol mL^{-1} between November 2005 (Figure 3-9) and May 2007. The physiological diversity of this biomass, i.e., the numbers of MOB, SRB, CHAB, and TNC, were evaluated in November 2006. In April 2007, the numbers of AA and SRB were determined.

Overall, the aerobic microbial numbers were somewhat higher in the waters from the KBU10002+8 group than in the groundwater or the other sample groups in the Prototype. The numbers of MOB and CHAB reached 970 and 4600 cells mL^{-1}, substantially higher than in the surrounding groundwater, which contained 8 and 2 cells mL^{-1}, respectively (Table 3-4). The same was observed regarding the numbers of SRB. In the Äspö groundwater, approximately 206 SRB mL^{-1} were detected. In the KBU10002+8 group, an average of 2540 SRB mL^{-1} were found in 2007. Furthermore, it was concluded that the number of SRB was increasing (Table 3-4). Of the AA, 25 mL^{-1} were found in the pore water inside the Prototype Repository, almost 200 times more than the 0.13 mL^{-1} AA found in the Äspö groundwater.

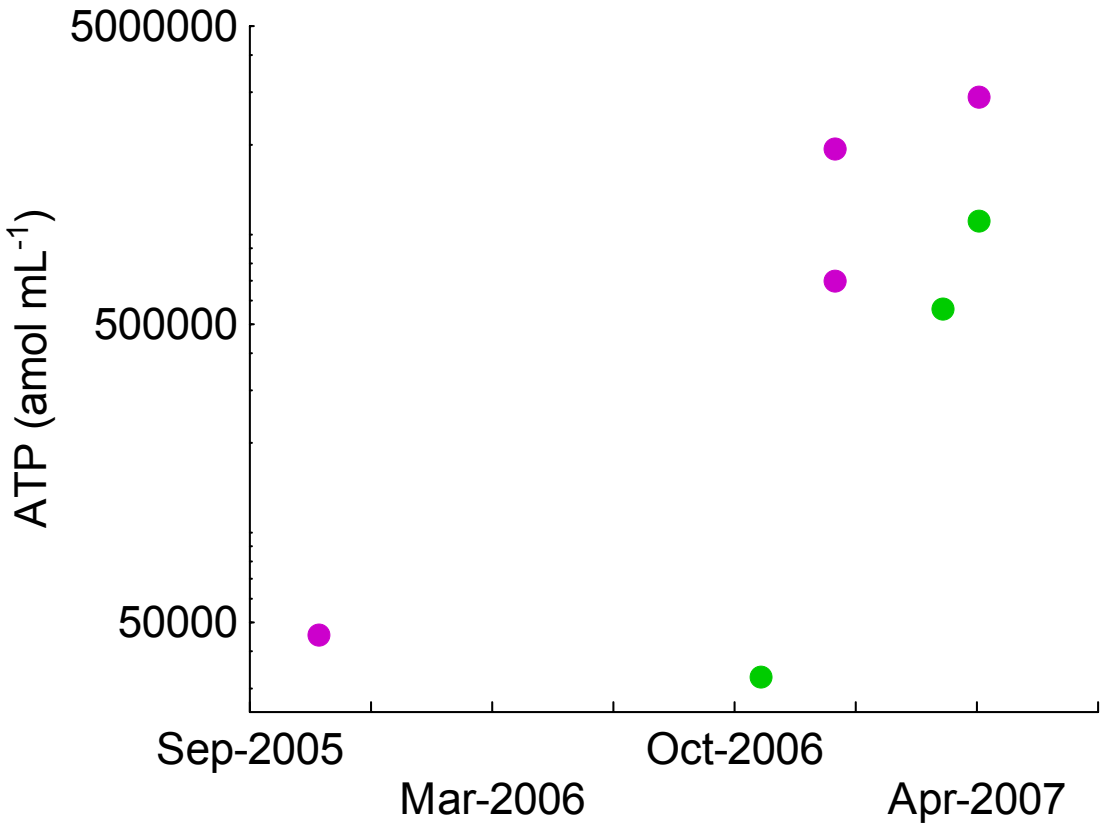


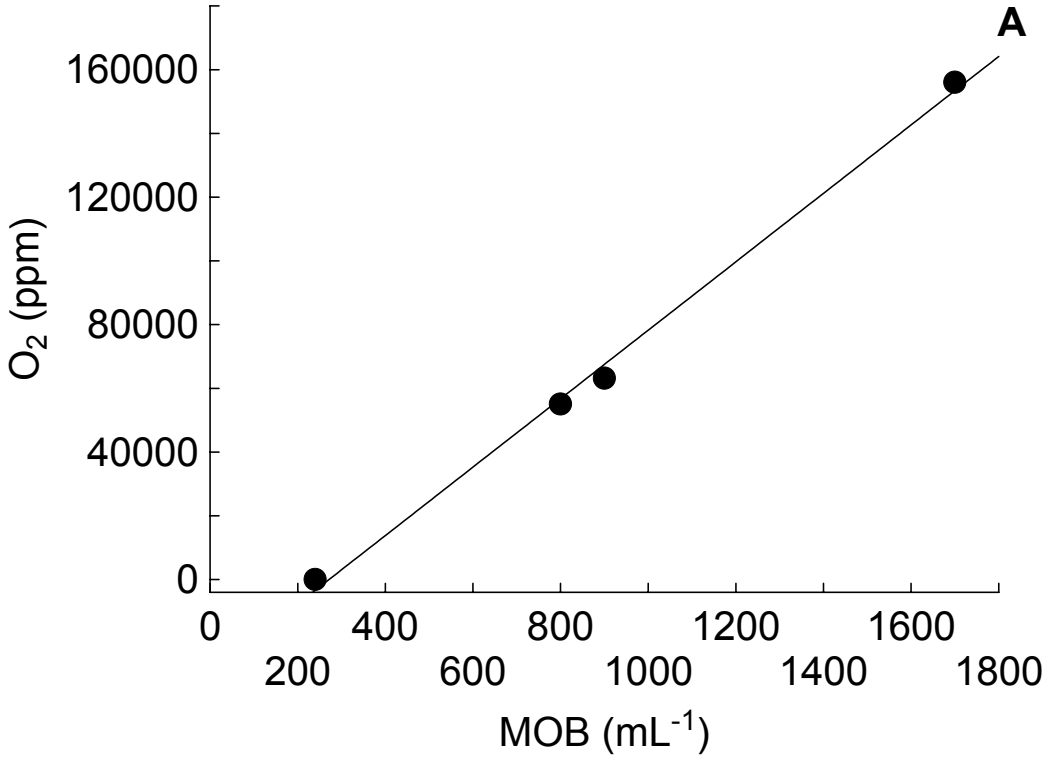
Figure 3-9. *Mean ATP (Adenosine Tri Phosphate) content of the pore water from the KBU10002+8 (green circles) and KFA01-04 (purple circles) sampling groups between November 2005 and April 2007. Please note the logarithmic y-scale.*

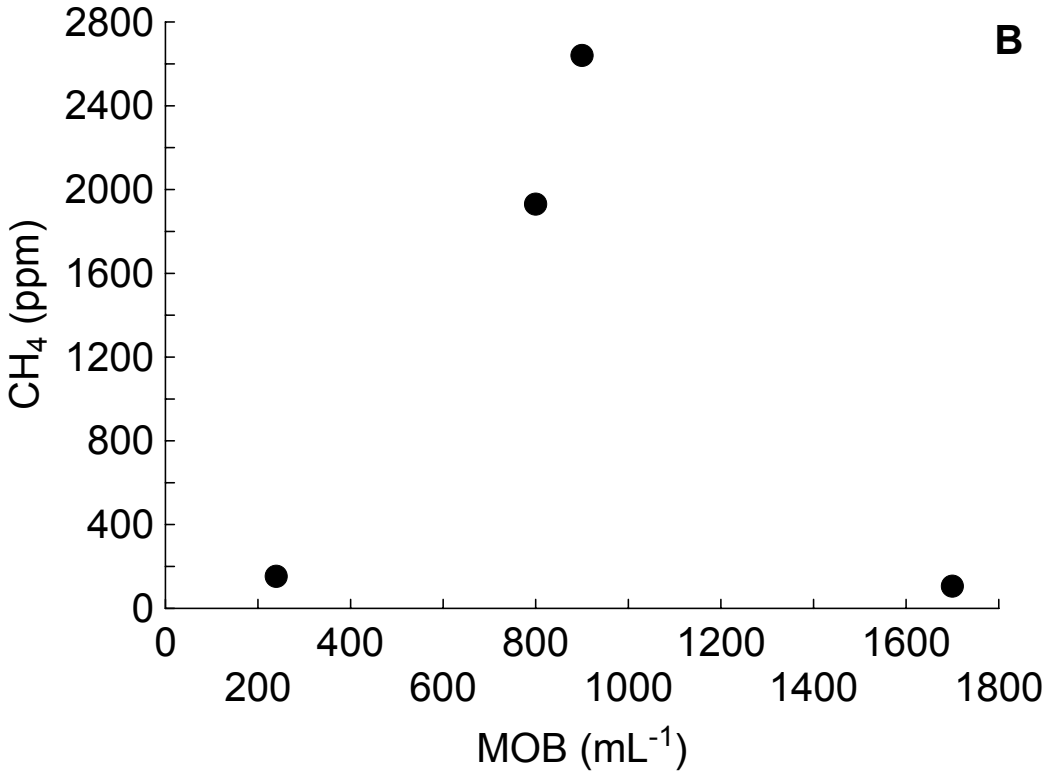
KBU10004 and KBU10006: In the KBU10004+6 group, three microbial analyses were performed in 2007. The ATP content at the sampling points was high, especially in May 2007, when the pore water contained 11,000,000 amoles ATP mL^{-1} (Table 3-4). This level was higher than those at many other sampling points in the Prototype and over 2000 times higher than in the surrounding groundwater. The numbers of AA and SRB were also analysed in 2007, and neither of the groups was detected.

KFA01, KFA02, KFA03, and KFA04: The microbial composition in the KFA01-04 group is also well characterized, as the group was analysed on 13 occasions. The ATP content at the sampling points increased from 40,000 to approximately 4,000,000 amoles mL^{-1} between November 2005 and spring 2007 (Figure 3-9). This level was almost 800 times higher than in the surrounding groundwater, and the detected biomass could to some extent consist of CHAB and MOB. In 2005, the numbers of CHAB and MOB mL^{-1} were determined to be 598 and 271 (Table 3-4). Both these numbers are between 50–100 times higher than those detected in the surrounding groundwater. In 2007, the numbers of SRB and AA were analysed, and on average 1 and 4.5 mL^{-1}, respectively, of such microbes were found. This means that the number of SRB was appreciably lower than in the surrounding groundwater. The number of AA, on the other hand, was slightly higher than in the surrounding groundwater, in which an average of 0.13 AA mL^{-1} were found.

3.4.3 Microbial processes coupled to the gas composition in the Prototype Repository

Based on the above (e.g., Figure 3-9), it was concluded that the pore water in the two sample groups KBU10002+8 and KFA01-04 have contained active microbial populations since at least 2005, when the microbial analyses were performed for the first time. To detect any significant correlation between the gas content in these sampling groups and the presence of the analysed microbes, we prepared scatter plots of the numbers of MOB (Figure 3-10, A–D), CHAB (Figure 3-11, A–D), AA (Figure 3-12, A–D), and SRB (Figure 3-13, A–D) and of the O_2, CH_4, H_2, and CO_2 contents of individual samples. Only samples in which the microbes were detected were included in the plots. We detected significant positive correlations between MOB and O_2 ($r^2 = 0.997$, $p = 0.002$), CHAB and CO_2 ($r^2 = 0.81$, $p = 0.1$), and AA and H_2 ($r^2 = 0.94$, $p = 0.03$) and a negative correlation between AA and CO_2 ($r^2 = 0.89$, $p = 0.06$). It was concluded that a very high concentration of H_2 was present when the MPN of SRB reached its maximum value of 5000 SRB mL^{-1}. Further indications that hydrogen could enhance sulphate reduction in the pore water are attached in Appendix C, where a report demonstrates that 60 mg L^{-1} of sulphide was produced at sampling point KFA01 within 6 weeks of high hydrogen levels being detected at this point.


A
160000
120000
80000
40000
0
O_2 (ppm)
0
400
800
1200
1600
200
600
1000
1400
1800
MOB (mL^{-1})


B
2800
2400
2000
1600
1200
800
400
0
CH_4 (ppm)
0
400
800
1200
1600
200
600
1000
1400
1800
MOB (mL^{-1})

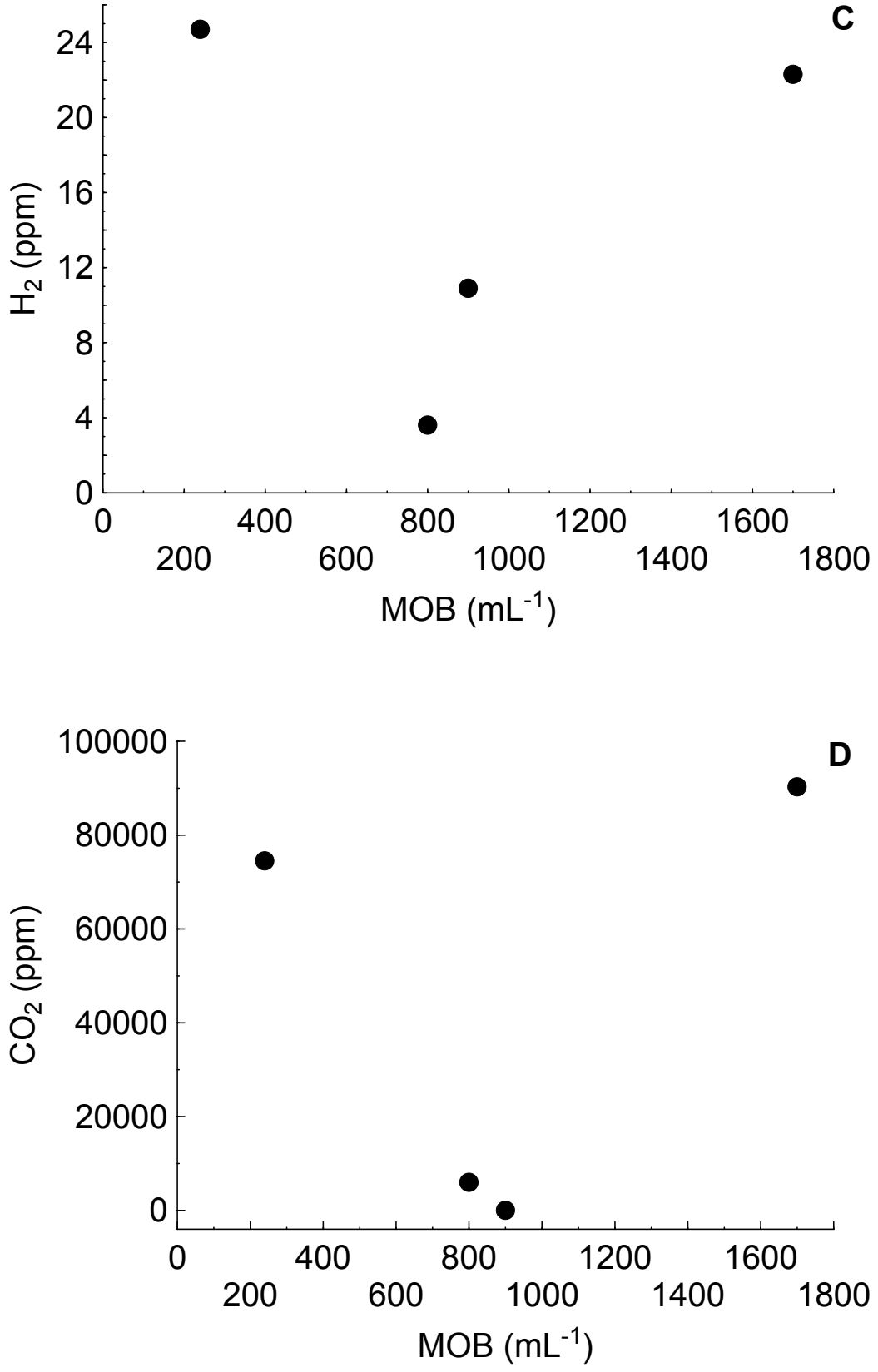
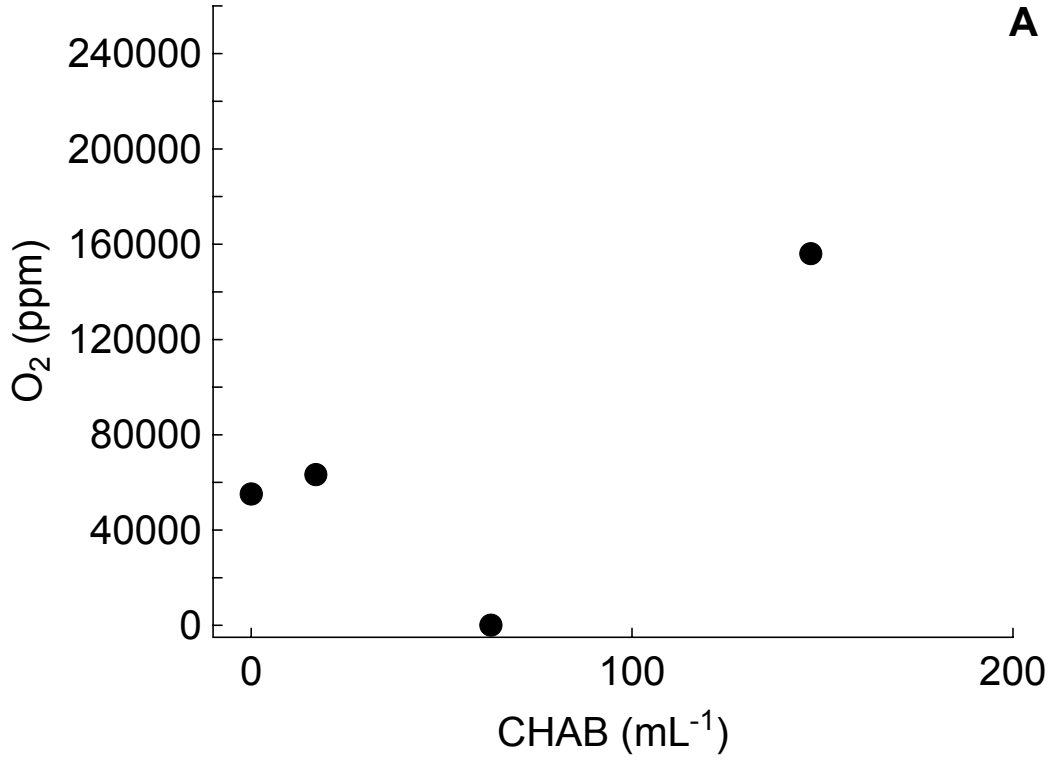


Figure 3-10. *The MPN of MOB(Methane-Oxidizing Bacteria) plotted against the A) O_2, B) CH_4, C) H_2, and D) CO_2 contents (in ppm of the total amount of extracted gas) of the pore water inside the Prototype Repository. A significant correlation between the number of MOB and the O_2 content was found ($r^2 = 0.997$, $p = 0.002$).*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160000
120000
80000
40000
0
O_2 (ppm)
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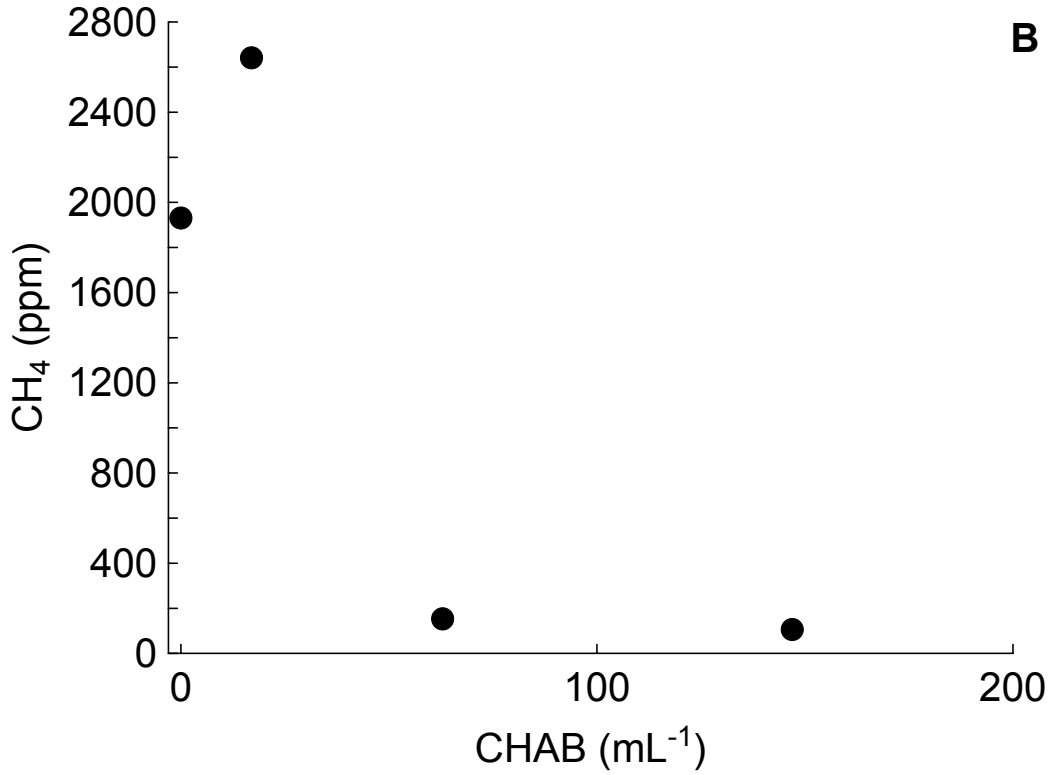
100
200
CHAB (mL^{-1})


B
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2400
2000
1600
1200
800
400
0
CH_4 (ppm)
0
100
200
CHAB (mL^{-1})

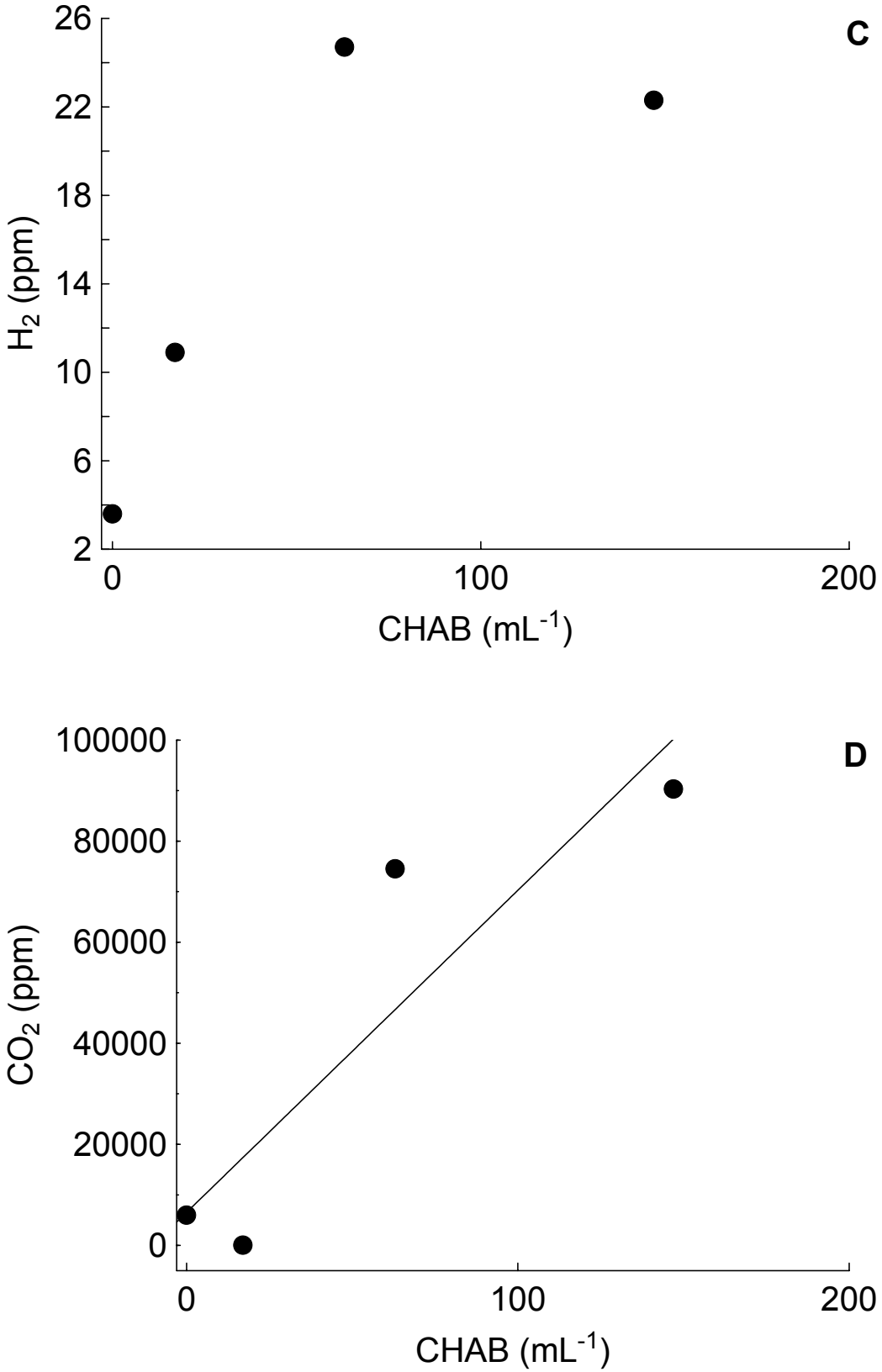
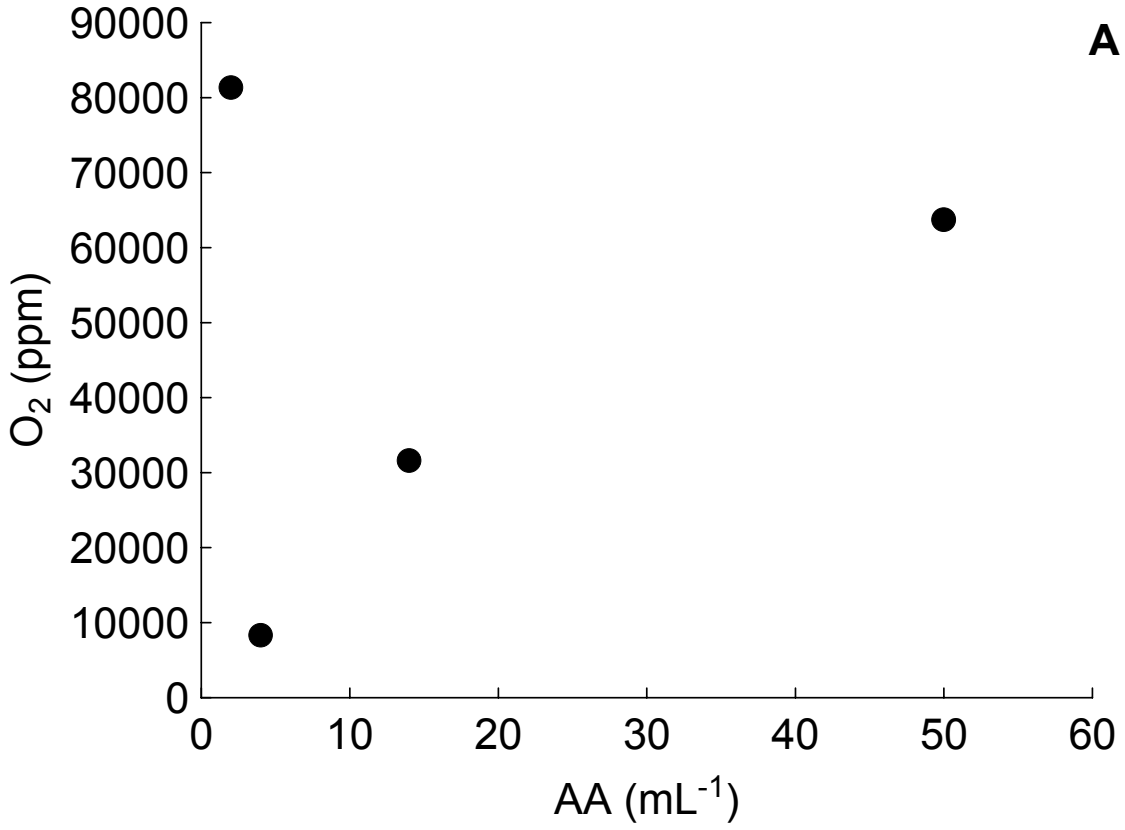
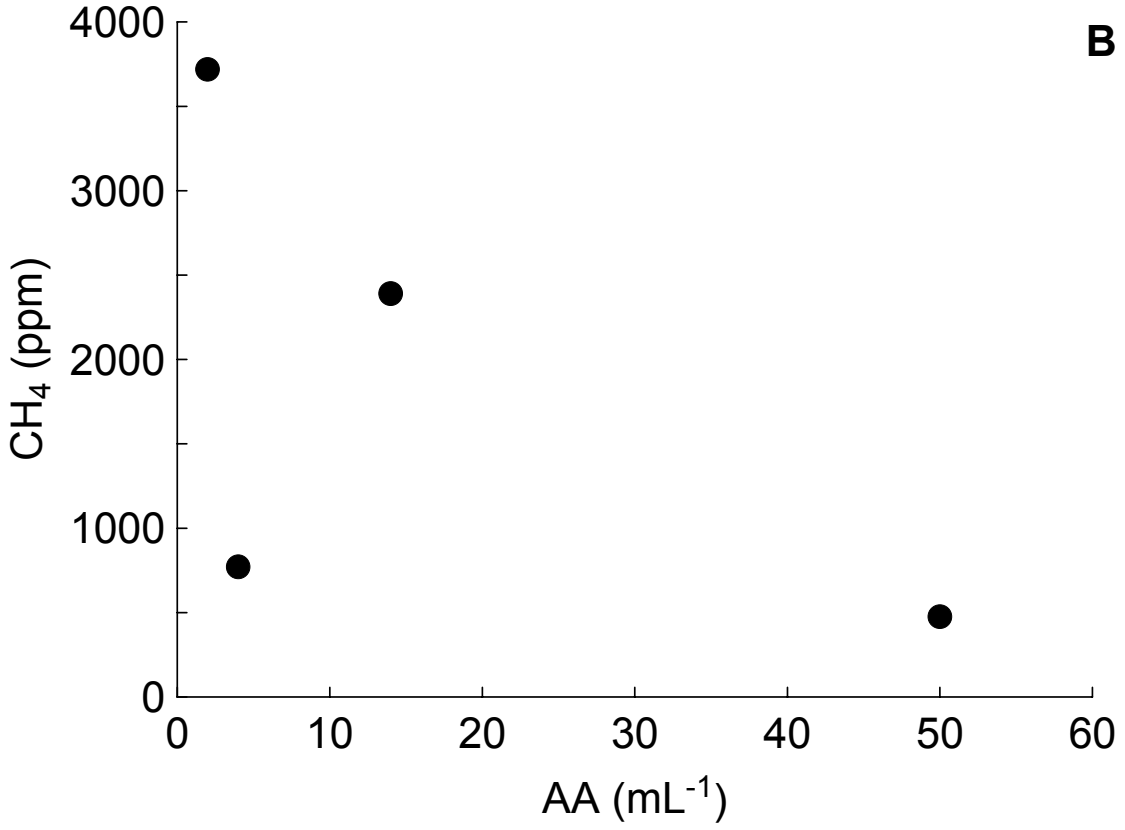


Figure 3-11. *The number of CHAB (Culturable Heterotrophic Aerobic Bacteria) plotted against the A) O_2, B) CH_4, C) H_2, and D) CO_2 contents (in ppm of the total amount of extracted gas)) of the pore water inside the Prototype Repository. A significant correlation was found between the number of CHAB and the CO_2 content ($r^2 = 0.81$, $p = 0.1$).*


A
90000
80000
70000
60000
50000
40000
30000
20000
10000
0
O_2 (ppm)
0
10
20
30
40
50
60
AA (mL^{-1})


B
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3000
2000
1000
0
CH_4 (ppm)
0
10
20
30
40
50
60
AA (mL^{-1})

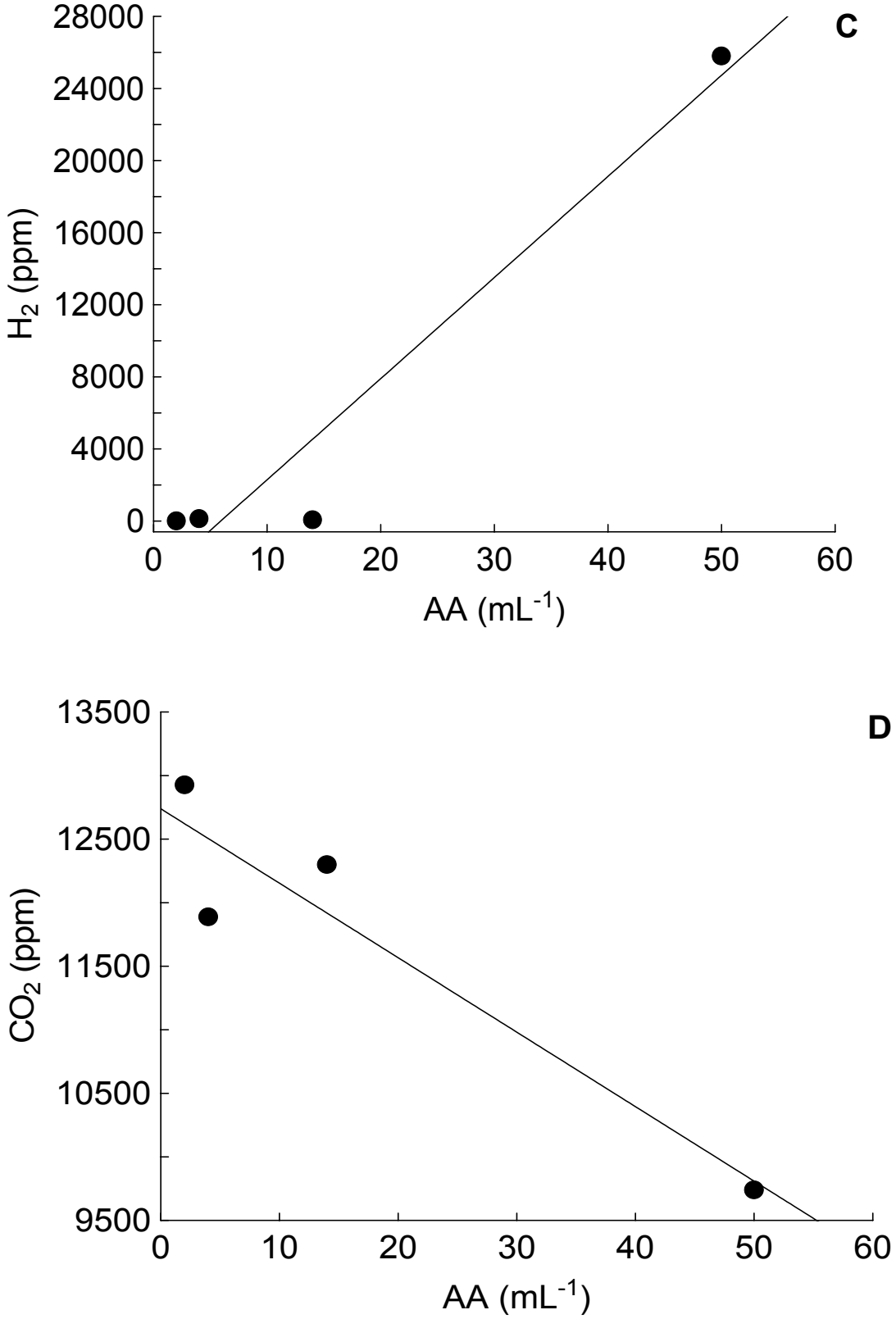
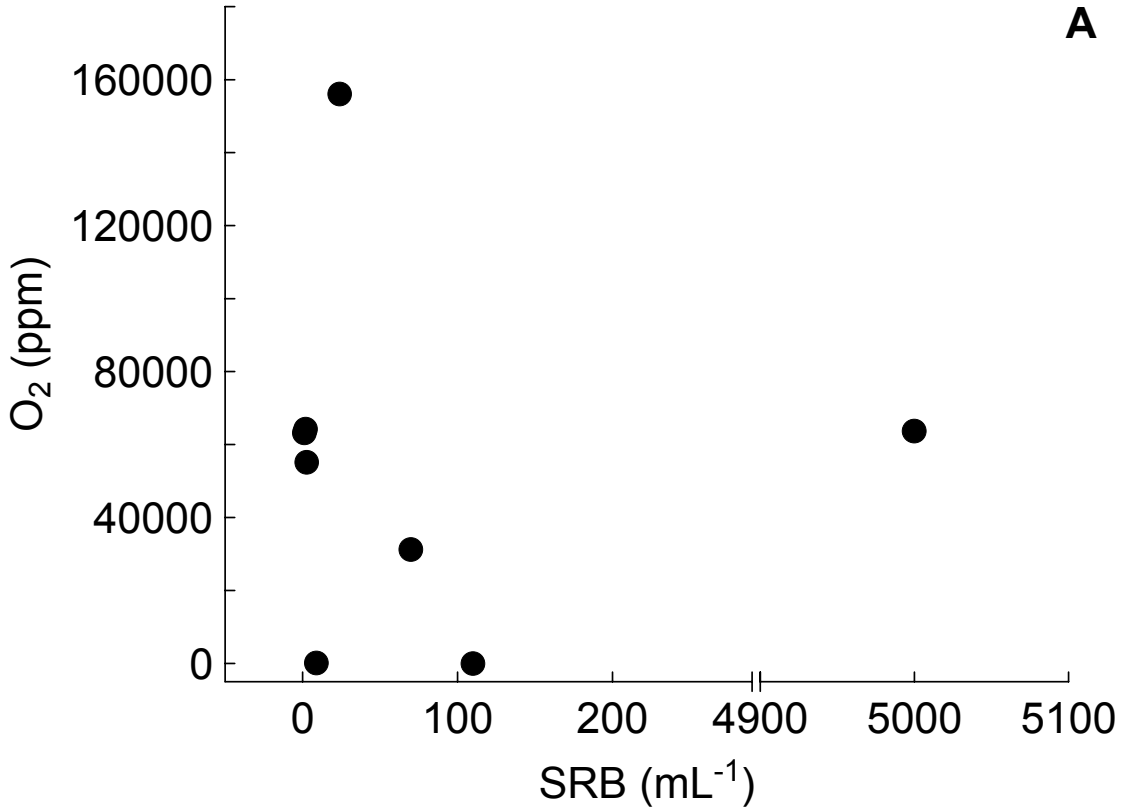
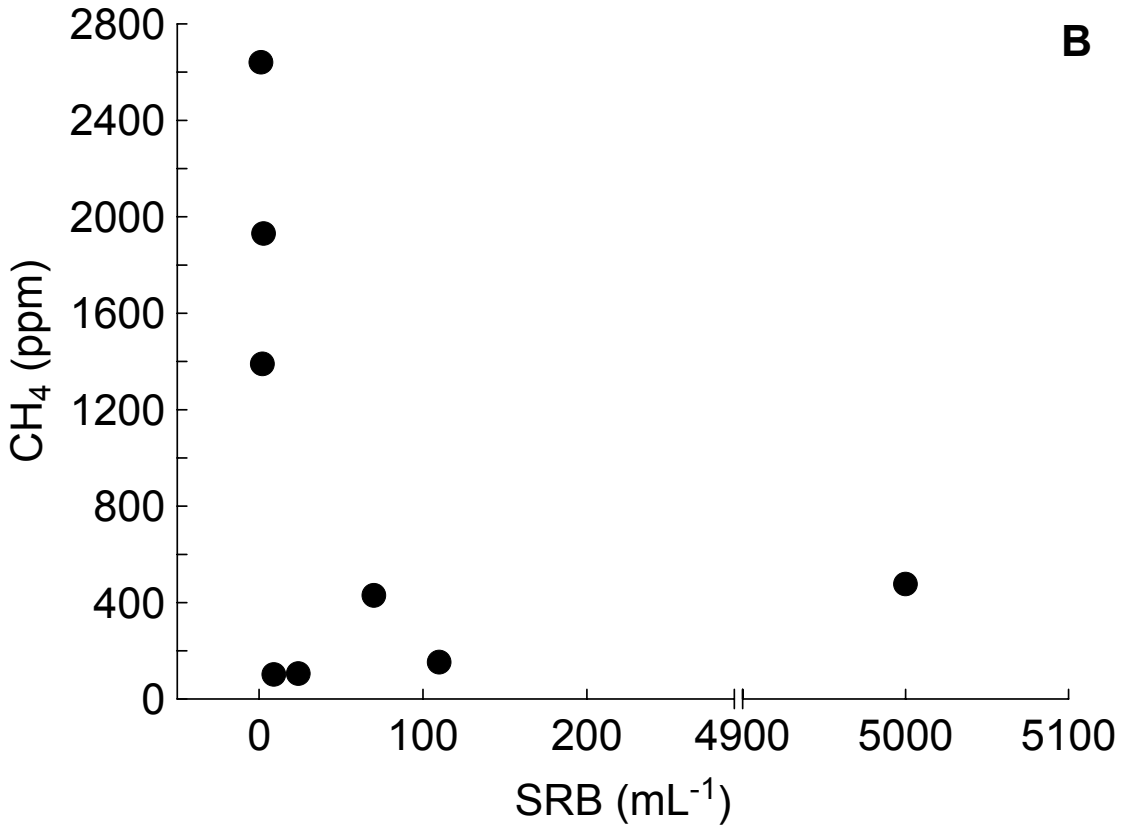


Figure 3-12. *The MPN of AA (Autotrophic Acetogens) plotted against the A) O_2, B) CH_4, C) H_2, and D) CO_2 contents (in ppm of the total amount of extracted gas)) of the pore water inside the Prototype Repository. Significant correlations were found between the number of AA and the contents of H_2 ($r^2 = 0.94$, $p = 0.03$) and CO_2 ($r^2 = 0.89$, $p = 0.06$).*


A
160000
120000
80000
40000
0
O_2 (ppm)
0
100
200
4900
5000
5100
SRB (mL^{-1})


B
2800
2400
2000
1600
1200
800
400
0
CH_4 (ppm)
0
100
200
4900
5000
5100
SRB (mL^{-1})

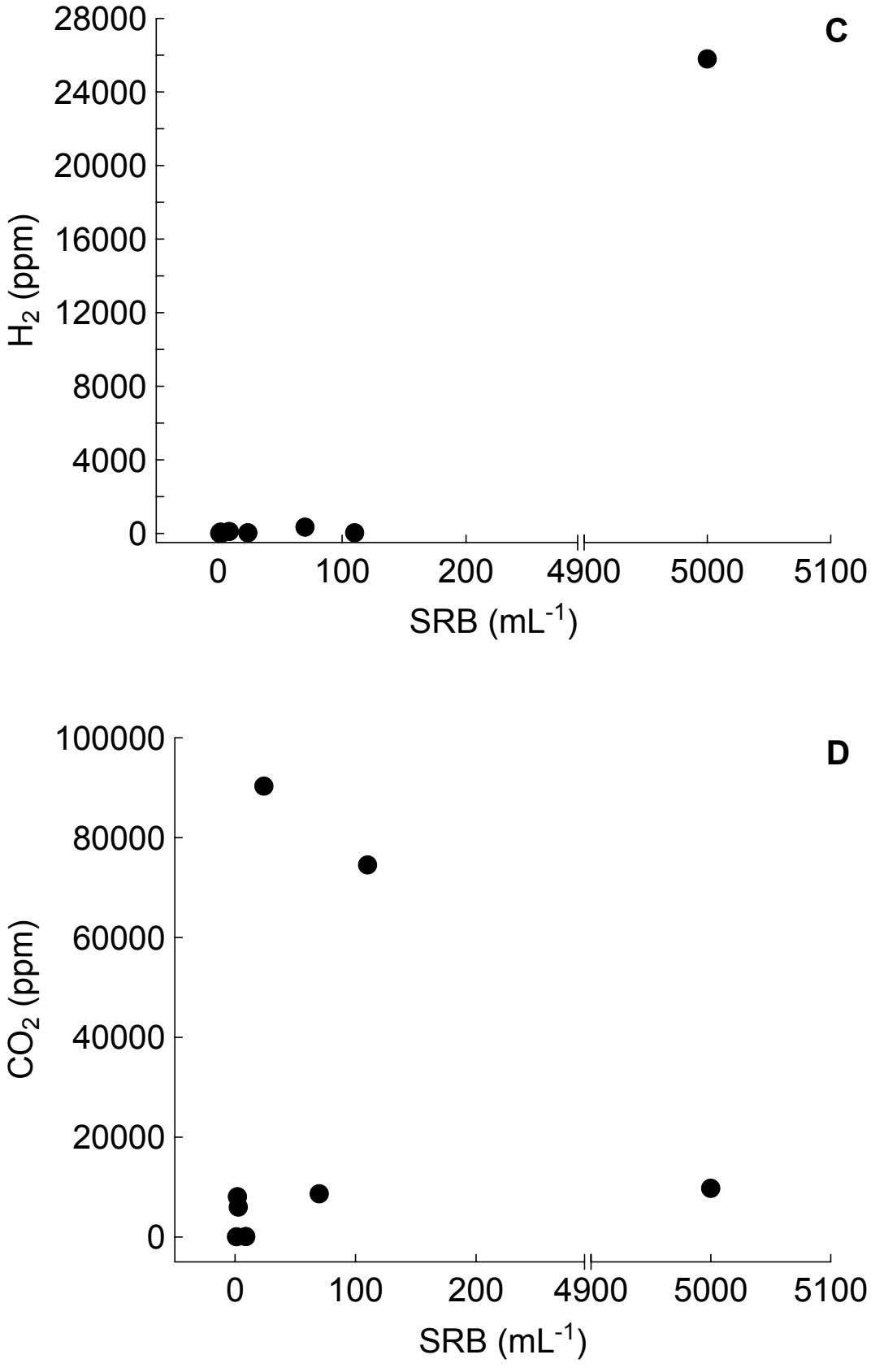


Figure 3-13. *The MPN of SRB (Sulphate-Reducing Bacteria) plotted against the A) O_2, B) CH_4, C) H_2, and D) CO_2 contents (in ppm of the total amount of extracted gas)) of the pore water inside the Prototype Repository. Please note the scale break in the X axis.*

3.4.4 Chemical composition:

In 2007, for the first time, partial class 5 data regarding the chemical composition of the pore water inside the Prototype Repository were received. The results of the chemical analyses of the pore water were compared with the means of data from 32 samplings made from 2003 to 2004 in Äspö groundwater boreholes (Sections 2.4.1 and 3.1) located near the Prototype (Table 2-1). Table 3-5 presents the mean values for the chemical composition of the Äspö groundwater and the pore water from each sample group inside the Prototype. All available enrichment factors of all compounds in the pore water relative to the levels of these compounds in the groundwater around the Prototype are shown in Figure 3-14. The individual data for each sampling of pore water inside the Prototype Repository are reported in the Appendix (Table 7-3).

KB513, KB514, KB613, and KB614: No or very little water was available from this group, which limited the amount of chemical data obtained. Nevertheless, four analyses were performed on water from two of the samples. As shown in Figure 3-14, the sulphate concentration in these samples was the highest found in the Prototype, 5550 mg mL^{-1} (Table 3-5), 16 times higher than in the surrounding groundwater (Figure 3-14). As well, the chloride, bromide, and fluoride concentrations were among the highest detected in the Prototype, though they were not as extreme as the sulphate concentrations (Table 3-5). Compared with the concentrations in the surrounding groundwater, these enrichment factors were 1.5–3 (Figure 3-14).

KBU10001, KBU10003, and KBU10007: Since water could not be extracted from these sampling groups in 2007 (Table 3-2), chemical analyses were not performed.

KBU10005: In April 2007, the chemical composition of the pore water in sampling group KBU10005 was examined. As shown in Figure 3-14 and Table 3-5, the water in KBU10005 was fairly similar to the groundwater in terms of salinity, pH, and sulphate and manganese concentrations, but differed from it in that it was depleted in iron and manganese and enriched in sodium and potassium (Table 3-5). Less calcium was present than in the groundwater, 24% of the amount in the groundwater (Table 3-5 and Figure 3-14). Many metals examined were present in concentrations of 0.1–100 μg L^{-1} (Table 3-5). In most cases, the concentrations of metals were higher in the pore water from the KBU10005 sampling point than in the groundwater outside the Prototype. The concentrations of molybdenum, rubidium, and zinc (Table 3-5) were especially high at 60.3, 80.8, and 92.2 μg L^{-1}, respectively. Of the actinides investigated, uranium was detected at a concentration of 19.2 μg L^{-1}, more than 100 times higher than in the groundwater (Figure 3-14). No lanthanides were found in KBU10005.

Table 3-5. The chemical composition of the pore water inside the Prototype Repository and of the surrounding groundwater, sampled 2003–2007.

Sample group	Sampling occasion	*n*	Na (mg L^{-1})	Stdev	Stdev %	K (mg L^{-1})	Stdev	Stdev %	Ca (mg L^{-1})	Stdev	Stdev %	Mg (mg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	32	1740	317	18	10.8	1.22	11	702	267	38	71.1	21.0	29
KB513-614	2007	2												
KBU10005	2007	1	2830			42.3			169			86.1		
KBU10002+8	2007	4	1750	104	6	13.7	0.49	4	713	80.1	11	79.1	6.11	8
KBU10004+6	2007	3	5760	767	13	126	19.9	16	43.0	53.9	126	80.6	42.3	52
KFA01-04	2007	10	2960	371	13	67.6	39.5	58	35.5	28.4	80	27.4	7.26	26

Sample group	Sampling occasion	*n*	Cl (mg L^{-1})	Stdev	Stdev %	SO_4 (mg L^{-1})	Stdev	Stdev %	Br (mg L^{-1})	Stdev	Stdev %	F (mg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	32	3850	707	18	346	107	31	20.5	7.24	35	0.83	0.66	80
KB513-614	2007	2	9100	3410	37	5500	2000	36	25.7	2.90	8	2.25	0.77	35
KBU10005	2007	1	4140			361			19.8			2.60		
KBU10002+8	2007	4	4300	484	11	383	60.9	16	19.0	2.09	11	1.65	1.38	83
KBU10004+6	2007	3	8080	539	7	1720	785	46	35.2	1.27	4	5.27	0.47	9
KFA01-04	2007	10	4210	631	15	639	358	56	20.2	2.10	10	2.03	1.45	71

Sample group	Sampling occasion	*n*	Si (mg L^{-1})	Stdev	Stdev %	Fe (mg L^{-1})	Stdev	Stdev %	Mn (mg L^{-1})	Stdev	Stdev %	Li (mg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	32	7.16	1.08	15	0.30	0.25	82	0.51	0.14	27	0.47	0.24	51.2
KB513-614	2007	2												
KBU10005	2007	1	11.3			0.003			0.10			0.27		
KBU10002+8	2007	4	6.60	1.26	19	0.002	0.0005	17	0.81	0.14	18	0.40	0.07	16
KBU10004+6	2007	3	21.0	1.48	7	0.04	0.03	79	0.08	0.11	134	0.41	0.07	18
KFA01-04	2007	10	9.53	4.94	52	0.01	0.005	64	0.11	0.06	53	0.08	0.03	36

Table 3-5, continued

Sample group	Sampling occasion	*n*	HCO_3^- (mg L^{-1})	Stdev	Stdev %	Sr (mg L^{-1})	Stdev	Stdev %	pH (mg L^{-1})	Stdev	Stdev %	S (mg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	31	141	60.9	43	12.1	4.52	37	7.46	0.09	1	110	34.5	31
KB513-614	2007	2							7.81	0.48	6			
KBU10005	2007	1				3.39			7.84			164		
KBU10002+8	2007	4				13.2	1.71	13	7.80	0.11	13	117	8.87	8
KBU10004+6	2007	3				1.24	1.51	122	8.47	0.30	4	587	238	40
KFA01-04	2007	10				0.46	0.40	86	7.83	0.45	6	220	130	59

Sample group	Sampling occasion	*n*	Al (µg L^{-1})	Stdev	Stdev %	Ba (µg L^{-1})	Stdev	Stdev %	Cd (µg L^{-1})	Stdev	Stdev %	Co (µg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	21				52.2	7.01	13	0.05	0.05	102			
KB513-614	2007	2												
KBU10005	2007	1	13.1			94.2			0.12			1.39		
KBU10002+8	2007	4	30.4	41.8	138	155	75.0	48	0.09	0.06	96	3.93	5.60	143
KBU10004+6	2007	3	13.9	16.9	121	43.8	60.8	139	0.11	0.19	173	3.13	4.79	153
KFA01-04	2007	10	69.4	176	254	46.1	21.5	47	0.06	0.11	180	2.04	1.82	89

Sample group	Sampling occasion	*n*	Cr (µg L^{-1})	Stdev	Stdev %	Cu (µg L^{-1})	Stdev	Stdev %	Hg (µg L^{-1})	Stdev	Stdev %	Mo (µg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	21							bd					
KB513-614	2007	2												
KBU10005	2007	1	1.68			3.40			bd			60.3		
KBU10002+8	2007	4	0.59	0.50	85	77.2	120	155	0.006	0.004	70	91.1	30.1	33
KBU10004+6	2007	3	1.44	1.13	79	14.7	14.3	97	0.005	0.004	89	329	106	32
KFA01-04	2007	10	0.59	0.36	60	6.59	12.8	194	0.003	0.003	81	191	118	62

Table 3-5, continued

Sample group	Sampling occasion	*n*	Ni (µg L^{-1})	Stdev	Stdev %	P (µg L^{-1})	Stdev	Stdev %	Pb (µg L^{-1})	Stdev	Stdev %	V (µg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	21										0.16	0.12	74
KB513-614	2007	2												
KBU10005	2007	1	9.50			29.8			2.02			5.66		
KBU10002+8	2007	4	175	300	171	36.8	30.9	84	0.59	0.51	86	0.42	0.29	69
KBU10004+6	2007	3	262	370	141	51.2	6.35	12	1.42	1.17	82	8.49	7.11	84
KFA01-04	2007	10	91.9	77.6	84	128	135	106	0.44	0.41	93	5.90	6.11	104

Sample group	Sampling occasion	*n*	Zn (µg L^{-1})	Stdev	Stdev %	La (µg L^{-1})	Stdev	Stdev %	Ce (µg L^{-1})	Stdev	Stdev %	Pr (µg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	21				0.14	0.17	119	0.15	0.19	124	bd		
KB513-614	2007	2												
KBU10005	2007	1	92.2			bd			bd			bd		
KBU10002+8	2007	4	74.7	28.1	37	0.10	0.12	116	0.08	0.13	155	0.08	0.02	200
KBU10004+6	2007	3	61.9	11.1	18	0.01	0.02	173	0.02	0.04	173	bd		
KFA01-04	2007	10	81.9	88.3	108	bd			bd			bd		

Sample group	Sampling occasion	*n*	Nd (µg L^{-1})	Stdev	Stdev %	Sm (µg L^{-1})	Stdev	Stdev %	Eu (µg L^{-1})	Stdev	Stdev %	Gd (µg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	21	0.05	0.06	118	bd			bd			bd		
KB513-614	2007	2												
KBU10005	2007	1	bd			bd			bd			bd		
KBU10002+8	2007	4	0.06	0.07	123	0.006	0.01	200	0.008	0.01	200	0.005	0.01	200
KBU10004+6	2007	3	0.02	0.03	173	bd			bd			bd		
KFA01-04	2007	10	bd			bd			bd			bd		

Table 3-5, continued

Sample group	Sampling occasion	*n*	Dy (µg L^{-1})	Stdev	Stdev %	Er (µg L^{-1})	Stdev	Stdev %	Rb (µg L^{-1})	Stdev	Stdev %	Y (µg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	21	bd			bd			32.3	7.36	23	0.20	0.08	37
KB513-614	2007	2												
KBU10005	2007	1	bd			bd			80.8			0.06		
KBU10002+8	2007	4	0.005	0.01	200	0.006	0.01	200	26.3	7.37	28	0.38	0.20	54
KBU10004+6	2007	3	bd			bd			141	22.0	16	0.07	0.02	34
KFA01-04	2007	10	bd			bd			82.2	12.0	15	0.02	0.02	112

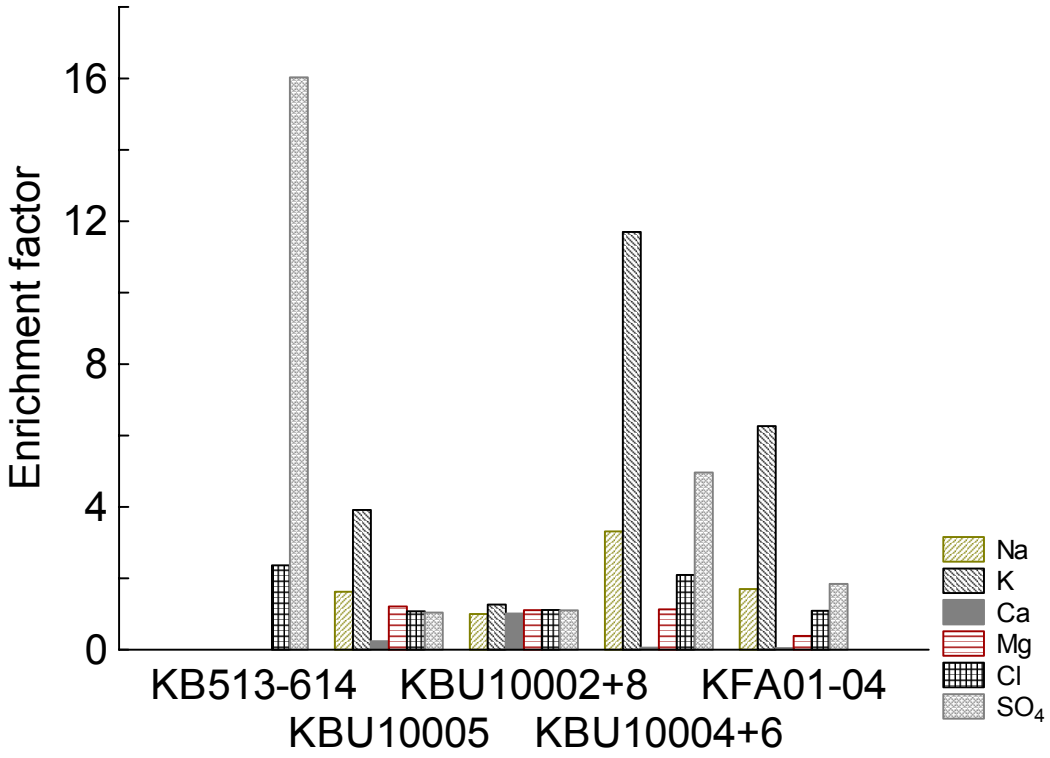
Sample group	Sampling occasion	*n*	Zr (µg L^{-1})	Stdev	Stdev %	Sb (µg L^{-1})	Stdev	Stdev %	Cs (µg L^{-1})	Stdev	Stdev %	Hf (µg L^{-1})	Stdev	Stdev %
Äspö groundwater	2003–2004	21	bd						3.01	1.05	35	bd		
KB513-614	2007	2												
KBU10005	2007	1	3.52			1.03			4.52			1.50		
KBU10002+8	2007	4	1.64	2.78	169	0.22	0.05	22	1.20	0.41	34	0.66	1.09	164
KBU10004+6	2007	3	1.31	1.25	96	0.66	0.21	32	7.30	2.82	39	0.50	0.50	99
KFA01-04	2007	10	0.34	0.55	164	1.17	0.4	34	6.24	2.69	43	0.14	0.25	182

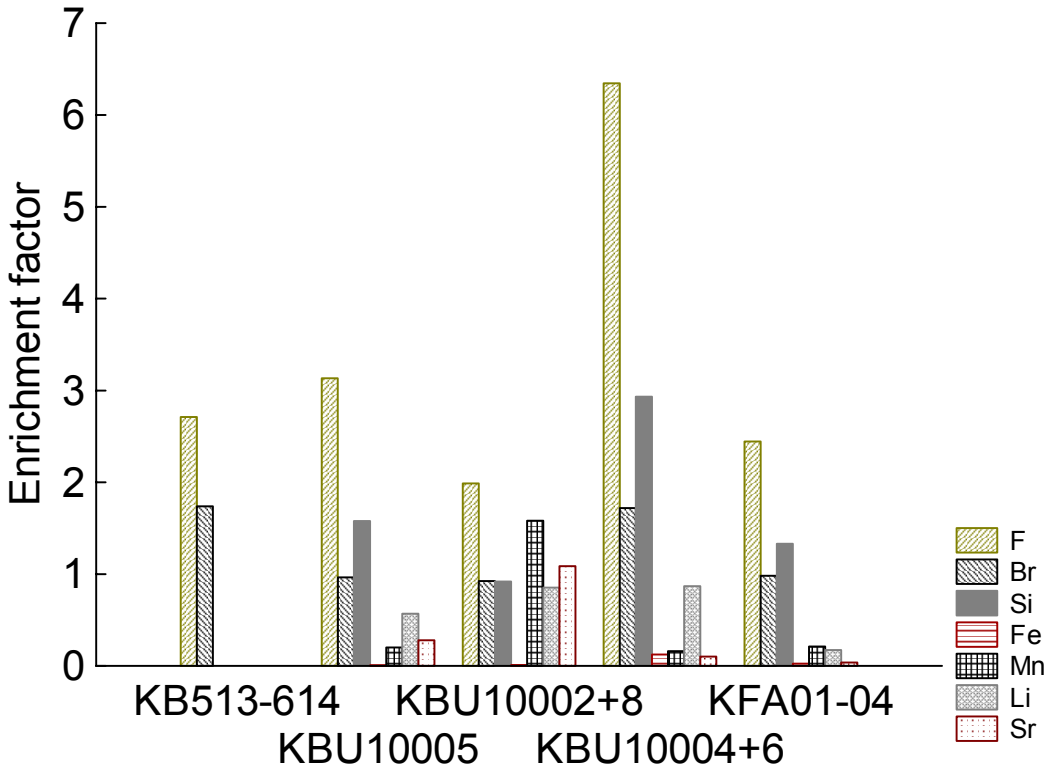
Sample group	Sampling occasion	*n*	Tl (µg L^{-1})	Stdev	Stdev %	U (µg L^{-1})	Stdev	Stdev %	Th (µg L^{-1})	Stdev	Stdev %	$^{10}B/^{11}B$ atomic	Stdev	Stdev %
Äspö groundwater	2003–2004	21	bd			0.14	0.13	93	bd			0.238	0.0005	0.2
KB513-614	2007	2												
KBU10005	2007	1	0.82			19.2			bd			0.239		
KBU10002+8	2007	4	0.04	0.03	70	3.80	2.43	3	bd			0.236	0.001	1
KBU10004+6	2007	3	0.44	0.38	87	66.1	57.1	87	bd			0.237	0.001	0.5
KFA01-04	2007	10	0.49	0.24	50	50.9	60.8	119	bd			0.237	0.003	1

KBU10002 and KBU10008: In spring 2007, the chemical composition of the pore water in sample group KBU10002+8 was examined on four occasions. The water in the KBU10002+8 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 3-5, Figure 3-14). Many of the metals examined were present in concentrations of 0.1–100 μg L^{-1} (Table 3-5). In most cases, the concentrations of metals were higher in the KBU10002+8 group than in the groundwater outside the Prototype Repository. The concentrations of barium, molybdenum, and zinc were especially high, at 155, 91.1, and 74.7 μg L^{-1}, respectively (Table 3-5. The concentration of the actinide uranium was approximately 30 times higher, 3.80 μg L^{-1}, than in the groundwater (Table 3-5, Figure 3-14). Several lanthanides were found in concentrations of approximately 10 ng L^{-1} in some of the pore water samples from KBU10002+8 (Table 3-5). In addition, sporadically high concentrations of nickel, aluminium, and copper were found in the pore water (Table 3-5).

KBU10004 and KBU10006: In spring 2007, the chemical composition of the pore water in sample group KBU10004+6 was examined on three occasions. The pore water in this group was different from that of the KBU10005 and KBU10002+8 groups (Table 3-5, Figure 3-14). The pore water had a pH that was one unit higher than that of the groundwater outside the Prototype Repository (8.47) and was twice as saline (Table 3-5). It contained five times as much sulphate, and was depleted in both iron and manganese (Figure 3-14). The concentrations of metals were higher in the pore water than in the groundwater outside the Prototype. The metals examined were present in concentrations of 0.1–100 μg L^{-1}, except for molybdenum and rubidium, which were more abundant, being present in concentrations of 329 and 141 μg L^{-1}, respectively (Table 3-5). As in other sampling groups inside the Prototype, the concentration of zinc was fairly high at 61.9 μg L^{-1} (Figure 3-14). The concentration of the actinide uranium was approximately 500 times higher, 66.1 μg L^{-1}, than in the groundwater (Table 3-5, Figure 3-14). Some lanthanides were found in concentrations of approximately 10 ng L^{-1} in some of the pore water samples from KBU10004+6 (Table 3-5). In addition, sporadically high concentrations of nickel and vanadium were found in the pore water (Figure 3-14).

KFA01, KFA02, KFA03, and KFA04: The chemical composition of the pore water in the KFA01-04 group was analysed on ten occasions in spring 2007 (Figure 3-14, Table 3-5). The pore water had a higher pH than the groundwater outside the Prototype Repository (7.8) and approximately as saline. It contained twice as much sulphate and was depleted in both iron and manganese (Figure 3-14). The concentrations of other metals were higher in the pore water than in the groundwater outside the Prototype. The metals examined were present in concentrations of 0.1–100 μg L^{-1}, except for molybdenum, which was more abundant at a concentration of 191 μg L^{-1} (Table 3-5). As in most of the other groups inside the Prototype, the zinc concentration was high (81.9 μg L^{-1}) relative to that in the surrounding groundwater (Figure 3-14). The concentration of rubidium was also high at 82.2 μg L^{-1} (Table 3-5). The concentration of the actinide uranium was over 360 times higher, 50.9 μg L^{-1}, than in the groundwater (Table 3-5, Figure 3-14). In addition, sporadically high concentrations of nickel and aluminium were found in the pore water (Table 3-5). No lanthanides were found in the KFA01-04 group.


Enrichment factor
16
12
8
4
0
KB513-614
KBU10002+8
KFA01-04
KBU10005
KBU10004+6
Na
K
Ca
Mg
Cl
SO4


Enrichment factor
7
6
5
4
3
2
1
0
KB513-614
KBU10002+8
KFA01-04
KBU10005
KBU10004+6
F
Br
Si
Fe
Mn
Li
Sr

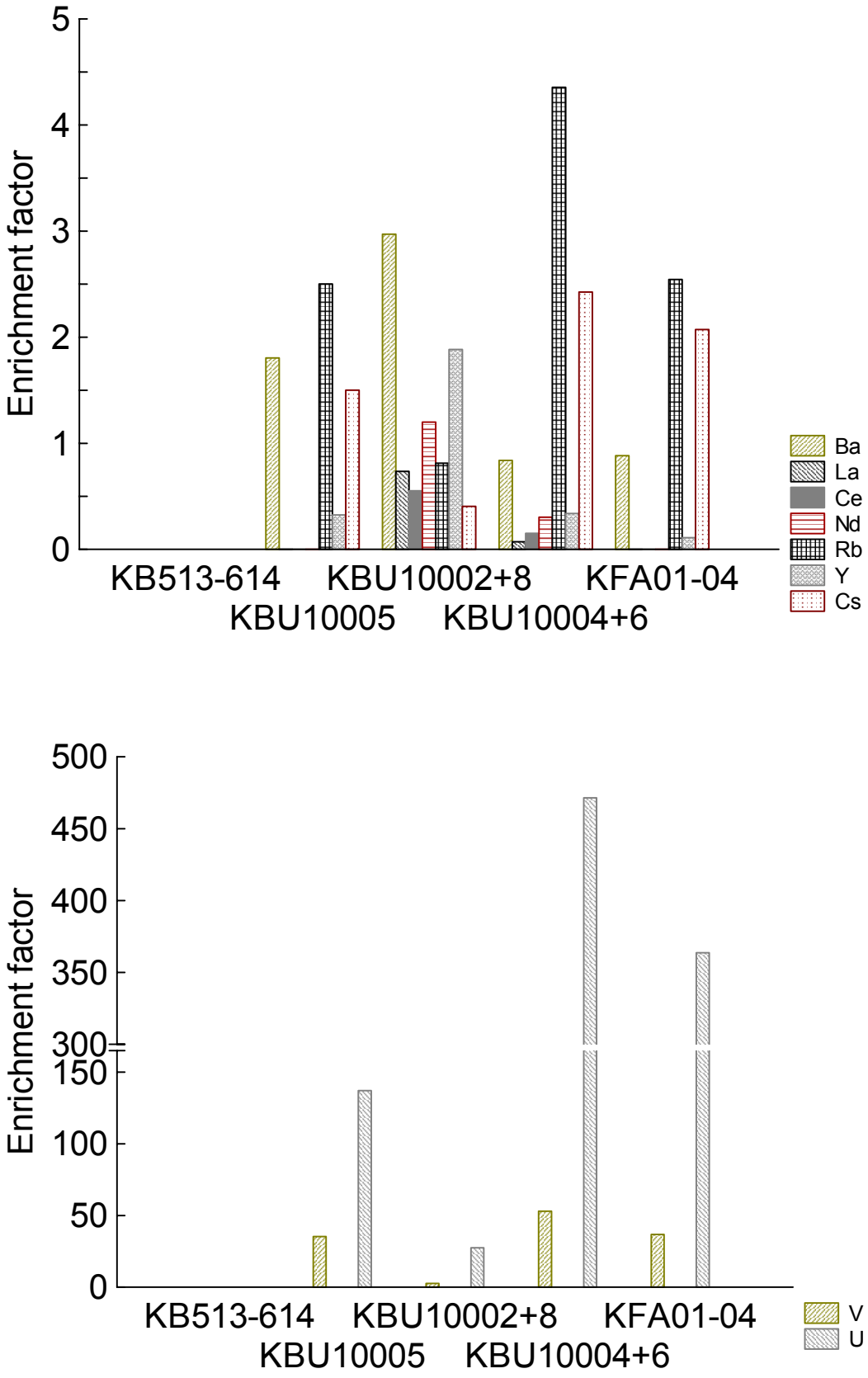


Figure 3-14. *Enrichment factors of various elements in the pore water inside the Prototype Repository in 2007 plotted against concentrations in the surrounding groundwater. Please note the scale break in the lower graph.*

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 evaluates the interplays between gas composition, chemistry, and microbial life, and the influence of the surrounding groundwater in the Prototype Repository. The report includes data produced by our laboratory from 2004 to 2007 from samplings at 16 hydrochemical sampling points in the bentonite buffer and the backfill in the Prototype. The pore water chemistry, gas content, pressure, and prerequisites for microbial life at the sampling points of the Prototype Repository were not uniform (Table 3-2, Table 3-3, Table 3-4, and Table 3-5). To monitor trends and to detect possible ongoing microbial processes, it was necessary to divide the 16 sampling points in the Prototype into seven sampling groups, as follows: 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. After making this grouping, patterns and developing trends in the Prototype Repository could more easily be discerned, so they can be continuously monitored.

4.1 Improvement of the sampling procedures

In December 2006, a new sampling procedure was developed and used when extracting water from the Prototype Repository. Starting at this time, sampling was conducted with pressure vessels made of pressure-resistant stainless steel (the sampling vessels were previously made of glass). Both types of sampling vessels were evacuated to a high vacuum before use, to enable extraction of water from bentonite-containing sampling points. The stainless steel vessels, however, could endure much higher pressure (>2 bar) than the glass vessels could, and could thus be left at the higher-pressure sampling points for the entire period necessary for water extraction. The improved sampling procedure worked very well, and it was now possible to extract water from nine of the 16 sampling points using the new technique. The other sampling points were probably either dry, had reached full compaction, or had broken sampling tubes (because they contained gas with the same composition as the tunnel air). In contrast, the sampling procedure that used vacuum-evacuated anaerobic serum flasks, permitted pore water extraction from only six of the 16 sampling points.

4.2 Sampling reproducibility

The gas composition and microbial composition differed depending on how much water had been extracted from the sampling point prior to sampling (Table 3-4). This fact further supported the finding that the environment inside the Prototype Repository was not uniform. There is also a possibility, especially from the inner section (section 1) of the Prototype, that water could have been taken from the PEEK sampling tube in the first samplings. To be able to compare the results of the different samplings, we therefore decided to use the same replicates in evaluating the results; for all sample groups except KBU10002+8, the first replicate was used; for the KBU10002+8 group, the third replicate was used.

The standard deviation as a percent of the mean value can be regarded as a measurement of how much the individual values in each sample group vary. A lower percent implies that the parameters behave the same throughout the group. Some of the parameters examined in the studies of the Prototype have particularly low such percentages: oxygen and nitrogen levels, ATP content, and amounts of sodium, sulphate, potassium, chloride, pH, magnesium, molybdenum, silica, rubidium, and antimony (Table 3-3, Table 3-4, and Table 3-5). The developing trends of these parameters can be regarded as stronger than those of parameters with higher standard deviation percentages; such weaker trends need to be further evaluated.

4.3 The biological activity in the Prototype Repository increases

The significance of examining the presence and activity of the microbes over time in the Prototype Repository can be visualized by the increase in ATP (Figure 3-9). ATP is a universal currency of free energy in all biological systems, and any increase in its levels indicates that the number of living cells inside the Prototype pore water is increasing. It has been demonstrated that increased microbial abundance in groundwater correlates very well with ATP increase (Eydal and Pedersen, 2007). When plotting the individual TNC and ATP data from the November 2005 and 2006 samplings (Table 7-2), we see that this is also true for the Prototype pore water (Figure 4-1).

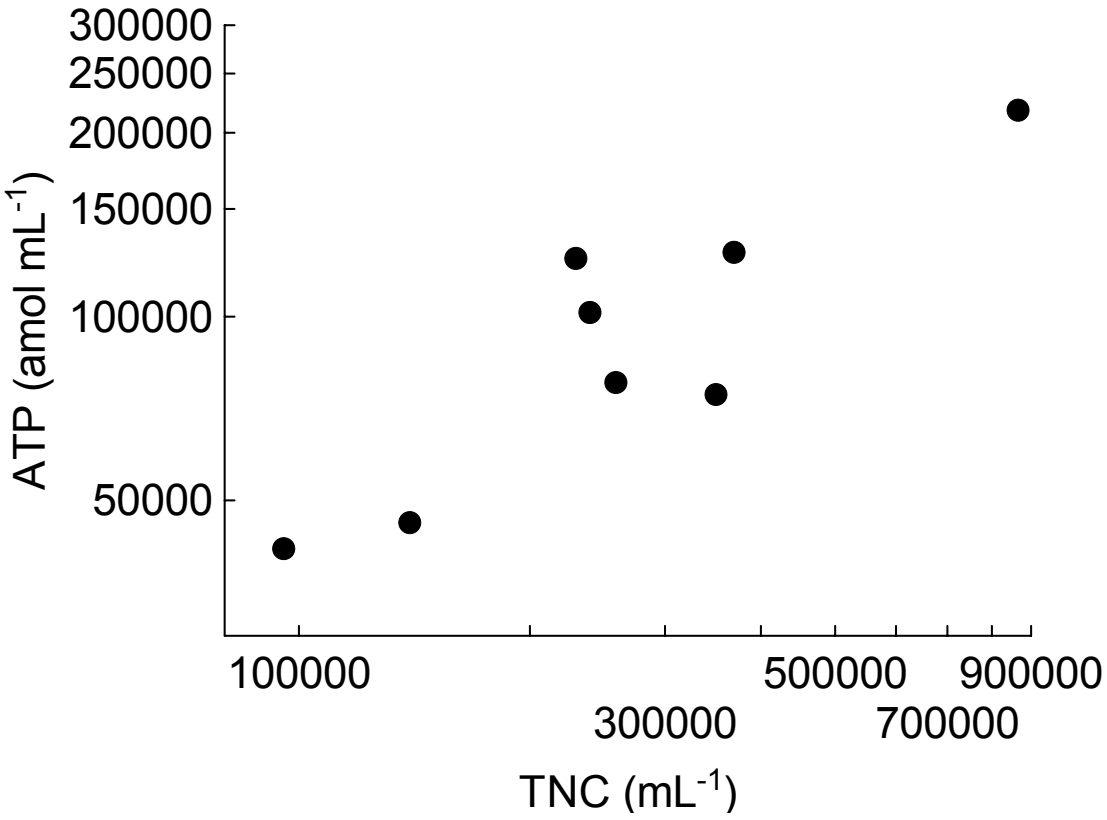


Figure 4-1. *ATP (Adenosine Tri Phosphate) content of the pore water plotted against the TNC (Total Number of Cells). The positive correlation is strong ($r^2 = 0.83$, $p = 0.0006$).* Please note the logarithmic scales.

Eydal and Pedersen (2007) demonstrated that a cell in the subsurface environment contains on average 0.1–1 amole of ATP, and that this value is fairly constant. By using the data from Figure 3-9 in the following equation, we can see that the biomass doubled between five and seven times over the period when ATP was measured in the pore water in the Prototype Repository:

$n = (\log N - N_0) / (\log 2)$

where n represents the number of doublings, N the present ATP value, and N_0 the initial ATP value.

With the ATP analyses, we established that there was an increase in microbial activity in the Prototype Repository over time. The rather large increase evident at the end of the sampling period (Figure 3-9), however, could be due to the change in sampling procedures made in late 2006, when we began to use pressure vessels to extract the pore water. It is therefore crucial to continue sampling using the new procedure to determine whether this increase continues over the years. To find out what kinds of microorganisms comprise this biomass, and its consequences for the Prototype environment, we can examine the MPN and gas data. This is easier said than done, however. Combining gas and microbial data can often be difficult, because many compounds produced by one kind of microbe are utilized by others (see section 1.3). One exception to this, at least inside the Prototype, is the oxygen content coupled to microbial activity. In the Prototype, there can only be microbial *consumption* of oxygen, because no microbes are likely present that can produce oxygen gas. On the other hand, we cannot exclude the possibility that oxygen is leaking into the Prototype from the surrounding tunnel.

4.4 Microbial decrease of oxygen in the Prototype Repository

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.

Overall, we see that the oxygen level generally decreased over time in the Prototype Repository (Figure 4-2). As of May 2007, it was possible to fill the pressure vessel with water from four of the seven sampling groups (Table 3-2), i.e., the KBU10002+8 group (located in the lower part of the backfill and near the canister in section 1, the inner section farthest from the tunnel), the KBU10004+6 group (located in the lower part of the backfill in section 1), the KBU10005 group (located in the upper part of the backfill in section 1), and the KFA01-04 group (located in the upper and lower parts of the backfill in section 2, the outer section closest to the tunnel). In Figure 4-2, the ATP contents of these pore waters are compared with the oxygen levels. The decrease in oxygen was accompanied by high ATP values, which suggests that microbial activity had an effect on the lowering oxygen levels.

Many types of microbes are able to decrease the oxygen concentration, since many microbes use this molecule when respiring; however, the amount of organic carbon present in a KBS-3-type repository will likely be too low (0.20–0.29%) to sustain most such microbes. If this should be the case, however, there are microbial strategies to get

around low amounts of organic carbon (see section 1.3), some of which were obviously used by microbes inside the Prototype. Two of these processes are methane oxidation and autotrophic acetogenesis. We know that MOB, AA, and CHAB were present in the pore water inside the Prototype (Figure 3-10, Figure 3-11, and Figure 3-12, Table 3-4) in numbers 120–2000 times greater than those in the groundwater outside it, i.e., they thrive in the Prototype Repository. Another explanation of the high microbial numbers inside the Prototype is that perhaps that no viral predation occurs inside the Prototype, unlike what occurs in the surrounding Äspö groundwater (Kyle et al., 2007).

MOB are microorganisms that consume oxygen when metabolizing methane (see section 1.3). When examining Figure 3-10, we see that the numbers of MOB were positively correlated with how the amount of oxygen present in the dissolved gas in the pore water, i.e., the presence of more oxygen promoted the growth of MOB. If we then look at the gas composition trends in the KBU10002+8 group (in which the most MOB were found, Table 3-4), we see that the methane in the gas phase in this group decreased from 0.7% to below 0.1% over time (Table 3-3). This clearly indicates the presence of active MOB inside the pore water in the Prototype Repository..

Other microbes, the AA, produce the organic compound acetate from carbon dioxide and hydrogen (see section 1.3). Low amounts of carbon dioxide in the gas phase were positively correlated with high numbers of AA (Figure 3-12). The AA were also positively correlated with high amounts of hydrogen (Figure 3-12). This means that carbon dioxide was depleted in pore water containing high numbers of AA, while hydrogen promoted extensive growth of AA. Any acetate produced can be used by CHAB, which also decrease the oxygen concentration when they respire (see section 1.3). The numbers of CHAB are positively correlated with the increase in carbon dioxide (Figure 3-11), which suggests that acetate was degraded and carbon dioxide produced (see section 1.3).

4.5 Microbial succession in pore water

MOB and CHAB, 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 the numbers occasionally exceeded 5000 mL^{-1} (Figure 3-13, Table 7-2), well above the number of the SRB in the Äspö groundwater nearest the Prototype (Table 3-4). Some SRB in the Äspö groundwaters were able to grow on both hydrogen and acetate (Motamedi and Pedersen, 1998), which could find their way into the buffer and backfill. A further 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, 2006). As the oxygen continues to decrease, the future 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.

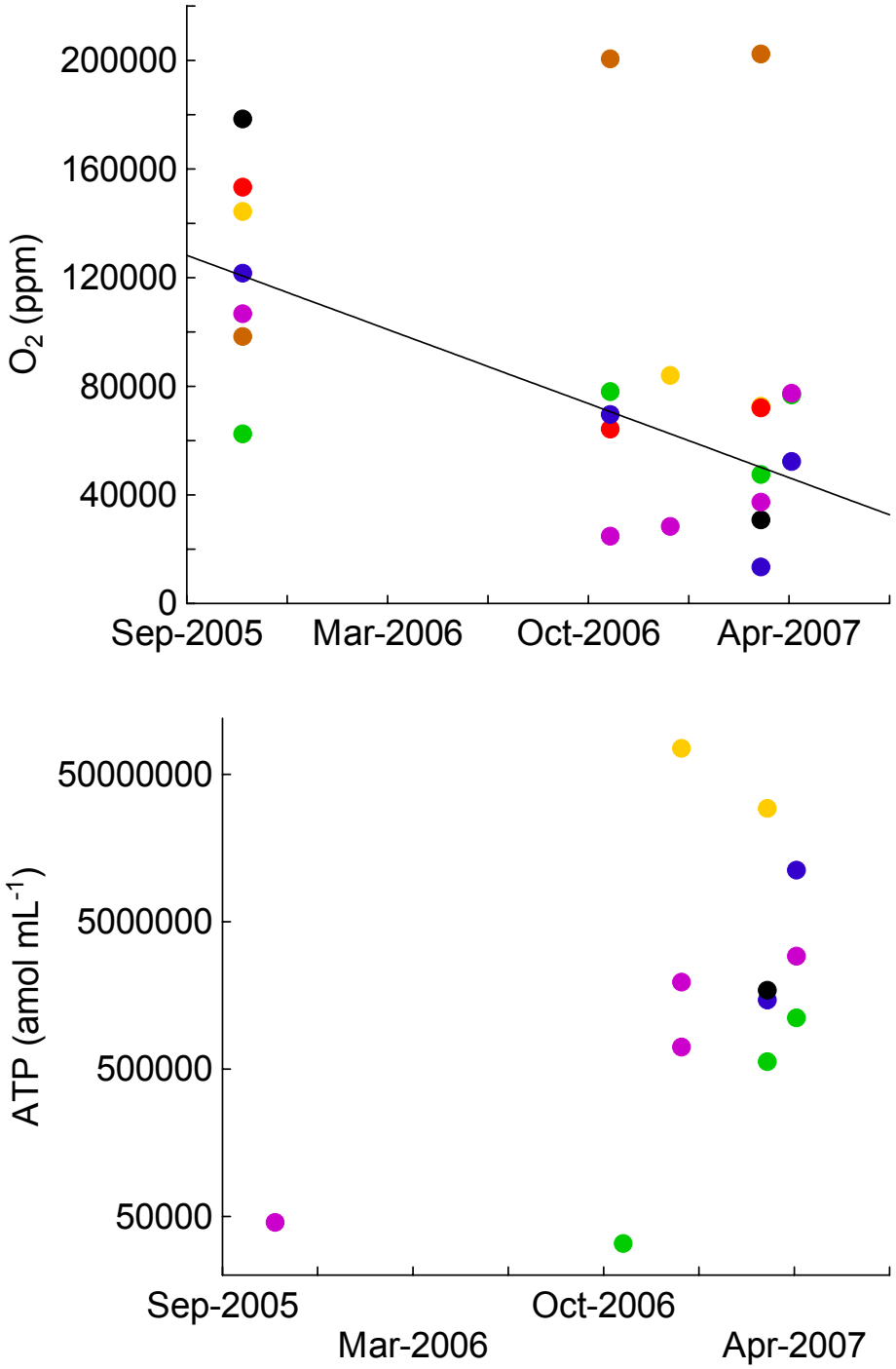


Figure 4-2. *Oxygen levels in ppm of the total amount of extracted gas between November 2005 and May 2007 in all sample groups from the Prototype Repository and ATP (Adenosin Tri Phosphate) contents in the sample groups from which water can be extracted over the same period. There was a negative correlation between oxygen level and ATP content over time ($r^2 = 0.56$, $p = 0.00008$). The sample groups are the KB513-614 (yellow circles), KBU10001 (red circles), KBU10003+7 (brown circles), KBU10002+8 (green circles), KBU10004+6 (blue circles), KBU10005 (black circles), and KFA01-04 (purple circles) groups. The KBU10003+7 group was excluded from the regression analysis because it was likely in contact with the tunnel air. Please note the logarithmic y-scale in the lower graph.*

4.6 Bentonite stimulates growth

Comparison of the KBU10002+8 group pore water and the surrounding groundwater from outside the Prototype Repository revealed that the chemistry was fairly similar in these two waters (Table 3-5). In addition, it was easy to extract water from the KBU10002+8 group. We therefore suspect that the hydrochemical sampling points in the KBU10002+8 group contain fairly much groundwater and that this suggests that the buffer in deposition hole 3, where sampling point KBU10008 is located, does not work optimally. In the KBU10002+8 group, the water pressure was approximately 3 bar in May 2007. According to Goudarzi and Johannesson (2006), the total pressure in the KBU10008 sampling point was approximately 4 bar and the backfill temperature approximately 25°C in late 2006. The KBU10002+8 group contained the highest numbers of MOB, AA, CHAB, and SRB in the Prototype (Table 3-4). The microbial abundance of all these metabolic groups is thus higher than in the surrounding groundwater, as shown in Table 3-4. The greater microbial abundance in this group than in the groundwater could mean that the bentonite/backfill material itself and/or the higher temperature in the backfill than in the groundwater stimulated the growth of these types of microbes, at least as long as the total pressure did not exceed 4 bar (which corresponds to a swelling pressure of 1 bar, given that the water pressure is 3 bar). We can establish whether the water-saturation phase will allow active microbial populations in the backfill and in the buffer/backfill interphase.

In the KFA01-04 group, the total pressure was considerably higher (6–7 bar) in late 2006 than in the KBU10002+8 group, according to Goudarzi and Johannesson (2006). The temperature in this group was approximately 20–30°C. The water pressure in this group was 5–7 bar in late 2006. This means that the swelling pressure here was approximately 1–2 bar. Comparing the overall water pressure and the water pressures at the individual sampling points, even at a swelling pressure of 2 bar we found viable microbes in the pore water from this group (Table 3-4, Figure 3-9).

4.7 Evaporation, mineral dissolution, and bacterial activity affects the pore water chemistry

Both abiotic and biotic processes affected the chemistry of the Prototype pore water.

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, KBU10005, and KFA01-04 groups (Figure 3-14, Table 3-5). 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); Figure 3-14 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 many of the sampling groups in the Prototype Repository (Figure 3-14, Table 3-5). Cristobalite dissolution can result in a small rise in Si concentration in the pore water (Luukkonen, 2007). Halite dissolution could explain the high chloride concentrations in the pore water (Luukkonen, 2007). Halite was not found in the analyses of the backfill and buffer material (Karnland, 2007), however, and the reason for the high chloride concentrations in the pore water remains to be discovered. One possibility is that the dry bentonite contains high amounts of Cl and F, which could dissolve in the pore water in the water-saturation phase (Luukkonen, 2007). The sampling points in the KB513-614 group were situated in the bentonite on top of the canisters in deposition holes 5 and 6. Here, the temperature reached 30°C in the buffer on top of the deposition holes. Very little water was extractable from this sampling group, though it did contain some water. Chemical analyses of the KB513-614 group indicated that the water was extremely saline and sulphate rich (Table 3-5), which could suggest that the bentonite contained Cl and F which was dissoluted. As well, the KBU10004+6 group, located in the lower part of the backfill in section 1 (the inner section farthest from the tunnel), contained fairly saline and sulphate-rich water (Figure 3-14, Table 3-5).

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., 2007b). Chelating agents have been reported for many of the compounds enriched in the Prototype pore water (compare Table 3-5 and Figure 3-14); i.e., V (Baysse et al., 2000), U (Moll et al., 2007a), and Cs (Wendling et al., 2005).

4.8 Concluding remarks

- Sampling methodology: Our methodologies for sampling and analysing pore water from the Prototype Repository worked very well. We thus have the unique opportunity to continue sampling water from the Prototype, monitoring further changes over the long term.

- Methanotrophy in the pore water: We observed that the biomass inside the pore water in the Prototype Repository was increasing at the same time as the oxygen content was decreasing (Figure 4-2). MOB were more abundant in oxygen-rich pore water (Figure 3-10). Besides oxygen, these microbes also use methane in their metabolism. In pore water containing high numbers of MOB, the methane was continuously decreasing (Figure 3-6, Table 3-3). Clearly, the MOB are active inside the Prototype.

- Heterotrophy in the pore water: The numbers of CHAB were also 100–2000 times higher in the Prototype Repository pore water than in water outside the Prototype (see section 3.4.2). A high number of CHAB indicates the microbial aerobic degradation of organic carbon. This process produces CO_2; consequently, high numbers of CHAB were correlated with high amounts of this gas (Figure 3-11). This organic carbon source inside the Prototype have to be be evaluated. Some organic carbon may come from the MX-80 Wyoming bentonite, which contains approximately 0.25% dissolved organic carbon (Svensson, 2008). Another potential source is acetate, which can be produced by the AA (see section 1.3) detected in the Prototype pore water at mean numbers up to 25 AA mL^{-1} (see section 3.4.2). The potential for these to produce acetate in situ remains to be determined.

- Sulphate reduction in the pore water: Appendix C presents a study of sulphide production in hydrogen-rich pore water. Up to 60 mg L^{-1} of sulphide was produced within 6weeksin pore water from the Prototype Repository. The SRB thus could produce extensive amounts of sulphide when they have access to an energy source, such as hydrogen (see section 1.3). In the Prototype pore water, 5000 SRB mL^{-1} were detected when the hydrogen content was high (Figure 3-13).When the oxygen becomes depleted inside the Prototype, the SRB probably will thrive even more than they do now, since SRB in general do not tolerate oxygen well.

- Mineral interactions: The chemical data for the pore water suggest that dissolution of the minerals cristobalite and gypsum occurs in the Prototype Repository (Figure 3-14, see section 4.7) (Luukkonen, 2007). The data also suggest that cation exchange occurs from the sodium and potassium to the magnesium and calcium in the interlayers of the montmorillonite (Figure 3-14, see section 4.7) (Luukkonen, 2007). In addition, evaporation at the warm interfaces between buffer and backfill might have occurred (see section 4.7). 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. Evaporation leading to high salinity might decrease the swelling capacity of the bentonite (see section 4.7).

- Bioligands: In iron-deficient environments, such as the Prototype Repository pore water (Figure 3-14, Table 3-5), 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 (see section 4.7). 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 500 times.

4.9 New perspectives and the importance of further sampling

- It is crucial to determine whether the biomass inside the Prototype Repository will continue to increase, and how this will affect the gas composition and the chemistry of the pore water. As the trends discussed in this report indicate, the oxygen will probably be depleted inside the Prototype Repository, at least partially due to the activity of MOB. As well, the presence of acetate, the organic carbon produced by AA, should be monitored, since the presence of organic carbon inside the buffer and backfill could promote microbial activity.

- When the Prototype Repository enters an anaerobic mode, the SRB should be carefully monitored. The substrate for SRB respiration is sulphate, so it is important to follow the chemical evolution of this compound in the pore water. Sulphate can be released during gypsum dissolution (Luukkonen, 2007), thus promoting SRB growth. Another parameter that should be monitored is sulphide concentration, since sulphide can compromise the safety of the copper canisters by corrosion and since we know that the SRB inside the Prototype can produce sulphide (Appendix C).

- The pore water chemistry inside the Prototype Repository displays signs of gypsum and cristobalite dissolution, cation exchange, and possibly evaporation. There are also reports of the microbial conversion of smectite to illite in the bentonite, performed by IRB at ambient temperatures and over a period of a few weeks (Kim et al., 2004). Such a process would affect the swelling capacity of the bentonite. IRB should thus be evaluated in further pore water samples.

- The production and presence of bioligands (which can dissolve many radionuclides) in the Prototype Repository pore water should be evaluated. The pore water chemical data indicate that several compounds (i.e., U, Cs, and V) for which bioligands or other chelators are known to exist (Wendling et al., 2005; Baysse et al., 2000; Moll et al., 2007a) are enriched.

- Newly developed molecular methods for examining the pore water in the Prototype Repository can be used to pinpoint the specific microbes most likely to survive in an actual storage facility of the KBS-3 type (see Appendix B). These methods can determine, for example, whether these microbes are MOB, AA, SRB, or IRB and/or can produce bioligands.

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7 Appendix A – Data

Table 7-1. Raw data from gas analyses, 2005–2007. The gas content is given in ppm of the total amount of extracted gas from each sample.

Sampling point	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)
KJ0050F01	2006-04-19		102	12.7	5060	2010	2.97	bd	152	bd	768
KJ0052F01	2006-03-23		595	11.1	10600	2430	2.26	bd	91.6	bd	894
KJ0052F03	2006-05-03		25	bd	9350	5560	2.79	bd	169	bd	769
KB513:1	2005-11-24	1.8	9.90	32.2	531	34.8	0.30	bd	bd	186	779
KB513:1	2007-04-17	0.4	5.20	72.3	3020	4590	bd	bd	16.3	59.9	925
KB514:1	2005-11-24	1.7	57.9	16.0	521	1780	1.50	bd	14.6	76.4	869
KB514:1	2007-01-24	1.1	68.1	14.3	617	5520	0.18	0.24	6.81	101	901
KB514:1	2007-04-17	0.5	52.0	16.7	2840	4850	bd	0.32	26.0	88.7	871
KB613:1	2005-11-24	2.2	32.4	22.5	322	908	0.20	0.30	7.45	135	829
KB613:1	2007-01-24		130	22.2	1780	16300	1.49	4.92	36.6	66.0	927
KB613:1	2007-04-17	0.6	2.50	17.8	3720	12900	7.72	1.21	74.3	81.3	857
KB614:1	2005-11-24	1.2	5.90	17.3	1310	72.3	0.50	bd	bd	178	776
KB614:1	2007-04-17	0.5	68.9	466	3180	3950	1.29	1.51	20.5	59.0	917
KBU10001:1	2005-11-24	1.3	7.00	23.0	1440	4930	2.10	2.10	bd	153	809
KBU10001:1	2006-11-09	3.2	3.50	12.7	20.0	4040	0.33	bd	bd	64.0	931
KBU10001:1	2007-04-17	2.6	1.80	4.90	10000	259	bd	bd	bd	71.7	918
KBU10001:1	2007-05-22	3.3									
KBU10001:2	2006-11-09	3.2	3.30	13.2	9760	306	0.56	bd	bd	70.6	917
KBU10002:1	2005-11-24	1.1									
KBU10002:1	2006-11-09	3.4	18.4	101	2140	38200	1.78	bd	bd	149	817
KBU10002:2	2006-11-09	3.4	18.9	83.6	688	25000	0.84	bd	bd	234	742
KBU10002:3	2006-11-09	3.4	22.3	64.1	105	90300	bd	bd	bd	156	762
KBU10002:3	2007-04-17	2.7	25800	14.9	476	9740	0.29	bd	bd	63.7	903
KBU10002:3	2007-05-22	3.3	11.1	183.3	360	8590	bd	bd	bd	83.1	910
KBU10002:4	2006-11-09	3.4	21.1	36.2	54	101000	1.1	bd	6.00	41.3	862
KBU10003:1	2005-11-24	1.3	227	10.9	29800	104	2.00	bd	bd	14.2	948
KBU10003:1	2007-04-17	1.1	2.20	13.9	15.0	792	bd	bd	bd	203	801
KBU10003:1	2007-05-01	0.9									
KBU10004:1	2005-11-24	1.9	9.90	13.5	327	774	1.2	bd	bd	126	821
KBU10004:1	2006-11-09	3.2	6.00	13.6	470	13700	1.63	bd	bd	22.4	966
KBU10004:1	2007-04-17	3.6	154	3.50	21.0	6630	bd	bd	bd	47.5	978
KBU10004:1	2007-05-22	3.6	1310	20.4	51.0	42900	bd	bd	bd	76.4	871
KBU10005:1	2005-11-24	1	9.60	15.2	320	25.3	bd	bd	bd	178	778
KBU10005:1	2007-04-17	0.3	7.90	154	39.0	11300	bd	1.16	bd	30.5	939
KBU10005:1	2007-05-22	0.4									
KBU10006:1	2005-11-24	1.9	7.30	13.2	176	1890	2.60	0.20	bd	117	837
KBU10006:1	2006-11-09	2.5	2.6	17.5	769	3770	1.54	0.1	bd	116	875
KBU10006:1	2007-04-17	3.6	29.3	52.6	71.0	7580	0.66	bd	bd	25.8	959
KBU10006:1	2007-05-22	1.2	13.9	308	290	7130	bd	bd	bd	27.9	954
KBU10007:1	2005-11-24	1	8.00	16.7	277	294	1.20	0.40	bd	18.2	772
KBU10007:1	2006-11-09	1.1	2.60	26.4	117	491	0.76	0.28	bd	200	790
KBU10007:1	2007-04-17	1.1	2.30	53.7	117	732	0.98	0.26	bd	201	799
KBU10007:1	2007-05-22	1									
KBU10007:2	2006-11-09	1.1	2.50	22.2	15.0	3350	0.05	bd	bd	153	845
KBU10008:1	2005-11-24	1.1	17.9	15.1	7050	778	0.90	bd	bd	62	901

Table 7-1, continued

Sampling point	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)
KBU10008:1	2006-11-09	3.4	14.6	65.5	49.0	30133	bd	bd	bd	264	708
KBU10008:2	2005-11-24	1.1									
KBU10008:2	2006-11-09	3.4	18.7	32.7	68.0	64600	bd	bd	17.7	10.1	876
KBU10008:3	2005-11-24	1.1									
KBU10008:3	2006-11-09	3.4	24.7	60.9	153	74500	0.5	bd	bd	bd	920
KBU10008:3	2007-04-17	3.7	339	130	429	8650	bd	bd	bd	31.2	963
KBU10008:3	2007-05-22	3.8	7.80	217	109	14200	bd	bd	bd	70.2	904
KBU10008:4	2005-11-24	1.1									
KBU10008:4	2006-11-09	3.4	45.0	44.2	7130	3950	0.50	bd	30.5	13.5	953
KFA01:1	2005-11-24	4.6									
KFA01:1	2006-11-29	6.9	42200	45.2	398	43600	1.08	bd	1380	53.7	837
KFA01:1	2007-01-24	7.8	73.2	95.5	496	82900	1.86	2.10	2.21	6.04	908
KFA01:1	2007-04-17	7.6	66.0	44.6	1390	8060	bd	bd	2.67	64.2	917
KFA01:1	2007-05-22	8.8	140	569	1750	3070	bd	bd	3.15	119	872
KFA02:1	2005-11-24	4.4	11.2	35.4	1700	337	0.70	bd	15.5	150	794
KFA02:1	2007-01-24	1.2	174000	50.1	715	59400	2.74	bd	24.1	32.6	903
KFA02:1	2007-04-17	7.6	129	7.10	771	11900	bd	bd	27.4	8.32	968
KFA02:1	2007-05-22	1.4									
KFA03:1	2006-11-09	4.6	628	15.5	1770	15400	1.25	0.31	2.00	4.54	973
KFA03:1	2007-01-24	2.2	61700	17.4	336	5250	0.86	0.21	bd	73.6	886
KFA03:1	2007-04-17	7.8	15.4	28.2	3950	6380	1.58	0.87	4.10	43.7	917
KFA03:1	2007-05-22	4.3									
KFA04:1	2005-11-24	4.8	10.9	16.2	2640	26.2	0.60	bd	7.55	63.2	883
KFA04:1	2006-11-29	6.6	744	13.4	59.6	25800	0.59	bd	1.25	14.5	983
KFA04:1	2007-01-24	7.4	119	151	601	262000	2.25	bd	3.96	bd	776
KFA04:1	2007-04-17	7.2	63.9	91.6	2390	12300	1.81	bd	2.32	31.6	945
KFA04:1	2007-05-22	8.6	18.7	13.6	1820	12400	bd	bd	0.88	35.5	948
KFA04:2	2005-11-24	4.8									
KFA04:3	2005-11-24	4.8									

Table 7-2. Raw data from microbial analyses, 1999–2007.

Sampling point	Sampling occasion	TNC (mL^{-1})	Stdev	n	ATP (amol mL^{-1})	Stdev	n	CHAB (mL^{-1})	Stdev	n	MPN SRB (mL^{-1})	Max	Min	MPN AA (mL^{-1})	Max	Min
KA3510 A	2006-11-08	13000	1300	3	7180	391	3	15.0	21.0	3	5.00	2.00	17.0			
KA3542 G01:2	2005-11-24	5100	840	6	3900	871	3	3.00		3	50	20.0	500			
KA3542 G01:2	2006-11-08	11000	1400	3	8460	332	3	bd		3	2.30	0.90	8.60			
KA3542 G01:3	2005-11-24	6300	3200	6				bd		3	50	20.0	500			
KA3542 G01:3	2006-11-08	12000	2400	3	6690	1020	3	bd		3	1700	700	4800			
KA3554 G01:1	2005-11-24	8900	3100	6				7		3	2.1	0.90	5.50			
KA3554 G01:1	2006-11-08	12000	1600	3	4780	333	3	27.0	23	3	3	1.00	12.0			
KA3554 G01:2	2005-11-24	4000	2700	6				3		3	5	2.00	15.0			
KA3554 G01:2	2006-11-08	8900	1300	3	7060	78.0	3	57.0	25	3	17	7.00	48.0			
KA3566 GO1:2	1999-03-01	99000														
KA3566 GO2:2	1999-03-01	82000									2400			bd		
KA3573 A:1	1999-03-01	58000									1.70			bd		
KA3573 A:2	1999-03-01	75000									24			bd		
KA 3573 A:2	2006-11-09	2800	240	3	2770	1820	3	bd		3	bd					
KA3600 F:1	1999-03-01	33000									13			bd		
KA3600 F:2-0	1999-03-01	52000									21			0.78		
KA3600 F:2-3	1999-03-01										13			bd		
KA3600 F:2	2006-11-09	2300	860	3	1240	324	3	bd		3	8.00	3	25.0			
KG0021 A01:2	2005-11-24	10000	5200	6	3920	1230	3	bd		3	0.20	0.10	1.10			
KG0021 A01:2	2006-11-08	28000	6700	3	5850	596	3	3.00	6.00	3	5	2.00	17.0			
KG0021 A01:3	2005-11-24	10000	3500	6	2720	519	3	bd		3	7	0.20	2.10			
KG0021 A01:3	2006-11-08	67000	3200	3	5390	504	3	3.00	6.00	3	0.80	0.30	2.40			
KB513	2007-04-17				9640000	370000	3									
KB613	2007-01-24				74200000	4930000	3									
KB613	2007-04-17				62700000	6110000	3				bd			2.00	1.00	11.0
KB614	2007-04-17				14275149	174046	3									
KBU10002:1	2006-11-09	140000	33000	3	45900	2240	3	133	31.0	3.00	bd					
KBU10002:2	2006-11-09	350000	23000	3	74300	4580	3	570	104	3	30.0	10.0	120			
KBU10002:3	2005-11-24	260000	84000	6	77500	2340	3	4600		3	9.00	4.00	25.0			
KBU10002:3	2006-11-09	96000	8000	3	41400	1690	3	147	55.0	3	24.0	10.0	94.0			
KBU10002:3	2007-04-17				47600	3400	3				5000	2000	17000	50.0	20.0	170

Table 7-2, continued

Sampling point	Sampling occasion	TNC (mL^{-1})	Stdev	n	ATP (amol mL^{-1})	Stdev	n	CHAB (mL^{-1})	Stdev	n	MPN SRB (mL^{-1})	Max	Min	MPN AA (mL^{-1})	Max	Min
KBU10002:3	2007-05-22				964000	8900	3									
KBU10003	2005-11-24	470000	250000	6				1230		3	bd					
KBU10004	2007-04-17				2020000	114000	3				bd			bd		
KBU10004	2007-05-22				11000000	686000	3							bd		
KBU10005	2007-04-17				1680000	56900	3				bd			bd		
KBU10006	2007-04-17				883000	45300	3				bd			bd		
KBU10008:1	2005-11-24	370000	61000	6	127000	2010	3	bd		3	0.20	0.10	1.10			
KBU10008:1	2006-11-09	230000	20000	3	124000	4210	3				bd					
KBU10008:2	2005-11-24	360000	49000	6				320000		3						
KBU10008:2	2006-11-09	240000	6900	3	101000	12400	3	9630	3360	3	130	50.0	390			
KBU10008:3	2006-11-09	94000	10000	3	24000	2340	3	63	15	3	110	40.0	300			
KBU10008:3	2007-04-17				1060000	33900	3				70.0	30.0	210	bd		
KBU10008:3	2007-05-22				1226000	47700	3									
KBU10008:4	2005-11-24	870000	490000	6	217000	3410	3	41000		3						
KFA01	2005-11-24				77500	2390	3	1060		3						
KFA01	2007-01-24				1820000	15500	3				bd			bd		
KFA01	2007-04-17				1060000	43300	3				2.00	1.00	11.0	bd		
KFA01	2007-05-22				3140000	334000	3									
KFA02	2007-01-24				1180000	39700	3									
KFA02	2007-04-17				1190000	115000	3				bd			4.00	1.00	17.0
KFA03	2005-11-24	410000	82000	6	55300	2800	3	1010		3	bd					
KFA03	2007-01-24				3650000	191000	3				bd			bd		
KFA03	2007-04-17				12900000	387000	3				bd			bd		
KFA04:1	2005-11-24				81500	13200	3	bd		3	2.70	1.20	6.70			
KFA04:1	2007-01-24				1070000	45400	3									
KFA04:1	2007-04-17				237000	54300	3				bd			14.0	6.00	35.0
KFA04:1	2007-05-22				2580000	254000	3									
KFA04:2	2005-11-24				11800	1430	3	17.0		3	1.20	0.50	2.90			
KFA04:3	2005-11-24							7.00		3	1.30	0.50	3.80			

Table 7-2, continued

Sampling point	Sampling occasion	MPN MOB (mL^{-1})	Max	Min	MPN HA (mL^{-1})	Max	Min	MPN IRB (mL^{-1})	Max	Min	MPM HM (mL^{-1})	Max	Min	MPN AM (mL^{-1})	Max	Min
KA3510A	2006-11-08	2.30	0.90	8.60												
KA3542 G01:2	2005-11-24	bd														
KA3542 G01:2	2006-11-08	2.30	0.90	8.60												
KA3542 G01:3	2005-11-24	bd														
KA3542 G01:3	2006-11-08	0.80	0.30	2.40												
KA3554 G01:1	2005-11-24	0.20	0.10	1.10												
KA3554 G01:1	2006-11-08	2.30	0.90	8.60												
KA3554 G01:2	2005-11-24	0.20	0.10	1.10												
KA3554 G01:2	2006-11-08	13.0	5.00	39.0												
KA3566GO1:2	1999-03-01															
KA3566GO2:2	1999-03-01				1200			3500			bd			24.0		
KA3573A:1	1999-03-01				2.20			13.0			bd			bd		
KA3573A:2	1999-03-01				49.0			92.0			bd			0.92		
KA 3573 A:2	2006-11-09	3	1	12												
KA3600F:1	1999-03-01				bd			79.0			bd			bd		
KA3600F:2-0	1999-03-01				24.0			33.0			bd			bd		
KA3600F:2-3	1999-03-01				2500			46.0			bd			bd		
KA3600F:2	2006-11-09	3.00	1.00	12.0												
KG0021 A01:2	2005-11-24	5.00	2.00	17.0												
KG0021 A01:2	2006-11-08	bd														
KG0021 A01:3	2005-11-24															
KG0021 A01:3	2006-11-08	0.20	0.10	1.10												
KB513	2007-04-17															
KB613	2007-01-24															
KB613	2007-04-17															
KB614	2007-04-17															
KBU10002:1	2006-11-09	1300	500	3900												
KBU10002:2	2006-11-09	1100	400	3000												
KBU10002:3	2005-11-24	24.0	10.0	94.0												
KBU10002:3	2006-11-09	1700	700	4800												
KBU10002:3	2007-04-17															
KBU10002:3	2007-05-22															

Table 7-2, continued

Sampling point	Sampling occasion	MPN MOB (mL^{-1})	Max	Min	MPN HA (mL^{-1})	Max	Min	MPN IRB (mL^{-1})	Max	Min	MPM HM (mL^{-1})	Max	Min	MPN AM (mL^{-1})	Max	Min
KBU10003	2005-11-24	23.0	9.00	86.0												
KBU10004	2007-04-17															
KBU10004	2007-05-22															
KBU10005	2007-04-17															
KBU10006	2007-04-17															
KBU10008:1	2005-11-24	800	300	2500												
KBU10008:1	2006-11-09	70.0	30.0	210												
KBU10008:2	2005-11-24	240	100	940												
KBU10008:2	2006-11-09	13.0	5.00	39.0												
KBU10008:3	2006-11-09	240	100	940												
KBU10008:3	2007-04-17															
KBU10008:3	2007-05-22															
KBU10008:4	2005-11-24	24.0	10.0	94.0												
KFA01	2005-11-24	13.0	5.00	39.0												
KFA01	2007-01-24															
KFA01	2007-04-17															
KFA01	2007-05-22															
KFA02	2007-01-24															
KFA02	2007-04-17															
KFA03	2005-11-24	bd														
KFA03	2007-01-24															
KFA03	2007-04-17															
KFA04:1	2005-11-24	800	300	2500												
KFA04:1	2007-01-24															
KFA04:1	2007-04-17															
KFA04:1	2007-05-22															
KFA04:2	2005-11-24	900	400	2500												
KFA04:3	2005-11-24	24.0	10.0	94.0												

Table 7-3. Raw data from chemical analyses of the Prototype Repository pore water, 2007.

Sample point	Sampling occasion	Na (mg L^{-1})	K (mg L^{-1})	Ca (mg L^{-1})	Mg (mg L^{-1})	Cl (mg L^{-1})	SO_4 (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})	pH
KB513	2007-04-17					11500	6960	2.80	37.7						8.15
KB613	2007-04-17					6690	4130	1.70	33.6						7.47
KBU10002	2007-04-17	1860	14.3	811	82.2	4420	358	bd	20.7	5.36	0.002	0.97	0.35	15.3	7.74
KBU10002	2007-05-23	1610	13.7	688	69.9	4140	361	2.60	19.8	5.63	0.003	0.80	0.35	12.4	7.67
KBU10004	2007-04-17	6260	133	7.15	105	8630	2380	5.10	35.7	19.3	0.06	0.02	0.35	0.23	8.63
KBU10004	2007-05-23	6150	142	16.7	105	8050	1920	4.90	36.2	22.0	0.004	0.02	0.38	0.52	8.67
KBU10005	2007-04-17	2830	42.3	169	86.1	4870	474	3.10	20.3	11.3	0.003	0.10	0.27	3.39	7.84
KBU10006	2007-04-17	4880	104	105	31.8	7550	850	5.80	33.8	21.7	0.05	0.21	0.49	2.98	8.12
KBU10008	2007-04-17	1780	13.1	732	82.5	4190	354	2.40	18.7	7.65	0.003	0.83	0.49	13.6	7.68
KBU10008	2007-05-23	1730	13.6	620	81.6	3710	344	1.10	16.1	7.69	bd	0.62	0.41	11.3	7.93
KFA01	2007-01-24	2460	173	32.1	26.7	4050	465	2.30	16.4	5.38	0.007	0.16	0.07	0.56	7.26
KFA01	2007-04-17	2580	81.6	35.8	24.0	2520	504	3.20	19.3	5.37	0.009	0.19	0.08	0.64	7.28
KFA01	2007-05-23	2640	79.5	37.2	20.6	3990	512	2.90	19.4	5.07	0.003	0.20	0.07	0.69	7.51
KF02	2007-01-24	2800	58.2	80.7	21.0	4590	361	bd	20.4	13.8	0.01	0.10	0.13	1.45	7.45
KF02	2007-04-17	2740	51.6	91.2	18.4	4420	345	bd	22.6	13.8	0.01	0.13	0.12	0.15	7.66
KFA03	2007-01-24	3490	49.3	9.06	39.0	4460	1340	3.10	19.0	3.18	0.004	0.05	0.04	0.15	8.44
KFA03	2007-04-17	3480	43.7	21.9	39.9	4400	1270	3.60	21.4	5.46	0.002	0.07	0.04	0.27	8.41
KFA04	2007-01-24	3100	48.7	15.2	28.6	4420	489	bd	18.2	14.2	0.01	0.09	0.08	0.23	8.19
KFA04	2007-04-17	3130	46.0	16.0	27.6	4590	530	2.80	21.6	15.1	0.02	0.08	0.10	0.27	8.12
KFA04	2007-05-23	3220	44.7	15.3	28.5	4640	573	2.40	23.2	13.9	0.003	0.03	0.09	0.24	8.00

Table 7-3, continued

Sample point	Sampling occasion	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})
KB513	2007-04-17													
KB613	2007-04-17													
KBU10002	2007-04-17	126	93.1	245	0.10	0.50	0.22	0.22	0.01	78.1	0.69	15.5	0.33	0.52
KBU10002	2007-05-23	106	9.77	185	0.03	1.64	1.19	54.7	bd	67.2	30.3	82.4	1.03	0.31
KBU10004	2007-04-17	730	6.93	8.51	bd	0.43	1.63	7.97	0.006	350	93.2	44.9	2.47	13.6
KBU10004	2007-05-23	719	33.2	8.99	bd	0.29	2.47	5.09	bd	214	6.99	51.0	1.65	11.5
KBU10005	2007-04-17	164	13.1	94.2	0.12	1.39	1.68	3.40	bd	60.3	9.50	29.8	2.02	5.66
KBU10006	2007-04-17	313	1.63	114	0.33	8.66	0.23	31.1	0.008	422	687	57.6	0.15	0.37
KBU10008	2007-04-17	122	8.76	114	0.13	12.3	0.14	0.96	0.007	84.1	625	19.9	bd	0.08
KBU10008	2007-05-23	114	9.95	76.3	bd	1.26	0.82	253	0.008	135	45.4	29.5	1.00	0.77
KFA01	2007-01-24	168	1.82	36.3	bd	1.33	0.28	0.76	0.002	95	169	9.51	0.23	2.08
KFA01	2007-04-17	176	43.9	43.8	0.12	4.59	0.25	1.40	0.008	119	191	31.4	bd	3.30
KFA01	2007-05-23	159	3.97	36.9	bd	4.05	0.92	3.93	bd	104	191	40.9	1.15	1.22
KF02	2007-01-24	134	29.9	68.4	bd	0.20	0.37	0.22	0.003	107	1.08	15.5	0.30	5.46
KF02	2007-04-17	107	3.13	78.8	0.03	4.97	1.03	1.13	bd	94.4	146	29.5	0.87	2.58
KFA03	2007-01-24	499	569	9.70	0.33	0.23	0.65	10.9	0.006	441	12.1	72.1	0.21	0.69
KFA03	2007-04-17	420	20.6	18.5	bd	0.81	0.89	0.71	0.002	330	10.4	151	0.88	0.74
KFA04	2007-01-24	178	7.77	53.0	bd	1.63	0.22	4.65	0.002	185	106	273	0.14	11.9
KFA04	2007-04-17	183	9.72	58.4	0.10	1.93	0.22	0.51	0.007	177	50.4	274	bd	13.6
KFA04	2007-05-23	179	3.84	57.0	bd	0.69	1.05	41.7	0.004	258	41.7	384	0.65	17.4

Table 7-3, continued

Sample point	Sampling occasion	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})
KB513	2007-04-17													
KB613	2007-04-17													
KBU10002	2007-04-17	56.0	0.03	bd	bd	bd	bd	0.03	bd	bd	bd	bd	bd	bd
KBU10002	2007-05-23	73.2	0.27	0.27	0.03	0.15	0.02	bd	0.02	bd	bd	bd	bd	bd
KBU10004	2007-04-17	74.7	0.03	0.07	bd	0.05	bd	bd	bd	bd	bd	bd	bd	bd
KBU10004	2007-05-23	54.8	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KBU10005	2007-04-17	92.2	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KBU10006	2007-04-17	56.5	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KBU10008	2007-04-17	54.7	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KBU10008	2007-05-23	115	0.12	0.06	bd	0.09	bd	bd	bd	bd	0.02	bd	0.02	bd
KFA01	2007-01-24	246	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KFA01	2007-04-17	29.2	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KFA01	2007-05-23	9.43	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KF02	2007-01-24	63.0	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KF02	2007-04-17	158	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KFA03	2007-01-24	49.5	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KFA04	2007-01-24	208	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KFA04	2007-04-17	21.1	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd
KFA04	2007-05-23	25.3	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd	bd

Table 7-3, continued

Sample point	Sampling occasion	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})	B^{10}/B^{11} Atomic
KB513	2007-04-17													
KB613	2007-04-17													
KBU10002	2007-04-17	bd	bd	bd	20.8	0.19	bd	0.20	1.22	bd	0.05	6.37	bd	0.2342
KBU10002	2007-05-23	bd	bd	bd	20.3	0.52	5.77	0.18	0.64	2.27	0.06	5.41	bd	0.2362
KBU10004	2007-04-17	bd	bd	bd	162	0.06	1.43	0.62	9.48	0.52	0.62	110	bd	0.2382
KBU10004	2007-05-23	bd	bd	bd	142	0.09	2.50	0.88	8.31	0.99	0.69	86.9	bd	0.2379
KBU10005	2007-04-17	bd	bd	bd	80.8	0.06	3.52	1.03	4.52	1.50	0.82	19.2	bd	0.2392
KBU10006	2007-04-17	bd	bd	bd	118	0.05	bd	0.47	4.12	bd	bd	1.12	bd	0.2361
KBU10008	2007-04-17	bd	bd	bd	36.0	0.21	bd	0.29	1.46	bd	bd	1.28	bd	0.2354
KBU10008	2007-05-23	bd	bd	bd	27.9	0.58	0.80	0.23	1.56	0.38	0.04	2.32	bd	0.2381
KFA01	2007-01-24	bd	bd	bd	83.1	0.01	bd	0.92	3.71	bd	0.52	6.03	bd	0.2371
KFA01	2007-04-17	bd	bd	bd	95.1	0.009	bd	1.04	4.45	bd	0.66	7.50	bd	0.2358
KFA01	2007-05-23	bd	bd	bd	79.0	0.009	1.70	0.87	3.46	0.75	0.65	5.66	bd	0.2399
KF02	2007-01-24	bd	bd	bd	94.8	0.01	bd	0.84	9.50	bd	0.45	8.39	bd	0.2355
KF02	2007-04-17	bd	bd	bd	101	0.02	0.27	0.77	9.22	0.12	0.49	3.46	bd	0.2372
KFA03	2007-01-24	bd	bd	bd	63.9	0.003	0.88	1.88	3.51	0.37	022	156	bd	0.2374
KFA04	2007-01-24	bd	bd	bd	76.6	0.02	bd	1.20	8.21	bd	0.55	52.0	bd	0.2351
KFA04	2007-04-17	bd	bd	bd	77.1	0.02	bd	1.14	8.10	bd	0.42	48.2	bd	0.2325
KFA04	2007-05-23	bd	bd	bd	83.6	0.09	0.36	1.18	8.69	0.12	0.93	60.9	bd	0.2378

8 Appendix B – Molecular analysis of microbes in the pore water from KB613

The pore water from borehole KB613 sampled in January 2007 contained visible microbial growth, and examination using light microscopy confirmed the observation. Figure 8-1 shows an image taken directly through the microscope (1000× magnification). To identify the microbes present in KB613 pore water, we used our molecular methods.

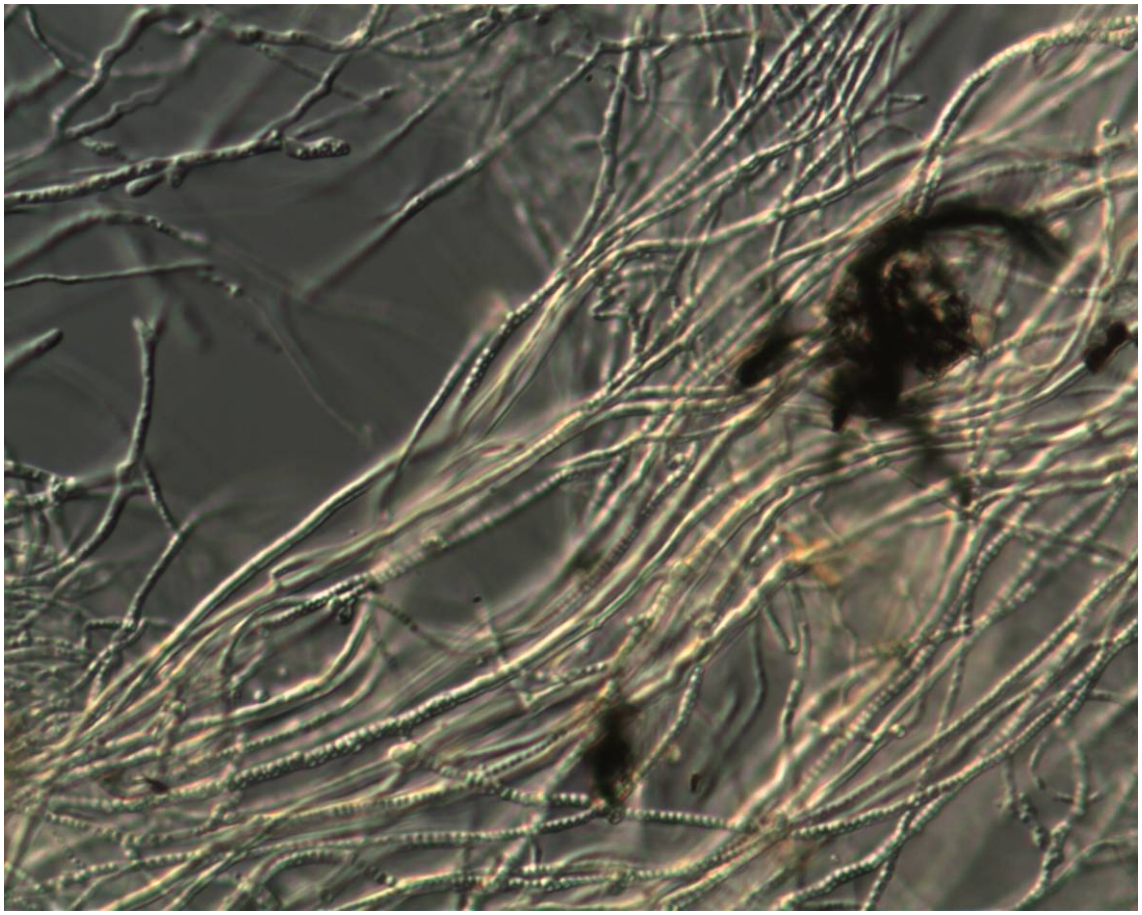

Figure 8-1. *The microbes growing in the pore water from borehole KB613.*

8.1 DNA extraction, PCR, and sequencing

DNA was extracted using the G+ protocol included in the DNeasy Blood and Tissue kit (no. 69504; QIAGEN, Solna, Sweden) according to the manufacturer's instructions. The 16S rRNA genes were partially amplified using the polymerase chain reaction (PCR) technique in 25-µL reactions with 12.5 µL of iProof HF Mastermix (no. 172-5310; Biorad, Sundbyberg, Sweden), 30 ng of DNA, and 10 pmol/µL of each of the universal 16SrDNA primers 530f (*Escherichia coli* numbering, 5´-GTG CCA GC(AC) GCC GCG G-3´) and 907R (*E. coli* numbering, 5´-CCG TCA ATT CCT TTR AGT TT-3´).

PCR amplification was performed using a Mycycler system (Biorad) with an initial denaturation step at 98°C for 30 s, followed by 30 repetitions at 98°C for 30 s, 60°C for 30 s, and 72°C for 40 s, concluding with a final extension at 72°C for 5 min. The chosen primers gave a PCR fragment of approximately 350 base pairs. The PCR products were checked in UV light in a Gel Doc XR system (Biorad) on a 1% ready-to-use agarose gel (no. 161-3010; Biorad). A band of the appropriate length was cut and the PCR product was eluted using a MinElute gel extraction kit (no. 28604; QIAGEN), according to the manufacturer's instructions. Sequence reaction and separation with gel electrophoresis were performed at MWG Biotech AG (Ebensberg, Germany) using the 907R primer. The sequence was compared with the GenBank database using the BLAST tool (www.ncbi.nlm.nih.gov/BLAST).

8.2 Sequence

The following sequence was reported from MWG:

CCTTAATGCGTTAGCTGCGGCACGGAATCCGTTGATAAGACCCCACACCTAGTGCCCAAC

GTTTACGGGGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCTCTTC

AGCGTCAGTATCGGCCCAGAGTGCTGCCTTCGCCGTTGGTGTTCCTCCTGATATCTGCGC

ATTTCACCGCTACACCAGGAATTCCACACTCCTCTACCGAACTCGAGTCACAGCAGTATC

GACTGGCGTCCCAAGGTTGAGCCTTGGATTTTCACAGTCGACTTACTGAACCGCCTAGGA

GCCCTTTACGCCCAATAATTCCGGACAACGCTCGCCCCCTACGTCTTACCGCGGCGGCTGG

The BLAST search (no. 071214) revealed that this sequence was 99% affiliated with that of an uncultured *Microthrix* sp. (RDBP accession no. DQ188340.1). This bacterium is known to form filamentous biofilms, for example, in sludge in wastewater treatment plants, i.e., fairly nutrient-rich environments.

9 Appendix C – Sulphide production in the pore water from sampling point KFA01

9.1 Sampling and results

On 29 November 2006, stainless steel pressure vessels were connected to sampling points KFA01 and KFA04 in the Prototype Repository. These vessels were retrieved on 11 December 2006 and analysed regarding the gas composition of the pore water. On this occasion, the pressure vessels were filled with pore water from the backfill in section 2 (the outer section closest to the tunnel). The gas analysis indicated that the water in KFA01 was very rich in hydrogen, as approximately 4% of the total gas in the water was hydrogen (Table 9-1). In KFA04, the hydrogen content was lower, at 0.07%.

Table 9-1. Results of the gas and sulphide analyses of the pore water extracted using pressure vessels from sampling points KFA01-04, KB514, and KB613, December 2006–January 2007. The gas content is given in ppm of the total amount of extracted gas from each sample.

Sampling point	Sampling date	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)	S^{2-} (mg L^{-1})
KFA01:1	2006-11-29	42200	45.2	398	43600	1.08	bd	1380	53.7	837	
KFA04:1	2006-11-29	744	13.4	59.6	25800	0.59	bd	1.25	14.5	983	
KFA01:1	2007-01-24	73.2	95.5	496	82900	1.86	2.10	2.21	6.04	908	56.8
KFA02:1	2007-01-24	174000	50.1	715	59400	2.74	bd	2.410	3.260	903	bd
KFA03:1	2007-01-24	61700	17.4	336	5250	0.86	0.21	bd	73.6	886	bd
KFA04:1	2007-01-24	119	151	601	262000	2.25	bd	3.96	bd	776	9.4
KB514:1	2007-01-24	68.1	14.3	617	5520	0.18	0.24	6.81	101	901	bd
KB613:1	2007-01-24	130	22.2	1780	16300	1.49	4.92	36.6	66.0	927	bd

The sampling was reproduced in the beginning of 2007. Sampling vessels were connected on 7 January 2007, and retrieved on 7 March 2007. In addition, sampling points KFA02 and KFA03 (also in the backfill in the outer section 2) and KB514 and KB613 (in deposition holes 5 and 6) were sampled. The pressure vessels from the backfill were completely water filled, the pressure vessel from KB613 was partially water filled, while sampling point KB513 delivered only gas. In addition, the sulphide concentrations were analysed using the methylene blue method according to the Swedish standard. In the pore water from sampling point KFA01, the hydrogen content was remarkably low at only 0.007% (Table 9-1); in contrast, the sulphide and carbon dioxide concentrations in this pore water were high, at 56.8 mg L^{-1} and 8%, respectively (Figure 9-1, Table 9-1). The sulphide and carbon dioxide concentrations in pore water from KFA04 were 9.4 mg L^{-1} and 26%, respectively (Table 9-1, Figure 9-1). The pore waters from sampling points KFA02, KFA04, and KB613 were also analysed for gas and sulphide. Sulphide was not found in these waters (Figure 9-1), but pore waters from KFA02 and KFA03 contained very high hydrogen concentrations, of 17% and 6% , respectively (Table 9-1).

Figure 9-1. *Sulphide analysis using the methylene blue method, performed on pore water from a reference and from sampling points KB613, KFA02, KFA03, KFA04, and KFA01. The intensity of the blue indicates how much sulphide is present in the sample; it can be converted to a concentration by measuring the absorbance on a spectrophotometer and subsequently comparing the results with a standard curve.*

9.2 Discussion

Very high hydrogen concentrations were reported repeatedly in section 2 (the outer section closest to the tunnel) of the Prototype Repository. The high hydrogen concentrations in some of the pore waters may be effects of corrosion of the instrumentation in the Prototype Repository. A real KBS-3 repository will not contain as much instrumentation, but will contain reinforcing barst and rock bolts, and thus the hydrogen content could be similar.

However, the data from KFA01 indicate that after the hydrogen gas content peaks, the sulphide production will be extensive and the carbon dioxide content of the gas phase will increase (Table 9-1). There is a microbiological explanation for this. Sulphate-reducing bacteria produce sulphide when respiring, and many of these bacteria can use hydrogen as an energy source. Thus, hydrogen can stimulate the growth of these microbes. These microbes also need a carbon source, which has yet to be discovered for the pore water in the Prototype Repository. An organic carbon source would result in an increase in the carbon dioxide content of the gas phase. We see that this happened in the pore water from the Prototype (Table 9-1). The organic carbon could be acetate, produced by acetogenic bacteria from carbon dioxide and hydrogen. Another possibility is that the backfill may contain enough organic carbon to support this extensive growth.