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The Microbe project

Achievements of a 10-year research programme

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September 2013

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Abstract

Microorganisms interact with their surroundings and sometime greatly modify the characteristics of their environments. Several such interactions may significantly influence the function of a future deep repository for spent nuclear fuel. Four specific microbial process areas of importance for proper repository functions were identified for study at the Microbe laboratory in separate projects, namely: microbial effects on the chemical stability of deep groundwater environments, bio-mobilization of radionuclides, bio-immobilization of radionuclides, and microbially induced corrosion of copper. The Microbe laboratory main and satellite sites in the Äspö tunnel and the outcome of the Microbe research programme are presented in this report. The results and conclusions of the Microbe project have been published in international peer-reviewed scientific journals. This report summarizes unpublished information about the investigated sites, achievements, and experiment results related to the published work, thereby fully reporting the realization of the Microbe project. The most important conclusions drawn during the Microbe project with implications for repository safety can be summarized as follows: The microbial reduction of sulphate can proceed at maximum rate in the presence of hydrogen at a concentration as low as approximately 1 μM. Viruses that attack bacteria (bacteriophages) exert a strong controlling effect on the total numbers of microorganisms in deep groundwater, which in turn will control the rates of microbial processes such as sulphate reduction. Microbial biofilms can reduce the sorption of radionuclides to rock surfaces. The swelling pressure and water content of buffer materials are strongly related to microbial survival and activity in these materials.

Sammanfattning

Mikroorganismer interagerar med sin omgivning på många olika sätt och de kan därigenom avsevärt påverka sin levnadsmiljö. Flera sådana interaktioner kan väsentligt påverka olika funktioner hos ett framtida djupförvar för använt kärnbränsle. Fyra specifika mikrobiella processer, som är viktiga för förvarets funktion, identifierades när projektet påbörjades och ledde till vidare studier i separata projekt vid Microbe-laboratoriet: mikrobiella effekter på den kemiska stabiliteten hos förvarsmiljön, bio-mobilisering av radionuklider, bio-immobilisering av radionuklider samt mikrobiellt inducerad korrosion av koppar. Ett sammandrag av Microbe-laboratoriets utformning, både dess huvud- och satellitplatser i Äspö-tunneln och de viktigaste resultaten av Microbe-forskningsprogrammet presenteras i denna rapport. En stor del av resultaten och slutsatserna från Microbe-projektet har redan publicerats i internationella expertgranskade vetenskapliga tidskrifter. Denna rapport sammanfattar opublicerad information om de undersökta platserna, experimentresultat samt slutsatser som kompletterar de publicerade arbetena. Microbe-forskningsprogrammet är med denna rapport fullständigt avrapporterat. De viktigaste slutsatserna som kan dras från Microbe-projekt med avseende på förvarets säkerhet kan sammanfattas enligt följande: Den mikrobiella reduktionen av sulfat kan ske i maximal hastighet i närvaro av löst vätgas ända ner till en koncentration så låg som 1 μM. Virus som angriper bakterier (bakteriofager) utövar en starkt begränsande effekt på det totala antalet mikroorganismer i djupa grundvatten, vilket i sin tur styr hastigheten med vilken mikrobiella processer, till exempel sulfatreduktion till sulfid, sker. Mikrobiella biofilmer kan minska sorption av radionuklider till bergytor. Svälltrycket och vatteninnehållet i buffertmaterialet är starkt kopplade till såväl mikrobiell överlevnad som till mikrobiell aktivitet i dessa lermaterial.

1 Introduction

The study of microbial processes in the laboratory makes valuable contributions to our knowledge of microbial processes in repository environments. However, results and conclusions suggested by laboratory studies must be tested in a repository-like environment for several reasons. First, at repository depth, the hydrostatic pressure reaches nearly 50 bars, a level very difficult to reproduce in the microbiology laboratory. This high pressure will influence chemical equilibria and the content of dissolved gases. Second, the geochemical environment of deep groundwater, on which microbial life depends and in turn influences, is complex. Dissolved salts and trace elements, and particularly the redox chemistry and the carbonate system, are characteristics that are very difficult to mimic in a microbiology laboratory. Third, natural ecosystems, such as those in deep groundwater, comprise a large number of microbial species distributed over many different communities. The laboratory is best suited for examining pure cultures, so the effects of consortia of many participating species in natural ecosystems cannot easily be investigated there. The limitations of laboratory investigations described above prompted the construction and set-up of an underground laboratory in the Äspö Hard Rock Laboratory (HRL) tunnel. The site, denoted the Microbe laboratory, was situated at a depth of 450 m from 1999 to 2010, when the field programme was terminated and the laboratory was dismantled to make way for an extension of the tunnel in which the Microbe laboratory was situated.

Microorganisms interact with their surroundings and sometime greatly modify the characteristics of their environments. Several such interactions may significantly influence the function of a future deep repository for spent nuclear fuel (Pedersen 2002). Four specific microbial process areas of importance for proper repository functions were identified for study at the Microbe laboratory in separate projects, namely: Microbial effects on the chemical stability of deep groundwater environments, bio-mobilization of radionuclides, bio-immobilization of radionuclides, and microbially induced corrosion of copper. The Microbe laboratory main and satellite sites in the Äspö tunnel and the outcome of the Microbe research programme are presented in this report.

1.1 Objectives

The major objectives for the Microbe project were to investigate:

- the influence of microbial processes on the long-term chemical stability of the repository environment;
- microbial processes relevant to the bio-mobilization and bio-immobilization of radionuclides under *in situ* conditions; and
- microbial processes that may induce corrosion of copper under conditions relevant to a deep repository for spent nuclear fuel.

1.2 Activities and events

A brief list of main activities, corresponding activity plans, and references to reports and papers that present the results and achievements of the Microbe project is given here. The positions of boreholes and sites discussed in this report are shown in Figure 1-1.

1999 Drilling and instrumentation of the Microbe site (Pedersen 2000).

2000 Installation of metal-free packers in three boreholes, i.e. KJ0050F01, KJ0052F01, and KJ0052F03, and a container with laboratory equipment (Pedersen 2005a).

2001 Development and installation of a system for extraction and analysis of gas (AP-TD-F82-02-17) (Pedersen 2005a).

2002 Development and installation of circulation systems with flow cells (AP-TD-F82-02-13) (Hallbeck and Pedersen 2008, Pedersen 2005a).

Hydrogeochemical characterisation of the Microbe groundwater (AP-TD-F82-02-14) (Pedersen 2005a).

Microbial sulphide production in bentonite under *in situ* buffer conditions (Masurat et al. 2010).

2003 Immobilization of trace elements on microbially produced iron oxides (AP-TD-F82-02-15) (Anderson and Pedersen 2003).

2004 Immobilization of trace elements on microbially produced iron oxides (AP-TD-F82-02-15) (Anderson et al. 2006b).

Immobilization of trace elements on microbial biofilms (AP-TD-F82-02-16) (Anderson et al. 2006a).

2005 Significant shift in groundwater salinity at the Microbe site due to drilling of KA3386A (Pedersen 2005b).

Immobilization of trace elements on microbial biofilms (AP-TD-F82-02-16) (Anderson et al. 2007).

2006 The influence of microbial activity on the reducing capacity of aquifers in granitic rock (AP-TD-F82-02-18) (Pedersen 2012a).

Analysis of corrosion of copper in compacted bentonite (Pedersen 2010).

Microbial mobilisation of trace elements from surfaces and minerals (AP-TD-F82-02-019) (Johnsson et al. 2006).

Discovery of large diversity and numbers of phages in Äspö groundwater (Kyle et al. 2008).

Investigation of microbial nitrate-reducing activity in groundwater at 450 m depth (Nielsen et al. 2006).

2007 Microbial mobilization of trace elements from surfaces and minerals (AP-TD-F82-02-019) (Johnsson et al. 2008).

Isolation of a bacteriophage lytic to sulphate-reducing bacteria and mapping of the diversity and distribution of Desulfovibrio aespoeensis in Äspö groundwater (Eydal et al. 2009).

2008 Drilling five holes at KA1362A for biofilm analysis, i.e. AP-TD-F82P1-02-010, -034, -035, -039, -049, and -050 (Jägevall et al. 2011).

2009 The influence of hydrogen, acetate, and lactate on microbial sulphate-reducing activity in deep groundwater (AP-TD-F82-02-18) (Persson et al. 2011).

2010 The influence of microbial activity on the reducing capacity of aquifers in granitic rock (AP-TD-F82-02-18) (Pedersen 2012b).

2011 The Microbe site and borehole installations at a depth of 450 m were dismantled to make way for a new tunnel.

1.3 Aim of this report

The most important results and conclusions of the Microbe project have been published in technical reports and international peer-reviewed scientific journals, as listed above. This report summarizes unpublished information about the investigated sites, achievements, and experiment results related to the published work, thereby fully reporting the realization of the Microbe project.

1.4 Data storage

Data concerning the above activities and plans have been reported to Sicada.

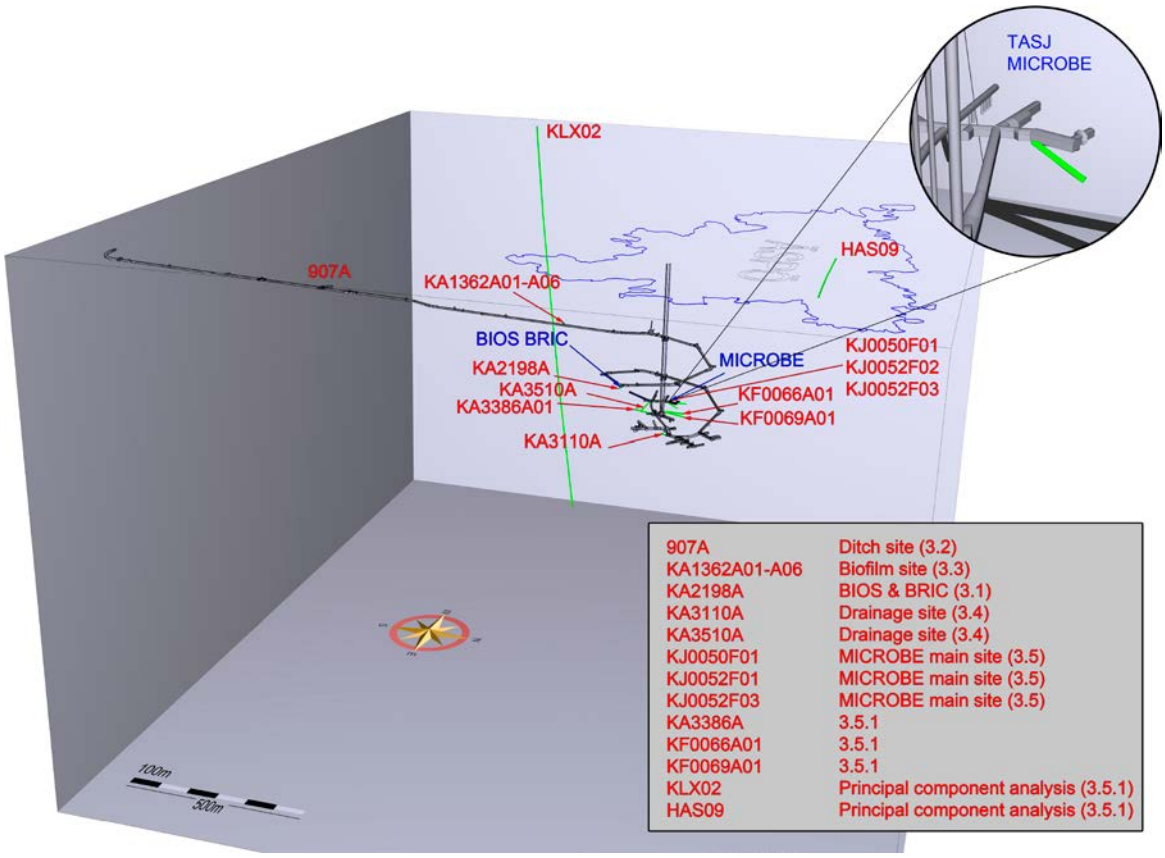

Figure 1-1. *The main sites and boreholes explored during the Microbe project. The sections of this report treating methods and results for particular sites and boreholes are specified in the text box.*

2 Description and characterization of the Microbe sites

2.1 The Bios site 2,200 m from the tunnel entrance

Organic surfaces and iron oxides have been identified as important factors in radionuclide transport modelling. Several microorganisms oxidize ferrous iron to ferric iron resulting in a mixture of organic material (i.e. microbes and their exudates) and iron oxides, here denoted biological iron oxides (Bios). Bios can be found everywhere along the Äspö HRL tunnel system. Bios are produced mainly by the stalk-forming bacterium *Gallionella ferruginea* (Hallbeck and Pedersen 2005) and related species. One particularly good tunnel site for investigations of trace element retention was identified 2,200 m from the tunnel entrance, on side A. At this point, a side vault reaches approximately 10 m into the host rock perpendicular to the tunnel; it had a borehole, core drilled on 15 November 1993, in its front end that delivered groundwater rich in ferrous iron and iron-oxidizing bacteria. The borehole has a diameter of 86 mm is 14.68 m long. It was used for rock tension measurements in 1993 and was thereafter abandoned. In 2001, it was chosen for the Microbe project and a packer was installed in its opening. The flow from this fully opened borehole was 0.8 L min^{-1} in June 2002; this flow rate dropped somewhat to approximately 0.6 L min^{-1} by June 2004.

The borehole was connected to two 2,000 × 300 × 250-mm artificial channels that mimic ditches in the tunnel (Figure 2-1). The channels have rock and artificial plastic supports that stimulate Bios formation (Figure 2-2). The retention of naturally occurring trace elements in the groundwater by the Bios was investigated.

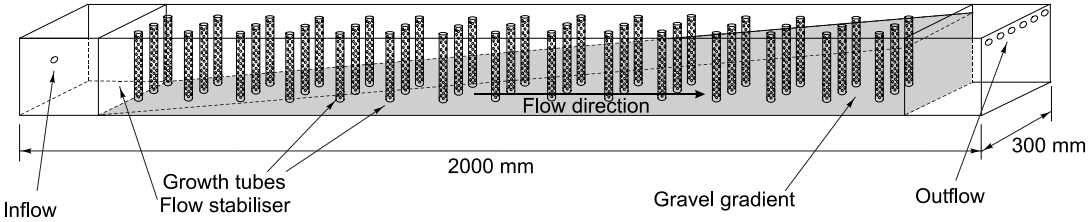

Figure 2-1. *Schematic of one of the two channels installed 297 m below sea level in the Äspö Hard Rock Laboratory tunnel site, 2,200 m from the tunnel entrance, for the investigation of trace element immobilization on Bios. The gravel gradient is indicated in grey.*

Figure 2-2. *Biofilms of the stalk-forming, iron-oxidizing bacteria that developed after five weeks on the plastic walls of the outlet channel chamber (300 × 250 mm) at the 2,200-m Bios site. The fluffy character of the Bios offers a huge surface area for trace element sorption. The material on the walls consists mainly of microbial cells, stalks, and metal oxides.*

2.1.1 Chemical characterization of KA2198A

The groundwater contained large amounts of ferrous iron and manganese relative to the small amount of sulphide (Table 2-1).

Table 2-1. Chemical composition of groundwater from KA2198A: main components.

Date	Na^+ mg L^{-1}	K^+ mg L^{-1}	Ca^{2+} mg L^{-1}	Mg^{2+} mg L^{-1}	HCO_3^- mg L^{-1}	Cl^- mg L^{-1}	SO_4^{2-} mg L^{-1}	S^{2-} mg L^{-1}
4 Feb 2002	1,430	39.8	186	137	219	2,780	280	–[a]
8 Nov 2006	1,530	42.3	269	148	202	3,050	337	0.023
	Br^- mg L^{-1}	F^- mg L^{-1}	Si^{4+} mg L^{-1}	Fe-ICP mg L^{-1}	Fe_{tot} mg L^{-1}	Fe^{2+} mg L^{-1}	Mn^{2+} mg L^{-1}	
4 Feb 2002	10.4	–	5.59	1.49	–	–	0.707	
8 Nov 2006	11.4	1.50	6.81	1.54	1.510	1.230	0.968	

[a] Not analysed.

2.1.2 Characterization of the Bios microbial community

The initial development and diversity of an *in situ* subsurface microbial community producing Bios was investigated at the initiation of biofilm growth, i.e. after two months, and after one year of undisturbed growth. Water chemistry data, samples of iron-encrusted biofilm material, and groundwater were collected from Bric (Bios reactor, *in situ*, continuous flow) apparatuses (Figure 2-1). Comparisons between the Bios Bric system and an anaerobic control (AC) Bric (Figure 2-3) revealed that water mixing at the inflow leads to profuse development of Bios related to a slightly elevated level of O_2 (up to 0.3 mg L^{-1} at the transition zone between Bios development and non-development) and elevated Eh (>120 mV) in the top 70 mm of water (Figure 2-4). Decreases in dissolved and particulate iron were connected to the visible appearance of Bios biofilms. The basic phylogenetic diversity of this site was evaluated using amplified ribosomal DNA restriction enzyme analysis (ARDRA), denaturing gradient gel electrophoresis (DGGE), and partial sequencing of 16S rDNA (Anderson et al. 2006b).

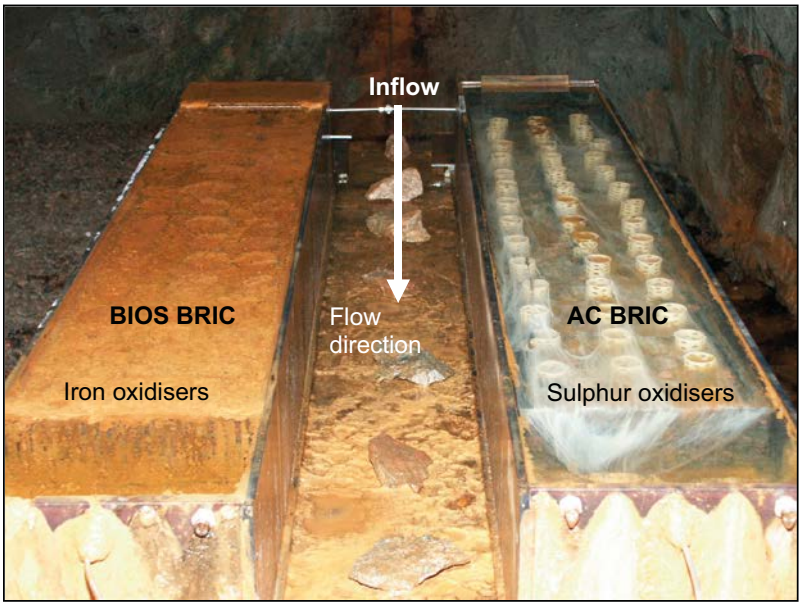

Figure 2-3. *The Bric apparatuses installed at the 2,200-m site. The Bric on the left is the Bios Bric and the Bric on the right is the AC Bric. The Brics are 2 m long, 300 mm wide, and 250 mm deep (see Figure 2-1) and are supplied with groundwater from a borehole that intersects a water-conducting fracture behind the rock face via borehole KA2198A. Note the prolific growth of sulphur-oxidizing bacteria in the AC Bric and the masses of iron oxide-stained biomass in the Bios Bric. This image shows 8 months of biofilm development (i.e. 4 months before sampling for phylogenetic analysis).*

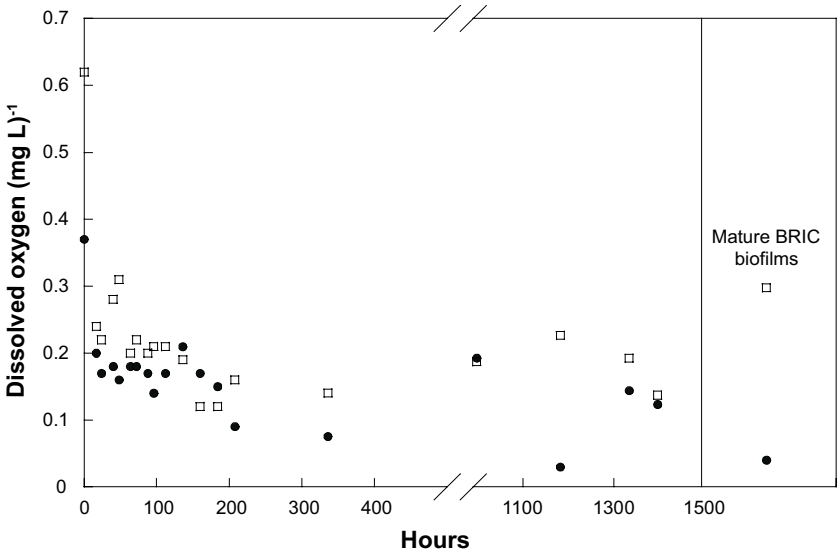

Figure 2-4. *Dissolved oxygen decline at a depth of 70 mm over 2,000 hours (three months) for the Bios Bric (□) and the AC Bric (●). Mature biofilms sampled after 8,700 hours (one year) indicate that the decrease in oxygen is preserved in the AC Bric but that oxygen levels can increase in the Bios Bric.*

From 67 clones that were positive for 16S rDNA inserts, a total of 42 ARDRA profiles were recognized, representing four bacterial phyla and 14 metabolic lifestyles. DGGE profiles indicated differences in the representative bacteria when considering either Bios biofilms or groundwater. DGGE profiles also indicated that the DNA extraction protocols and any PCR biases were consistent. Bacterial metabolic groups associated with indirect metal adsorption and reduction along with bacteria utilizing many alternative electron acceptors were strongly represented in the clones. The study indicated that the microbial diversity of Bios was large. The importance of understanding Bios composition and growth dynamics has increased because of the radionuclide sorbing capacity of Bios. The Bios in the Äspö HRL tunnel have radiation levels above the background level, due to their accumulation of naturally occurring radionuclides in the groundwater that seeps into the tunnel.

2.2 The 907-m ditch site

At 907 m from the tunnel entrance, on side A, a small vault with a rescue chamber supports a ditch containing groundwater rich in ferrous oxides and iron-oxidizing bacteria (Figure 2-5). This ditch was used as a natural analogue to the artificial Bios channels at 2,200 m. Growth characteristics of the Bios were compared between the two sites.

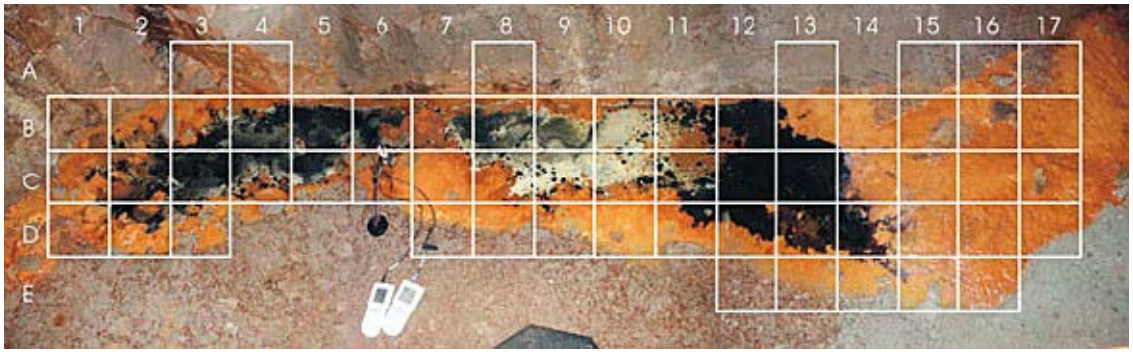

Figure 2-5. *The 907-m ditch site is characterized by a shallow, elongated pond that overflows into the main tunnel ditch to the right of the image. The brown material is Bios. The black colour is from iron sulphide precipitates and the white material is calcium carbonate. The overlaid grid created an organized environment and enabled pH, oxygen, and redox measurements in three dimensions. Field instruments are shown at the bottom of the figure. Grid size is approximately 250 × 250 mm.*

2.3 Drill site for analysis of biofilms on fracture surfaces

In spring 2008, a total of 13 potential drill sites for the analysis of biofilms in aquifers were located along the tunnel 170–2,600 m from the tunnel entrance at points where the tunnel intersected water-conducting fractures. The final selected site was situated at the end of a 4-m-wide, 16.3-m-long side tunnel (relative to the centre line of the 5-m-wide main tunnel) on the left side of the main tunnel (facing downwards), 1,362 m from the entrance and 186 m underground. At this site, drilling was considered safe, fulfilling the regulatory requirements for drilling and working safety. Quantifying and sequencing the collected and extracted DNA from the tunnel fractures indicated abundant microbial populations of high diversity in this area of the tunnel. Finally, the chance to sample multiple fracture surfaces exposed to flowing water was considered very good.

The inner wall of the vault was found to constitute a main vertical fracture surface that ran parallel to the tunnel and perpendicular to the side tunnel. Drilling was done horizontally 2 m above the tunnel floor with five drill holes at an angle of approximately 45° relative to the side tunnel in a folding fan shape to reach the main fracture from different directions from the side tunnel face (Figure 2-6). Drill cores were obtained in approximately 100-cm sections, and the outflow of groundwater was registered after the retrieval of each core. The total lengths of the five drill holes were 90, 220, 200, 373, and 467 cm and the drill cores were denoted KA1362A-2, -3, -4, -5, and -6, respectively. Structures in each core were identified as possibly water-conducting fractures based on appearance and increase in water flow, and were sampled for biofilm analysis.

2.4 Effect of borehole drainage on microbial numbers

A relationship between the drainage volume and the biomass in groundwater was noted during experiments at the Microbe site. This effect was studied in more detail in three boreholes (Figure 2-7). The packer configuration differed, with a low packed-off volume of 10 mL in KJ0052F01 (Figure 2-9) and large volumes >5 L in KA3110A and KA3510A. The biomass numbers decreased by approximately one order of magnitude in the large-volume boreholes, while the amount of biomass in the KJ0052F01 did not change over the drained volume. These results indicated that the amount of biomass generally decreases with increasing drained volume.

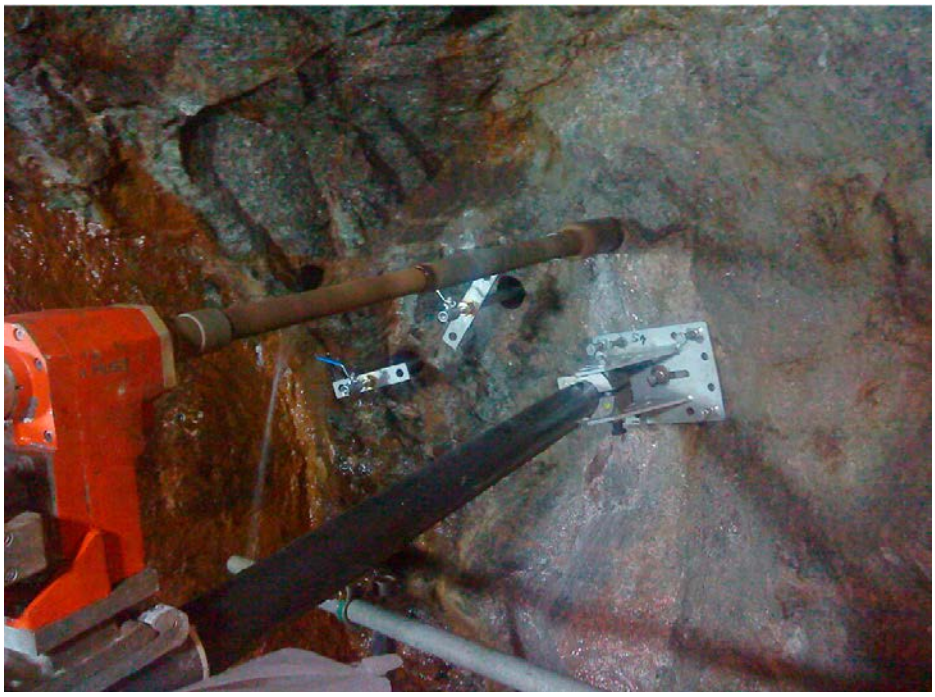

Figure 2-6. *Drilling of KA1362A-4.*

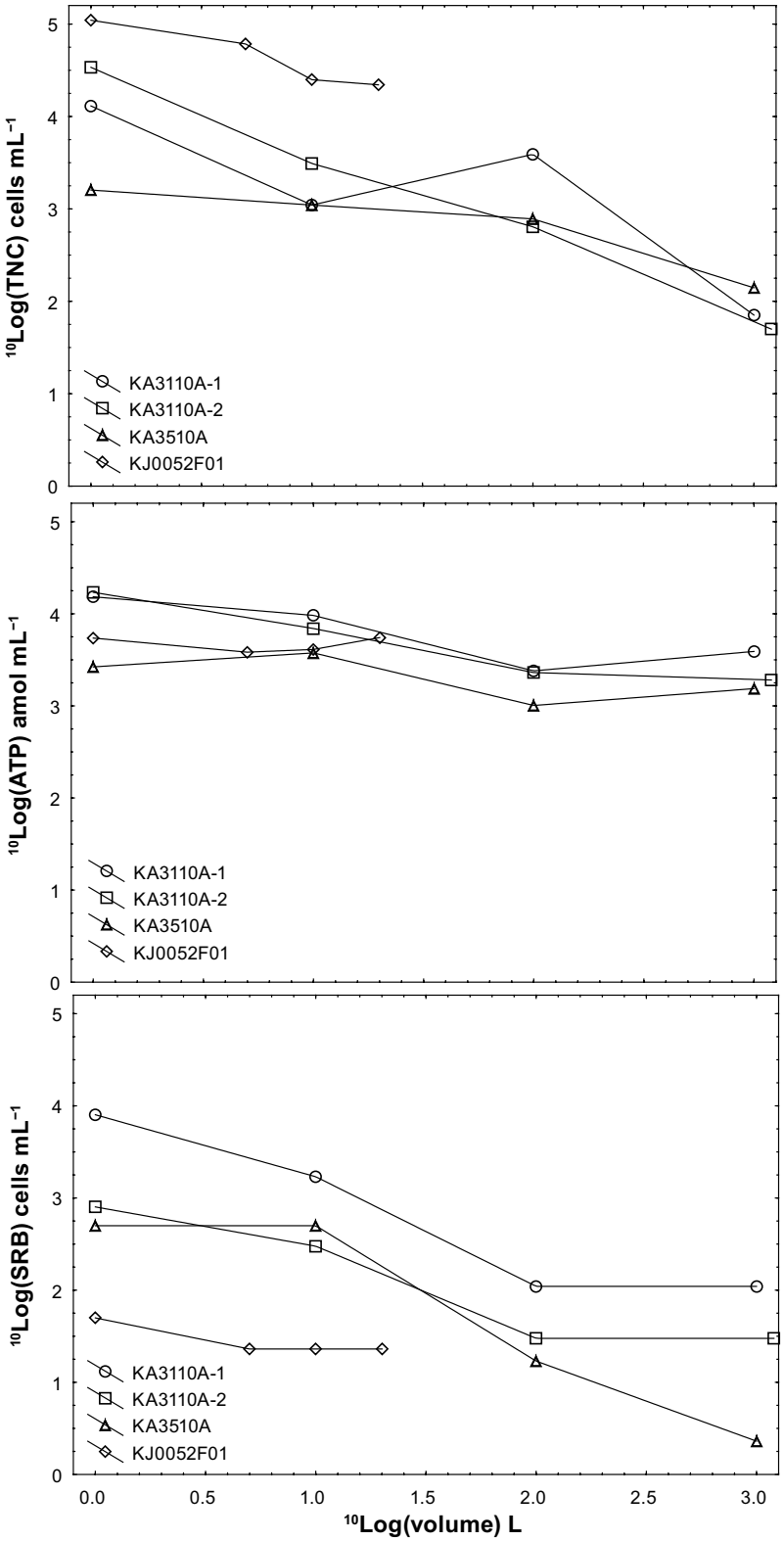

Figure 2-7. *The effect of the drainage volume of three boreholes on the total number of cells (TNC), ATP concentration, and the most probable number of sulphate-reducing bacteria (SRB).*

2.5 The Microbe site at a depth of 450 m in the F tunnel

Triple-tube drilling was used in drilling KJ0050F01, KJ0052F01, and KJ0052F03 in May 1999 (Figure 2-8). KJ0050F01 reached a length of approximately 50 m and had a diameter of 76 mm. One water-conducting feature was found 12.7 m into the hole; it had a flow of 0.4 L min^{-1} and the formation pressure was 2.6 MPa as of December 2004. KJ0052F01 reached a length of approximately 50 m and had a diameter of 76 mm. One water-conducting feature was found 43.8 m into the hole; it had a flow of 0.9 L min^{-1} and the formation pressure was 3.15 MPa as of December 2004. KJ0052F03 reached a length of approximately 10 m and had a diameter of 76 mm. One water-conducting feature was found 9.3 m into the hole; it had a flow of 1.5 L min^{-1} and the formation pressure was 2.6 MPa as of December 2004. Details can be found elsewhere (Pedersen 2000).

The water-conducting feature in each of the three Microbe boreholes was sectioned off with a double packer with a sealing length of 1,000 mm. The material chosen for the hard body parts was PEEK. Polyurethane rubber (Shore 90) with a thickness of 6 mm was used for the expansion part. Teflon-coated stainless steel casings (green) were used to attach the rubber to the PEEK bodies (Figure 2-9). A dummy body was installed between the packers to minimize the void borehole volume; it had an outer diameter of 73 mm, leaving a 1.5-mm slot between the borehole wall and the dummy. Two PEEK tubes (outer dimension 1/8", inner dimension 2 mm) were installed so that water could be circulated through the packed-off section. The sections inside and outside the packers were connected with polyamide tubing to manometers and valves on the outside. Guard packers and expansion systems for the packers were also installed.

A steel container measuring 6.0 × 2.5 × 2.7 m (length, width, height) was installed in the F tunnel at the 450-m level (Figure 2-8). The lab environment was kept stable by a climate control system that maintained the temperature at the chosen level, 22°C ± 0.5°C, and the humidity at 40‰ ± 5‰. The container was equipped with laboratory benches and instrumentation for microbiological research. A total of six flow cell circulation (FCC) systems were installed in the container, three of which utilized PEEK tubing (Figure 2-10). Three more FCCs were later constructed using stainless steel tubing (Figure 2-11). An on-line system for measuring E_h was installed in 2006 (Figure 2-12). The instrumentation is described in detail elsewhere (Pedersen 2005a, 2012a).

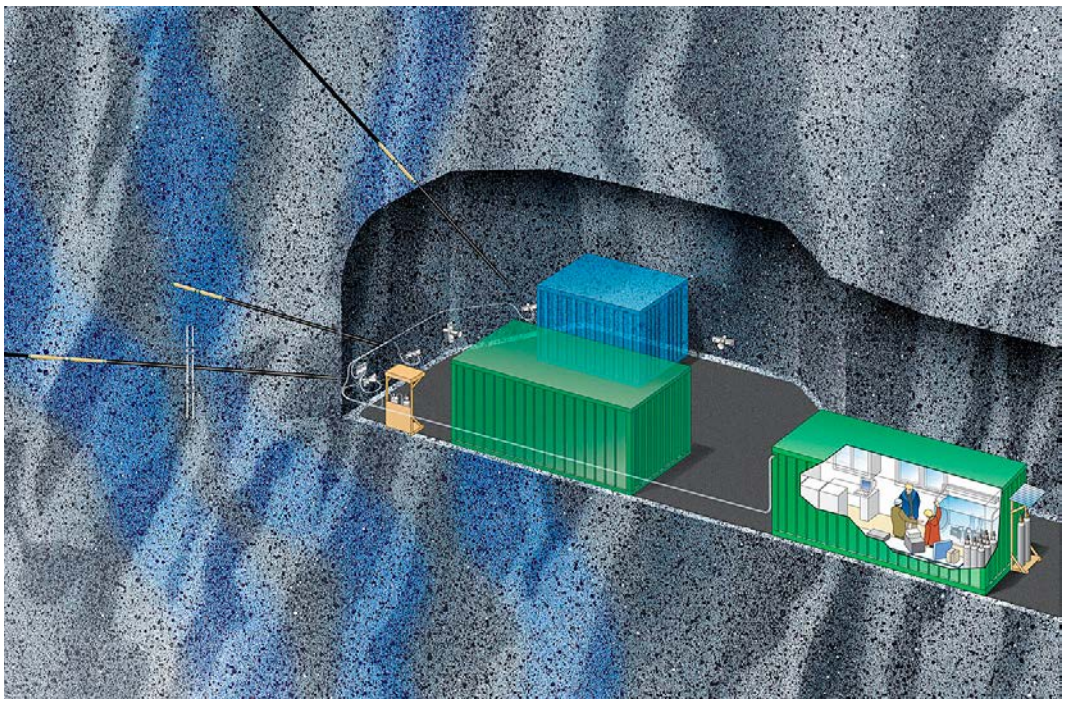

Figure 2-8. *An artist's view of the Microbe 450-m site and the metal-free packer configuration. The laboratory (on the right) was situated in a steel container and connected to three discrete fractures in the rock matrix. PEEK tubing connected the systems in the lab with the groundwater (see text for details). The drawing shows boreholes KJ0052F01, KJ0052F03, and KJ0050F01 (left to right).*

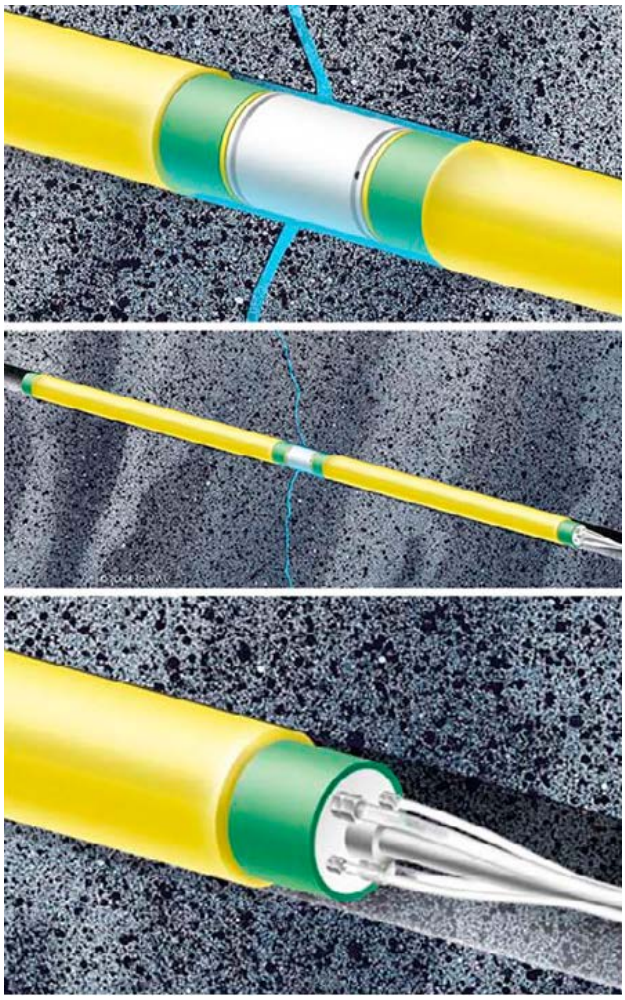

Figure 2-9. *The packer system. The yellow sections are expandable polyurethane packers, while the green rings are Teflon-coated stainless steel casings. The grey components are made of PEEK, as are the 1/8-inch (outer diameter) sampling tubes. Groundwater sampling and circulation are mediated via two small holes opposite each other in the grey portion in the middle of the packer assembly (the dark spot in the top drawing). The depicted section is 100 mm long.*

Figure 2-10. *The microbiology laboratory environment at the 450-m site was originally equipped with three circulation systems with PEEK tubing (right), an anaerobic box (centre-left), an in situ gas extractor, and a Kappa 5 gas chromatograph (right of the anaerobic box).*

Figure 2-11. *Three new circulation systems with stainless steel tubing were added to the Microbe laboratory in 2006.*

Figure 2-12. *Two pairs of pressure-resistant redox electrodes installed on-line on the circulating groundwater in one of the stainless steel circulation systems.*

2.5.1 Evolution of chemical composition of the groundwater at the Microbe site

The Microbe research programme's constituent projects were run over a long time and required stable groundwater conditions. The placement of the Microbe laboratory in the F tunnel of the Äspö HRL assured such conditions until 2002. However, the drilling of boreholes KF0066A01 and KF0069A01 in the search for a proper location for the Äspö Pillar Stability Experiment (APSE) in May–June 2002 changed this situation. The long-term trends of chloride and sulphate concentrations in the Microbe boreholes are shown in Figure 2-13. There was a slow increase in these concentrations from 1999 to 2004 in KJ0050F01 and KJ0052F01 since drilling these boreholes. The data are sparse and it is impossible to evaluate whether this increase was continuous or more transitory in nature.

There is a possibility that the drilling of KF0066A01 and KF0069A01 early in 2003 may have short-circuited the Microbe boreholes. The chloride concentrations measured in August 2003 in KF0066A01 and KF0069A01 were 11,652 and 10,809 mg L^{-1}, respectively. Consequently, an accelerating up-coning effect was observed. A third APSE borehole was drilled at NASA 3384, denoted KA3386A01. This borehole intersects the formation from which the Microbe boreholes got their groundwater. Large-diameter boreholes were drilled in January 2005 at the 450-m level for the In Situ Corrosion Testing of Miniature Canisters (MiniCan). This drilling intersected shallow fractures near the tunnel wall that short-circuited KA3386A01 with the MiniCan boreholes. A significant drainage of groundwater started from the Microbe formation. Over less than a day in January, pressure drops of up to 300 KPa were registered in the Microbe boreholes.

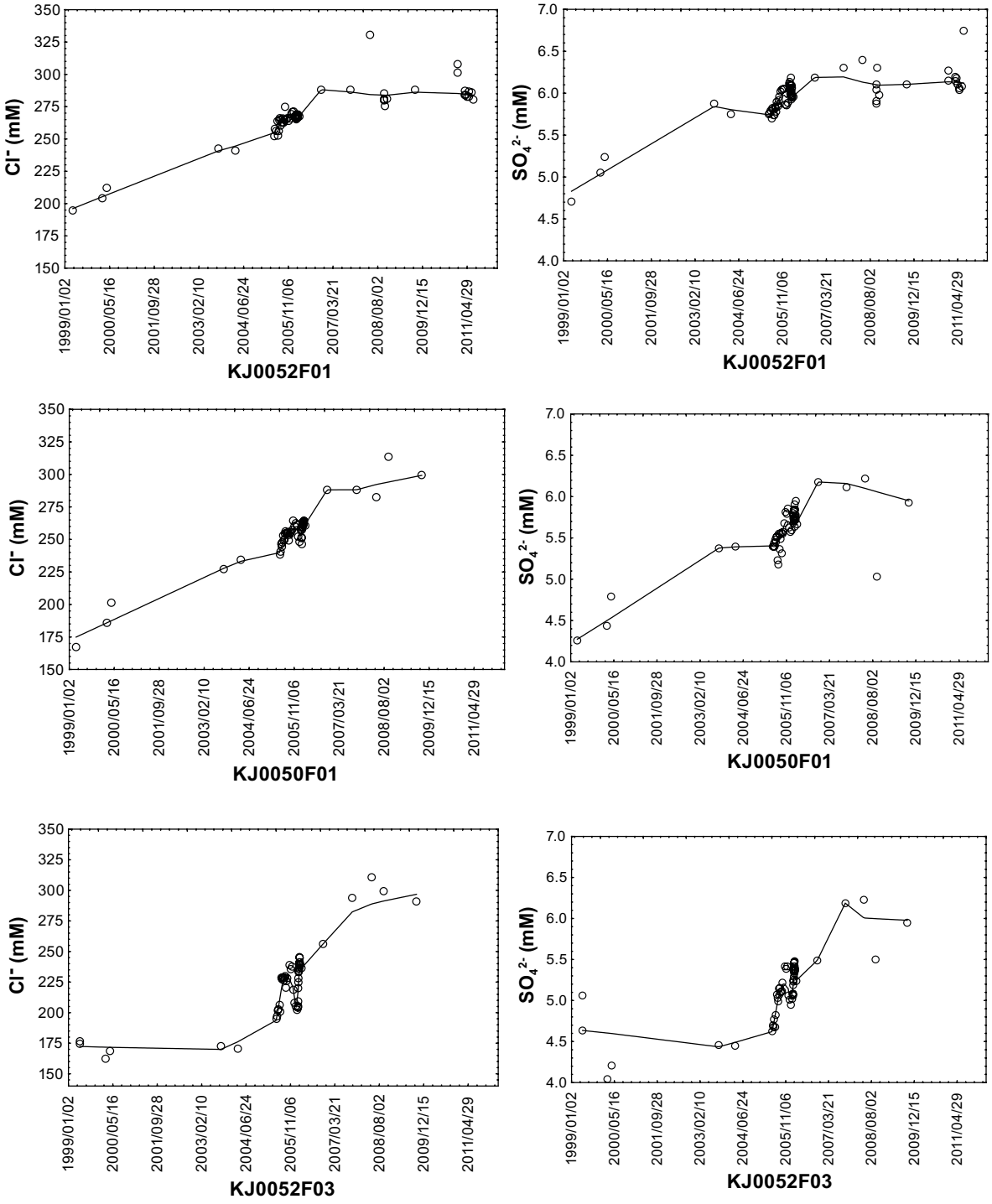

Figure 2-13. *Evolution of dissolved chloride and sulphate in the three boreholes at the Microbe site.*

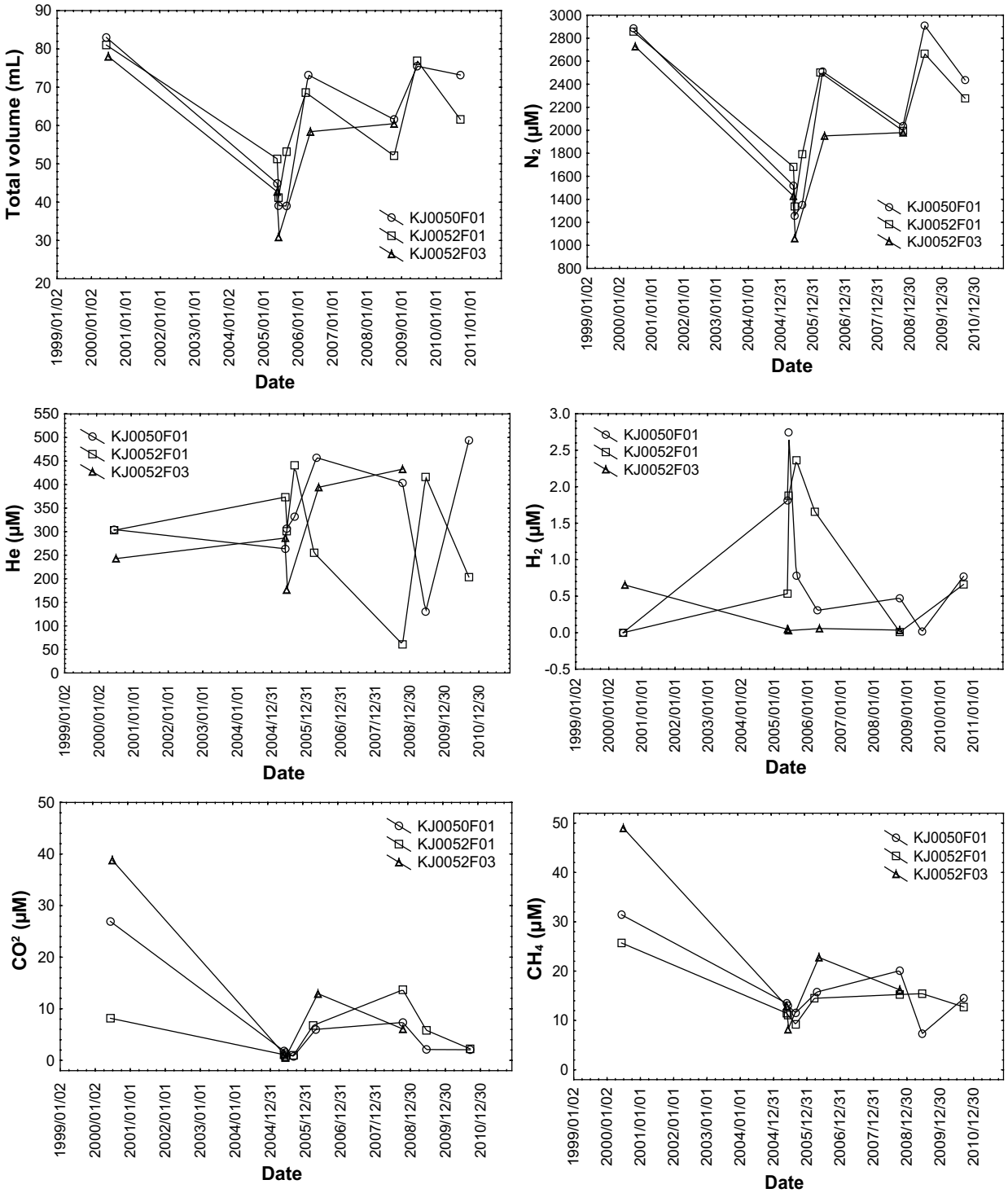

Figure 2-14. *Evolution of gases in the three boreholes at the Microbe site.*

Between January and November 2005, more than 15,000 m^3 of groundwater was drained from KA3386A01. The outflow was 0.8 L s^{-1} from January to the end of May, when this borehole was closed; after closure, the outflow stabilized at 0.5 L s^{-1}. The inflow to the tunnel increased by approximately 30% from 3,179 m from the tunnel entrance and downwards due to the drilling operations at the MiniCan site. This is a very significant drainage. As there was a large pressure drop in the Microbe boreholes that corresponded to the offset of this drainage, it can be concluded that the Microbe groundwater formation experienced strong drainage from January to November 2005. A completely new mixing and groundwater situation developed in 2005, as implied by principal component analysis (PCA) (Figure 2-15), indicating that the MiniCan disturbance was very strong

for KJ0052F03 and for KJ0050F01 and KJ0052F01. The KA3386A01 borehole was equipped with packers in December 2005 that blocked the drainage and stopped the increase in salinity. Further details can be found elsewhere (Pedersen 2005b).

The MiniCan drilling activities significantly changed the amount of dissolved gases from that observed directly after drilling (Figure 2-14). The amount of total gas decreased in 2005 when KA3386A01 was flooding and increased again when the outflow was stopped. Nitrogen was the main gas in all three boreholes, followed by helium. There were increased traces of hydrogen and of carbon dioxide and methane during the drainage event. The analytical procedures for the gases are described elsewhere (Pedersen et al. 2008).

Principal component analysis (PCA)

The evolution of groundwater is generally strongly related to present and past flow conditions. Throughout this continuous process, the type of infiltrating water as well as the water existing in the rock change in composition. The solute and isotopic content of groundwater can be interpreted as resulting from geochemical reactions between the groundwater and the minerals it contacts, from the mixing of groundwater types of different origins (and hence different chemical signatures), or from a combination of both processes. Software tools to address these topics are under development in the international waste management community. One such tool is the multivariate mixing and mass balance (M3) model developed by SKB (Laaksoharju et al. 1999). M3 is an interpretative technique for performing cluster analysis (using multivariate principal component analysis) in order to simplify and summarize the obtained groundwater data, identify waters of different origins, infer the mixing ratio of these mixing reference waters (end-members) to reproduce each sample's chemistry, identify any deviation between the chemical measurements of each sample and the theoretical chemistry from the mixing calculation, and interpret these deviations as resulting from groundwater reactions. The constituents used for the modelling are major components (i.e. Na, K, Ca, Mg, HCO_3, Cl, and SO_4) and stable isotopes (i.e. δ^2H and $\delta^{18}O$). These components are known to contain most of the information variability in groundwater data. The boreholes analysed were KJ0050F01, KJ0052F01, and KJ0052F03.

The selected end-members identified from the PCA (Figure 2-15) for the current modelling are as follows:

Deep saline water, which represents the brine type of water found in KLX02 at a depth of 1,631–1,681 m, with a measured Cl content of 47,200 mg L^{-1}; brines are waters with significantly higher salinity than ocean water (Cl content of 19,800 mg L^{-1});

Baltic Sea water, which represents modern Baltic Sea water in the bay around Äspö Island;

Littorina Sea water, which represents the past Baltic sea water composition during the salinity maximum recorded during the Littorina Sea Stage (8000 BP);

Altered meteoric water, which represents dilute shallow groundwater of the type found in the HAS09 borehole (Äspö Island) from a depth of 0–40 meters above sea level; and

Glacial water, which is a precipitation water in which the stable isotope values ($\delta^{18}O$ = –21 SMOW and δ^2H = –158 SMOW) are based on the values of $\delta^{18}O$ measured in surface calcite deposits, interpreted as sub-glacial precipitates, collected from various geological formations on the west coast of Sweden; the water represents a possible meltwater from the last glaciation >13,000 years BP.

Principal component analysis results

The PCA results compare the geochemical composition in April 2000 with that in September 2003 and in January and November 2006. The Microbe groundwater samples lie in the central area of the PCA plot shown in Figure 2-15. This indicates that these waters do not have an extreme groundwater composition, but are instead affected by the mixing of several reference waters. The plot shows that all three Microbe groundwater samples have moved towards a deeper brine signature. KJ0050F01 displays the largest move and has approached the position of KJ0052F01, suggesting that deep groundwater is up-coning towards the Microbe sites.

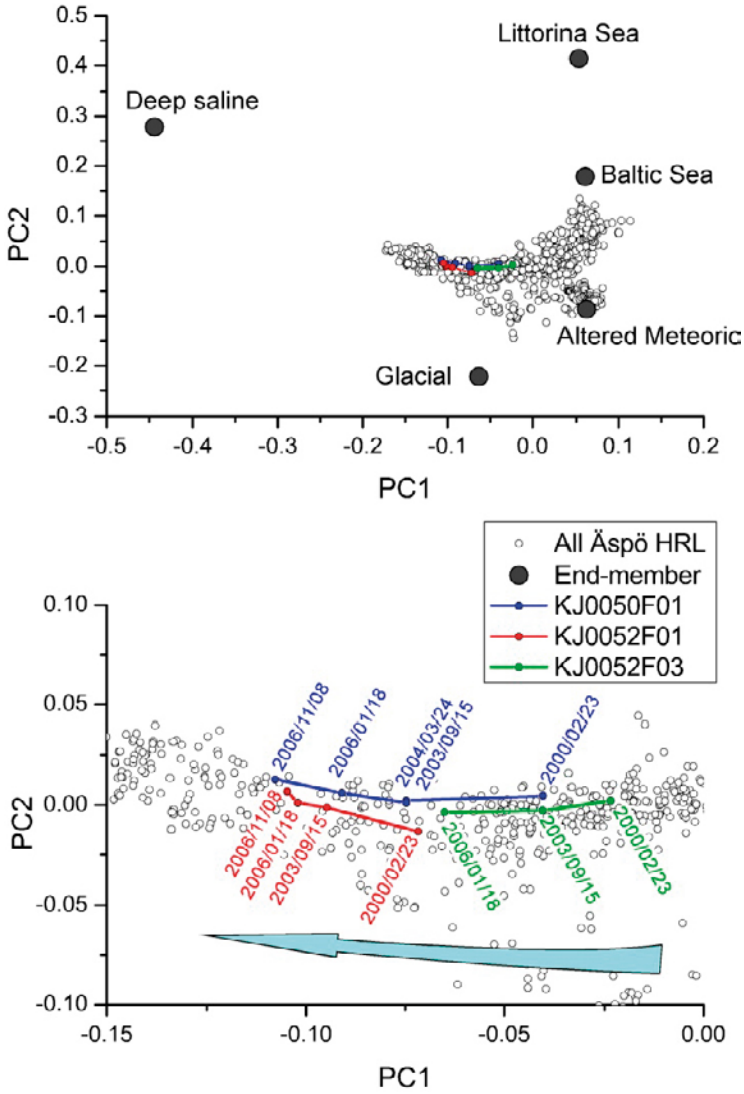

Figure 2-15. *Principal component plot showing groundwater chemical data from the Microbe 450-m site in comparison with data from the entire Äspö HRL and the end-members (upper graph); the sampling dates are specified in the bottom graph, where the scale of the principal components has been increased.*

2.5.2 Evolution of the numbers of microorganisms in groundwater at the Microbe site boreholes

The analytical protocols for microorganisms can be found elsewhere (Hallbeck and Pedersen 2008). In 2005, most numbers decreased to varying degrees due to the KA3386A01 draining event described above for groundwater chemistry (Figure 2-16 and Figure 2-17). The numbers eventually recovered to values similar to those observed before the draining event.

The total number of cells (TNC) was generally highest in KJ0052F01 followed by KJ0050F01 and KJ0052F03 water, except in 2005 when the draining event caused changes in all numbers. This between-borehole trend was valid for many of the cultivable physiological groups of microorganisms. The numbers of nitrate- and sulphate-reducing bacteria (NRB and SRB, respectively), acetogens, and methanogens were highest in KJ0052F01 water on most sampling occasions over the six years of measurement.

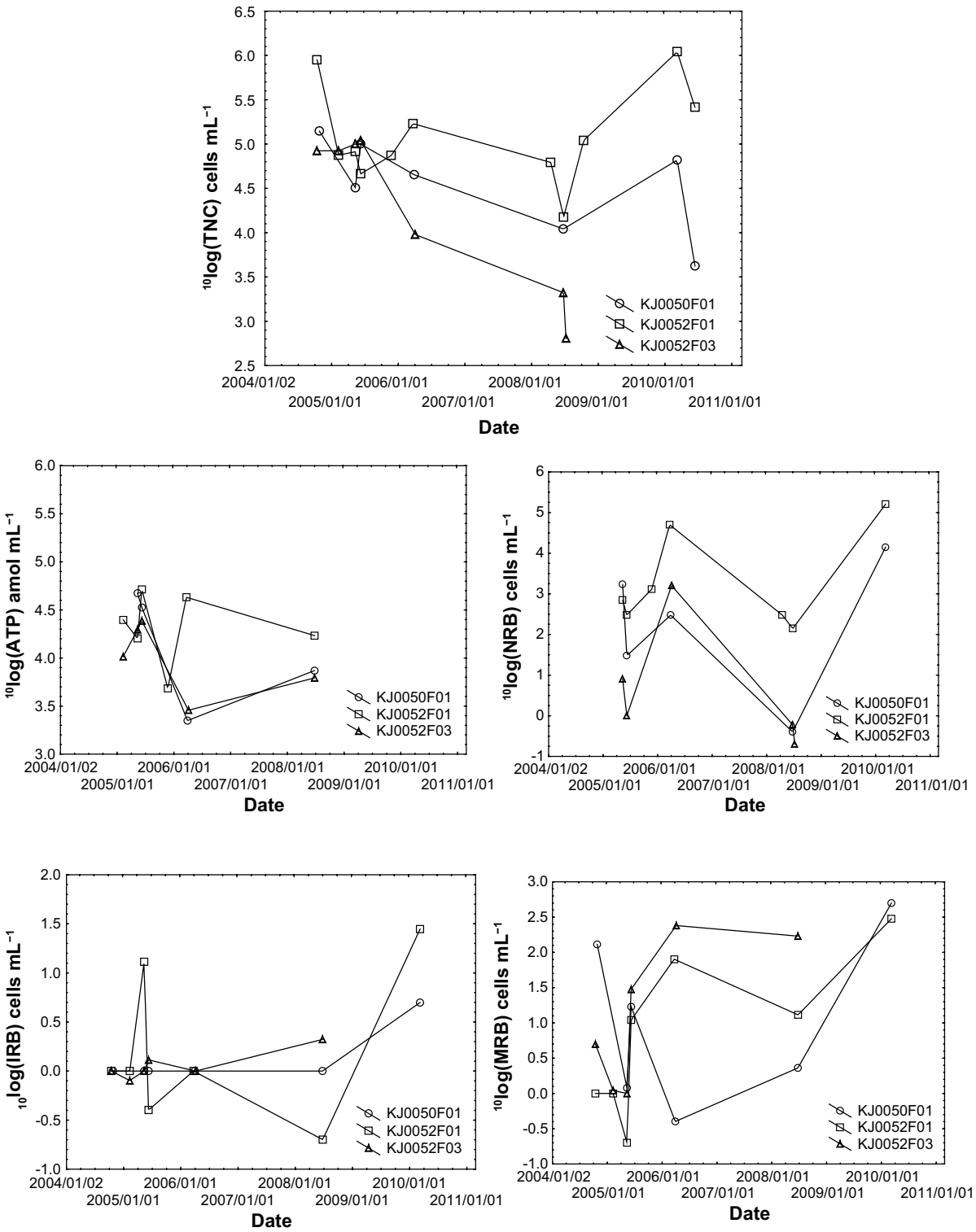

Figure 2-16. *Evolution of total cell numbers, ATP, and cultivable microorganisms in the three boreholes at the Microbe site.*

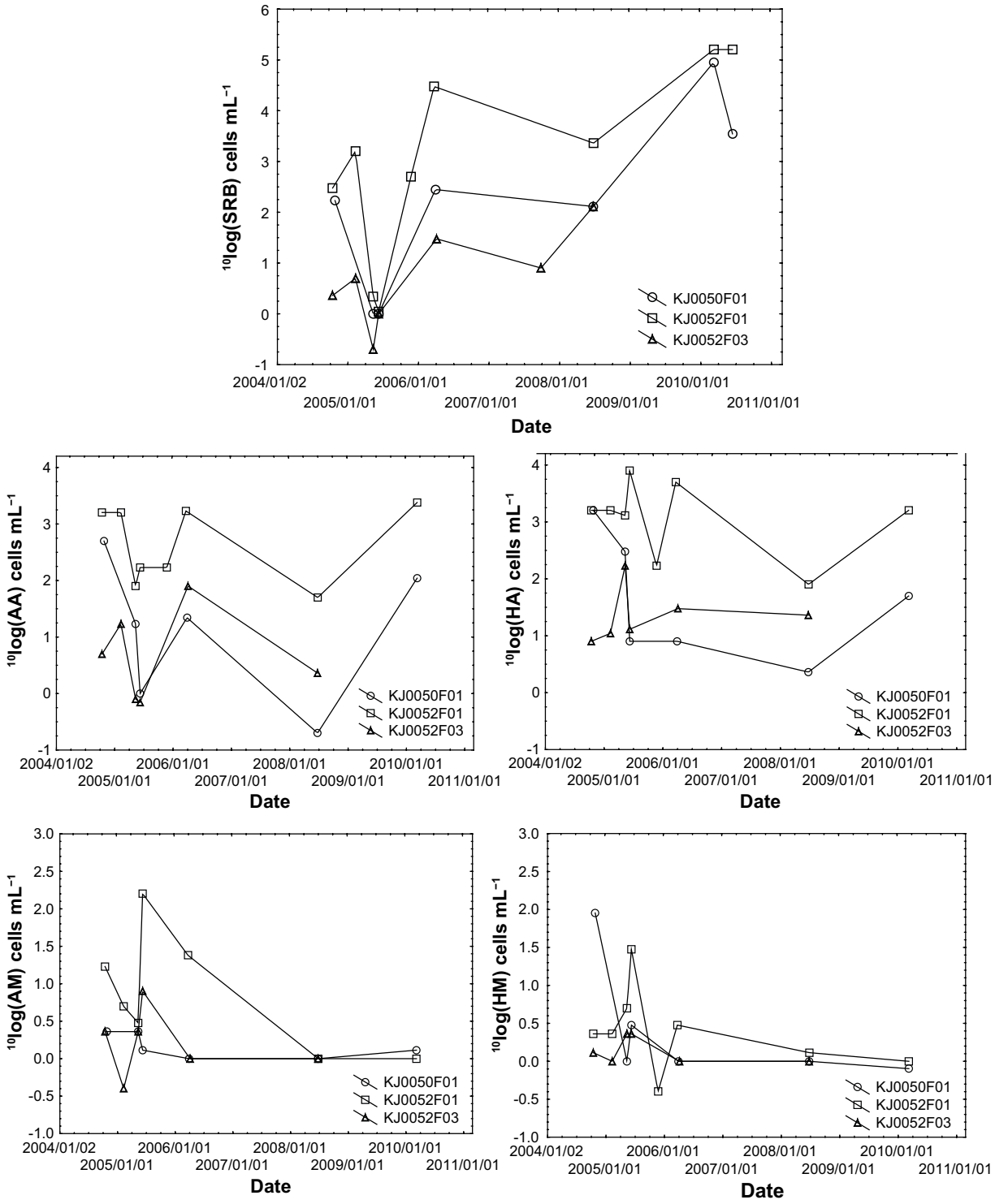

Figure 2-17. *Evolution of cultivable microorganisms in the three boreholes at the Microbe site.*

2.5.3 Evolution of the concentration of sulphide in groundwater at the Microbe site boreholes

Due to the corrosive nature of sulphide, microbial reduction of the sulphur in sulphate to sulphide has been a concern in radioactive waste disposal for decades. Sulphide was analysed in groundwater from the three Microbe boreholes over the whole project period of more than 10 years. For most of this time, sulphide concentrations were low, or below the limit of detection. However, soon after the event when KA3386A01 drained the Microbe groundwater (3.5.1), there was a significant increase

in the sulphide concentration in groundwater from all three boreholes (Figure 2-18), the increase being largest in KJ0052F01 and smallest in KJ0052F03. Relatively soon after the drainage was stopped, the sulphide concentrations dropped back to low values or values below detection.

The increase in sulphide concentration followed an increase in the hydrogen concentration (Figure 2-18) and was highest in KJ0052F01, where the concentration of hydrogen also was the highest. During the drainage period, the numbers of SRB decreased and were at or below the limit of detection (0.2 cells mL^{-1}); however, soon after the drainage was stopped, SRB increased rapidly to numbers that equalled or exceeded those observed before the drainage. These results are in line with results indicating that SRB numbers and, in particular, their sulphide-producing activity are boosted by hydrogen (Pedersen 2012b).

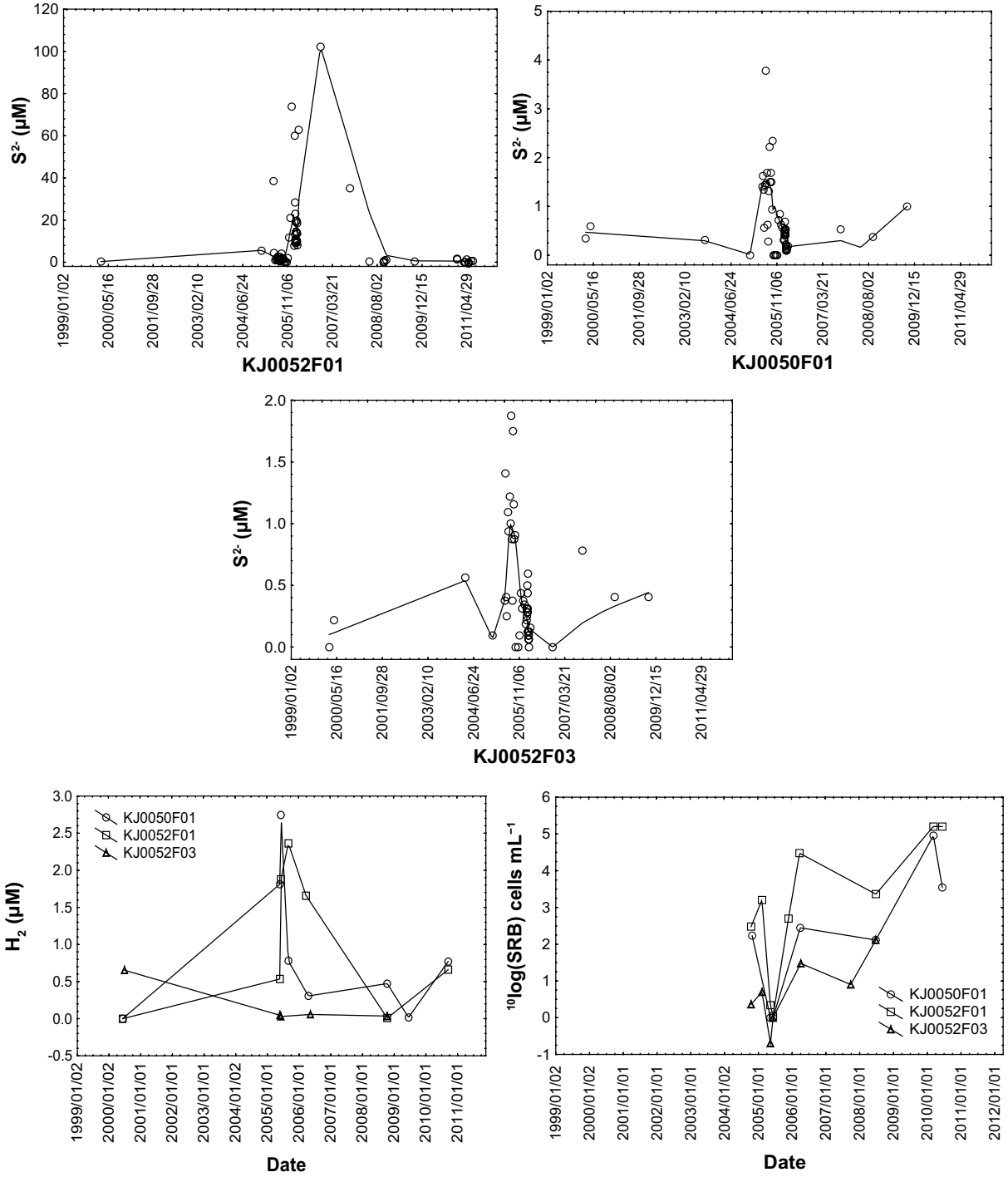

Figure 2-18. *The concentrations of sulphide and hydrogen in the three Microbe boreholes and the most probable numbers of cultivable sulphate-reducing bacteria over 12 years.*

The drainage caused an up-coning of deeper groundwater, as indicated by the PCA analysis (Figure 2-15). Hydrogen is known to increase in concentration with depth in groundwater; therefore, the hydrogen concentration could be expected to increase in the Microbe groundwater due to the inflow of deeper, more saline groundwater. However, when the drainage was stopped, hydrogen was not replenished and the concentration decreased again, likely due to its consumption by SRB.

Taken together, the drainage incident became a good learning experience that demonstrated that the flow and mixing of different groundwater end-members can significantly affect microbial abundance and activity in deep groundwater. Not only did the drainage event influence microbial sulphate-reducing activity and the numbers of SRB, but most of the analytical results for biomass and microorganism numbers were significantly influenced by the drainage as well (Figure 2-16 and Figure 2-17). The drainage had a generally negative effect on the microbial numbers that, with few exceptions, decreased significantly. Similarly, microbial numbers were found to decrease during borehole drainage (Figure 2-7). Consequently, if aquifers are drained before sampling for microbial analysis, the results will likely underestimate the *in situ* numbers. This conclusion agrees with the fact that microorganisms often attach and form biofilms in flowing water. Prolonged drainage will force microorganisms to attach, thereby lowering the observed numbers in the sampled groundwater, relative to the numbers present before the drainage or pumping commenced.

3 Experiments with circulation systems

A total of 23 experimental configurations were investigated with the circulation systems (Figure 2-11) during the research programme; each configuration is presented in Table 3-1. Nine of the experiments and their results have been published as original scientific work, and three of the experiments and their results have been reported in SKB R publications (Persson et al. 2011). The remaining eleven experiments and results were previously unpublished. Here, the focus is on the unpublished and unreported experiments and results. Detailed descriptions of the experimental configurations can be found in the published literature listed in Table 3-1 and elsewhere (Pedersen 2005a, Hallbeck and Pedersen 2008).

3.1 Effect of H_2 and lactate: experiments 1–3

These results have previously been reported briefly (Persson et al. 2011). The experiments were performed in the laboratory with groundwater from KA3110A in the Äspö HRL tunnel. The groundwater was collected and transported under pressure in 4-L expansion vessels as described elsewhere (Pedersen 2013). These experiments were intended mainly to test and develop methods. We needed to test and develop sampling methodology and analytical protocols. The first system (Experiment 1, Table 3-1) was kept as an untreated control, and the analysed parameters did not change significantly over the experimental time of 48 days (Figure 3-1). In the second system (Experiment 2, Table 3-1), hydrogen was added, which induced some sulphate reduction to sulphide. The concomitant small increase in sulphate may be due to the dissolution of gypsum in the bentonite that was connected to these circulations as described elsewhere (Persson et al. 2011). In the third system (Experiment 3, Table 3-1), lactate was added, which was mainly oxidized to acetate and carbon dioxide by SRB with a concomitant reduction of sulphate to sulphide (Figure 3-1). The TNC increased almost one order of magnitude and the pH rose from 7.2 to 9, likely due to proton consumption by the sulphate-reduction process with H_2 ($4\ H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4\ H_2O$). In summary, the methods and the protocols worked as expected and were implemented in the following experiments.

3.2 Effect of H_2 and acetate: experiments 4–6

These experiments have been published (Pedersen 2012a). Briefly described, three parallel-flow cell cabinets with PEEK tubing were configured to allow observation of the effect on microbial metabolic activity of adding 3 mM hydrogen or 2.4 mM acetate, compared with an untreated control. Hydrogen addition reduced the generation time four-fold to two weeks, doubled the sulphide production rate, and increased acetate production by approximately 50%. The acetate addition induced acetate consumption. The studied microbial processes appeared to proceed very slowly in terms of volume and time, though the results suggest that individual cells could be very active. Lytic bacteriophages were hypothesized to have caused this contradictive observation. Phages may consequently significantly reduce the rates of subterranean microbial processes. Furthermore, the results suggested that hydrogen from corroding underground constructions could induce significant local microbial activity, and that the low concentrations of hydrogen often observed in pristine subterranean environments may support slow but sustainable microbial activity in deep groundwater.

3.3 Effect of H_2 and acetate: experiments 7–11

PEEK tubing was originally chosen for the circulation systems to avoid any metals that could interfere with the delicate radionuclide experiments that were initially performed (Anderson et al. 2006a, 2007). During experiments 1–6, gases were observed to diffuse through the PEEK tubing used; therefore, three new circulation systems were constructed with stainless steel tubing instead of PEEK. The Microbe site had three boreholes and it was important to evaluate the usefulness of groundwater from these boreholes, because at the start of the Microbe project, the chemistry and

microbiology differed greatly between them (Figure 2-15, Figure 2-16, and Figure 2-17). KJ0052F03 was found to communicate readily with another borehole, KJ0052F02, which was drilled for the CHEMLAB experiments, and was therefore abandoned for circulation experiments.

The results of experiments 10 and 11 indicated that hydrogen alone did trigger a significant reduction of sulphate to sulphide, both with and without the addition of acetate. Again, just as in experiment 3, pH increased, probably as a result of the proton-consuming sulphate-reduction process. The results of experiments 7–11 also demonstrated the stainless steel configuration to be superior to the PEEK systems. Helium, methane, and hydrogen concentrations decreased much more slowly in the stainless steel systems than in the PEEK systems (Figure 3-2). The concentration of sulphide increased rapidly in the KJ0052F01 circulation system while sulphide could not be detected in the KJ0050F01 system. It was therefore decided to change the groundwater types, and KJ0052F01 groundwater was hereafter directed to the stainless steel circulations and KJ0050F01 was directed to the PEEK circulations.

3.4 Effect of H_2 and acetate: experiments 12–23, days 0–61

Glass surfaces were used in experiments 1–11 as a support for the attachment and growth of microorganisms in groundwater. The use of flat surfaces made it possible to observe and count the microorganisms that had attached. It can be argued that the natural rock surfaces in groundwater may appear differently to microorganisms and that microbial diversity and numbers may differ between glass and rock. Therefore, crushed rock from the drill core obtained when drilling KJ0052F01 was used as support for microorganism attachment and growth in circulations connected to KJ0052F01 in experiments 12–14 and 18–20. Glass surfaces were kept for experiments with KJ0050F01 groundwater.

Fennoscandian groundwater at repository depth is anaerobic, reducing in character, and populated by a large diversity of obligate and facultative anaerobic microorganisms. Concentrations of H_2 and carbon monoxide are often 0.01–1 µM and of dissolved organic carbon (DOC) and methane 0.01–1 mM. Microbial activity involving these electron and energy donors may help keep deep groundwater anaerobic and reduced. Therefore, new experiments were performed in which H_2 was added in concentrations of 0.1–10 mM to a sulphate-reducing community attached to crushed rock in KJ0052F01 groundwater under a pressure of 2.0 MPa and *in situ* geochemical conditions. The results have been published elsewhere (Pedersen 2012b). Briefly described, the experiments reported a threshold concentration of approximately 1 µM H_2 at which H_2-dependent sulphate reduction ceased despite the presence of DOC and acetate, suggesting that H_2 was needed for sulphate-reducing activity. The $\delta^{13}C$ values of acetate and DOC data suggested that organic material was degraded to acetate via a heterotrophic process. New pressure-resistant micro-sensors for measuring E_h indicated an H_2-concentration-dependent decrease in E_h. The investigated community rapidly mitigated the increase in E_h caused by repeated additions of 0.1–0.2 mM pulses of O_2 as long as H_2 was available. The results imply that sulphate reduction to sulphide with H_2 may dominate sulphate-rich groundwater, which may have implications for metallic underground constructions.

Parallel with the KJ0052F01 experiments, a similar configuration was set up with KJ0050F01 groundwater in circulations with PEEK tubing and glass surfaces. The results from KJ0050F01 are shown in Figure 3-3 in comparison with the data obtained from the KJ0052F01 experiment. These experiments were conducted in two phases. The first phase, lasting from days 0 to 61, operated under anaerobic conditions. During the second phase, the experiments were disturbed by repeated introductions of O_2. The results of the O_2 treatments are discussed below in Section 5.5.

The experiments confirmed that the stainless steel systems retained the gases helium and hydrogen much longer than did the PEEK systems (Figure 3-3); methane was not influenced in the same way. A general observation based on all data was that microbial activity in the KJ0050F01 circulations was much slower than in the KJ0052F01 circulation. For example, the sulphide and acetate formation rates differed significantly. Because hydrogen had escaped from the KJ0050F01 circulations after approximately 40 days, sulphate reduction slowed.

3.5 Effect of O_2: experiments 12–23, days 62–96

The safe implementation of various spent nuclear fuel repository concepts relies on an O_2-free, reduced environment, because O_2 is corrosive of copper and iron canisters and because some radionuclides, such as Np, Pu, Tc, and U, are more soluble and mobile under oxic than under anoxic and reduced conditions. O_2 is introduced with air into the repository during the open phase; at closure, some of this air will be captured in repository voids. Other means of O_2 intrusion are infiltration of oxygenated water from the ground surface and from melting ice during periods of glaciation, when the intrusion of diluted and oxygenated groundwater can be significant (Hallbeck 2010). A good understanding of the effects of O_2 on subterranean microbial ecosystems and vice versa is therefore required for the safety analysis of future SNF repositories.

Both the KJ0050F01 and the KJ0052F01 experiments revealed that the O_2 pulses had significant effects: the TNC dropped (not shown), while DOC and E_h rose (Figure 3-3). The circulation with the highest concentration of hydrogen and rate of sulphide production resisted O_2 pulses more than did the other circulations. Consequently, the work demonstrated that O_2 pulses may cause the rapid collapse of deep microbial ecosystems. However, O_2 intrusion into deep groundwater layers can occur only via intruding surface water, which is a slow transport process. Such intruding water usually carries organic material and a surface-related microbial diversity well adapted to O_2. By the time intruding surface water reaches SNF repository depths, any O_2 will have long been reduced. In general, microbial processes will reduce O_2, either directly via aerobic respiration or via chemical processes, following the microbial production of reduced chemical species such as ferrous iron and sulphide.

Table 3-1. List of experiments performed with the circulation systems in the Microbe laboratory.

No.	Experimental configuration	Treatment	Source of groundwater	Circulation system	Surface type	Publication
1	Effect of H_2 and lactate	Untreated control	KA3110A	PEEK	Glass surfaces	Persson et al. (2011)
2	Effect of H_2 and lactate	10 mM H_2	KA3110A	PEEK	Glass surfaces	Persson et al. (2011)
3	Effect of H_2 and lactate	14 mM lactate	KA3110A	PEEK	Glass surfaces	Persson et al. 2011)
4	Effect of H_2 and acetate	Untreated control	KJ0052F01	PEEK	Glass surfaces	Pedersen (2012a)
5	Effect of H_2 and acetate	2.3 mM acetate	KJ0052F01	PEEK	Glass surfaces	Pedersen (2012a)
6	Effect of H_2 and acetate	3 mM H_2	KJ0052F01	PEEK	Glass surfaces	Pedersen (2012a)
7	Effect of H_2 and acetate	12 mM H_2	KJ0050F01	Stainless steel	Glass surfaces	Unpublished
8	Effect of H_2 and acetate	16 mM acetate, 12 mM H_2	KJ0050F01	Stainless steel	Glass surfaces	Unpublished
9	Effect of H_2 and acetate	Untreated control	KJ0050F01	Stainless steel	Glass surfaces	Unpublished
10	Effect of H_2 and acetate	4 mM H_2	KJ0052F01	PEEK	Glass surfaces	Unpublished
11	Effect of H_2 and acetate	16 mM acetate, 4 mM H_2	KJ0052F01	PEEK	Glass surfaces	Unpublished
12	Effect of H_2	10 mM H_2	KJ0052F01	Stainless steel	Crushed rock	Pedersen (2012b)
13	Effect of H_2	1 mM H_2	KJ0052F01	Stainless steel	Crushed rock	Pedersen (2012b)
14	Effect of H_2	0.1 mM H_2	KJ0052F01	Stainless steel	Crushed rock	Pedersen (2012b)
15	Effect of H_2 and acetate	10 mM H_2	KJ0050F01	PEEK	Glass surfaces	Unpublished
16	Effect of H_2 and acetate	21 mM acetate, 10 mM H_2	KJ0050F01	PEEK	Glass surfaces	Unpublished
17	Effect of H_2 and acetate	Untreated control	KJ0050F01	PEEK	Glass surfaces	Unpublished
18	Effect of O_2 on experiment 12	10 mM H_2 + O_2	KJ0052F01	Stainless steel	Crushed rock	Pedersen (2012b)
19	Effect of O_2 on experiment 13	1 mM H_2 + O_2	KJ0052F01	Stainless steel	Crushed rock	Pedersen (2012b)
20	Effect of O_2 on experiment 14	0.1 mM H_2 + O_2	KJ0052F01	Stainless steel	Crushed rock	Pedersen (2012b)
21	Effect of O_2 on experiment 15	10 mM H_2, O_2	KJ0050F01	PEEK	Glass surfaces	Unpublished
22	Effect of O_2 on experiment 16	21 mM acetate, 10 mM H_2 O_2	KJ0050F01	PEEK	Glass surfaces	Unpublished
23	Effect of O_2 on experiment 17	Untreated control + O_2	KJ0050F01	PEEK	Glass surfaces	Unpublished

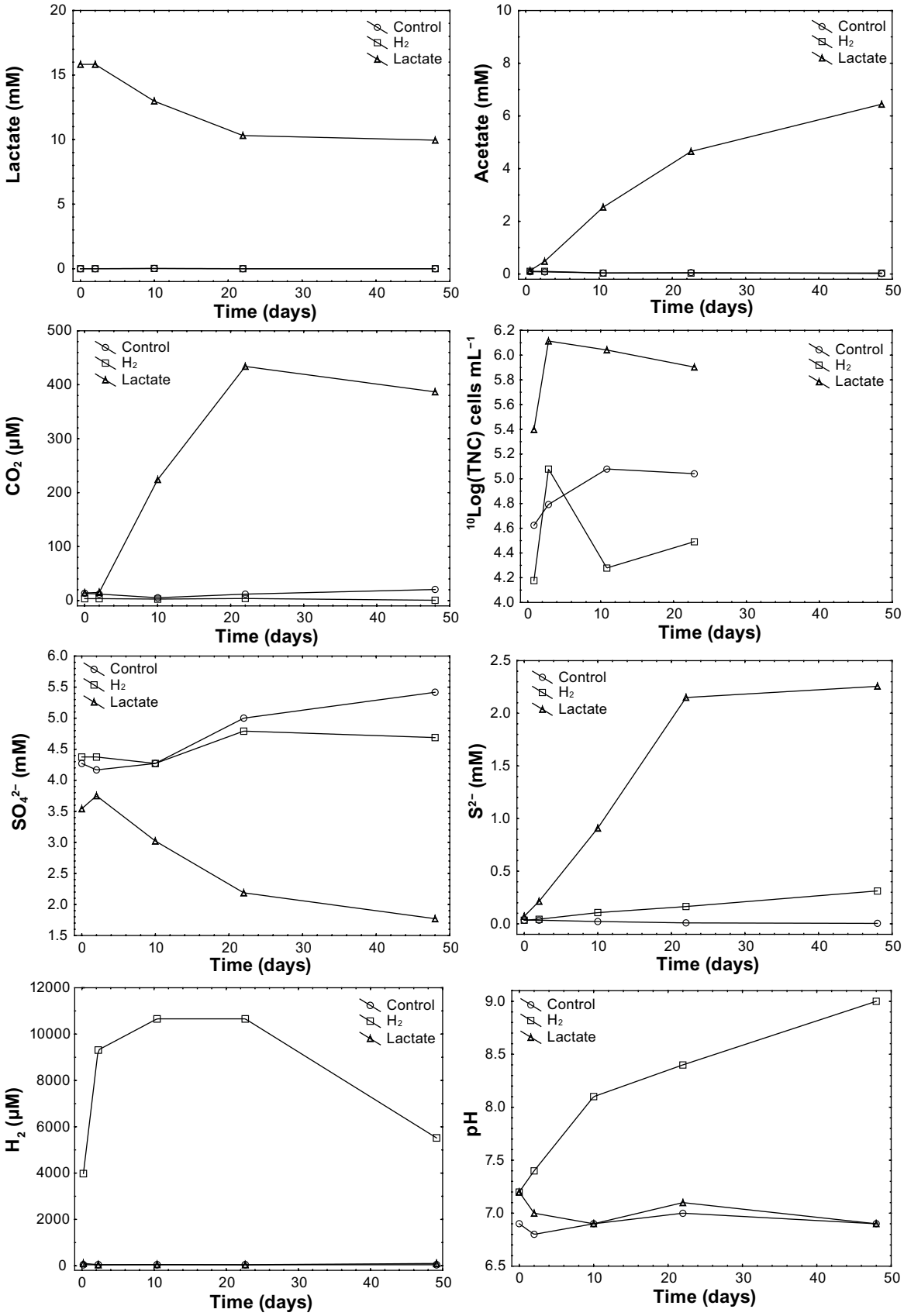

Figure 3-1. *The effect of hydrogen or lactate on chemistry, dissolved gases, and total numbers of bacteria in circulations with KA3110A groundwater. Details are presented in Table 3-1, experiments 1–3.*

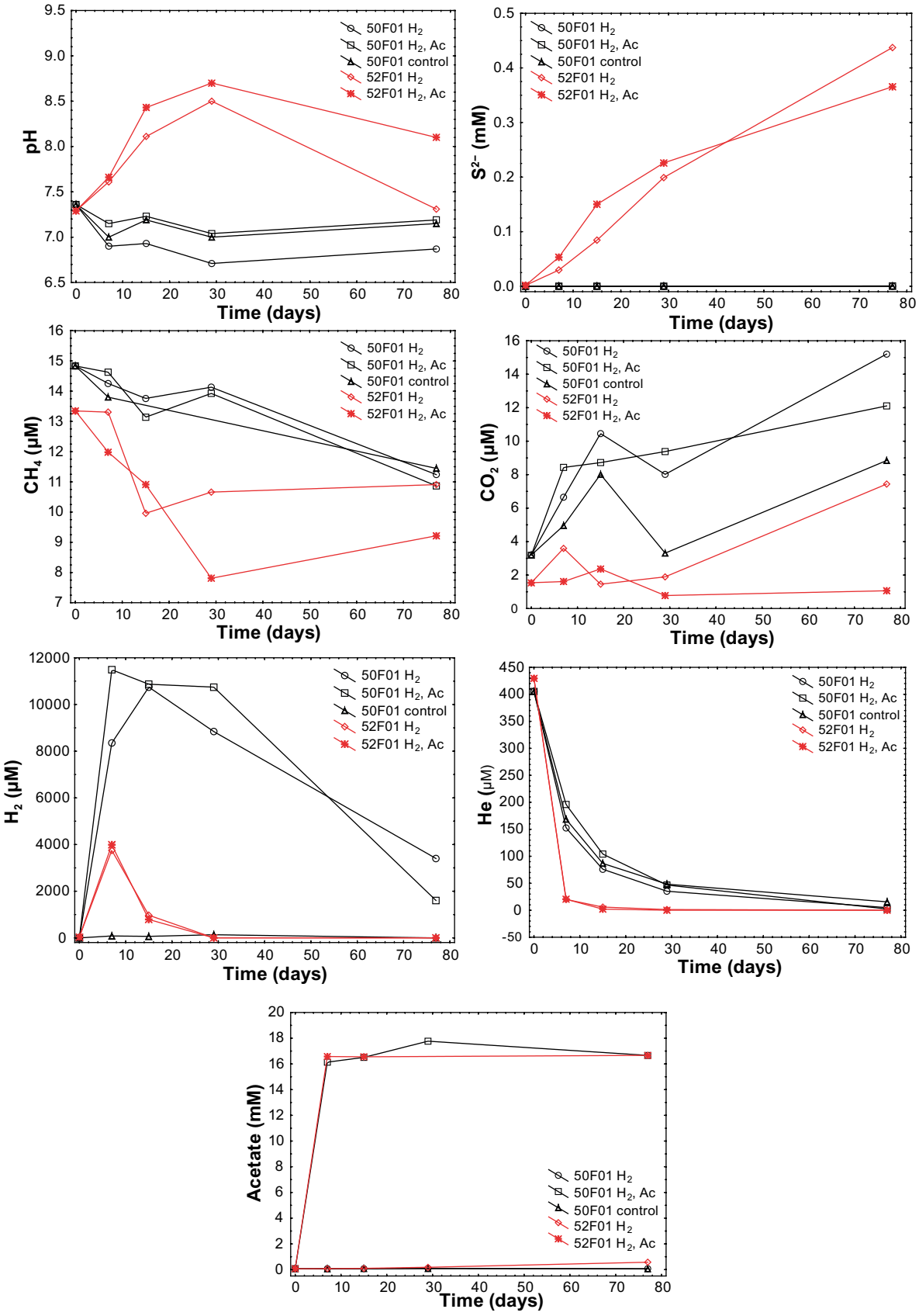

Figure 3-2. *The effect of hydrogen and/or acetate on chemistry and dissolved gases in circulations with KJ0052F01 groundwater. Details are presented in Table 3-1, experiments 7–11.*

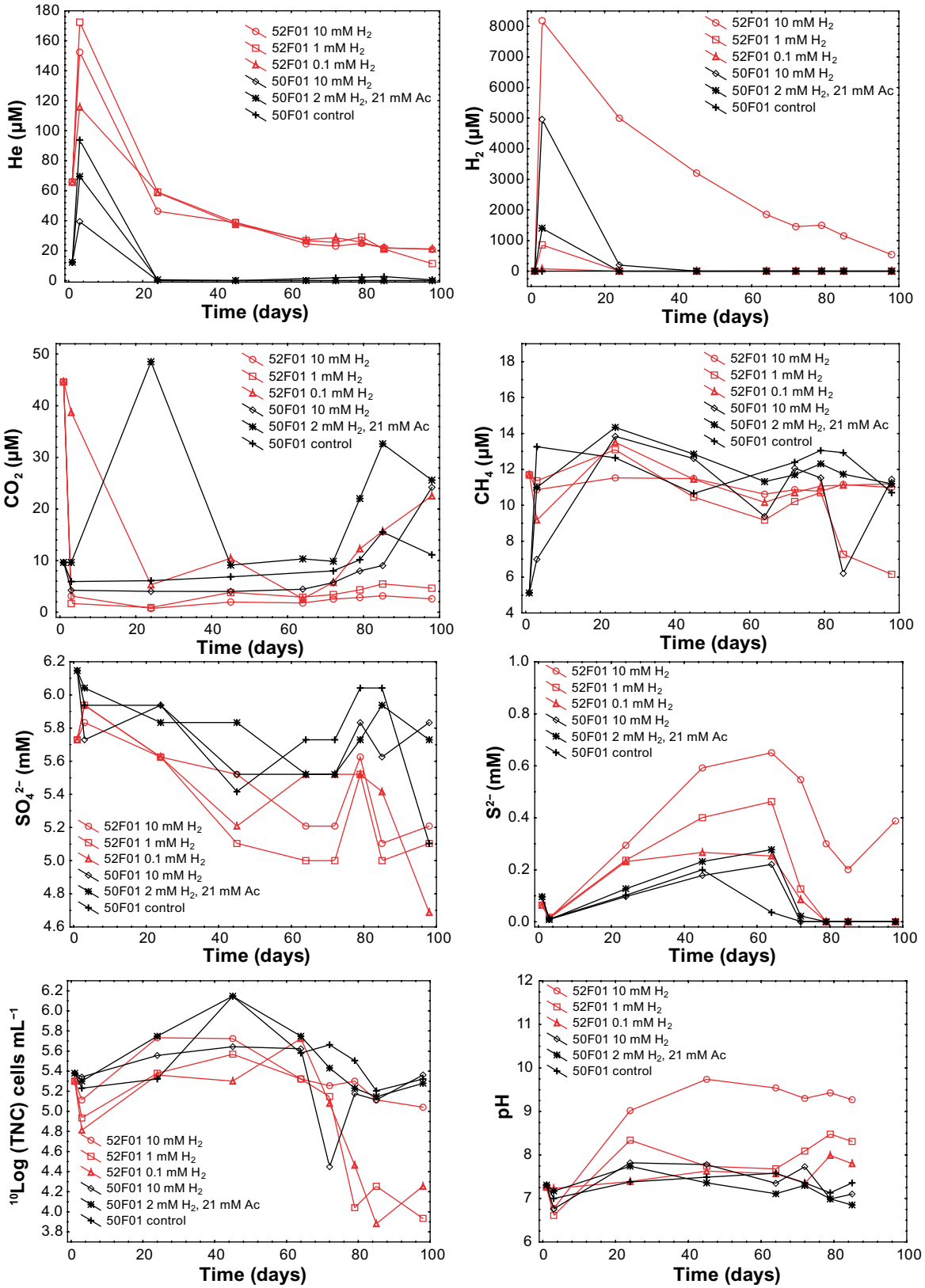

Figure 3-3. *The effect of hydrogen, hydrogen + acetate, and oxygen on chemistry, dissolved gases, E_h, organic carbon, and total numbers of cells in circulations with KJ0052F01 and KJ0050F01 groundwater. Pulses of 0.1–0.2 µM O_2 were added on days 62, 70, 77, and 96. Details are presented in Table 3-1, experiments 12–23.*

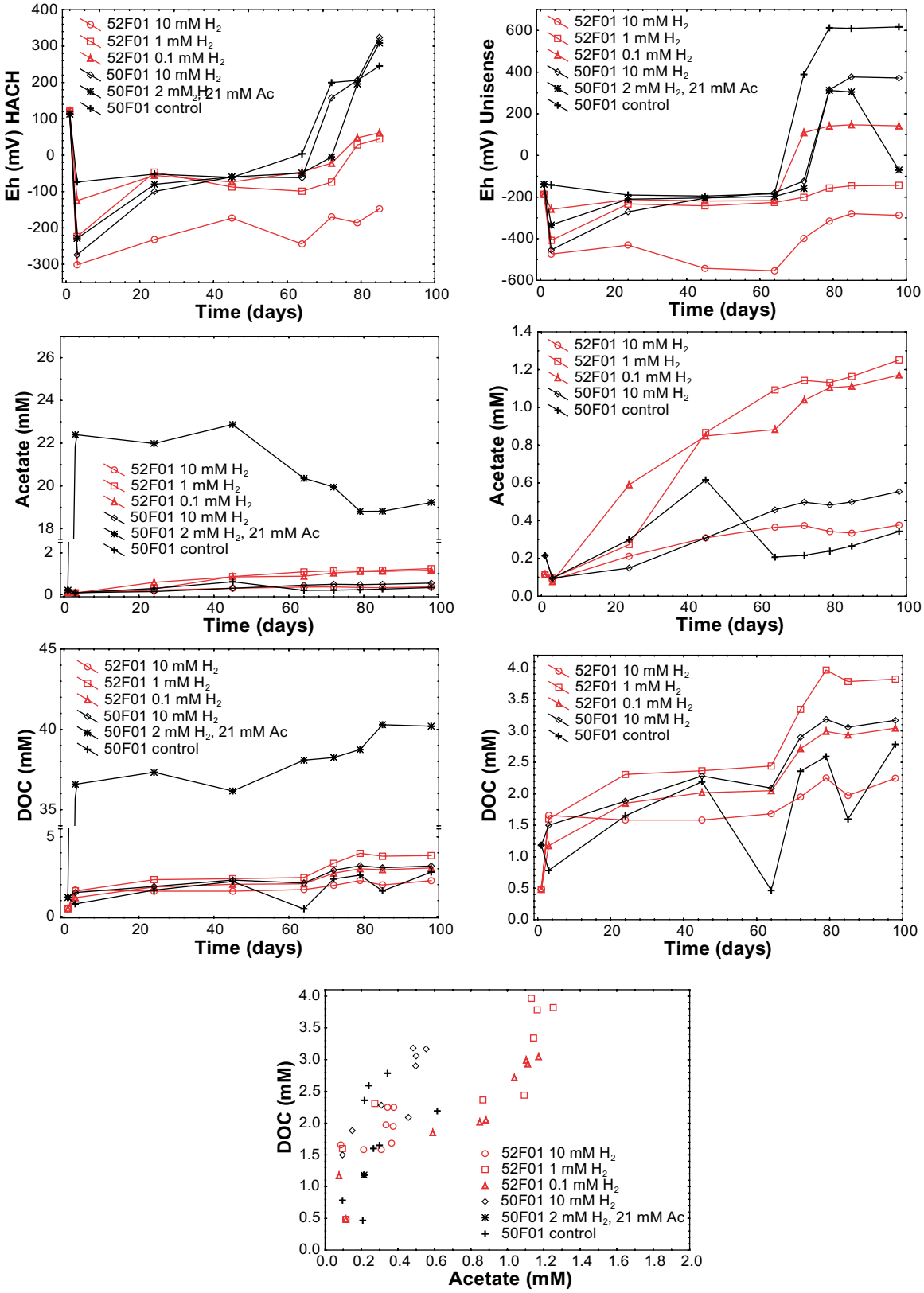

Figure 3-3. *Continued: The effect of hydrogen, hydrogen + acetate and oxygen, on chemistry, dissolved gases, E_h, organic carbon, and total numbers of cells in circulations with KJ0052F01 and KJ0050F01 groundwater. Pulses of 0.1–0.2 µM O_2 were added on days 62, 70, 77, and 96. Details are presented in Table 3-1, experiments 12–23.*

4 Acquired knowledge for a competent safety case

The Microbe project activities have generated new knowledge. The most important conclusions with implications for a competent safety case are summarized below. In addition, many new methods, techniques, and pieces of equipment for investigating microbial processes under repository conditions have been developed and disseminated in publications.

- The microbial reduction of sulphate can proceed at maximum rate in the presence of hydrogen at a concentration as low as approximately 1 μM (Pedersen 2012b).
- Viruses that attack bacteria (bacteriophages) exert a strong controlling effect on the total numbers of microorganisms in deep groundwater, which in turn will control the rates of microbial processes such as sulphate reduction (Eydal et al. 2009).
- Microbial biofilms can reduce the sorption of radionuclides to rock surfaces (Anderson et al. 2006a).
- The swelling pressure and water content of buffer materials are strongly related to microbial survival and activity in these materials (Masurat et al. 2010, Pedersen 2010).

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References

SKB's (Svensk Kärnbränslehantering AB) publications can be found at www.skb.se/publications.

Anderson C R, Pedersen K, 2003. *In situ* growth of *Gallionella* biofilms and partitioning of lanthanides and actinides between biological material and ferric oxyhydroxides. Geobiology 1, 169–178.

Anderson C, Pedersen K, Jakobsson A-M, 2006a. Autoradiographic comparisons of radionuclide adsorption between subsurface anaerobic biofilms and granitic host rocks. Geomicrobiology Journal 23, 15–29.

Anderson C R, James R E, Chi Fru E, Kennedy C B, Pedersen K, 2006b. *In situ* ecological development of a bacteriogenic iron oxide-producing microbial community from a subsurface granitic rock environment. Geobiology 4, 29–42.

Anderson C, Jakobsson A-M, Pedersen K, 2007. Influence of *in situ* biofilm coverage on the radionuclide adsorption capacity of subsurface granite. Environmental Science & Technology 41, 830–836.

Eydal H S C, Jägevall S, Hermansson M, Pedersen K, 2009. Bacteriophage lytic to *Desulfovibrio aespoeensis* isolated from deep groundwater. The ISME Journal 3, 1139–1147.

Hallbeck L, 2010. Principal organic materials in a repository for spent nuclear fuel. SKB TR-10-19, Svensk Kärnbränslehantering AB.

Hallbeck L E, Pedersen K, 2005. Genus I. Gallionella Ehrenberg 1838 166AL. In. Brenner D J, Krieg N R, Staley J T (eds). Bergey's manual of systematic bacteriology. Vol 2, The proteobacteria (Part C). The Alpha-, Beta-, Delta and Epsilonproteobacteria. 2nd ed. New York: Springer, 880–886.

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

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

Johnsson A, Ödegaard-Jensen A, Jakobsson A-M, Ekberg C, Pedersen K, 2008. Bioligand-mediated partitioning of radionuclides to the aqueous phase. Journal of Radioanalytical and Nuclear Chemistry 277, 637–644.

Jägevall S, Rabe L, Pedersen K, 2011. Abundance and diversity of biofilms in natural and artificial aquifers of the Äspö Hard Rock Laboratory, Sweden. Microbial Ecology 61, 410–422.

Kyle J E, Eydal H S C, Ferris F G, Pedersen K, 2008. Viruses in granitic groundwater from 69 to 450 m depth of the Äspö hard rock laboratory, Sweden. The ISME Journal 2, 571–574.

Laaksoharju M, Skårman C, Skårman E, 1999. Multivariate mixing and mass balance (M3) calculations, a new tool for decoding hydrogeochemical information. Applied Geochemistry 14, 861–871.

Masurat P, Eriksson S, Pedersen K, 2010. Microbial sulphide production in compacted Wyoming bentonite MX-80 under *in situ* conditions relevant to a repository for high-level radioactive waste. Applied Clay Science 47, 58–64.

Nielsen M E, Fisk M R, Istok J D, Pedersen K, 2006. Microbial nitrate respiration of lactate at *in situ* conditions in ground water from a granitic aquifer situated 450 m underground. Geobiology 4, 43–52.

Pedersen K, 2000. Äspö Hard Rock Laboratory. The Microbe site. Drilling, instrumentation and characterisation. SKB IPR-00-36, Svensk Kärnbränslehantering AB.

Pedersen K, 2002. Microbial processes in the disposal of high level radioactive waste 500 m underground in Fennoscandian shield rocks In Keith-Roach M J, Livens F R (eds). Interactions of microorganisms with radionuclides. Amsterdam: Elsevier, 279–311.

Pedersen K, 2005a. Äspö Hard Rock Laboratory. The Microbe framework. Site descriptions, instrumentation, characterisation. SKB IPR-05-05, Svensk Kärnbränslehantering AB.

Pedersen K, 2005b. Äspö Hard Rock Laboratory. Microbe. Analysis of microorganisms and gases in Microbe groundwater over time during MiniCan drainage of the Microbe water conducting zone. SKB IPR-05-29, Svensk Kärnbränslehantering AB.

Pedersen K, 2010. Analysis of copper corrosion in compacted bentonite clay as a function of clay density and growth conditions for sulfate-reducing bacteria. Journal of Applied Microbiology 108, 1094–1104.

Pedersen K, 2012a. Subterranean microbial populations metabolize hydrogen and acetate under *in situ* conditions in granitic groundwater at 450 m depth in the Äspö Hard Rock Laboratory, Sweden. FEMS Microbiology Ecology 81, 217–229.

Pedersen K, 2012b. Influence of H_2 and O_2 on sulphate-reducing activity of a subterranean community and the coupled response in redox potential. FEMS Microbiology Ecology 82, 653–665.

Pedersen K, 2013. Metabolic activity of subterranean microbial communities in deep granitic groundwater supplemented with methane and H_2. The ISME Journal 7, 839–849.

Pedersen K, Arlinger J, Eriksson S, Hallbeck A, Hallbeck L, Johansson J, 2008. Numbers, biomass and cultivable diversity of microbial populations relate to depth and borehole-specific conditions in groundwater from depths of 4–450 m in Olkiluoto, Finland. The ISME Journal 2, 760–775.

Persson J, Lydmark S, Edlund J, Pääjärvi A, Pedersen K, 2011. Microbial incidence on copper and titanium embedded in compacted bentonite clay. SKB R-11-22, Svensk Kärnbränslehantering AB.