Äspö Hard Rock Laboratory

The microbe site

Drilling, instrumentation and characterisation

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Göteborg University

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SKB

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ABSTRACT

A test plan for the MICROBE site (Pedersen, 2000a) was established in February 1999. A set of microbiology research tasks for the performance assessment of high level radioactive waste (HLW) disposal has been identified. Those with a potential for study at the MICROBE site are microbial production and consumption of gases, microbial influence on radionuclide migration and microbial corrosion of copper.

The major objectives for the MICROBE site are: 1) Assays of microbial activity in groundwater at *in situ* conditions. 2) Establishment of data on hydrogen generation and hydrogen flow in granitic rock environments. This is of importance for the understanding of the redox stability of the host rock. 3) Set up of experiment where the engineered barriers, bentonite, backfill and copper can be investigated for the influence of microorganisms at realistic and controlled conditions. 4) Generation of accurate data on the rates of microbial reactions under repository conditions.

The boreholes for MICROBE were drilled in May 1999 in the J-niche at Äspö HRL, 450 m underground. Three boreholes were produced for MICROBE and two additional boreholes have been drilled in the J-niche for CHEMLAB. Triple-tube drilling technique with a core retriever was used to minimise the exposure of the core to drill water and to deliver the core intact, also when multi-fractured rock was penetrated. The MICROBE boreholes are instrumented with a metal free packer system. It has been constructed to have the smallest possible void volume.

The groundwater chemistry and microbiology results are compared with data from other sites using principal component analysis. Data of specific interest for microbiology, i.e. carbonate and dissolve organic carbon, dissolved gases and stable isotopes have been compared with data obtained from other sites in the Fennoscandian shield. The goal with this comparison was to understand how the MICROBE site boreholes, and also the CHEMLAB site boreholes, compare with other sites. The MICROBE groundwater data generally plot in the middle of the ranges observed for the Fennoscandian shield sites studied, showing that the MICROBE site represents a prevalent groundwater situation at repository depth.

Imminent activities at MICROBE concern the design of a system that allows circulation of groundwater under full formation pressure. This system should enable work with microbes at very close to *in situ* conditions. It will hopefully be operative during 2001. A system for sensible measurement of hydrogen and other gases will also be developed.

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1 THE MICROBE SITE

1.1 Background

A test plan for the MICROBE site (Pedersen, 2000a) was established in February 1999. A set of microbiology research tasks for the performance assessment of high level radioactive waste (HLW) disposal has been identified. Those with a potential for study at the MICROBE site are:

- Microbial production and consumption of gases. Will bacterial production and consumption of gases like carbon dioxide, hydrogen, nitrogen and methane influence the performance of repositories?

- Microbial influence on radionuclide migration. To what extent can bacterial dissolution of immobilised radionuclides and production of complexing agents increase radionuclide migration rates? Will attached microbes retard migrating radionuclides?

- Microbial corrosion of copper. Bio-corrosion of the copper canisters, if any, will be a result of microbial sulphide production. Two important questions arise: Can sulphide-producing bacteria survive and produce sulphide in the bentonite surrounding the canisters? Can bacterial sulphide production in the surrounding rock exceed a performance safety limit?

1.2 Objectives

The major objectives for the MICROBE site are:
- To assay microbial activity in groundwater at *in situ* conditions. Their influence on redox conditions, radionuclide migration and gas composition and consumption will be in focus.

- To establish data on hydrogen generation and flow in granitic rock environments. The flow of hydrogen from where it is produced will determine the possible rate of long-term microbial subterranean activity.

- To enable experiment where the engineered barriers (bentonite, backfill and copper) can be investigated for the influence of microorganisms at realistic and controlled conditions with a significant knowledge about the microbiology of the groundwater used.

- To generate accurate data about rates of microbial reactions at repository conditions, for performance assessment calculations.

1.3 The site location - the J-niche

The tasks in 1.1 have been addressed in a range of projects, of which several are ongoing. Important conclusions have been obtained based on laboratory and field data. While some results seem very solid with general applicability, others are pending inspection at *in situ* conditions. This is especially true for data generated at the laboratory only. *In situ* generated data must be obtained for microbial activities anticipated in the far- and near-field environment at realistic HLW repository conditions. This can only be achieved at an underground site, developed for microbiological research, using circumstantial protocols for contamination control during drilling and operation. An *in situ* site allows experiments at

natural pressure with relevant gas content in groundwater, which is of great importance for microbial activity and very complicated to obtain *in vitro*. Such a site was drilled in May 1999 in the J-niche at Äspö HRL, 450 m underground (Figure 1-1). The J-niche tunnel has a diameter of 6-9 m and is located at about 450 m depth (Figure 1-2). The length of the niche is 20 m. Three boreholes were produced for MICROBE. Two additional boreholes have been drilled in the J-niche for CHEMLAB. Groundwater data from those two adjacent boreholes have been included in this report when available.
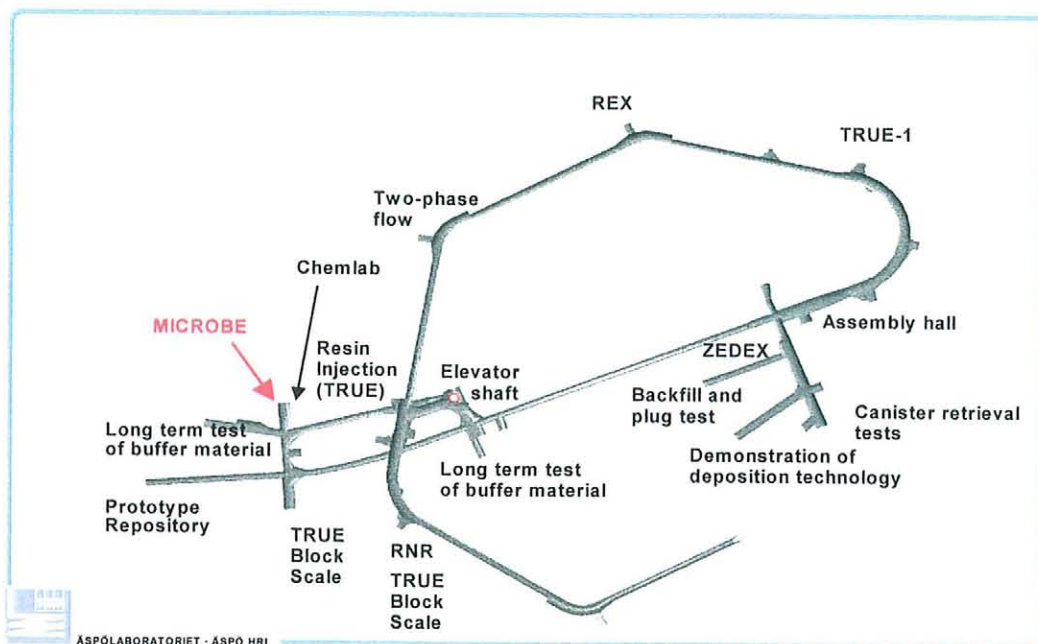
Figure 1-1. *The microbiology site, MICROBE, located in the J-niche of the Äspö Hard Rock Laboratory tunnel system.*
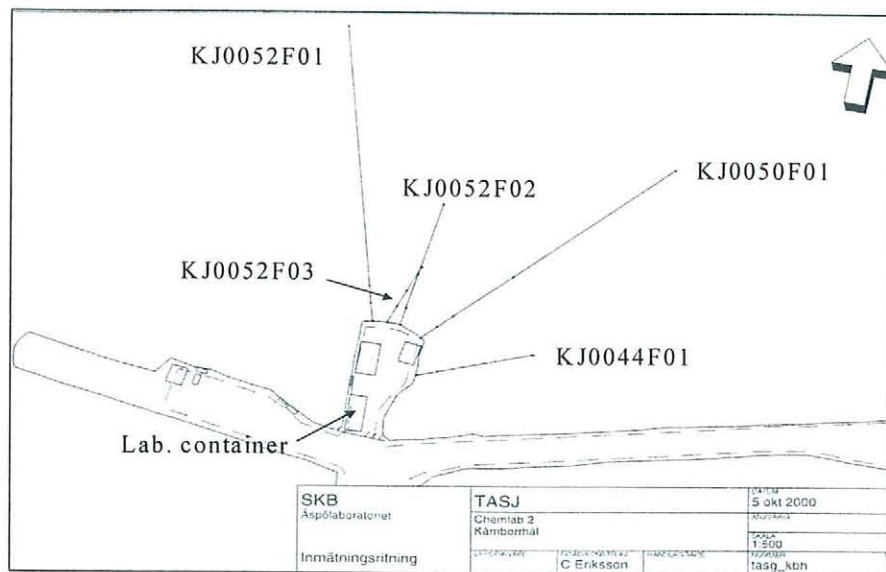
Figure 1-2. *The J niche with the MICROBE boreholes KJ0050F01, KJ0052F01 and KJ0052F03, and the CHEMLAB boreholes KJ0044F01 and KJ0052F02.*

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2 DRILLING AND INSTRUMENTATION OF BOREHOLES

2.1 Quality plan

A quality plan was established for the drilling of the MICROBE boreholes. The CHEMLAB boreholes (KJ0044F01, KJ0052F02) were drilled during the same campaign: *"Drilling of KJ0052F01, KJ0052F02, KJ0050F01, KJ0044F01 for the RNR experiments and microbiology sampling in the J-niche QP TD F58-99-015"*. KJ0052F03 was added to the campaign after the Quality Plan (QP) was written, but the drilling followed what was decided for the other two MICROBE boreholes. Triple tube drilling was applied (Figure 2-1). Triple-tube drilling technique with the use of a core retriever was used to minimise the exposure of the core to drill water and to deliver the core intact, also when multi-fractured rock is penetrated. The drill tube protected the drill core from contact with aquifer systems

Figure 2-1. *The triple tube drilling principle.*

intersected during drilling. The split tube kept intersected fractures intact, with also small pieces of rock in their original place. Steam and alcohol cleaning was applied to minimize potential contamination, as described in the QP.

2.2 Borehole characters

2.2.1 KJ0044F01

This borehole was drilled for the CHEMLAB-RNR experiments.

2.2.2 KJ0050F01

This borehole reaches a length of approximately 50 m and has a diameter of 76 mm. One water-conducting feature was found at 12.7 m depth. It has a flow of 0.4 L per minute and the formation pressure is 29 kg cm^2.

2.2.3 KJ0052F01

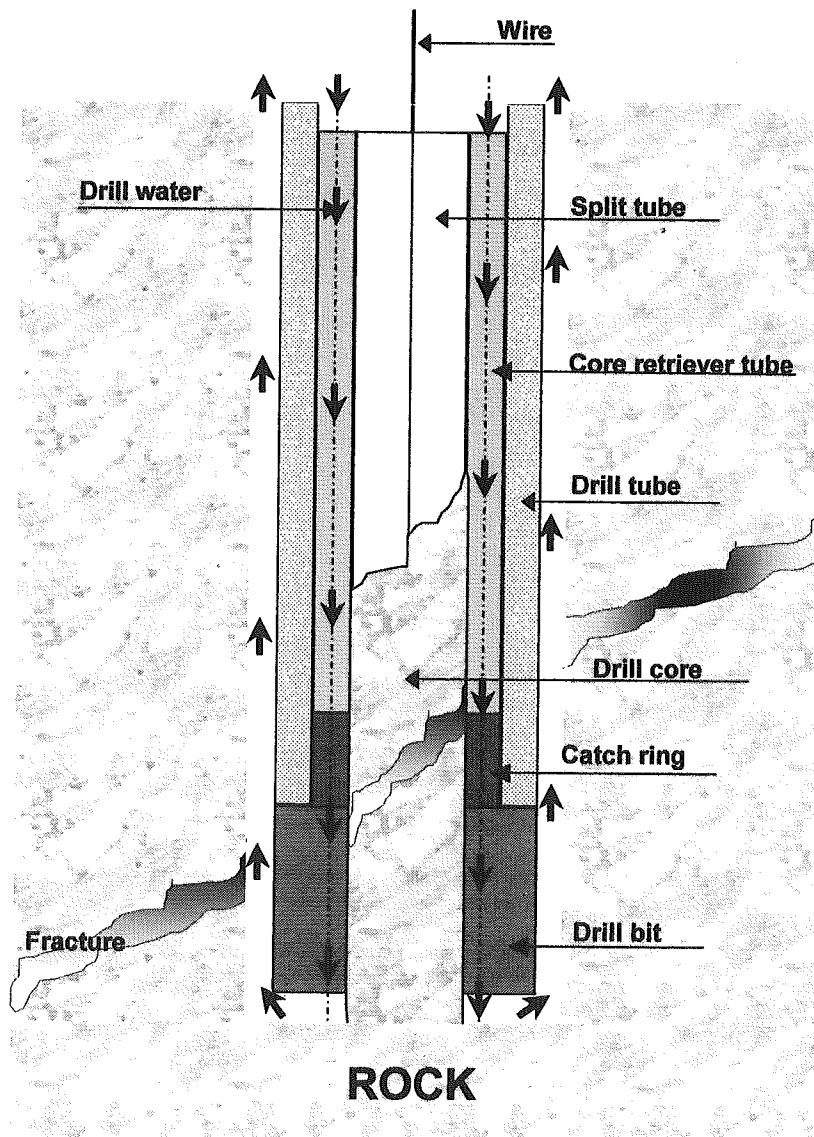This borehole reaches a length of approximately 50 m and has a diameter of 76 mm. One water-conducting feature was found at 43.8 m depth. It has a flow of 0.9 L per minute and the formation pressure is 29 kg cm^2.

2.2.4 KJ0052F02

This borehole was drilled for the CHEMLAB-RNR experiments. This borehole has a hydraulic communication with KJ0052F03.

2.2.5 KJ0052F03

This borehole reaches a length of approximately 10 m and has a diameter of 76 mm. One water-conducting feature was found at 9.3 m depth. It has flow of 1.5 L per minute and the formation pressure is 26 kg cm^2. The borehole intersects a fracture 20 cm below where KJ0052F02 intersects the same fracture.

2.3 Instrumentation of boreholes and the site

The MICROBE boreholes are instrumented with packers that do not expose metal to the groundwater. It was deemed important to have the smallest possible void volume in the systems. The instrumentation and the site container are described below.

2.3.1 Packer system

One water-conducting feature was found in each of the MICROBE boreholes. They were sectioned of with a double packer with a sealing length of 1000 mm per packer. The material chosen for the hard body parts was PEEK. Polyuretan rubber (Shore 90) with a thickness of 6 mm was used for the expansion part. Teflon (green) coated stainless steel casings were used to attach the rubber to the PEEK bodies (Figure 2-2). Dummy bodies were installed to minimize the void borehole volume. They have an outer diameter of 73 mm, leaving a 1.5 mm slot between the borehole wall and the dummy. Two PEEK tubings (dimension outer:1/8" / inner: 2 mm) were installed so that water can be circulated through the packed off section, if needed. 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. One expansion vessel was installed for all three packer systems.
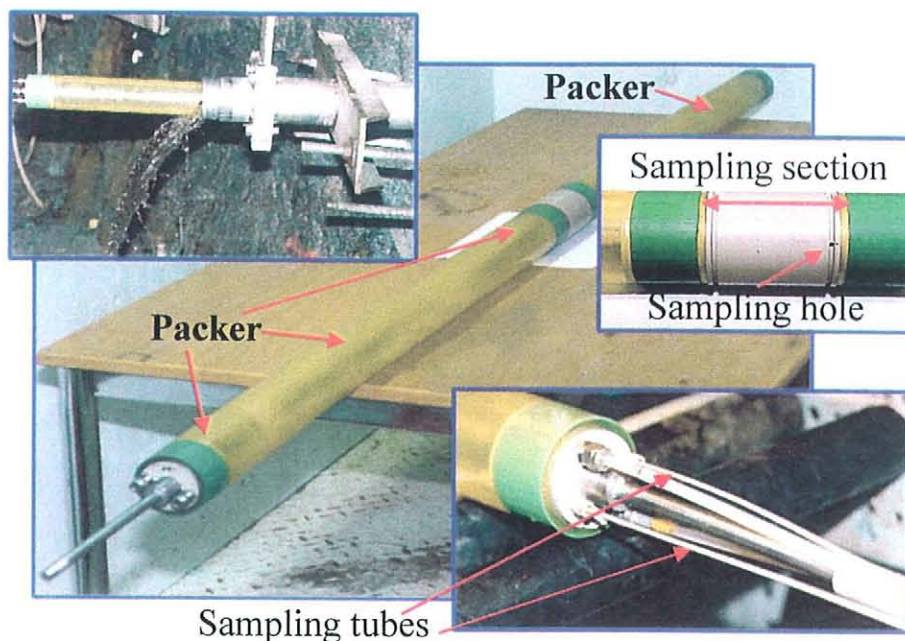
Figure 2.2. The packer system. The yellow sections are expansible poly-uretan packers, green rings are the Teflon coated stainless steel casings. The grey parts are the PEEK bodies. The sampling tubes are made of 1/8" (outer diameter) PEEK.

2.3.2 Laboratory container

A container, 7.5 x 2.5 m was placed in the J-niche (Figure 1-2). The container is equipped with a COY anaerobic glove box with three pairs of gloves. The container will be equipped progressively, in accordance with the experiments that are started.

2.4 The rock and the targeted fractures

BIP mapping and core logging were performed. The results can be found in SICADA.

3 GROUNDWATER CHARACTERISATION RESULTS

3.1 Groundwater chemistry

Groundwater was sampled according to the activity information in table 3-1a. Chemical analysis was performed according to the Class V protocols. The chemical main constituents, environmental isotopes and trace elements of the groundwater are shown in tables 3-1b-f.

Table 3-1a. Activity history of the groundwater chemistry analysis.

Idcode	Secup[a] m	Seclow[a] m	Sample No	Sampling date	Class No	Project	Northing Äspö96 (m)	Easting Äspö96 (m)	Elevation Äspö96 (m)
KJ0044F01	2,17	17,26	3154	2000-02-23	5	Chemlab	7310.7	1986.4	-448.3
KJ0050F01	12,64	12,84	3153	2000-02-23	5	Microbe	7321.4	1980.7	-448.2
KJ0052F01	43,7	43,90	3155	2000-02-23	5	Microbe	7360.1	1960.7	-450.5
KJ0052F02	2,14	21,42	3157	2000-02-23	5	Chemlab	7327.1	1971.2	-447.4
KJ0052F03	9,23	9,43	3156	2000-02-23	5	Microbe	7324.6	1970.3	-447.6

[a]) Secup and Seclow is upper and lower limit of the sampled section, respectively.

Table 3-1b. Chemical composition of the groundwater – main constituents.

Idcode	Sample	Na mg/L	K mg/L	Ca mg/L	Mg mg/L	HCO_3 mg/L	Cl mg/L	SO_4 mg/L	SO_4-S mg/L	Br mg/l	F mg/L	Si mg/L	Fe-ICP mg/L
Measurement uncertainty		5%	5%	5%	5%	5%	5%	10%	5%	10%	10%	5%	5%
KJ0044F01	3154	2310	10,4	2080	47,8	28	7030	451	162	45,9	1,59	5,5	0,056
KJ0050F01	3153	2260	9,61	1890	59,3	64	6580	426	155	44,4	1,40	6,2	0,143
KJ0052F01	3155	2370	7,73	2140	47,1	11	7230	485	175	47,6	1,01	5,1	0,049
KJ0052F02	3157	2430	9,07	2410	51,6	28	8130	487	170	55,4	1,59	5,6	0,137
KJ0052F03	3156	2060	8,76	1460	64,7	71	5750	388	138	34,2	1,97	6,4	0,165

Table 3-1c. Chemical composition of the groundwater – main constituents (continued).

Idcode	Sample	Fe_{tot} mg/L	Fe^{2+} mg/L	Mn mg/L	Li mg/L	Sr mg/L	pH pH unit	El. Cond. mS/m	Smpl Flow L/min	Drillwater %	DOC Mg/L	S_2 mg/L	NH_4-N mg/L	PO_4-P mg/L
Measurement uncertainty		10%	10%	5%	5%	5%	0.1 unit	5%			0.1 mg/L	10%	20%	5%
KJ0044F01	3154	0,067	0,043	0,39	1,58	35,1	7,2	1840	0,85	0,14	42,3	0,01	0,05	-
KJ0050F01	3153	0,144	0,125	0,50	1,38	32,1	7,3	1800	0,40	0,16	3,1	0,01	0,11	-
KJ0052F01	3155	0,052	0,049	0,29	1,49	36,1	7,8	1930	0,90	0,16	1,4	0.00	0,03	-
KJ0052F02	3157	0,112	0,107	0,43	1,76	40,5	7,4	2200	0,28	0,07	2,8	-0,06	0,06	-
KJ0052F03	3156	0,170	0,170	0,48	1,03	25,1	7,4	1610	1,50	0,27	3,5	-0,04	0,10	-

Table3.1d. Chemical composition of the groundwater – environmental isotopes.

Idcode	Secup[a] m	Seclow[a] m	Sample	Sampling date	δ^2H dev SMOW	$\delta^{18}O$ dev SMOW	^3H TU
Measurement uncertainty:					1 unit	0.2 unit	1 unit
KJ0044F01	2,17	17,26	3154	2000-02-23	-74,6	-10,3	3,7
KJ0050F01	12,64	12,84	3153	2000-02-23	-75,1	-10,3	4,4
KJ0052F01	43,7	43,9	3155	2000-02-23	-86,2	-11,7	1,0
KJ0052F02	2,14	21,42	3157	2000-02-23	-87,6	-11,1	1,5
KJ0052F03	9,23	9,43	3156	2000-02-23	-74,7	-9,70	3,9

a) Secup and Seclow is upper and lower limit of the sampled section, respectively.

Table 3-1e. Chemical composition of the groundwater – trace elements.

Idcode	Sample	U ug/L	Th ug/L	Sc ug/L	Rb ug/L	Y ug/L	In Ug/L	Cs ug/L	Ba ug/L	La ug/L	Tl ug/L	Ce ug/L	Pr ug/L	Nd ug/L
Measurement uncertainty:						5-20% depending on element								
KJ0044F01	3154	0,026	-0,4	0,475	33,1	0,318	-0,020	3,32	74,1	0,14	0,128	0,100	-0,020	0,030
KJ0050F01	3153	0,018	-0,4	0,412	27,5	0,257	-0,020	3,31	68,7	0,19	0,156	0,173	-0,020	0,056
KJ0052F01	3155	0,020	-0,4	0,702	29,1	0,415	-0,020	4,24	68,5	0,34	0,121	0,272	0,025	0,096
KJ0052F02	3157	0,064	-0,4	0,776	28,9	0,397	0,031	4,14	74,6	0,34	0,127	0,264	0,022	0,080
KJ0052F03	3156	0,040	-0,4	0,39	28,3	0,329	0,021	3,17	60,9	0,18	0,112	0,189	-0,020	0,061

Table 3-1f. Chemical composition of the groundwater – trace elements.

Idcode	Sample	Sm ug/L	Eu ug/L	Ho ug/L	Er ug/L	Tm ug/L	Yb ug/L	Lu ug/L
Measurement uncertainty					5-20% depending on element			
KJ0044F01	3154	-0,02	-0,02	-0,02	-0,02	-	-0,02	-0,02
KJ0050F01	3153	-0,02	-0,02	-0,02	-0,02	-	-0,02	-0,02
KJ0052F01	3155	-0,02	-0,02	-0,02	-0,02	-	-0,02	-0,02
KJ0052F02	3157	-0,02	-0,02	-0,02	-0,02	-	-0,02	-0,02
KJ0052F03	3156	-0,02	-0,02	-0,02	-0,02	-	-0,02	-0,02

Gas composition

The contents of gas, the amounts of specific gases and the volumes extracted are shown in figure 3-2a-b.

Table3-2a. Content of gas in the J-niche boreholes – specific gases.

Borehole	Sample date	Analysis date	N_2	H_2	He	Ar	CO_2	CH_4	C_2H_2	C_2H_4	C_2H_6	C_3H_6	C_3H_8
			ml/L	µl/L	ml/L	ml/L	ml/L	ml/L	µl/L	µl/L	µl/L	µl/L	µl/L
KJ0044F01	000629	000706	77.6	6	9.27	0.78	0.37	0.92	<0.05	<0.05	0.3	<0.09	<0.09
KJ0050F01	000608	000609	70.7	<3	7.46	0.70	0.66	0.77	<0.05	<0.05	0.3	<0.09	<0.09
KJ0052F01	000608	000609	70.0	<3	7.42	0.80	0.20	0.63	<0.05	<0.05	0.2	<0.09	<0.09
KJ0052F02	000629	000706	44.1	10	7.0	0.78	0.75	1.10	<0.03	<0.03	0.2	<0.06	<0.06
KJ0052F03	000629	000706	66.8	16	5.95	0.69	0.95	1.2	<0.04	<0.04	0.2	<0.08	<0.08

Table3-2b. Content of gas in the J-niche boreholes – volume of gas and water extracted.

Borehole	Sample date	Analysis date	Volume gas ml	Volume water extracted g
KJ0044F01	000629	000706	91	345
KJ0050F01	000608	000609	83	382
KJ0052F01	000608	000609	81	380
KJ0052F02	000629	000706	55	334
KJ0052F03	000629	000706	78	381

3.1.1 Stable isotopes

Stable isotopes of the carbon containing gases were analysed. The results are shown in Table 3-3.

Table3-3. Carbon isotope analysis of carbon dioxide and methane extracted from the J-niche boreholes.

Borehole	Sample date	IFE no GEO	$CH_4\ \delta^{13}C$ ‰ PDB	$CO_2\ \delta^{13}C$ ‰ PDB
KJ0044F01	000629	20001392	-58.1	-21.5
KJ0050F01	000608	20001393	-50.0	-21.0
KJ0052F01	000608	20001394	-50.6	-23.3
KJ0052F02	000629	20001395	-62.0	-21.9
KJ0052F03	000629	20001396	-51.1	-28.1

3.2 Microbiology

The groundwater to be analysed was collected directly from the borehole tubing after sampling for chemistry. This gave the requested flushing of a borehole before microbiology sampling. The sample was transferred directly to anaerobic glass serum bottles for further preparation in the lab on ground. To ensure the efficiency of the equipment sterilization, control samples were analysed. These were sample bottles and the media used. Sterile water was sampled as control water and processed as samples.

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3.2.1 Direct Counting

Total numbers of cells in the groundwater samples were counted using a stain that stain DNA and RNA, allowing cells to be stained specifically with minimal background. Samples were stained according to the acridine orange (AO) direct count method. The filters were rinsed twice with 1.0 ml of 0.2 μm filtered double distilled water to dissolve salt crystals prior to staining for 10 minutes with AO. Cells were counted on an Olympus BH-2 microscope with blue filters. Results were calculated as an average of two prepared filters, with sample standard deviation as the error.

3.2.2 Culturing media

Bacterial growth media were designed using 10% groundwater sampled and shipped to the lab prior to sampling. This allows media to be designed that accurately mimic the actual groundwater situation, giving the microbes a growth medium with minimal changes from their natural environment.

Media are prepared anaerobically, according to the Hungate method. Media contain (g/l): NH_4Cl, 0.4; NaCl, 2.8; KH_2PO_4, 0.01; KCl, 0.02; Na_2SO_4, 0.002; $MgCl_2 \cdot 6H_2O$, 0.6; $CaCl_2$, 1.7; $FeCl_2 \cdot 4H_2O$, 0.001; $NaHCO_3$, 1.68; Cysteine $HCl \cdot H_2O$, 0.25; $Na_2S \cdot 9H_2O$, 0.25; resazurin, 0.2 mg; Element solution, 10 ml; Vitamin solution, 5 ml; 0.2μm filter sterilized groundwater from the corresponding borehole depth, 100 ml and double distilled water up to 1000 ml. The element solution contained (g/l): Nitrilotriacetic acid, 12.8; $FeSO_4 \cdot 7H_2O$, 0.1; $MnCl_2 \cdot 4H_2O$, 0.1; $CoCl_2 \cdot 6H_2O$, 0.17; $CaCl_2 \cdot 2H_2O$, 0.1; $ZnCl_2$, 0.1; $CuCl_2$, 0.02; H_3BO_2, 0.01; $Na_2MoO_4 \cdot 2H_2O$, 0.01; NaCl, 1.0; and Na_2SeO_3, 0.01. The vitamin solution contained (mg/l): Biotin, 2; Folic acid, 2; Pyridoxine B_6, 0.1; Thiamine HCl B_1, 5; Riboflavin B_2, 5; Nicotinic acid, 5; Pantothenic acid, 5; Cyanocobalamine B_{12}, 0.1; p-aminobenzoic acid, 5; and Lipoic acid (Tioctic), 5. The pH of the media was adjusted to borehole pH, 7.3 to 7.5, after autoclaving.

The medium for each groundwater sample was divided into different anaerobic bottles, and substrates for different physiological groups of microorganisms were added to each anaerobically. The medium for autotrophic methanogens (AM) contained no additions. The medium for heterotrophic methanogens (HM) contained 10 mM acetate, 10 mM trimethylamine (TMA), 50 mM methanol and 74 mM formate. The medium for autotrophic acetogens (AA) contained 50 mM 2-bromethanesulfonic acid (BESA) as an inhibitor of methanogenesis. The medium for heterotrophic acetogens (HA) contained 50 mM BESA, 10 mM TMA, 74 mM formate, and 2 g/l yeast extract. The medium for sulphate reducing bacteria (SRB) contained 14 mM Na_2SO_4 and 6 mM lactate. The medium for iron reducing bacteria (IRB) contained 7 g/l amorphous iron and 11 mM lactate.

3.2.3 Most Probable Number

The different types of media were dispensed anaerobically in 9 ml aliquots into sterile test tubes with N_2 in the gas phase, which were used to determine the most probable number (MPN) of each physiological group of *Bacteria* or *Archaea* in each groundwater. Three types of negative controls were prepared: with medium only, with addition of 1 ml 0.2 μm filter sterilized groundwater, and inoculated with 1 ml groundwater and immediately killed with 2% formaldehyde. The AM and AA tubes were gassed with 2 bar overpressure oxygen free H_2. Tubes were incubated on their sides at 17°C.

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After 3 to 5 months incubation, tubes were analysed for products of metabolism. The gas phase of the methanogenic tubes (AM and HM) were analysed for presence of methane by gas chromatography. The acetogenic tubes (AA and HA) were also analysed as negative controls for methanogenesis. Acetate was analysed with a kit (Boehringer Mannheim, Mannheim Germany) which detects acetate by an enzymatic and UV method. SRB tubes were analysed for production of hydrogen sulphide by adding 0.1 ml of the culture to 2 ml of 5 mM $CuSO_4$ solution. If the solution turns brown, sulphide was produced in the culture. IRB tubes were analysed for both total and ferrous iron using a spectrophotometric ferrozine method.

Table 3-4. The results from direct counting (AODC) and MPN counting of microbes in the packed of MICROBE borehole sections.

Borehole	Sample date	Analysis date	AODC	IRB	SRB	HA	AA	HM	AM
			cells/ml						
KJ0050F01	000301	000608-000811	14000	24	360	24	<0.1	<0.1	<0.1
KJ0052F01	000301	000608-000811	2300	2.3	9.4	24	<0.1	<0.1	<0.1
KJ0052F03	000229	000608-000811	20000	36	1100	0.23	0.26	<0.1	<0.1

4 THE MICROBE SITE COMPARED TO OTHER SITES

4.1 Groundwater chemistry

The groundwater chemistry results can be compared with data from other sites in different ways. First, principal component analysis were performed. Secondly, data of specific interest were compared, i.e. carbonate and dissolve organic carbon, dissolved gases and stable isotopes. The data have been compared with data obtained from other sites in the Fennoscandian shield depicted in Table 4-1. As these data have been compiled over a long time period, their appearance in the comparison figures varies according to availability of data. However, the goal with this comparison was to reflect a view of how the MICROBE site, and also the CHEMLAB site boreholes compare with the general groundwater situation.

4.1.1 Multivariate mixing and mass balance calculations (M3) - Method

The evolution of the groundwater is generally strongly related to the present and past flow conditions. As this is a continuous process, the type of infiltrating water as well as the already existing water in the rock change their compositions. The solute and isotopic content of groundwater samples can be interpreted as either the result of geochemical reactions between the groundwater and the minerals it contacts, or as the mixing of groundwater types of different origins (and hence different chemical signatures), or as 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) (Laaksoharju et al., 1995; Laaksoharju and Skårman, 1995; Laaksoharju et al., 1999) model developed by SKB. M3 is an interpretative technique used to perform a 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 deviations between the chemical measurements of each sample and the theoretical chemistry from the mixing calculation, interpret these deviations as resulting from groundwater

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Table 4-1. Sites information comprising all boreholes in igneous rock investigated for microbiology since 1987. Detailed information about the boreholes and the rock formation studied can be obtained elsewhere (Pedersen, 2000b). F = boreholes in Finland; S = boreholes in Sweden.

SITE	Year	Borehole	Depths (m)	
Hålö (S)	1992-1996	HBH01, HBH02	10-45	(2 levels)
Hästholmen (F)	1997-1998	HH-KR1, HH-KR2, HH-KR3, HH-KR4, HH-KR5, HH-KR6	65-943	(6 levels)
Kivetty (F)	1997-1998	KI-KR5, KI-KR13	497-721	(2 levels)
Laxemar (S)	1988-2000	KLX01, KLX02	100-1700	(7 levels)
Olkiluoto (F)	1998-1999	OL-KR3, OL-KR4, OL-KR8, OL-KR9, OL-KR-10	248-863	(7 levels)
Palmottu (F)	1998-1999	R302, R337, R387	32-309	(5 levels)
Romuvaara (F)	1998-1999	RO-KR10, RO-KR11	543-564	(2 levels)
Stripa (S)	1987-1991	V1, V2	799-1240	(4 levels)
Ävrö (S)	1987	KAV01	420-924	(4 levels)
Äspö (S)	1988-1996	KAS02, KAS03, KAS04	129-1002	(11 levels)
Äspö Hard Rock Laboratory (HRL) tunnel (S)	1992-2000	KR0012, KR0013, KR0015, SA813B, SA923A, SA1062A, HA1327B, SA1420A, KA2511A, KA2512A, KA2858A, KA2862A, KA3005A, KA3010A, KA3067A, KA3105A, KA3110A, HD0025A, KA3385A, KA3539G, KA3548A01, KA3600F, KJ0050F01, KJ0052F02, KJ0052F03	68-450	(25 levels)
11 sites	**1987-1999**	**53 boreholes**	**10-1700**	**(75 levels)**

reactions. The data set used for this analysis is listed in table 4-2. Boreholes analysed were KJ0044F01, KJ0050F01, KJ0052F01, KJ0052F02 and KJ0052F03.

Groundwater chemical data from these boreholes are compared with other chemical data from Fennoscandian shield sites. The objectives of this analysis were to answer the following questions: 1) What type of water is found in the MICROBE site boreholes? 2) What can (tentatively) be said about the origin of the groundwater? The M3 model consists of three steps, where the first step is a standard multivariate analysis (Principal Component Analysis = PCA), followed by mixing and finally by mass balance calculations. Multivariate techniques are useful in gathering information from many chemical variables the information is used to model the mixing and reactive processes affecting the obtained groundwater composition. Here, the first two steps were applied, namely PCA and mixing calculations. The aim of the M3 model is to compare the measured groundwater samples by creating an ideal mixing model for the site. The groundwater constituents that cannot be explained by mixing may be described by reactions such as biogeochemical reactions. The model thus operates in an opposite way to many standard models that resolve the contribution from reactions prior to mixing.

Table 4-2. The chemical data used for the M3-PCA analysis.

	Idcode	Secup m	Seclow m	Sample	Sampling date	Na mg/L	K mg/L	Ca mg/L	Mg mg/L	HCO$_3$ mg/L
				Measurement uncertainty:		5%	5%	5%	5%	5%
1	KJ0044F01	16.00	17.26	3154	2000-02-23	2310	10.4	2080	47.8	28
2	KJ0050F01	12.64	12.84	3153	2000-02-23	2260	9.61	1890	59.3	64
3	KJ0052F01	43.70	43.90	3155	2000-02-23	2370	7.73	2140	47.1	11
4	KJ0052F02	15.00	21.42	3157	2000-02-23	2430	9.07	2410	51.6	28
5	KJ0052F03	09.23	09.43	3156	2000-02-23	2060	8.76	1460	64.7	71

	Idcode	Secup M	Seclow m	Sample	Sampling date	Cl mg/L	SO$_4$ Mg/L	δ^2H SMOW	δ^{18}O SMOW	^{3}H TU
				Measurement uncertainty:		5%	10%	1 unit	0.2 unit	1 unit
1	KJ0044F01	16.00	17.26	3154	2000-02-23	7030	451	-74.6	-10.3	3.7
2	KJ0050F01	12.64	12.84	3153	2000-02-23	6580	426	-75.1	-10.3	4.4
3	KJ0052F01	43.07	43.90	3155	2000-02-23	7230	485	-86.2	-11.7	1.0
4	KJ0052F02	15.00	21.42	3157	2000-02-23	8130	487	-87.6	-11.1	1.5
5	KJ0052F03	09.23	09.43	3156	2000-02-23	5750	388	-74.7	-9.7	3.9

4.1.2 PCA Results

The results from the PCA analysis are based on the MICROBE data set (Table 4-2) together with geochemical data from Fennoscandian shield sites. The constituents used for the modelling are major components (Na, K, Ca, Mg, HCO$_3$, Cl and SO$_4$), tritium (^{3}H) and stable isotopes (δ^2H and δ^{18}O). It is known that these components contain most of the variability of the information in groundwater data. The variability for the first and second principal components shown in Figure 4-1 is 70% which means that the first and second principal components summarize 70% of the groundwater data information. The weight for the different elements is shown in the equations for the first and second principal components respectively. From these weights the importance of the individual elements for the total analysis can be tracked. The MICROBE groundwater samples plot in the middle of Figure 4-1, showing that those waters do not have an extreme groundwater composition but the samples are affected by mixing of several reference waters. The selected reference waters identified from the PCA (Figure 4-1) for the current modelling are:

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Brine reference water, which represents the brine type of water found in KLX02:1631-1681m, with a measured Cl content of 47200 mg/l. Brines are waters with significantly higher salinity than ocean water (Cl content of 19800 mg/l).

Baltic Sea reference water, which represents modern Baltic Sea water, with ocean water as the saline component.

Altered marine reference water, which represents seawater altered by bacterial sulphate reduction (Laaksoharju (ed.), 1995). This water type is obtained in the HRL tunnel below marine sediments (SA0813B: 5.6-19.5m).

Precipitation reference water, which represents dilute shallow groundwater of the type found in HLX06(871103): 45-100m.

Glacial reference water, which is a precipitation water composition where the stable isotope values ($\delta^{18}O$ = -21 SMOW and δ^2H = -158 SMOW) are based on measured values of $\delta^{18}O$ in the calcite surface deposits, interpreted as sub-glacial precipitates, collected from different geological formations on the west coast of Sweden (Tullborg and Larson, 1984). The water represents a possible melt water composition from the last glaciation >13000 years BP.
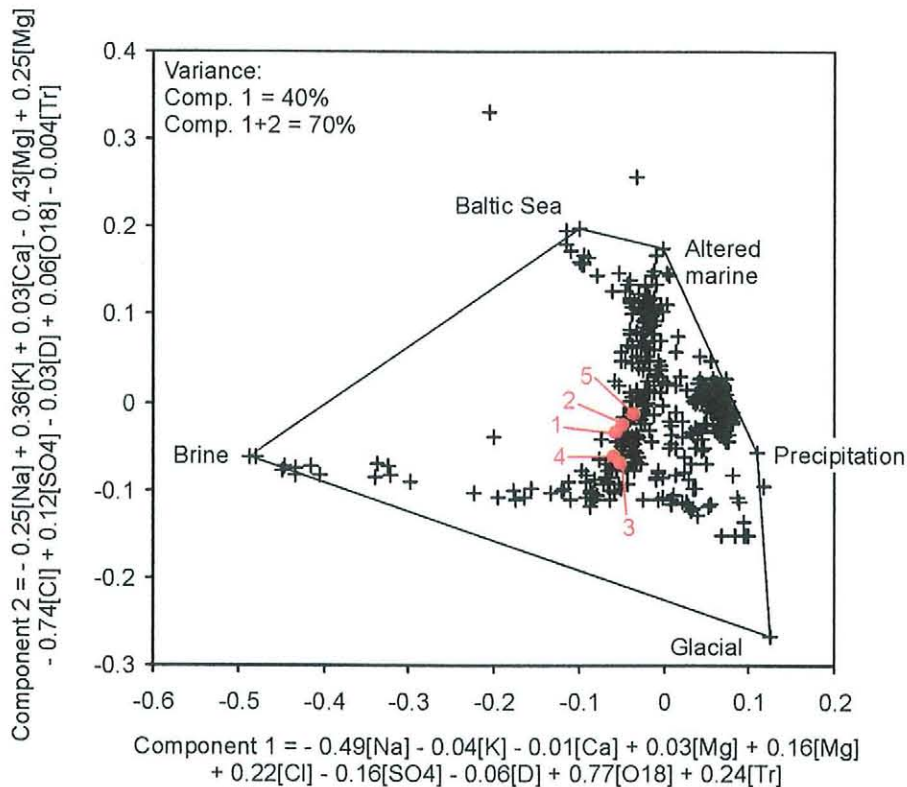

Figure 4-1. Principal Component plot showing groundwater chemical data from the MICROBE and CHEMLAB sites (red) in comparison to data from Fennoscandian field sites (black +). The samples from the investigated sites plot in the middle of the PCA-plot indicating that these waters may be affected by a possible mixing contribution from several reference waters such as glacial, brine and precipitation. The plot is based on seven major components, tritium and stable isotopes (δ^2H and $\delta^{18}O$). The first and second principal components account for 70% of the variability in the data set analysed.

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4.1.3 Mixing portions

The calculated mixing portions of the MICROBE groundwaters using the M3 concept are shown in Table 4-3. The portions describe how large the influence of the different reference waters may be on the observed water. The mixing portions describe a possible mixing situation in the bedrock in relation to the chosen reference waters. The MICROBE samples 1-4 plot in the middle of the PCA-plot indicating that these waters may be affected by a possible mixing contribution from several reference waters where glacial, brine and precipitation type of water dominate (Figure 4-1). This is also shown in the mixing portions for the data set (Table 4-3). The samples contain between 18 and 21% brine water and between 29 and 38% glacial water in the sample 2 the portion of precipitation water is 22%. The MICROBE sample 5, KJ0052F03, seems to be even more influenced by precipitation, which is the largest single component in this water (35%).

Table 4-3 PCA mixing modelling results (a mixing portion >10% is regarded as significant, the uncertainties are ±10% from the reported mixing portions).

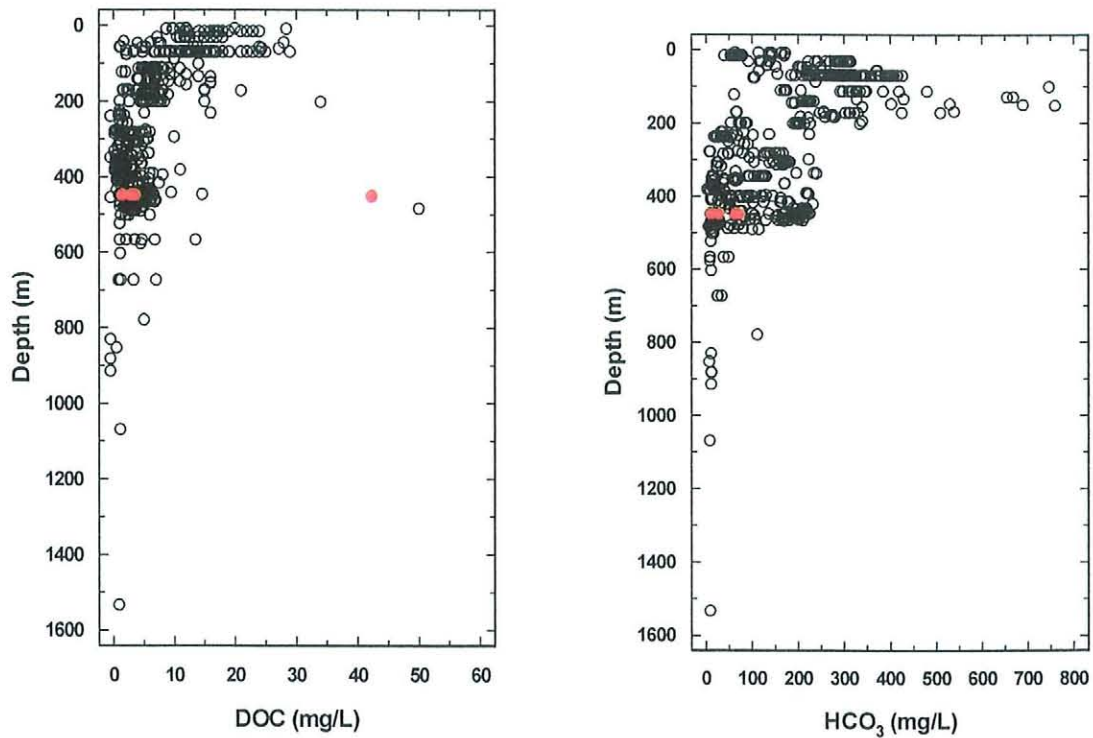|	Idcode	Secup	Seclow	Sample	Sampling date	Brine	Glacial	Precipitation	Altered marine	Baltic Sea
		m	M			%	%	%	%	%
1	KJ0044F01	16.00	17.26	3154	2000-02-23	19%	29%	17%	17%	17%
2	KJ0050F01	12.64	12.84	3153	2000-02-23	18%	26%	22%	18%	18%
3	KJ0052F01	43.7	43.9	3155	2000-02-23	21%	38%	14%	14%	14%
4	KJ0052F02	15.00	21.42	3157	2000-02-23	21%	36%	14%	14%	14%
5	KJ0052F03	9.23	9.43	3156	2000-02-23	16%	17%	35%	16%	16%

Figure 4-2. The content of DOC and HCO$_3$ in MICROBE and CHEMLAB boreholes (red circles, and all other DOC and HCO$_3$ data for Fennoscandian field sites (Table 4-1).
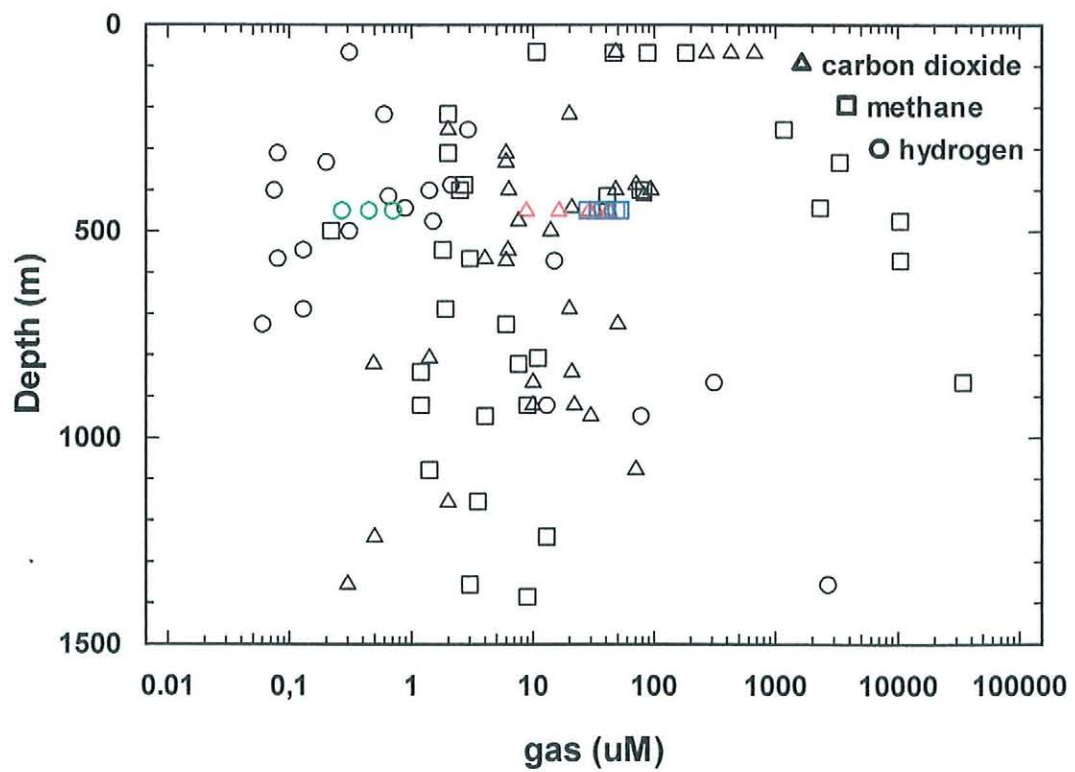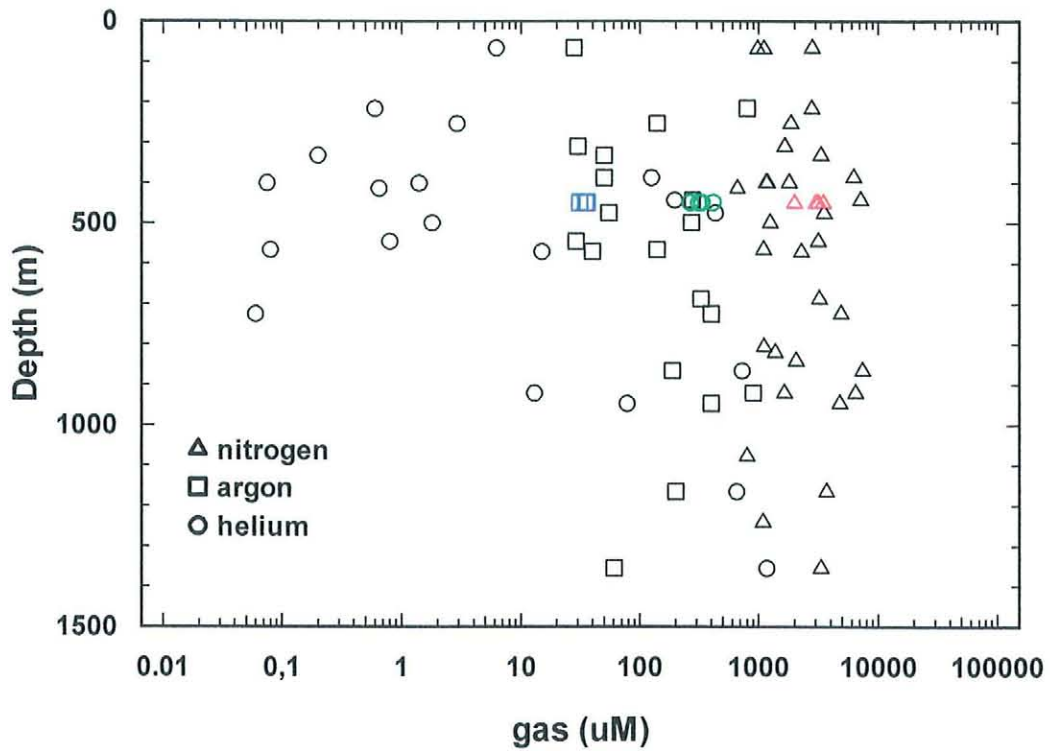
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Figure 4-3. *The content of dissolved gases in MICROBE and CHEMLAB boreholes (blue, green and red symbols), and all other gas data for Fennoscandian field sites (Table 4-1).*

4.1.4 Carbon content

Figure 4-2 shows the content of dissolved organic carbon (Table 3-1c) and inorganic carbon (Table 3-1b) in relation to data sampled for other Fennoscandian field sites. With exception for KJ0044F01, all data plot within the range of obtained data. We have no obvious explanation for the extreme DOC value in KJ0044F01 (table 3-1c), except for some kind of contamination. This borehole is used for CHEMLAB 2, and it is possible that the operation of the lab may have contaminated the borehole. Future analysis will show if this high DOC content is consistent in KJ0044F01.

4.1.5 Gas content and composition

The gas content of six gases from Table 3-2a is shown in Figure 4-3 in relation to gas data from other Fennoscandian field sites (Table 4-1). Nitrogen plot in the middle of the range of gas data obtained, while argon plot among the lower and helium among the higher values obtained. Carbon dioxide and hydrogen also plot average in the ranges while methane is in the upper part of the range observed. For methane, the very high data are from Oilkiluoto in Finland, and this site is not representative for methane in general, as judged from the other sites studied.

4.2 Microbiology

4.2.1 Total numbers of microorganisms

The total numbers of microorganisms plot in the lower range of what has been observed at the 500 m level. Two levels around 800 m in the Stripa borehole V2 showed similar values over a 4 years sample period (Pedersen and Ekendahl 1992).
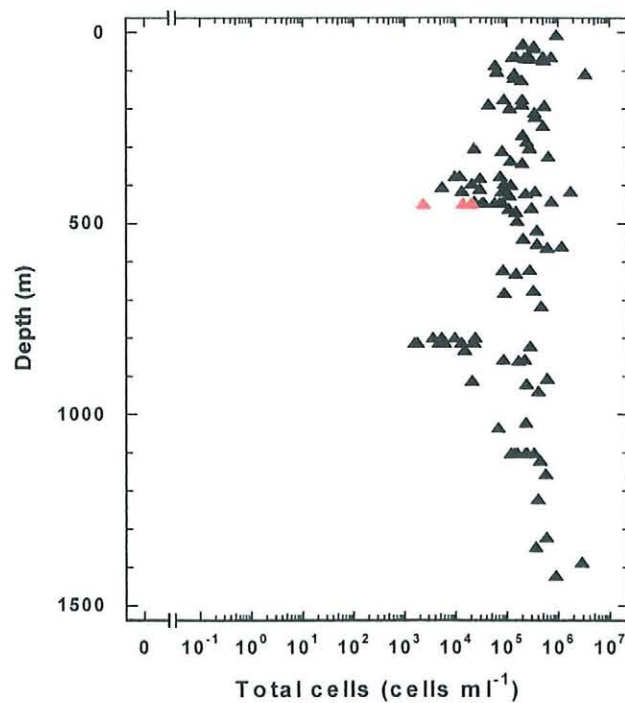

Figure 4-4. *Total number of microorganisms in the three MICROBE site boreholes (red symbols) compared to total numbers obtained.*
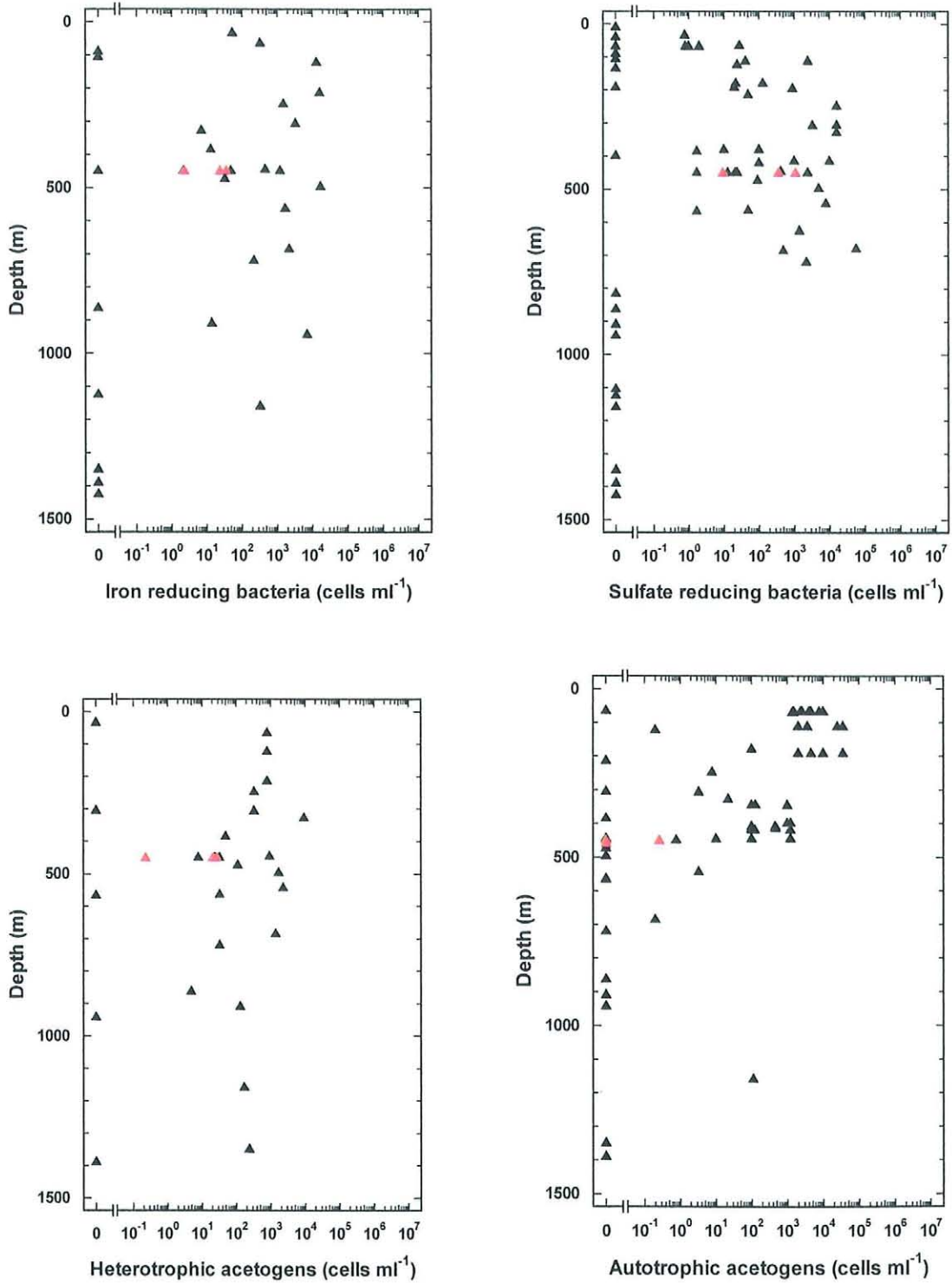
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Figure 4-5. Most probable numbers of physiological groups of microorganisms observed at the MICROBE site (red triangles). The figures show the average total number of each microbe group. Each observation consists of an MPN determination with three or five parallel tubes in the dilution series. Black triangles show data from other sites (confer Table 4-1).
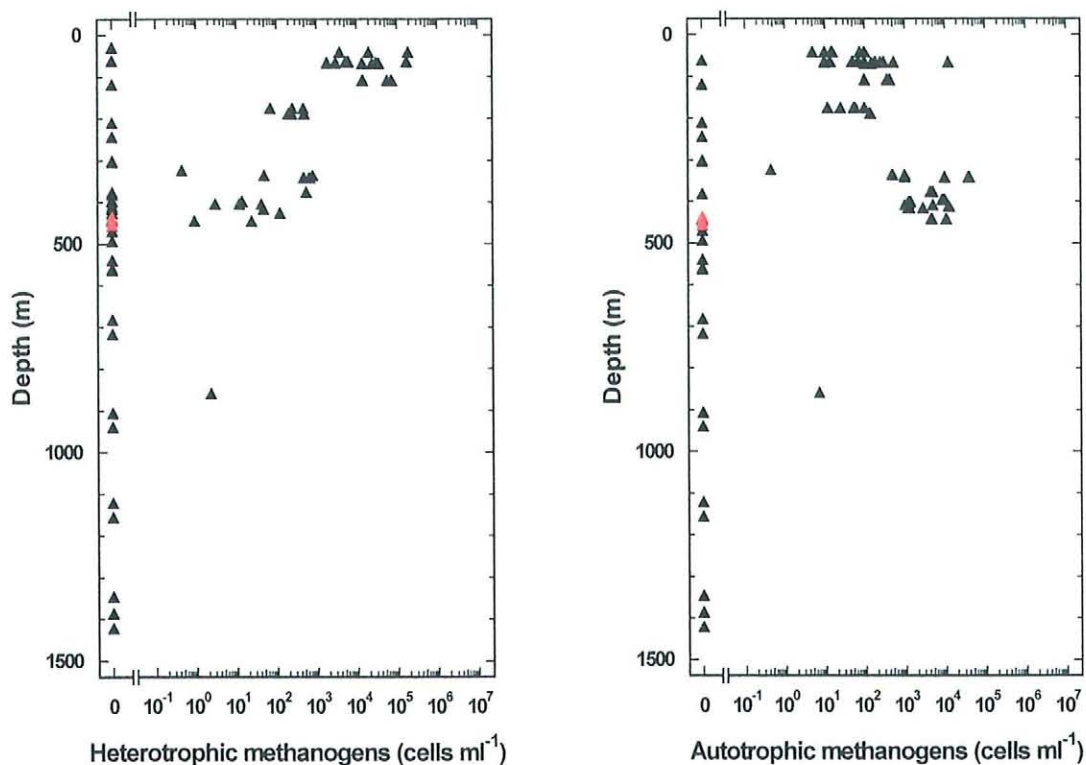
21

Figure 4-5. *(continued) Most probable numbers of physiological groups of microorganisms observed at the MICROBE site (red triangles). The figures show the average total number of each microbe group. Each observation consists of an MPN determination with three or five parallel tubes in the dilution series. Black triangles show data from other sites (confer Table 4-1).*

The MPN numbers of physiological groups of microbes are shown in figure 4-5. IRB, SRB and acetogens plot in the mid to lower range of what has been observed earlier. Culture for methanogens did not show positive results. It is not yet clear if this is due to actual lack of methanogens at the MICROBE site, or if we had problems with the suitability of the media used.

4.2.2 Stable isotopes

The stable isotope data (Fig. 4-6) indicates that the methane found is biogenic. This result does not contradict the absence of methanogens in the MPN analysis since it is possible that the methane was produced elsewhere in the rock system.
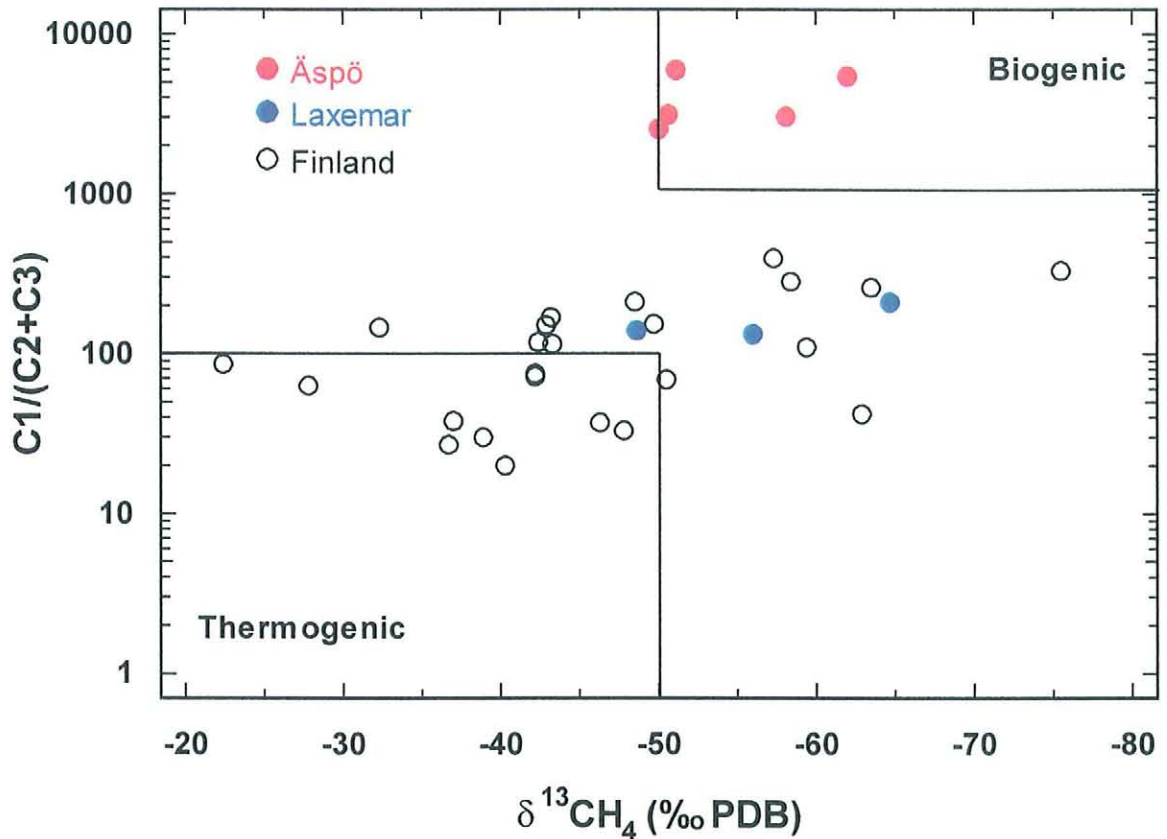
Figure 4-6. *Stable isotope values of* ^{13}C *in methane dissolved in the MICROBE and CHEMLAB site groundwaters (Table 3-3) compared to data obtained from other deep Fennoscandian groundwater (Table 4-1). The isotope value is plotted versus the quote of methane gas and the sum of ethane and propane (Table 3-3). Biogenic and thermogenic boxes indicate typical values for methane produced by microorganisms and deep mantle reactions, respectively.*

5 SITE DEVELOPMENT

A system that allows circulation of groundwater under full formation pressure is presently being designed. This system should enable work with microbes at very close to *in situ* conditions. It will hopefully be operative early spring 2001. A system for sensible measurement of hydrogen and other gases will be developed. We will use a reduction gas detector (Trace Analytical, USA), having a detection limit for hydrogen, carbon monoxide and methane close to 1 ppb. This system will be used for characterisation of hydrogen generation and flow in granitic rock environments. The results will be compared with theoretical calculations performed at KTH, Stockholm.
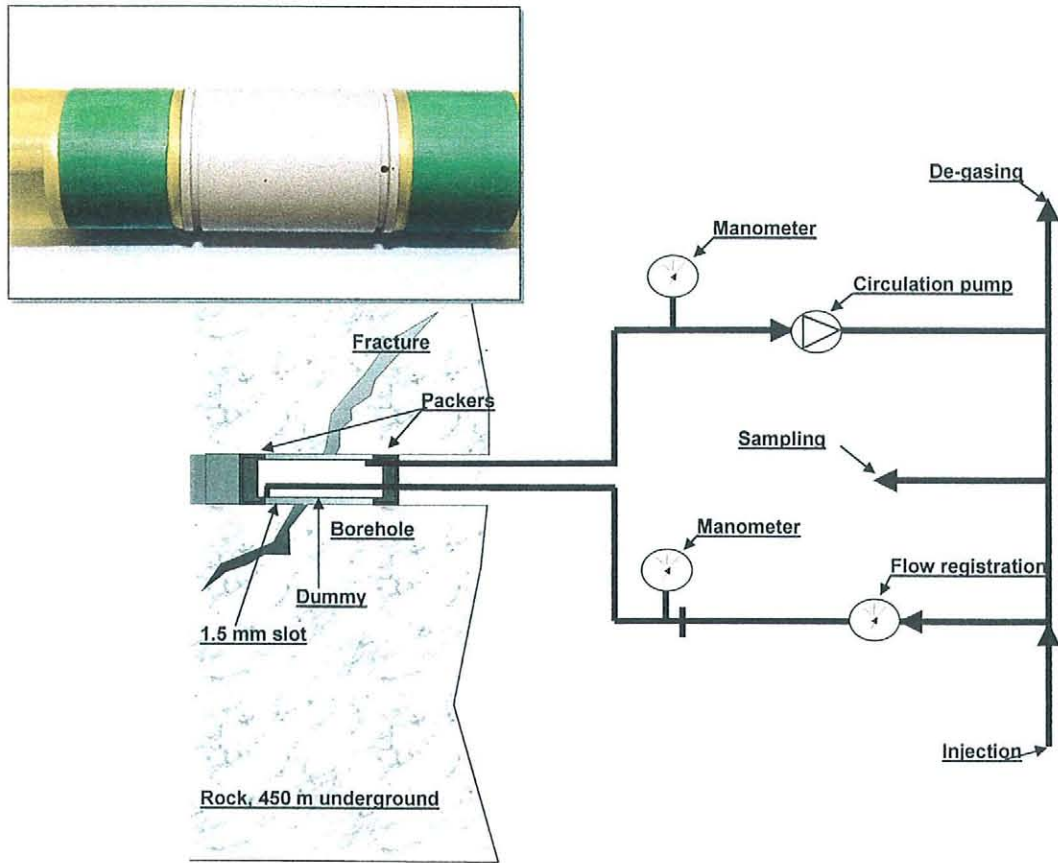
Figure 5-1. *The circulation system that is under development. (compare with figure 2-2).*

6 REFERENCES

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