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# Forsmark site investigation

# Total numbers and metabolic diversity of microorganisms in borehole KFM01A and KFM02A

## Results from three investigated sections, 110.1-120.8 m and 176.8-183.9 m in KFM01A and section 509.0-516.1 m in KFM02A

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# Contents

# 1 Introduction

This document reports performance of and results from microbe investigations in boreholes KFM01A and KFM02A within the site investigation programme in Forsmark /1/. The work was conducted according to the activity plans AP PF 400-03-08 and AP PF 400-03-09 (SKB internal controlling documents). The report presents microbiological data from three borehole sections:

- KFM01A, 110.1–120.8 m,
- KFM01A, 176.8–183.9 m,
- KFM02A, 509.0–516.1 m.

The sampling was carried out in February, April and October 2003 within the hydrochemical characterisation activities in KFM01A and KFM02A performed in accordance with activity plans AP PF 400-02-38 and AP PF 400-03-38 (SKB internal controlling documents) respectively. The sampling and the downhole sampling equipment are described in /2, 3/. Subsequent laboratory work was performed during 8–10 weeks after the samples reached the laboratory.

The flushing water used during the core drilling of the boreholes may cause contamination by foreign bacteria and thereby affect the in situ microbiological conditions. Control of the microbe content in the flushing water was performed during drilling of the two boreholes. The results are reported in /4, 5/.

# 2 Objective and scope

Microorganisms have been demonstrated in all investigated Fennoscandian shield groundwaters, at depths ranging from surface to 1700 m /6/. Active microorganisms influence the groundwater geochemistry /7/ and the redox potential /8/. Therefore, a full understanding of the geochemical situation in deep groundwater requires knowledge about presence, diversity and activity of microorganisms.

The microbiological analysis program was carried out according to protocols developed during previous investigations of Finnish groundwaters /9, 10/. They include determination of the total number of cells in the groundwater and a statistical cultivation method for numbering the most probable number (MPN) of cultivable metabolic groups of microorganisms. They are manganese, iron and sulphate reducing bacteria (MRB, IRB and SRB), autotrophic and heterotrophic acetogens and autotrophic and heterotrophic methanogens. A PVB sample container was filled with groundwater /2, 3/ and shipped to the laboratory in Göteborg within 4–6 h. Sub-sampling for analysis was performed immediately at arrival of the PVB vessel.

# 3 Equipment

## 3.1 Equipment for transfer of sample from the PVB vessel

The transfer of sample from the PVB vessel to the culturing tubes required a procedure that did not expose the sample to oxygen. This was solved by the design of an adapter (no 4 in Figure 3-1) that could be attached to the inlet/outlet of the PVB sampler (no 3 in Figure 3-1). Portions of 10 ml sample were distributed to nitrogen flushed Hungate tubes as shown in Figure 3-1. The pressurized PVB sampler automatically ejected sample when the sampling valves were opened (6 and 7 in Figure 3-1).

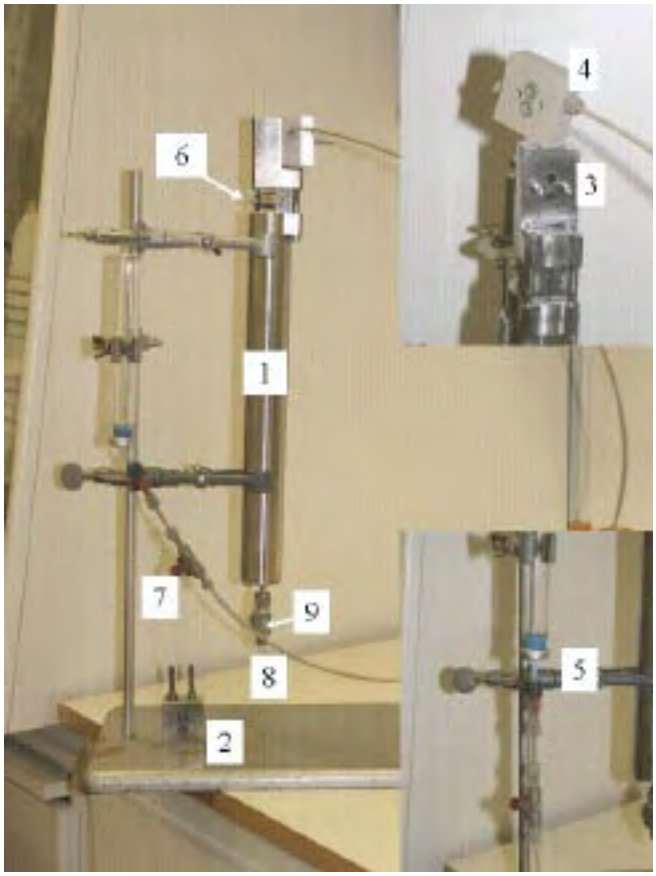


***Figure 3-1****. This setup was designed for oxygen-free transfer of samples from the PVB vessel (1) to nitrogen flushed, stoppered Hungate tubes (5). 1, PVB vessel; 2, transportation seal; 3, inlet/ outlet of the PVB; 4, PEEK sampling device; 5, transfer of sample to Hungate tubes, 6, PVB valves; 7, PEEK sampling valve; 8, PEEK sampling tube; 9, PVB pressure valve.*

## 3.2 Equipment for most probable number determination

The preparation of anaerobic media required an anaerobic box and a gas bench for mixing and delivery of gas mixes ands gases for growth as described in detail in the activity plans. Typically, the preparation time for one sample delivery corresponded to about two weeks full time work in the laboratory. The dilution and inoculation of samples for analysis of metabolic groups followed a well defined procedure, depicted in Figure 3-2. One set of 45 tubes was used for each analysis. Incubation at about 17°C was performed next. Finally, each tube was analyzed for presence of metabolic products typical for the respective metabolic group cultivated. They were: manganese reducing bacteria: $Mn^{2+}$, iron reducing bacteria: $Fe^{2+}$, sulphate reducing bacteria: $S^{2-}$, autotrophic and heterotrophic acetogens: acetate, autotrophic and heterotrophic methanogens: methane.

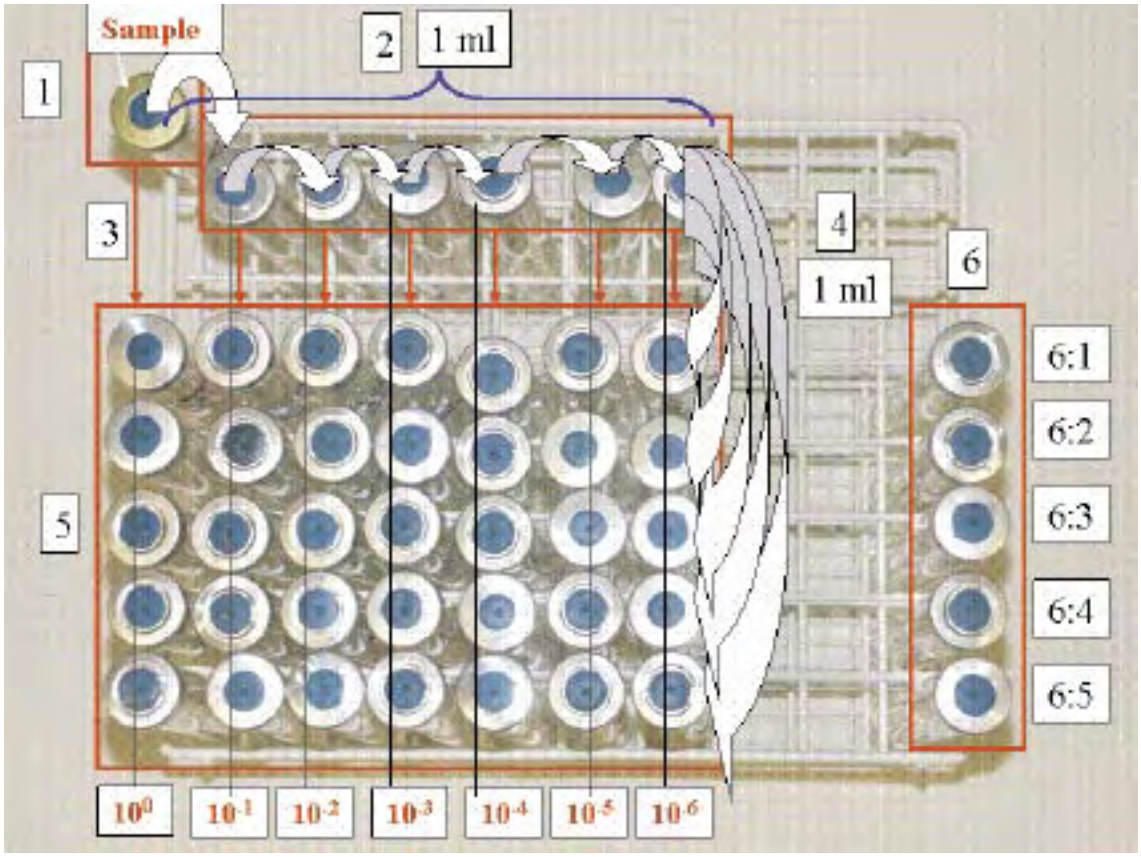


***Figure 3-2.*** *The procedure for a most probable number determination. The Hungate tube with sample is used as the source for inoculation (1). A serial dilution was performed first (2). Thereafter, sub-samples were transferred (3–4) to the growth tubes (5) and to control tubes (6).*

# 4 Performance

The microbial characterizations were performed according to activity plans AP AP PF 400-03-08 and AP PF 400-03-09. Details can be obtained from the appendices attached to those plans.

## 4.1 Sample transport

Sample transport went very well and all samples arrived in time for analysis.

## 4.2 Preparations of media

The media includes a redox indicator that turns pink when the redox potential goes above –40 mV (relative a $H_2$ electrode). Such tubes are not used if they appear. This guarantees anoxic cultivation conditions. Controls for the media and the inoculation procedure are included (Figure 3-2, 6-1 to 6-5).

## 4.3 Analysis

Acetate producing microorganisms were unfortunately not analysed in KFM01A. This was due to a problem with the acetate analysis kit resulting in negative values irrespective of the acetate concentration. New kits were applied on the cultures from KFM02A.

The sensitivity of the thermal conductivity detector (TCD) for methane was found to be too low for detection of autotrophic methanogens in KFM01A. This problem was solved for the KFM02A samples, by application of a flame ionisation detector (FID).

## 4.4 Nonconformities

The activity was performed according to the activity plans AP PF 400-03-08 and AP PF 400-03-09 with the following deviations:

- Acetate producing microorganisms were not analysed in KFM01A.
- Autotrophic methanogens were not detected in KFM01A due to not low enough detection limit of methane.

# 5 Data handling and interpretations

## 5.1 Analyses and interpretation

The total numbers of microorganisms are counted on duplicate filtration filters from three or four sample tubes. Each filter is regarded as one independent observation. The mean of 6–8 filters from 3–4 tubes is calculated and reported with the standard deviation (SD and the number of observations (n).

The MPN procedure results in a scheme with tubes that score positive or negative growth. Combinations of three dilutions are used to calculate the most probable number of respective group, as described elsewhere /11/.

## 5.2 Nonconformities

The data handling and interpretations were performed according to the activity plans AP PF 400-03-08 and AP PF 400-03-09 without any deviations that can affect the quality of the data.

# 6 Results

The total numbers of cells were similar in all three sections, and averaged at $5.2\times10^4$ cells $ml^{-1}$ (Table 6-1) which is similar to what has been observed elsewhere in deep groundwater /6, 7/.

There was a clear trend towards decreasing numbers for all groups of cultivable microorganisms with depth. The shallowest section, KFM01A 110–120 m, showed a very large proportion of cultivable cells. In addition, this was the only section with detectable manganese reducing bacteria. Low numbers of heterotrophic methanogens were found, but heterotrophic acetogens were absent.

Autotrophic microorganisms were not detected. Those groups of organisms are very difficult to cultivate. It is possible that the sampling and transportation procedures are detrimental to their viability. But, it is certainly also possible that those organisms are absent in the sample.

**Table 6-1. Total number of cells in the analysed sections of KMF01A and KMF02A.**

| Borehole (section) | Cells ml–1<br>Total number of cells | Standard deviation | Number of observations |
|---|---|---|---|
| KFM01A<br>(110.1–120.8) | 5.8x104 | ± 2.5x104 | 8 |
| KFM01A<br>(176.8–183.9) | 3.9x104 | ± 2.3x104 | 7 |
| KFM02A<br>(509.0–516.6) | 5.9x104 | ± 2.4x104 | 6 |

**Table 6-2. Most probable number (MPN) of metabolic groups of microorganisms in KMF01A, section 110.1–120.8.**

| Metabolic groups | Cells ml–1<br>MPN | Lower – upper<br>95% confidence limits |
|---|---|---|
| Iron reducing bacteria | 4000 | 1000–15000 |
| Manganese reducing bacteria | 3000 | 1000–13000 |
| Sulphate reducing bacteria | 1.2 | 0.5–2.9 |
| Autotrophic methanogens | n.a.* | – |
| Heterotrophic methanogens | 1.2 | 0.5–2.9 |
| Autotrophic acetogens | n.a. | – |
| Heterotrophic acetogens | n.a. | – |

* not analysed

**Table 6-3. Most probable number (MPN) of metabolic groups of microorganisms in KMF01A, section 176.8–183.9.**

| Metabolic groups | Cells ml–1 MPN | Lower – upper 95% confidence limits |
|---|---|---|
| Iron reducing bacteria | 4 | 2–14 |
| Manganese reducing bacteria | < 0.2 | – |
| Sulphate reducing bacteria | 0.2 | 0.1–1.1 |
| Autotrophic methanogens | n.a.* | – |
| Heterotrophic methanogens | 1.7 | 0.7–4.0 |
| Autotrophic acetogens | n.a. | – |
| Heterotrophic acetogens | n.a. | – |

* not analysed

**Table 6-4. Most probable number (MPN) of metabolic groups of microorganisms in KMF02A, section 509.0–516.6.**

| Metabolic groups | Cells ml–1 | MPN Lower – upper 95% confidence limits |
|---|---|---|
| Iron reducing bacteria | 11 | 4–30 |
| Manganese reducing bacteria | < 0.2 | – |
| Sulphate reducing bacteria | 1.4 | 0.6–3.5 |
| Autotrophic methanogens | < 0.2 | – |
| Heterotrophic methanogens | 0.8 | 0.3–2.4 |
| Autotrophic acetogens | 0.9 | 0.3–2.4 |
| Heterotrophic acetogens | 160 | 60–530 |

**Table 6-5. The percentage of the total number of cells (Table 6-1) cultured with MPN (Tables 6-2–6-4) in the analysed sections in KMF01A and KFM02A.**

| Borehole (section, m) | Cells cultured (%) MPN | Lower – upper 95% confidence limits |
|---|---|---|
| KMF01A (110.1–120.8) | 12.0 | 3.5–48.3 |
| KMF01A (176.8–183.9) | 0.015 | 0.005–0.32 |
| KMF02A (509.0–516.6) | 0.29 | 0.11–0.96 |

# 7 Conclusions

The data obtained compare well with earlier obtained data, using similar sampling and analysis methods /6–11/.

- The total numbers of cells plot as average numbers if compared to the database for the Fennoscandian shield.
- IRB were higher than usually observed in the shallowest borehole section.
- MRB has not been tested for earlier. The numbers were high in the 110.1–120.8 m section. MRB was not detected at the deep levels.
- SRB seem to be ubiquitous. They are always found, as here, down to depths about 500–600 m. Here, the numbers were lower than commonly observed.
- Heterotrophic methanogens proliferate on one- and two-carbon compounds that should show up as TOC in the chemistry analysis. They appeared at all levels. Autotrophic methanogens could not be demonstrated at the 509.0–516.1 m level.
- Acetogens have been found in high numbers in the Äspö area /6, 7/ and heterotrophic acetogens were common in deep boreholes /9, 10/. Heterotrophic acetogens proliferate on one-carbon compounds that should show up as TOC in the chemistry analysis. Their presence in KMF02A was expected.
- The percentages of cultivable cells were unusually high in KMF01A at 110.1–120.8 m. Else the percentages compare well with earlier obtained data /6–11/ where the majority of the values distributed in the interval between 0 and 1%.

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