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Forsmark site investigation

Total numbers and metabolic diversity of microorganisms in borehole KFM03A

Results from section 448–453 m

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Contents

1 Introduction

This document reports the performance and results from microbe investigations in the near-vertical borehole KFM03A within the site investigation programme in Forsmark /1/. The work was conducted according to the activity plan AP PF 400-04-37 (SKB internal controlling document). The report presents microbiological data from borehole section 448–453 m.

The sampling was carried out in April 2004 within the hydrochemical characterisation activities in KFM03A according to the activity plan AP PF 400-04-63 (SKB internal controlling document). The sampling and the down-hole sampling equipment are described in /2/. Subsequent laboratory work was performed during 8–10 weeks after the samples reached the laboratory. Table 1-1 gives the data references to where the obtained results are stored.

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 three boreholes, KFM01A, KFM02A and KFM04A but not KFM03A. The results from these other boreholes are reported in /3, 4/.

Table 1-1. Data references.

Subactivity	Database	Identity number
Microbe investigation	SICADA	Field note no Forsmark 253

2 Objective and scope

Microorganisms have been demonstrated in every groundwater investigated in the Fennoscandian shield, at depths ranging from surface to 1,700 m /5/. Active micro-organisms influence the groundwater geochemistry /6/ and the redox potential /7/. 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 groundwater /6, 8, 9/. They include determination of the total number of cells in the groundwater (AODC) and a statistical cultivation method for numbering the most probable number of cultivable metabolic groups of micro-organisms (MPN). These are manganese, iron and sulphate reducing bacteria, autotrophic and heterotrophic acetogens and autotrophic and heterotrophic methanogens. Since the methodology to investigate microbes improves over time, this report also includes a new method to measure the total amount of micro-organisms, ATP measurements.

3 Equipment

3.1 Equipment for transfer of sample from the PVB vessel

A PVB sample container was filled with groundwater /2/ and sent to the laboratory in Göteborg within 4–6 h. Sub-sampling for analysis was performed immediately at arrival of 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 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).

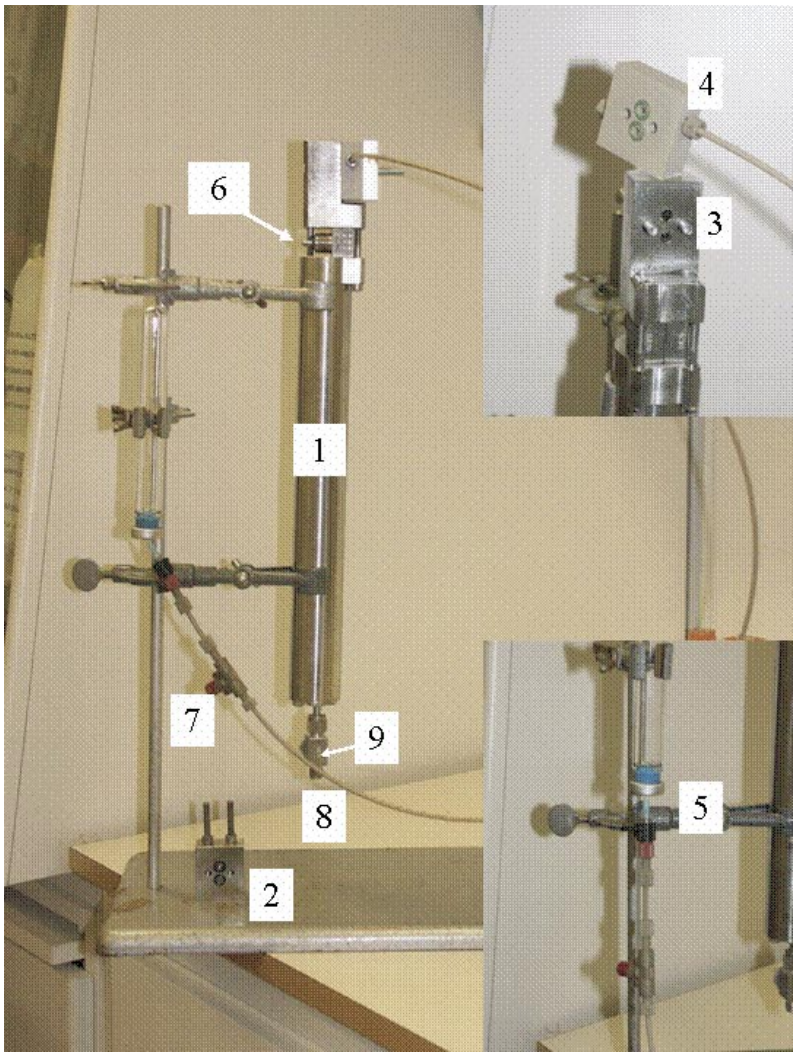

Figure 3-1. *This setup was designed for oxygen-free transfer of samples from the PVB vessel (1) to nitrogen flushed, stopped Hungate tubes (5). 1, PVB vessel; 2, transportation seal; 3, inlet/outlet of the PVB; 4, PEEK sampling device; 5, transfer of sample to Hungate tubes, 6, PVB valves; 7, PEEK sampling 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 mixtures and 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. Those were: manganese reducing bacteria: Mn^{2+}, iron reducing bacteria: Fe^{2+}, sulphate reducing bacteria: S^{2-}, autotrophic and heterotrophic acetogens: acetate and autotrophic and heterotrophic methanogens: methane.

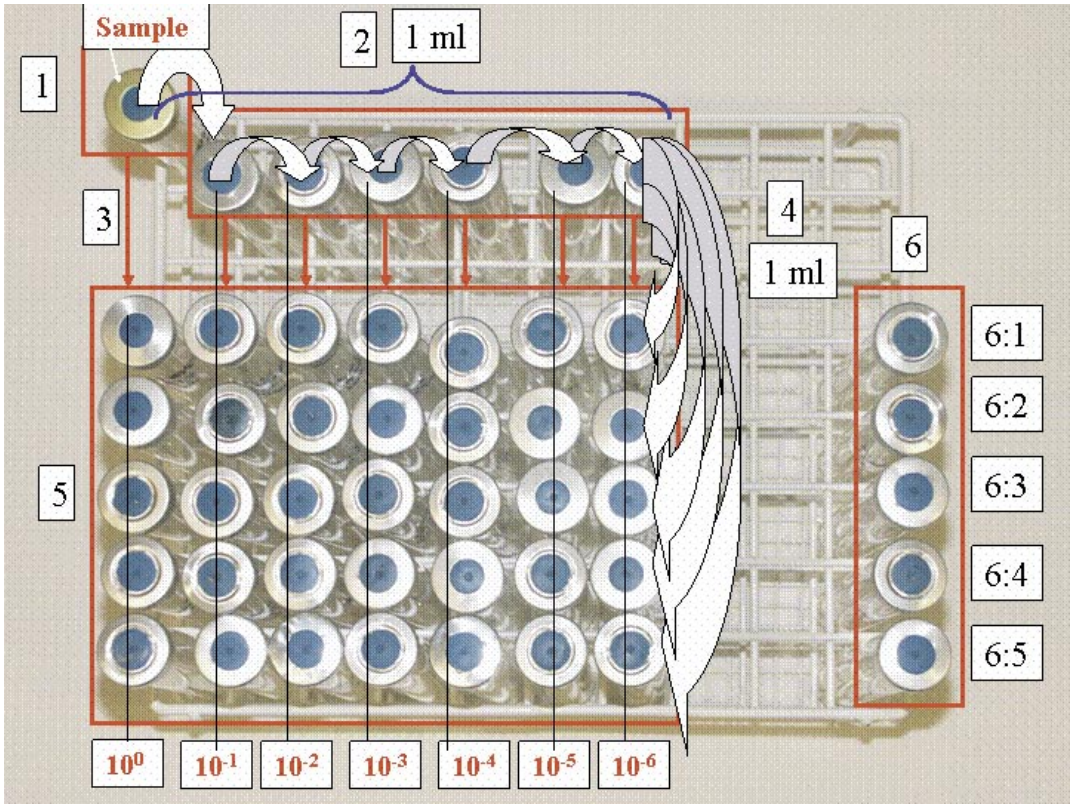

Figure 3-2. *The procedure for a "most probable number" determination. The Hungate tube with sample is used as the source for inoculation (1). A serial dilution was performed first (2). Thereafter, sub-samples were transferred (3-4) to the growth tubes (5) and to control tubes (6).*

4 Performance

The microbial characterizations were performed according to activity plans AP 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 included 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 were included (Figure 3-2, 6-1 to 6-5).

4.3 Analysis

From this section ATP measurements were used as a complement to the AODC for the total number of cells.

5 Data handling

5.1 Analyses and interpretation

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

The ATP measurements were performed three times for each sample from the different depths. The mean of the three samples was calculated and reported with the standard deviation (SD).

The MPN procedure results in a scheme with tubes that score positive or negative for growth. Combinations of three dilutions are used to calculate the most probable number of respective group, as described elsewhere /10/.

6 Results

The mean value for the total numbers of cells was 1.0×10^5 cells ml^{-1} (Table 6-1) which is similar to what has been observed elsewhere in deep groundwater /5, 6/.

The total number of cells derived from the ATP method gave a somewhat higher value than obtained from AODC although the MPN values indicated a low activity in this section (Table 6-1).

The percentage cultivable cells were low at this section with 0.28%. The most abundant group of organisms were the heterotrophic methanogens and acetogens with 90 ml^{-1} for both groups (Table 6-2). Of the respiring bacteria the manganese reducers were dominating with 70 ml^{-1}. Iron- and sulphate reducers were present but in low numbers (Table 6-2). Autotrophic methanogens and acetogens were found in very low numbers only.

Table 6-1. Total number of cells in the analysed section of KMF03A.

Borehole (section)	Total counts (cells ml^{-1})					
	AODC	Standard deviation	Number of observations	ATP	Standard deviation	Number of observations
KFM03A (448–453 m)	1.0×10^5	$\pm 1.8 \times 10^4$	5	2.8×10^5	$\pm 8 \times 10^3$	3

Table 6-2. Most probable number (MPN) of metabolic groups of microorganisms in KMF03A, section 448–453 m.

Metabolic groups	Cells ml^{-1}	
	MPN	Lower – upper 95% confidence limits
Iron reducing bacteria	11	4–30
Manganese reducing bacteria	70	30–210
Sulphate reducing bacteria	17	7–48
Autotrophic methanogens	0.7	0.7–4.6
Heterotrophic methanogens	90	30–290
Autotrophic acetogens	0.8	0.3–2.4
Heterotrophic acetogens	90	30–290

Table 6-3. The percentage of the total number of cells (Table 6-1) cultured with MPN (Table 6-2) in the section 448–453 m in KMF03.

Borehole (section, m)	Cells cultured (%)	
	MPN	Lower – upper 95% confidence limits
KFM03A (448–453 m)	0.28	0.11–0.88

7 Conclusions

The data obtained compare well with earlier obtained data, using similar sampling and analysis methods /5–9/.

- The total numbers of cells plot as average numbers if compared to the database for the Fennoscandian shield.
- The ATP measurements in this section gave a somewhat high total number of cells value. This would indicate a high metabolic activity in the section but the MPN numbers could not verify this.
- The amount of cultivable organisms in section 446–453 was low.
- MRB together with heterotrophic methanogens and acetogens showed the highest MPN numbers.
- SRB and IRB were present but in low numbers.
- Autotrophic organisms were very few in this section.

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