P-04-89

Forsmark site investigation

Total numbers and metabolic diversity of microorganisms in borehole KFM03A

Results from three investigated borehole sections, 639.0 – 646.1m, 939.5 – 946.6 m and 980.0 – 1001.2 m.

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June 2004

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ISSN 1651-4416
SKB P-04-89

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Keywords: Groundwater, Microorganisms, Iron reduction, Sulphate reduction, Acetogen, Methanogen, Total number, Forsmark, AP-PF-400-03-09, Field note No. Forsmark 237.

This report concerns a study which was conducted for SKB. The conclusions and viewpoints presented in the report are those of the authors and do not necessarily coincide with those of the client.

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Contents

1 Introduction

This document reports performance and results from microbe investigations in borehole KFM03A within the site investigation programme in Forsmark /1/. The work was conducted according to the activity plan AP PF 400-03-09 (SKB internal controlling document). The report presents microbiological data from three borehole sections:

- KFM03A, 639.0–646.1 m
- KFM03A, 939.5–946.6 m
- KFM03A, 980.0–1001.2 m

The sampling was carried out in December 2003 and in February and March 2004 within the hydrochemical characterisation activities in KFM03A according to the activity plan AP PF 400-03-63 (SKB internal controlling document). The interval at 448.0-455.6 m was sampled in April as the last section in KFM03A and the microbe results will be included in a second version of this report. 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.

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

Table 1-1. Data references.

Subactivity	Database	Identity number
Microbe investigation	SICADA	Field Note No. 237

2 Objective and scope

Microorganisms have been demonstrated in every groundwater investigated in the Fennoscandian shield, at depths ranging from surface to 1700 m /5/. Active micro-organisms influence the groundwater geochemistry /5/ and the redox potential /2/. 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 /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 and two new physiological groups of bacteria are included in the MPN analyses; methane oxidizing bacteria, type I and II.

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.

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 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).

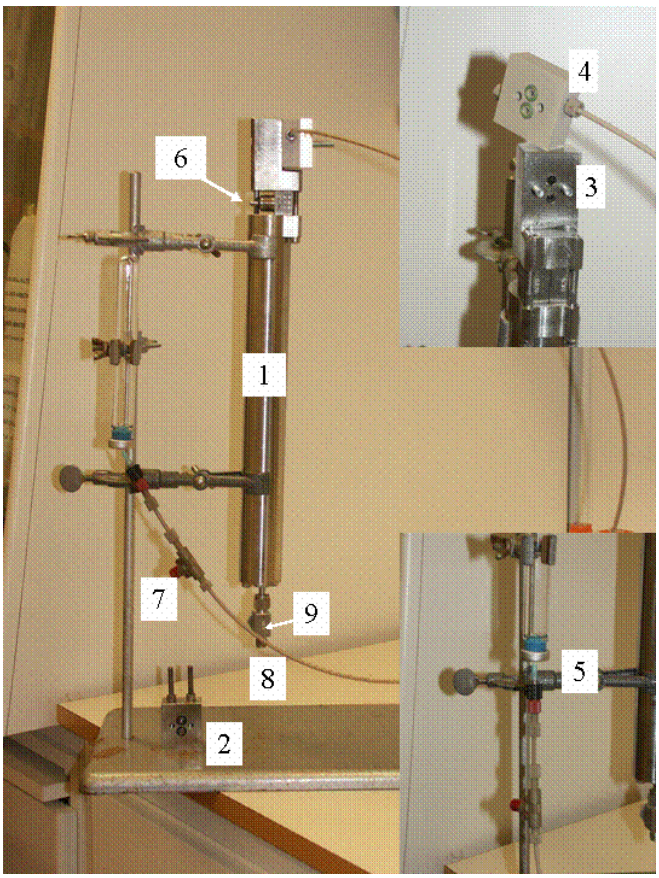

Figure 3-1. *This setup was designed for oxygen-free transfer of samples from the PVB vessel (1) to nitrogen flushed, stopped Hungate tubes(5). 1, PVB vessel; 2, transportation seal; 3, inlet/outlet of the PVB; 4, PEEK sampling device; 5, transfer of sample to Hungate tubes, 6, PVB valves; 7, PEEK sampling 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, autotrophic and heterotrophic methanogens: methane and methane oxidizing bacteria, group I and II, turbidity and molecular probes.

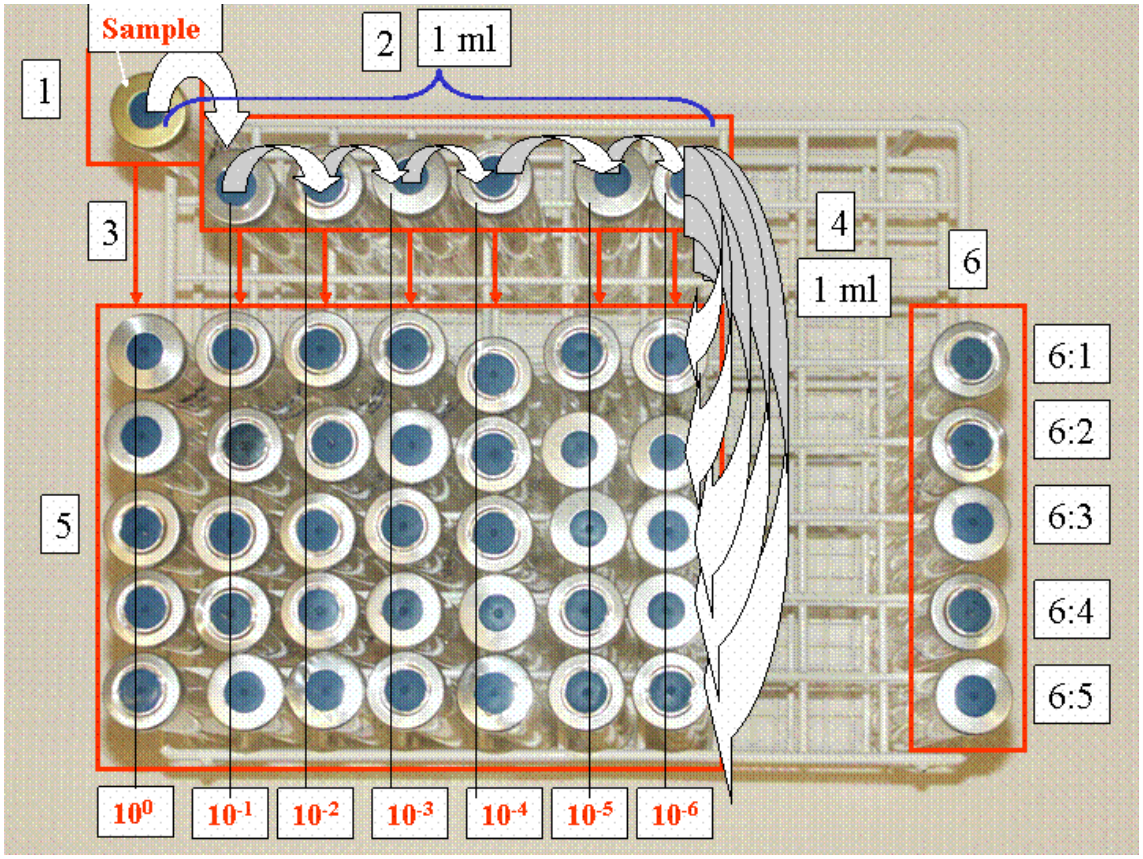

Figure 3-2. *The procedure for a "most probable number" determination. The Hungate tube with sample is used as the source for inoculation (1). A serial dilution was performed first (2). Thereafter, sub-samples were transferred (3-4) to the growth tubes (5) and to control tubes (6).*

4 Performance

The microbial characterizations were performed according to activity plan AP PF 400-03-09. Details can be obtained from the appendices attached to this plan.

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 now on and in the following investigations MPN for methane oxidizing bacteria is included together with ATP measurements as a complement to the AODC for the total number of cells. ATP was not analyzed in section 980.0 – 1001.2 m.

5 Nonconformities

The microbe studies in groundwater from three sections of KFM03A was conducted according to the activity plan AP PF 400-03-09 without nonconformities.

6 Data handling

6.1 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 growth. Combinations of three dilutions are used to calculate the most probable number of respective group, as described elsewhere /10/.

7 Results

The total numbers of cells were similar in all three sections, and averaged at 5,3 x 10^4 cells ml^{-1} (Table 7-1) which is similar to what has been observed elsewhere in deep groundwater /5, 6/.

The total number of cells derived from the new ATP method correlates well with the number obtained from AODC at the shallowest depth. In the section 939.5-946.1 m, however, the ATP value gives a higher total number of cells probably due to a very high activity in this section. The high percentage cultivable cells at this depth, 48 % (Table 7-5) also indicate this.

The middle section, 939.5-946.1 m, which is more close to the deepest, 980.0–1001.2 m, showed a very large proportion of cultivable cells. They were mostly sulphate reducers and acetogens. The other two sections had portions of cultivable cells more in accordance with previously reported values.

Iron reducing bacteria were found only in the shallowest section but in low numbers. Manganese reducers were low in numbers or absent in all three sections.

Methanogens were found in low numbers in all sections. They were mostly heterotrophic. The highest number of heterotrophic methanogens was found in middle section even though only 17 per ml.

Table 7-1. Total number of cells in the analysed sections in KFM03A.

Borehole (section)	Total counts (cells ml^{-1})					
	AODC	Standard deviation	Number of obser-vations	ATP	Standard deviation	Number of obser-vations
KFM03A (639.0 – 646.1 m)	2.1 x 10^4	-	1	4.6 x 10^4	±3 x 10^4	3
KFM03A (939.5 – 946.6 m)	6.1 x 10^4	±1.6 x 10^4	6	2.4 x 10^5	±2.5 x 10^4	3
KFM03A (980.0 – 1001.5 m)	5.8 x 10^4	±4.2 x 10^3	6	n.a. *	-	-

** not analysed*

Table 7-2. Most probable number (MPN) of metabolic groups of microorganisms in KFM03A, section 639.0 – 646.1 m.

Metabolic groups	Cells ml^{-1}	
	MPN	Lower - upper 95 % confidence limits
Iron reducing bacteria	22	10-58
Manganese reducing bacteria	1.7	0.7-4.6
Sulphate reducing bacteria	30	10-120
Autotrophic methanogens	0.4	0.1-0.7
Heterotrophic methanogens	1.3	0.5-3.8
Autotrophic acetogens	11	4-30
Heterotrophic acetogens	17	8-41
Methane oxidizers, type I	<0.2	-
Methane oxidizers, type II	<0.2	-

Table 7-3. Most probable number (MPN) of metabolic groups of microorganisms in KFM03A, section 939.5 – 946.6 m.

Metabolic groups	Cells ml^{-1}	
	MPN	Lower - upper 95 % confidence limits
Iron reducing bacteria	<0.2	-
Manganese reducing bacteria	1.2	0.5-2.9
Sulphate reducing bacteria	5000	2000-20000
Autotrophic methanogens	<0.02	-
Heterotrophic methanogens	17	7-48
Autotrophic acetogens	900	300-2900
Heterotrophic acetogens	23000	9000-86000
Methane oxidizers, type I	23.0	9.00-86.0
Methane oxidizers, type II	2.00	1.00-10.0

* not analysed

Autotrophic micro-organisms were detected in all three sections with as much as 900 autotrophic acetogens in section 939.5-946.6 m. This section was the latest sampled and the relatively higher amounts of cultivable organisms in this section might to some extent be triggered by an improvement introduced in the cultivation technique for acetogens and methanogens, i.e. flushing of all syringes with hydrogen sulphide.

Methane oxidizers have not been included earlier in the site investigation. They were present in the two deepest sections and it was type I that had the highest numbers with 35 per ml in section 980.0-1001.2 m.

Table 7-4. Most probable number (MPN) of metabolic groups of microorganisms in KFM03A, section 980.0 – 1001.2.

Metabolic groups	Cells ml^{-1}	
	MPN	Lower - upper 95 % confidence limits
Iron reducing bacteria	<0.2	-
Manganese reducing bacteria	<0.2	-
Sulphate reducing bacteria	24	10-94
Autotrophic methanogens	<0.02	-
Heterotrophic methanogens	5	2-17
Autotrophic acetogens	30	10-130
Heterotrophic acetogens	2.2	0.9-5.6
Methane oxidizers, type I	35.0	16.0-82.0
Methane oxidizers, type II	0.70	0.20-2.1

Table 7-5. The percentage of the total number of cells (Table 7-1) cultured with MPN (Tables 7-2 – 7-4) in the analysed sections in KFM03A.

Borehole (section, m)	Cells cultured (%)	
	MPN	Lower – upper 95 % confidence limits
KFM03A (639.0 – 646.1 m)	0.40	0.16-1.2
KFM03A (939.5 – 946.6 m)	48	18-164
KFM03A (980.0 – 1001.2 m)	0.16	0.06-0.57

8 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 amount of cultivable organisms in section 939.5-946.1 m was higher than usually observed. The most abundant organisms were sulphate reducers and autotrophic and heterotrophic acetogens.

- The ATP measurements in section 639.5-646.6 m gave a good agreement with the AODC method. In section 939.5-946.1 m, the ATP method gave a higher total number of cells probably related to a high microbial activity at this depth.

- Numbers of IRB were highest in section 639.5-646.6 m but still low in comparison with some shallower ground water.

- Very low numbers of MRB were found in all sections.

- In section 939.5-946.1 m, SRB were among the proliferating organisms. They were also present at the other two investigated depths but in much lower numbers.

- The largest amount of methanogens was found in section 939.5-946.1 m and was mostly heterotrophic. Heterotrophic methanogens were detected in the other sections. Autotrophic methanogens could be detected in tiny amounts only in section 639.5-646.6 m.

- Heterotrophic acetogens were found in very high numbers in section 939.5-946.1 m but also a large amount of autotrophic acetogens could be cultivated from this section. The acetogens was the dominating group of organisms at this level and their numbers were about 50% of the total number of cells counted. Both types of acetogens were found in the other sections investigated but in small amounts.

- For the first time methane oxidizers were included in the investigation. Type I was found in the two deepest sections but very few type II could be detected in these sections. In the shallowest section none of them was found.

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