P-04-276

Oskarshamn site investigation

Total numbers and metabolic diversity of microorganisms in borehole KSH01A

Results from three investigated sections, 158.5–167 m, 245–261.6 m and section 548–565 m

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March 2004

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ISSN 1651-4416
SKB P-04-276

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Keywords: Groundwater, Microorganisms, Iron reduction, Sulphate reduction, Acetogen, Methanogen, Total number, Oskarshamn, Simpevarp.

Abstract

A geochemical characterisation of groundwater is included in the site investigation of Simpevarp, and within this a microbial part. The microbial part consists of determination of the total amount of microorganisms together with a method to determine the amount of organisms that belong to different physiological groups, “the most probable number method” (MPN). The investigation included 7 different groups, iron-, manganese- and sulphate-reducing bacteria, auto- and heterotrophic methanogens and auto- and heterotrophic acetogens. Samples were taken from three sections in the borehole KSH01A, 156.5–167 m, 245–261.6 m and 548–565 m.

The total amount of microorganisms varied between 7.2×10^4 and 1.4×10^5 with a decrease with depth. This corresponds with earlier observations from the Fennoscandian Shield. In the section 156.5–167 m, sulphate-reducing bacteria dominated, 160 per milliliter groundwater, but neither methanogens nor acetogens were analysed du to problems with the analyses. In the section 245–261.6 m, heterotrophic methanogens and acetogens were dominating. Values for autotrophic acetogens were missing here. The deepest section had few organisms in general but acetogens, both auto- and heterotrophic showed the highest values with 30 and 17 per milliliter groundwater, respectively.

Sammanfattning

I den geokemiska karakteriseringen av grundvatten i samband med platsundersökning i Simpevarp ingår en mikrobiell del. Denna del omfattar bestämning av den totala mängden mikroorganismer samt en metod att bestämma mängden av olika fysiologiska grupper av mikroorganismer. Metoden kallas ”most probable number” (MPN). I undersökning ingick de 7 olika grupperna, järn-, mangan och sulfatreducerande bakterier, auto- och heterotrofa metanogener och auto- och heterotrofa acetogener. Provtagningarna gjordes i 3 sektioner i borrhålet KSH01A, 156,5–167 m, 245–261,6 m och 548–565 m.

Det totala antalet mikroorganismer varierade mellan 7.2×10^4 och 1.4×10^5 och minskade med djupet vilket tidigare observerats i grundvatten från den Fennoskandiska skölden. I sektionen 156,5–167 m dominerade sulfatreducerande bakterier, 160 per milliliter, men varken metanogener eller acetogener analyserades här på grund av problem med analyserna. I sektionen 245–261,6 m var heterotrofa metanogener och acetogener de dominerande grupperna. Värden för autotrofa acetogener saknades här. Den djupaste sektionen innehöll få organismer generellt men acetogener, både auto- och heterotrofer, visade det högsta antalet, 30 respektive 17 per milliliter grundvatten.

Contents

1 Introduction

This document reports performance and results from the activity “Fullständig kemi-karakterisering med mobilt fältlaboratorium i KSH01A”. The work was conducted according to the activity plan AP PS 400-02-033. The report presents microbiological data from three borehole sections:

- KSH01A, 156.5–167 m.
- KSH01A, 245–261.6 m.
- KSH01A, 548–565 m.

The field work was carried out in April, May and September 2003, respectively. Subsequent laboratory work was performed during 8–10 weeks after the samples reached the laboratory.

2 Objective and scope

Microorganisms have been demonstrated in all investigated Fennoscandian shield groundwaters, at depths ranging from surface to 1,700 m /1/. Active microorganisms influence the groundwater geochemistry /2/ and the redox potential /3/. 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 /4, 5/. They include determination of the total number of cells in the groundwater and a statistical cultivation method for numbering the most probable number of cultivable metabolic groups of microorganisms. They are iron, manganese and sulphate reducing bacteria, autotrophic and heterotrophic methanogens and autotrophic and heterotrophic acetogens. A PVB sample container was filled with groundwater and shipped to the laboratory in Göteborg within 4–6 h. Subsampling 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).

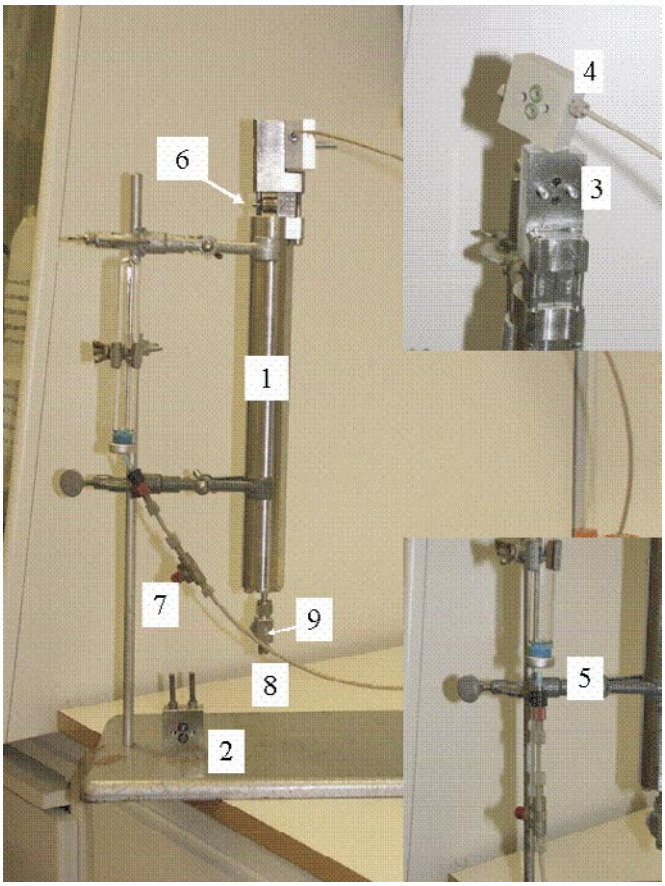

Figure 3-1. *This setup was designed for oxygen-free transfer of samples from the PVB vessel (1) to nitrogen flushed, stoppered Hungate tubes (5). 1, PVB vessel; 2, transportation seal; 3, inlet/outlet of the PVB; 4, PEEK sampling device; 5, transfer of sample to Hungate tubes, 6, PVB valves; 7, PEEK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

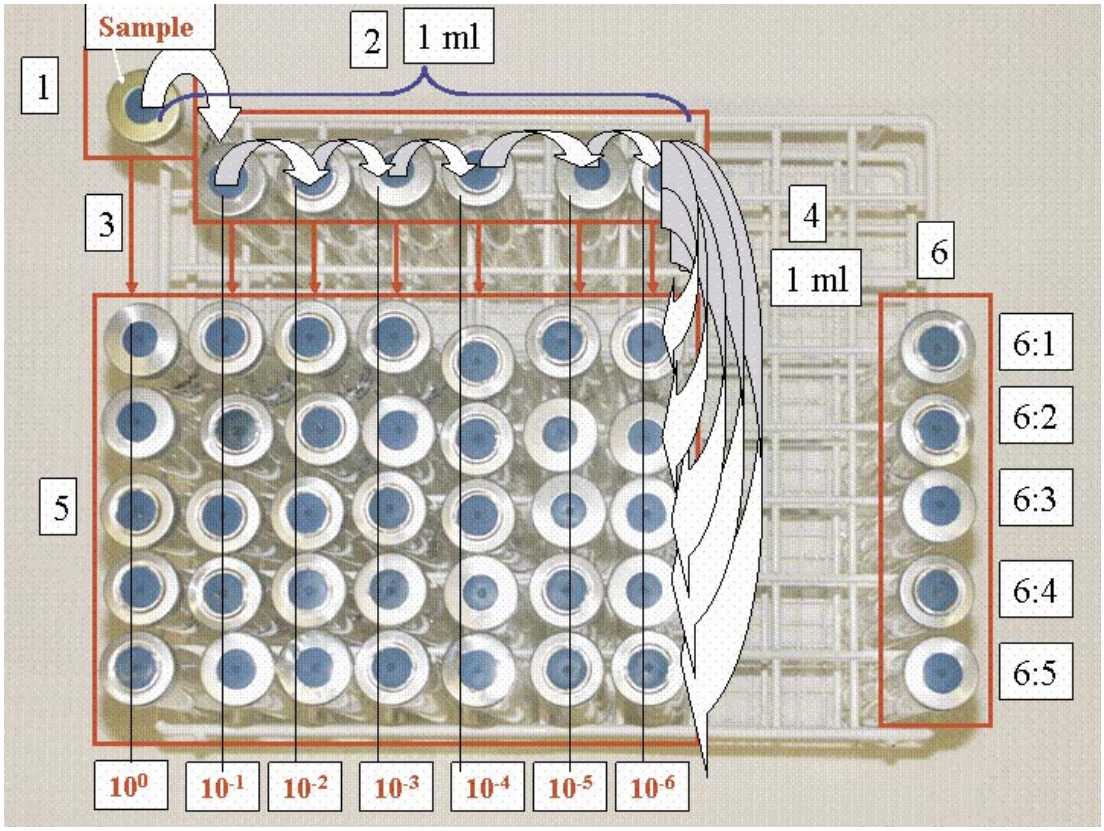

Figure 3-2. *The procedure for a most probable number determination. The Hungate tube with sample is used as the source for inoculation (1). A serial dilution was performed first (2). Thereafter, sub-samples were transferred (3–4) to the growth tubes (5) and to control tubes (6).*

4 Performance

The microbial characterizations were performed according to approved protocols. Details can be obtained from the laboratory at Göteborg University.

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, tubes 6-1 to 6-5).

4.3 Analysis

Acetate producing microorganisms were unfortunately not analysed in section 156.6–167 m. 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 the other two sections.

The sensitivity of the thermal conductivity detector (TCD) for methane was found to be too low for detection of autotrophic methanogens in section 156.6–167 m. This problem was solved for following samples, by application of a flame ionisation detector (FID).

5 Data handling

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 /6/.

6 Results

The total numbers of cells decreased about 50% from the highest to the lowest sampled section (Table 6-1). The numbers observed averaged at 10^5 cells ml^{-1} and were within the range of what has been observed elsewhere in deep groundwater /1 and 2/.

All physiological groups tested for, except autotrophic methanogens, were present at one or several of the investigated sections (Tables 6-2 to 6-4). Iron reducing bacteria were present at low but significant numbers in all sections. Sulphate reducing bacteria were found in relatively high numbers, also at all levels, while manganese reducers were only found in the shallowest section. Heterotrophic methanogens and acetogens were present in the two deepest sections, and they appeared in significant numbers which in many cases were higher than obtained earlier in deep bore hole samples /5/. This may be due to variations in the characteristics of the different groundwaters investigated. It may also be related to our continuous development of our MPN techniques, rendering a better sensitivity and cultivability. Due to the technical problems with the analytical procedures (4.3), methanogens and acetogens could net be tested for in section 156.6–167 m.

The percentage of total number of cells (Table 6-1) that could be cultured with the MPN technique varied from 0.085 up to 1.6 (Table 6-5). Those percentages compare well with earlier obtained data where the majority of the values were distributed in the interval between 0 and 1% /5/.

Table 6-1. Total number of cells in the analysed sections in KSH01A.

Borehole (section)	Cells ml^{-1}		Number of observations
	Total number of cells	Standard deviation	
KSH01A (156.5–167)	1.4×10^5	0.58×10^5	8
KSH01A (245–261.6)	1.0×10^5	0.15×10^5	6
KSH01A (548–565)	7.2×10^4	0.093×10^5	6

Table 6-2. Most probable number (MPN) of metabolic groups of microorganisms in KSH01A, section 156.5–167 m.

Metabolic groups	Cells ml^{-1}	
	MPN	Lower–upper 95% confidence limits
Iron reducing bacteria	2.1	0.9–5.5
Manganese reducing bacteria	3.3	1.5–7.7
Sulphate reducing bacteria	160	60–530
Autotrophic methanogens	n.a.*	–
Heterotrophic methanogens	n.a.	–
Autotrophic acetogens	n.a.	–
Heterotrophic acetogens	n.a.	–

*not analysed.

Table 6-3. Most probable number (MPN) of metabolic groups of microorganisms in KSH01A, section 245–261.6 m.

Metabolic groups	Cells ml^{-1}	
	MPN	Lower–upper 95% confidence limits
Iron reducing bacteria	2.1	0.9–5.5
Manganese reducing bacteria	< 0.2	–
Sulphate reducing bacteria	22	10–58
Autotrophic methanogens	< 0.2	–
Heterotrophic methanogens	700	300–2,100
Autotrophic acetogens	n.a.*	n.a.
Heterotrophic acetogens	900	300–2,900

*not analysed

Table 6-4. Most probable number (MPN) of metabolic groups of microorganisms in KSH01A, section 548–565 m.

Metabolic groups	Cells ml^{-1}	
	MPN	Lower–upper 95% confidence limits
Iron reducing bacteria	3.3	1.5–7.7
Manganese reducing bacteria	< 0.2	–
Sulphate reducing bacteria	35	16–82
Autotrophic methanogens	< 0.2	–
Heterotrophic methanogens	2.6	1.2–6.3
Autotrophic acetogens	30	10–130
Heterotrophic acetogens	17	8–41

Table 6-5. The percentage of the total number of cells (Table 6-1) cultured with MPN (Tables 6-2 to 6-4) in the analysed sections in KSH01A.

Borehole (section, m)	Cells cultured (%)	
	MPN	Lower–upper 95% confidence limits
KSH01A (156,5–167)	0.12	0.043–0.38
KSH01A (245–261.6)	1.6	0.61–5.1
KSH01A (548–565)	0.085	0.051–0.37

7 Conclusions

The data obtained compare well with earlier obtained data, using similar sampling and analysis methods /1–5/.

- The total numbers of cells plot as average numbers if compared to the database for the Fennoscandian shield.
- IRB were lower that usually observed.
- MRB has not been tested for earlier. We have, this far, one more observation of MRB, in Forsmark, also in a shallow borehole.
- SRB seem to be ubiquitous. They are always found, as here, down to depths about 500–600 m. Deeper, earlier investigations show low or negative values.
- Methanogens have been demonstrated common in the Äspö area /1–2/. Their presence in KSH01A was expected. Heterotrophic methanogens proliferate on one- and two-carbon compounds that should show up as TOC in the chemistry analysis.
- Acetogens have been found in high numbers in the Äspö Area /1–2/ and heterotrophic acetogens were common in deep boreholes /4–5/. Heterotrophic acetogens proliferate on one-carbon compounds that should show up as TOC in the chemistry analysis. Their presence in KSH01A was expected.
- The percentages of cultivable cells compare well with earlier obtained data where the majority of the values distributed in the interval between 0 and 1%.

8 References

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