Appendix 1

LOT A2 – Bacteria part

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1. SVENSK SAMMANFATTNING

”Long term test of buffer material” (LOT) är en försöksserie som startades upp vid Äspö Hard Rock Laboratory (HRL) 2000. Där testas modeller och hypoteser angående stabiliteten av bentonitbufferten som skall användas vid ett eventuellt KBS3 slutförvar av kärnbränsle. I LOT försöken har ett antal kopparcylindrar med värmare placerats i block av bentonit och värmts till en temperatur av 90°C. LOT försöket inkluderar även så kallade A-försök under förhöjd temperatur (130°C), för att accelerera de processer som kan anses skadliga för bentoniten. LOT paketen har tidigare provtagits efter ett år och nu efter fem-sex år har de testats igen.

En del skadliga processer i bentoniten kan orsakas av mikrober. I denna rapport beskrivs mikrobiologiska analyser vad gäller totalt 20 punkter i bentoniten i toppen (block 38) och mitten (block 19-20) av LOT paket A2. Detta paket har lagrats fem-sex år nere i berget i Äspötunneln runt en 130°C varm kopparvärmare sedan starten av LOT försöket. Genom att odla bakterier från bentoniten i specifika medier kunde följande bakteriella parametrar undersökas: Det totala antalet av odlingsbara heterotrofa aeroba mikroorganismer, sulfatreducerande bakterier och bakterier som producerar det organiska ämnet acetat (acetogener). Även ATP innehållet i bentoniten bestämdes. Dessutom odlades bakterier från bentoniten upp i syrefritt sulfatreducerarmedium. I dessa kulturer undersöktes närvaro av de biologiskt producerade ämnena sulfid och acetat, eftersom dessa potentiellt kan ha negativ inverkan på kopparkapseln i ett KBS3 slutförvar. Resultaten jämfördes till viss del med data över densitet, temperatur och vatteninnehåll från Clay Technology AB. Resultaten visade att det fanns viabla sulfatreducerande och acetogena bakterier i storleksordningen 10^0-10^2 och odlingsbara aeroba bakterier i storleksordningen 10^3-10^5 g^{-1} bentonit efter fem år nere i berget, så länge temperaturen och densiteten inte varit för hög och/eller vatteninnehållet för lågt. Mikroorganismer med flera olika morfologier kunde hittas i kulturerna med bentonit.

När förekomsten av bakterier undersöks i bentoniten är det viktigt att den provtas på rätt sätt. Syre är giftigt för många bakterier som lever i syrefria miljöer, däribland sulfatreducerande bakterier. För att utesluta att antalet av dessa bakterier underskattas är det viktigt att inte syre tränger in i bentoniten. Därför slussades bentoniten in i en box med syrefri atmosfär kort efter provtagning. En annan faktor som kan ge underskattade resultat är uttorkning. Genom att svepa in bentoniten i steril aluminiumfolie och plasta in den skyddades bentoniten mot detta. Samtidigt skyddades den även från kontaminering av bakterier från omgivningen. Protokollen för provtagning fungerade tillfredställande. Det var möjligt att provta bentoniten sterilt, vilket kunde visas genom att det inte växte i alla bentonitprover utan bara i de prover där bakterierna överlevde de omgivande förhållandena i bentoniten under lagringen i A2 paketet. Att sulfatreducerande bakterier växte i odlingsmediet visar att bentoniten provtogs under syrefria förhållanden.

2. ENGLISH ABSTRACT

”Long term test of buffer material” (LOT) is a series of experiments which was initiated at Äspö Hard Rock Laboratory (HRL) in 2000, where hypotheses and models regarding the stability of bentonite buffer in a possible KBS3 repository for nuclear waste are tested. In the LOT experiments a number of copper canisters with heaters have been placed in blocks with bentonite and heated to a temperature of 90°C. The LOT experiments also include so-called A-tests where the temperature in the copper canister heater has been elevated to 130°C to accelerate the processes that are harmful to the bentonite buffer. The LOT parcels have previously been sampled after one year. After five-six years of storage they are now tested again.

Some harmful processes in the bentonite can be caused by bacteria. In this report, the bacterial analyses regarding 20 sample points in the bentonite at the top (block 38) and middle (block 19-20) of the LOT A2 parcel are described. This parcel has been stored underground in the Äspö tunnel around a 130°C warm heater since the start of the LOT experiments five-six years ago. By culturing bacteria from the bentonite in specific media the following bacterial parameters could be examined: The total amount of culturable heterotrophic aerobic bacteria, sulphate-reducing bacteria, and bacteria that produce the organic compound acetate (acetogens). The ATP content in the bentonite was also determined. In addition, bacteria from the bentonite were cultured in different sulphate-reducing media. In these cultures, the presence of the biotic compounds sulphide and acetate was investigated, since these have potentially negative effect on the copper canister in a KBS3 repository. The results were to some extent compared to density, water content, and temperature data provided from Clay Technology AB. The results showed that 10^0-10^2 viable sulphate-reducing and acetogenic bacteria and 10^3-10^5 heterotrophic aerobic bacteria g^{-1} bentonite were present after five years of storage in the rock, as long as the ambient temperature and density have not been too high, or the water content in the bentonite too low. Bacteria with several morphologies could be found in the cultures with bentonite.

When the presence of bacteria in the bentonite is investigated, it is important that the sample procedures are accurate. Oxygen is toxic to many of the bacteria that thrive in anaerobic groundwater, among them sulphate-reducing bacteria. To avoid underestimation of the number of sulphate-reducing bacteria in the bentonite it is important to exclude oxygen during sampling. Therefore, the bentonite was put in an anaerobic chamber shortly after sampling. Another factor that gives underestimated results is desiccation. By wrapping the bentonite in sterile aluminium foil and plastic bags it was possible to avoid this. The aluminium foil also served as protection against contamination of bacteria from the surroundings. The sampling procedures worked accurately. It was possible to sample the bentonite under sterile conditions, which was proved by the fact that bacteria only grew were the ambient conditions during storage in the A2 parcel allowed this. Presence of sulphate-reducing bacteria proved that the bentonite was sampled without intrusion of oxygen in levels toxic to these organisms.

3. METHODS

3.1 Sampling of bentonite from the LOT A2 parcel

Sampling

The bentonite from the LOT A2 parcel was sampled according to AP TD F62-06-010. Presence of bacteria was investigated in bentonite from the middle and top part of the LOT A2 parcel part in the blocks 19-20 and 38, respectively. These two bentonite blocks were wrapped in sterile aluminium foil and placed in the anaerobic box (COY Laboratory Products, Grass Lake, MI, USA) immediately after sampling. Inside the box, a triplicate of bentonite pieces of approximately 1-2 g each was collected by means of a hammer, a sterile chisel and a sterile knife. Triplicates in one cm intervals from each block were collected at a distance of 0-9 cm from the copper surface.

Preparation of enrichment culture media and inoculation

Bacteria from the bentonite were enriched in anaerobic growth media supplied with sulphate. Two types of sulphate-reducing enrichment media were produced; One medium with an organic carbon and energy source and one with inorganic carbon and energy sources. Both media contained a basal salt solution (l^{-1} milli-Q water); 7 g NaCl, 1g $CaCl_2 \times H_2O$, 0.67 g KCl, 1 g NH_4Cl, 0.15 g KH_2PO4, 0.5 g $MgCl_2 \times 6H_2O$, 3 g $MgSO_4 \times 6H_2O$. The salt solution was autoclaved and cooled under a N_2/CO_2 (80/20%) atmosphere for 1 h. After that the following solutions were added: 10 ml trace element solution, 60 ml 1M $NaHCO_3$ solution, 10 ml yeast extract solution, 10 ml vitamin solution, 1 ml thiamine solution, 1 ml vitamin B_{12} solution, 5 ml iron stock solution, 2 ml resazurin solution, 10 ml cystein-HCl solution, and 10 ml Na_2S solution. Five ml of 50% lactate solution (l^{-1} milli-Q water) was added to the anaerobic enrichment media with organic energy and carbon source. The pH was set to 6.5-7.5 and the medium was added in 45 ml aliquots to N_2/CO_2 (80/20%) filled 120-ml serum flasks sealed with butyl rubber stoppers. The serum flasks and an additional set of sterile rubber stoppers were placed in an anaerobic box. The serum flasks were opened inside the box and 1 g bentonite pieces from each sample point were put in the anaerobic media with and without lactate, respectively. The serum flasks were resealed with new stoppers and removed from the anaerobic box. To the cultures without lactate, H_2/CO_2 at 2 bars above atmospheric pressure (80/20%) was added as energy and carbon source. The enrichment cultures were left in room temperature overnight for the bentonite to disperse in the medium. After that they were vigorously shaken and incubated at 30°C for six weeks.

Sampling of bentonite for ATP determination

The third piece from each sampling point was put in a sterile 50-mL Falcon tube for ATP analysis. The tube was removed from the anaerobic box and 10 ml of B/S extraction solution (BioThema AB, Handen, Sweden) was added. The bentonite was dispersed in the extraction solution over night and frozen at -20°C until analysis.

3.2 Presence of bacteria in the bentonite from the LOT A2 parcel

The microbial metabolites sulphide and acetate was analysed in the enrichment cultures after six weeks of incubation. The enrichment cultures were also analysed with microscopy to examine the presence of bacteria and confirm that the metabolites were of biotic origin.

Microscopy

The supernatants from the enrichment cultures were diluted 20 times in sterile water and 0.1 ml were filtered onto 0.2 um pore size filters stained black (Osmonics, Minnetonka, MN, US) and stained with acridine orange (10 mg l^{-1}) for 7 min. Bacteria on the filters were observed in an inverted microscope (Nikon Diaphot 300, Teknooptik AB, Göteborg, Sweden) at 1000 times magnification using blue light (390-490 nm) and photographed.

Sulphide analysis

The sulphide concentration was determined in enrichment cultures with lactate and H_2/CO_2 according to Widdel and Bak (1992). Approximately 1 ml of the supernatant from each enrichment culture was carefully withdrawn with an anaerobic syringe and needle and 0.1 ml was added to 2 ml of 5 mM $CuSO_4$. If sulphide was present in the sample, a brown precipitate (CuS) was formed. The absorbance of the solution was measured spectrophotometrically (Genesys 10 UV, Thermo electron corporation, Waltham, MA, USA) at λ 480 nm and the concentration was determined with an external standard curve. Sulphide is reported if bacteria were present in the cultures. The background in medium without growth was approximately 40 mg l^{-1}.

Acetate analysis

The acetate concentration was determined in the enrichment cultures with H_2/CO_2. A sample from the supernatant from each enrichment culture was carefully withdrawn with an anaerobic syringe and needle. The acetate concentration in the supernatant in the bentonite enrichment cultures was determined spectrophotometrically with a kit (Boehringer Mannheim, Mannheim, Germany) which detects acetate by an enzymatic method. Acetate is reported if bacteria were present in the cultures. The background in medium without growth was approximately 6 mg l^{-1}.

3.3 Enumeration of bacteria from the bentonite in the LOT A2 parcel

As mentioned above, the enrichment cultures were left at room temperature overnight after inoculation to allow the bentonite to disperse in the medium. After dispersion of the bentonite, the enrichment cultures were used as inocula for analysis of the number of viable culturable heterotrophic aerobic bacteria (CHAB) originating from the bentonite, as well as most probable number (MPN) of sulphate-reducing bacteria (SRB) and autotrophic acetogens (AA).

Culturable heterotrophic aerobic bacteria (CHAB)

In the CHAB analysis 0.1 ml from each of the enrichment cultures was spread on a triplicate of agar plates, which contained (l^{-1} milliQ water); peptone 0.5 g, yeast extract 0.5 g, starch 0.25 g, Na-acetate 0.25 g, $CaCl_2 \times 2H_2O$ 0.2 g, K_2HPO_4 0.1 g, NaCl 10 g, trace metal solution 1 ml, agar 15 g. After one week of incubation at room temperature the number of colonies was counted. The mean and the standard deviation for the plates from both enrichment cultures from each sample point were calculated.

MPN analysis of sulphate-reducing bacteria (SRB)

In the MPN analysis of SRB 1 ml of the original enrichment cultures was inoculated into five Hungate tubes with the sulphate-reducing media with lactate. After six weeks of incubation, the sulphide concentration in the MPN tubes were determined as described above. Tubes with three

times as high sulphide concentrations compared to the medium with no growth were regarded as growth positive. MPN of SRB with 99% confidence levels according to Cornish and Fischer were calculated using a computer program described in Klee (1993).

MPN analysis of autotrophic acetogens (AA)

In the MPN analysis of AA 1 ml of the original enrichment cultures was inoculated into five Hungate tubes with sulphate-reducing media added with H_2/CO_2 but with the exclusion of 3 g $MgSO_4 \times 6H_2O$. After six weeks of incubation, the acetate concentration in the MPN tubes were determined as described above. Tubes with three times as high acetate concentrations compared to medium with no growth were regarded as growth positive. MPN of AA with 99% confidence levels according to Cornish and Fischer were calculated using a computer program described in Klee (1993).

3.4 ATP content in the bentonite in the LOT A2 parcel

The ATP in the approximately 1 g of bentonite was extracted with 10 ml of B/S extraction solution. The ATP content in the extract was analyzed with the BioThema ATP Biomass Kit HS (BioThema AB, Handen, Sweden) and a Sirius FB12 luminometer (Berthold Detection Systems, Pfortzheim, Germany). The method used was modified from Eydal and Pedersen (2006) which has previously been described in TD-06-01. The ATP concentration was calculated based on the average of three measurements as follows:

$$\text{amol ATP g}^{-1}\text{ bentonite} = \frac{I_{smp} - I_{bkg}}{I_{(std + smp - bkg)} - I_{(smp - bkg)}} \times DF/B \times SF$$

Where:

I: *light intensity measured as relative light units*

smp: *sample*

bkg: *background*

std: *standard*

B: *g bentonite*

DF/B: *dilution factor to obtain amount of ATP g^{-1} bentonite = 100/B*

SF: *shift factor from pmol to amol = 10^6*

The bentonite interfered with the ATP analysis. Therefore, a calibration curve of ATP measurements with B/S extraction solution, bentonite (1 g/10 ml) and five different known amounts of ATP was prepared and compared to B/S extraction solution with the same ATP amounts but without bentonite. Using this calibration curve, the measurements in the bentonite samples were translated to the corresponding ATP amounts without the bentonite interference.

4. RESULTS AND EVALUATION

4.1 Sampling

Figure 1 schematically shows where the bentonite samples for microbiological analysis from the LOT A2 parcel (Figure 1) were extracted. Samples were taken from block 38 on the top of the A2 parcel (called TOP LOT) and block 19-20 in the middle (called MID LOT). The sample preparation was performed under anaerobic conditions with sterile equipment, which assured accurate sampling of the bentonite. The corresponding density, temperature and water content data for these positions is shown in Figure 2.

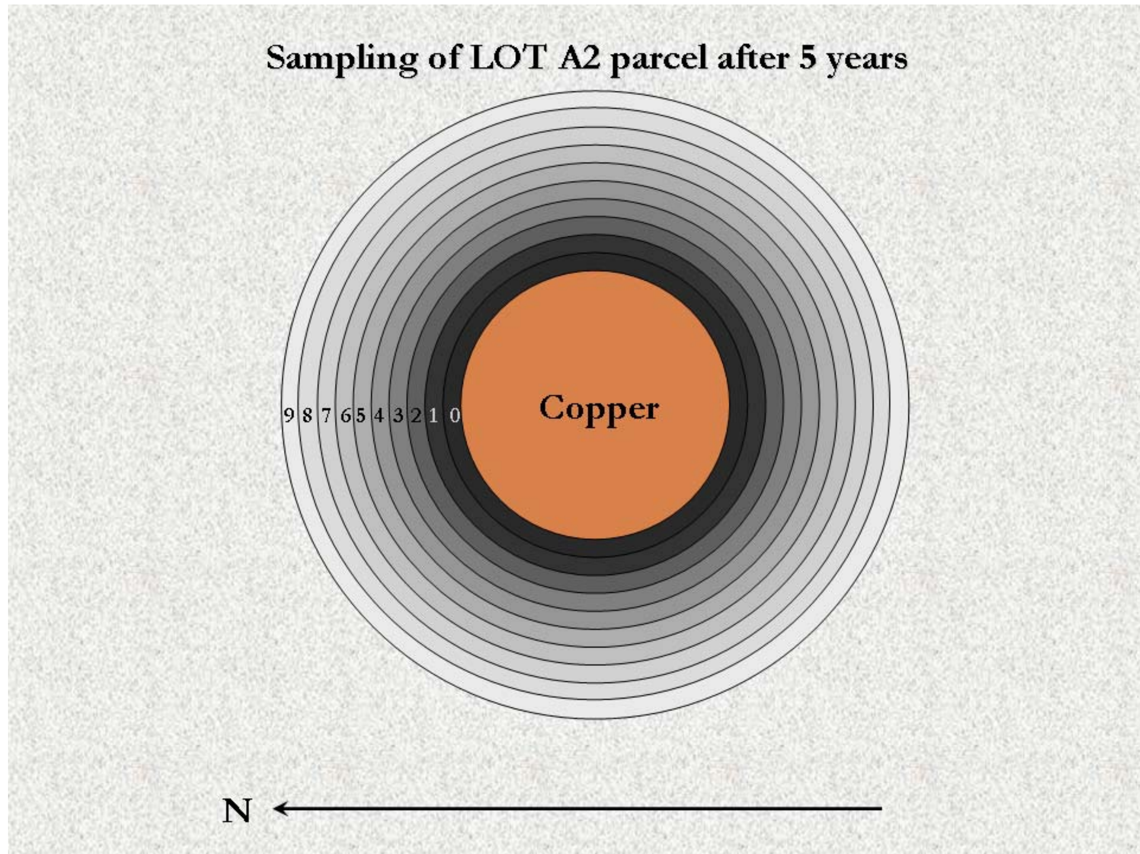

Figure 1. *Schematic picture over the location of the samples from TOP LOT (block 38) and MID LOT (block 19-20) in the LOT A2 parcel.*

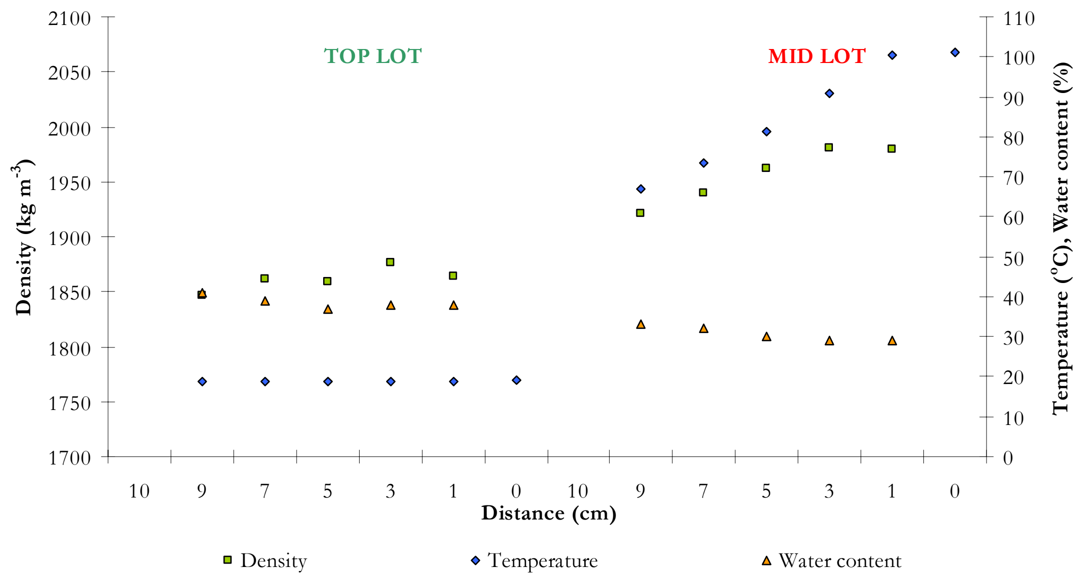

Figure 2. *Density, temperature, and water content at 0-9 cm distance from the copper surface in the TOP LOT and MID LOT bentonite samples in the A2 parcel (data from Clay Technology AB).*

4.2 Presence of bacteria in the bentonite from the LOT A2 parcel

Enrichment cultures were prepared to examine if viable bacteria were present in the sampled bentonite. In Figure 3 the concentrations of sulphide in the bentonite enrichment cultures with addition of the organic carbon source lactate are shown. When the sulphide concentrations from TOP LOT and MID LOT are investigated, it is apparent that the elevated sulphide concentrations (54-507 mg l^{-1}) could be found in most of the TOP LOT samples. Bacteria with various morphologies were found in all these enrichment cultures. In Figure 4, images from microscopy analysis of two of these cultures are shown.

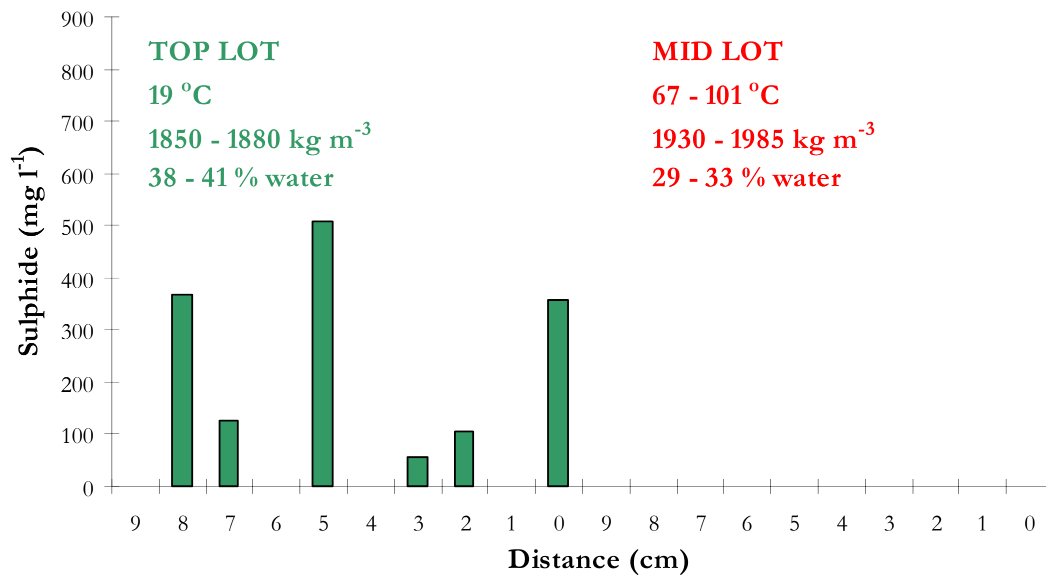

Figure 3. *Sulphide concentrations in the anaerobic enrichment cultures inoculated with TOP LOT bentonite (green) and MID LOT bentonite (red) originating 0-9 cm from the copper in the A2 parcel. The medium for the enrichments contained lactate as carbon and energy source. The bentonite cultures were incubated six weeks at 30°C prior to analysis.*

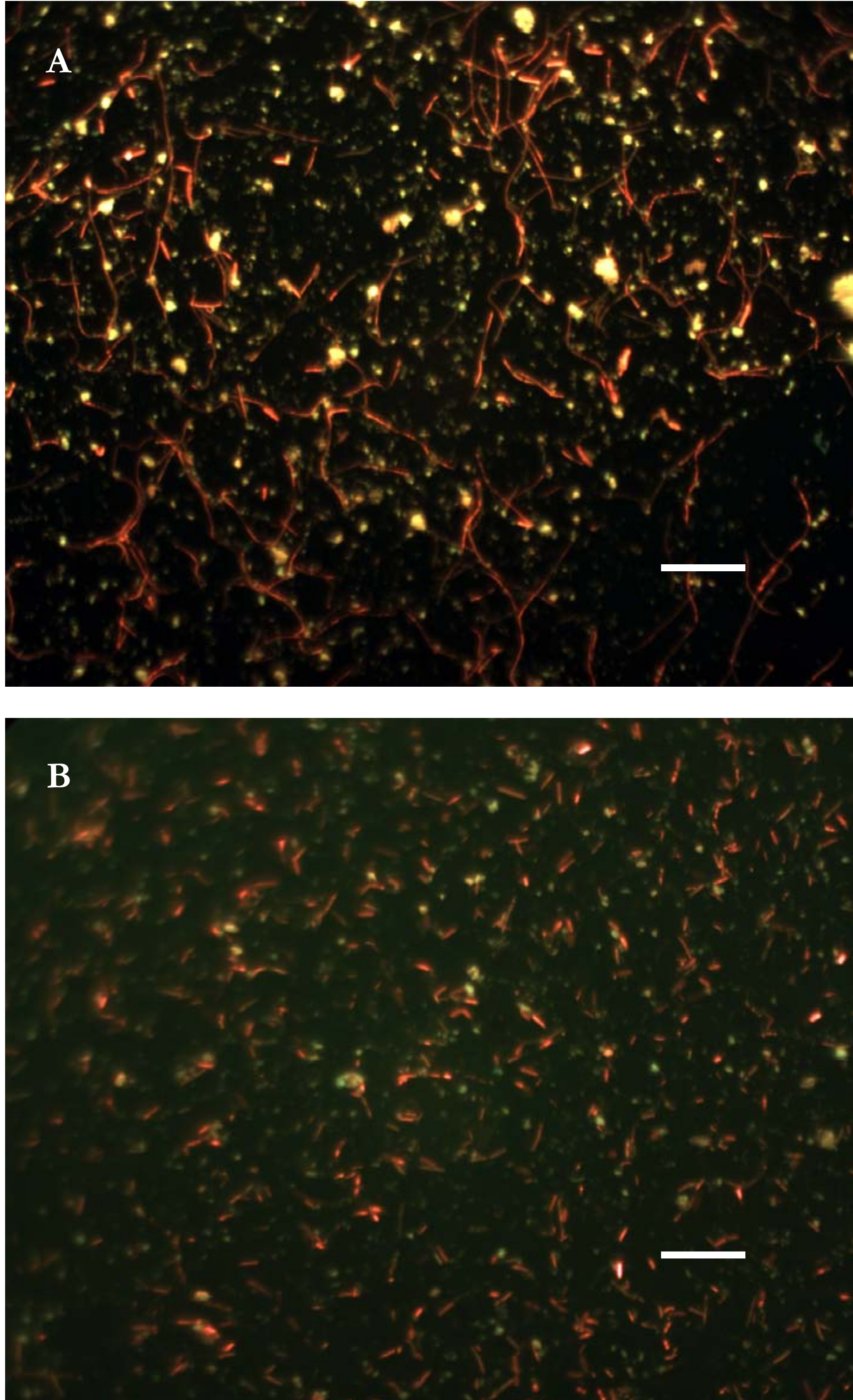

Figure 4. *Images from the bentonite enrichment cultures with lactate added as energy and carbon source: A) A sulphide and acetate containing culture that was inoculated with TOP LOT bentonite situated 8 cm from the copper surface (~1850 kg m*$^{-3}$*, temperature ~20 °C, and water content of 39%). B) A sulphide and acetate containing culture that was inoculated with TOP LOT bentonite situated 0 cm from the copper surface (density ~1865 kg m*$^{-3}$*, temperature ~20 °C, and water content of 38%). The white bar represents 10 µm.*

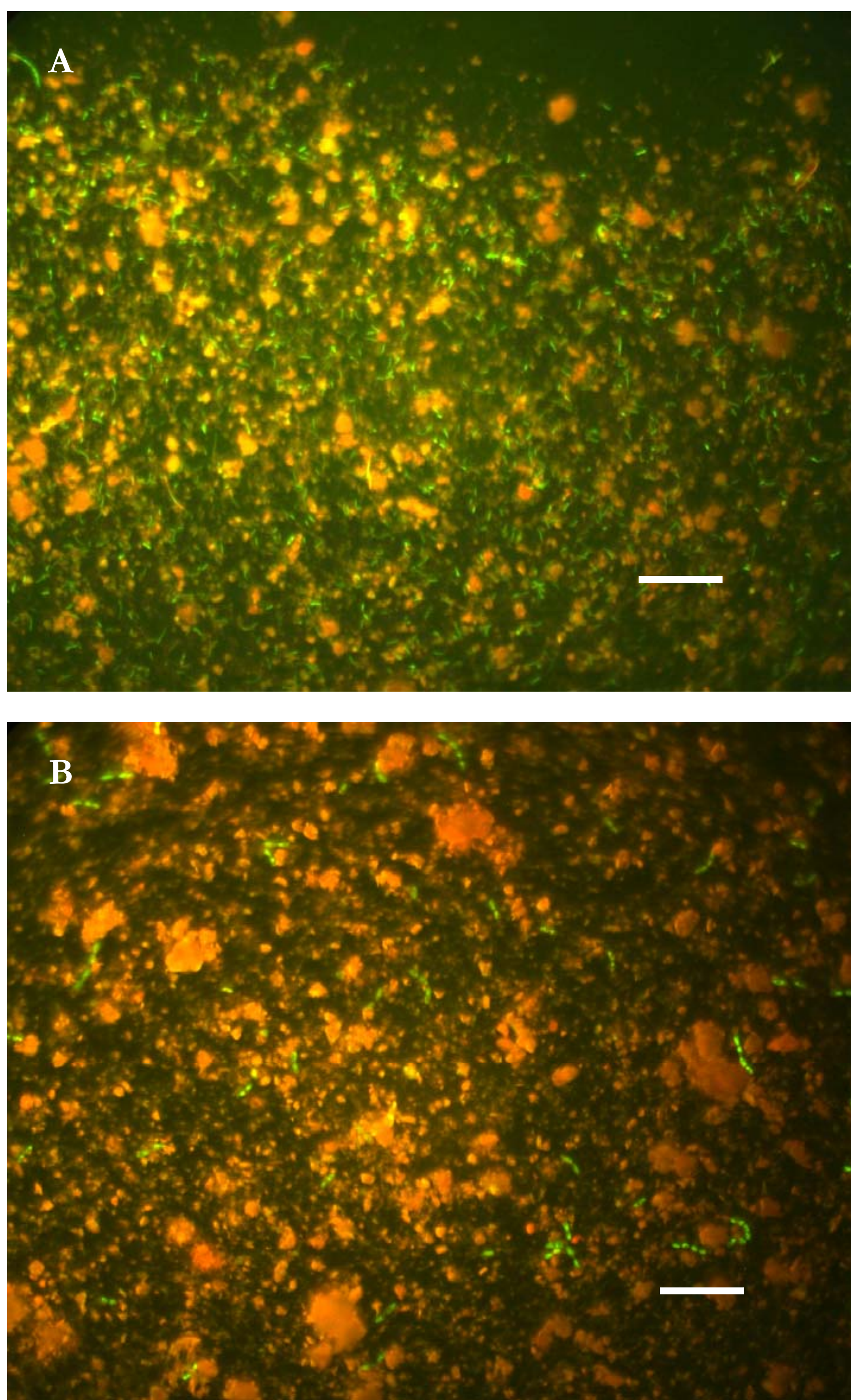

Figure 5. *Images from the bentonite enrichment cultures with H_2/CO_2 added as energy and carbon source for autotrophic growth: A) a sulphide and acetate containing culture that was inoculated with TOP LOT bentonite situated 1 cm from the copper surface (~1850 kg m^{-3}, temperature ~20 °C, and water content of 38%) B) A sulphide containing culture that was inoculated with MID LOT bentonite situated 9 cm from the copper surface (~1830 kg m^{-3}, temperature ~70 °C, and water content of 33%). The white bar represents 10 µm.*

In Figure 6 the concentrations of acetate in the bentonite enrichment cultures with addition of the inorganic energy and carbon sources H_2/CO_2 are shown. Acetate, ranging from 23 to 318 mg l^{-1}, was found in all the cultures with TOP LOT bentonite except one. Concomitant growth of bacteria with various morphologies was evident in all these samples. In Figure 5 a, an image from microscopy analysis of one of these cultures is shown. In difference to the TOP LOT samples, acetate was not detected in any of MID LOT samples.

In Figure 7 the concentrations of sulphide in the bentonite enrichment cultures with addition of the inorganic energy and carbon sources H_2/CO_2 are shown. Elevated sulphide concentrations with concomitant presence of bacteria were detected in some of the enrichment cultures with TOP LOT bentonite (48-204 mg l^{-1}). Since acetate is a carbon source used by many sulphate-reducing bacteria (Madigan *et al.* 2000) and acetate was produced in the TOP LOT bentonite enrichment cultures (Figure 6), the sulphide in these cultures might have originated from acetate metabolism by SRB. Another possibility for SRB to gain carbon and energy is to use inorganic energy and carbon sources, H_2 and CO_2, respectively. This might have been the case in the MID LOT culture shown in Figure 5 b. Acetate was not detected in this culture. In this sample, the density was <1950 kg m^{-3}, the temperature ~ 70°C and the water content ~ 29°C.

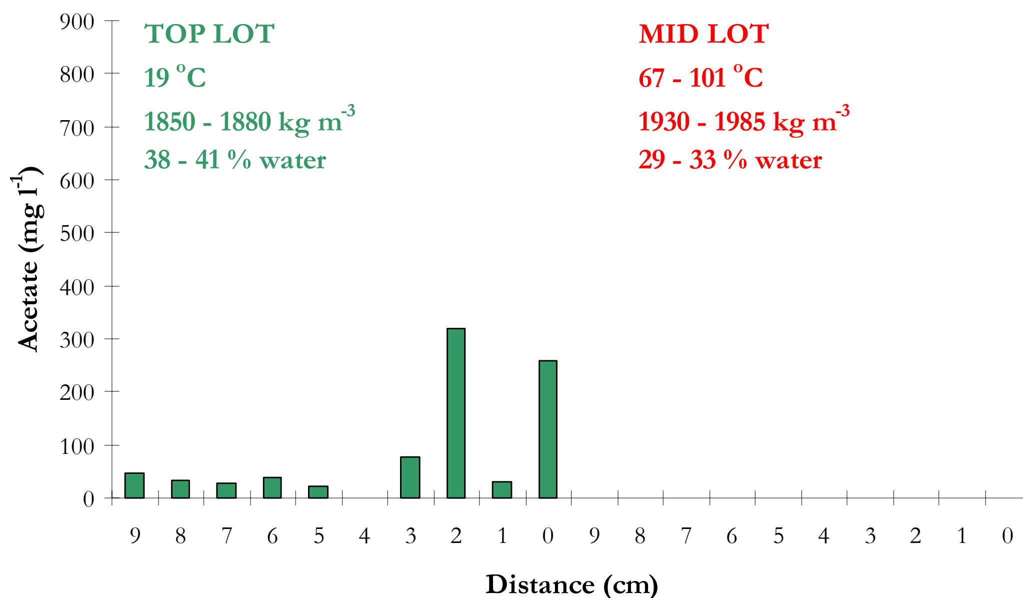

Figure 6. *Acetate concentrations in the anaerobic enrichment cultures inoculated with TOP LOT bentonite (green) and MID LOT bentonite (red) originating 0-9 cm from the copper in the A2 parcel. The medium for the anaerobic enrichments contained H_2/CO_2 as energy and carbon sources for autotrophic growth. The bentonite cultures were incubated six weeks at 30°C prior to analysis.*

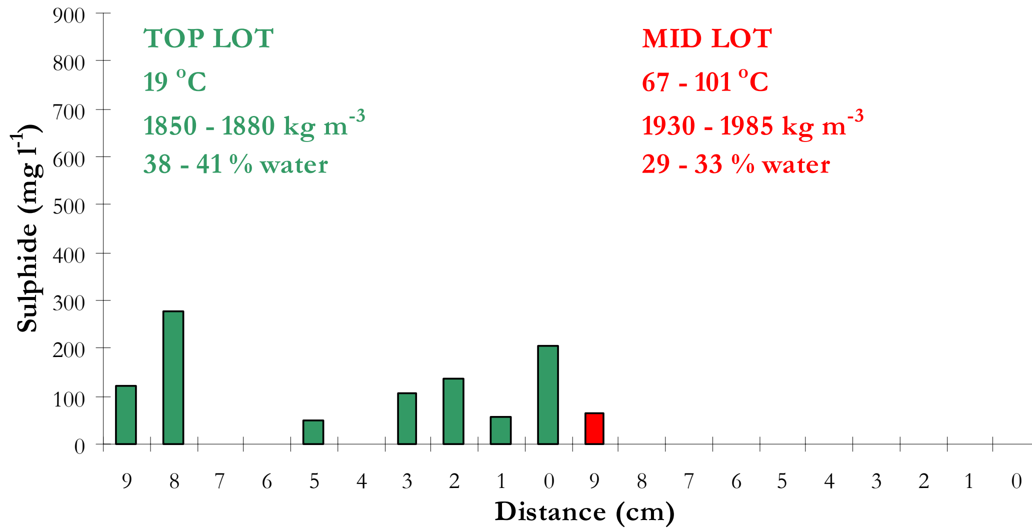

Figure 7. *Sulphide concentrations in the anaerobic enrichment cultures inoculated with TOP LOT bentonite (green) and MID LOT bentonite (red) originating 0-9 cm from the copper in the A2 parcel. The media for the anaerobic enrichments contained H_2/CO_2 as energy and carbon sources for autotrophic growth. The bentonite cultures were incubated six weeks at 30°C prior to analysis.*

In Table 1, presence of bacteria and metabolic products in the TOP LOT and MID LOT bentonite are compared to the density, temperature and water content at the sites in the A2 parcel from where the sampled bentonite originated. The results show that bacteria were more abundant in the TOP LOT than in MID LOT samples, where the temperature and density during storage in the rock were lower and the water content higher.

Table 1. *Comparison of temperature, density, and water content in the sampled bentonite and bacterial growth in enrichment cultures with TOP LOT and MID LOT bentonite from the LOT A2 parcel after six weeks of incubation at 30°C. + growth – no growth*

Measurement	Position	Carbon and energy source	Distance from Cu (cm)									
			9	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**0**
Temperature (°C) [a]	TOP LOT	-	19		19		19		19		19	19
Density (kg m^{-3}) [a]	TOP LOT	-	1847		1862		1859		1879		1864	
Water content (%)[a]	TOP LOT	-	33		30		29		29		29	
Sulphide [b]	TOP LOT	Lactate	-	+	+	-	+	-	+	+	-	+
Growth of bacteria [d]	TOP LOT	Lactate	+	+	+	+	+	+	+	+	+	+
Acetate[c]	TOP LOT	H_2/CO_2	+	+	+	+	-	+	+	+	+	+
Sulphide [b]	TOP LOT	H_2/CO_2	+	-	-	-	+	-	-	+	+	+
Growth of bacteria [d]	TOP LOT	H_2/CO_2	+	+	+	+	+	+	+	+	+	+
Temperature (°C) [a]	MID LOT	-	67		73		81		91		101	101
Density (kg m^{-3}) [a]	MID LOT	-	1928		1955		1969		1978		1982	
Water content (%)[a]	MID LOT	-	39		41		37		38		38	
Sulphide[b]	MID LOT	Lactate	-	-	-	-	-	-	-	+	-	-
Growth of bacteria [d]	MID LOT	Lactate	-	-	-	-	-	-	-	-	-	-
Acetate[c]	MID LOT	H_2/CO_2	-	-	-	-	-	-	-	-	-	-
Sulphide [b]	MID LOT	H_2/CO_2	+	-	-	-	-	-	-	-	-	-
Growth of bacteria [d]	MID LOT	H_2/CO_2	+	-	-	-	-	-	-	-	-	-

[a] *data from Clay Technology AB,* [b] *detection limit for sulphide 1.3 mg l^{-1},* [c] *detection limit for acetate 15 mg l^{-1},* [d] *analysed with microscopy*

4.3 Enumeration of bacteria from the LOT A2 parcel

CHAB, SRB and AA numbers above detection limit were detected in several TOP LOT bentonite samples (Figure 8-10). In these, the density was 1850-1880 kg m^{-3}, the temperature ~ 20°C and the water content 38-41 %.

In the samples originating from MID LOT bentonite, only one sample (located 4 cm from the copper canister) showed CHAB numbers above detection limit. In this sample, the density was >1950 kg m^{-3}, the temperature >80°C and the water content ~ 30%.

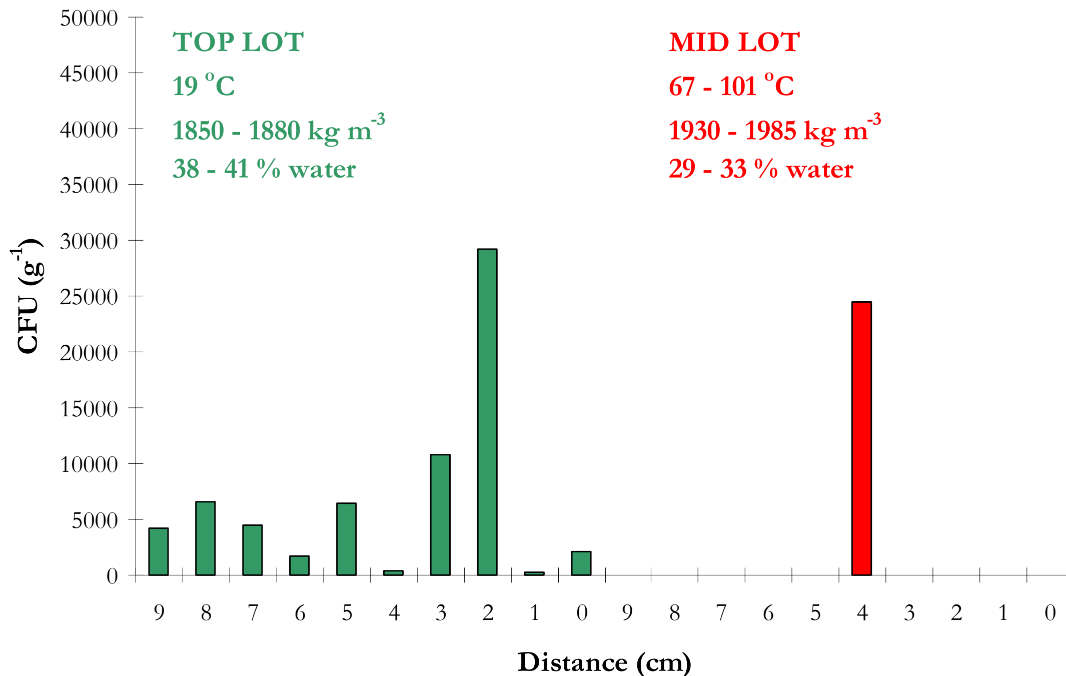

Figure 8. *Culturable heterotrophic aerobic bacteria in the TOP LOT and MID LOT bentonite originating 0-9 cm from the copper in the A2 parcel after one day of dispersion of the bentonite in the anaerobic media. The numbers are means of the counts from the bentonite enrichment cultures with and without addition of organic carbon (n=2).*

In Table 2, the quantitative results of bacteria in the sampled bentonite are shown. Between 10^0-10^2 g^{-1} AA and SRB bentonite were found. Compared to the groundwater in the area where 10^1-10^3 ml^{-1} AA and SRB can be detected (Pedersen 2000), these numbers are lower. Nonetheless, it is obvious that both AA and SRB were present in almost all samples from the TOP LOT bentonite.

As shown in Table 1 and Figure 3-7, growth of bacteria with concomitant presence of sulphide and/or acetate was found in all TOP LOT (at 0-9 cm distance from the copper) and one MID LOT (at 9 cm distance from the copper) from the bentonite enrichment culture. However, growth was not always detected in the in the tubes in the MPN analysis. Theoretically, the numbers of SRB in these samples would be ~1-5 g^{-1} bentonite. These samples are illustrated with dotted staples in Figure 7 and 8.

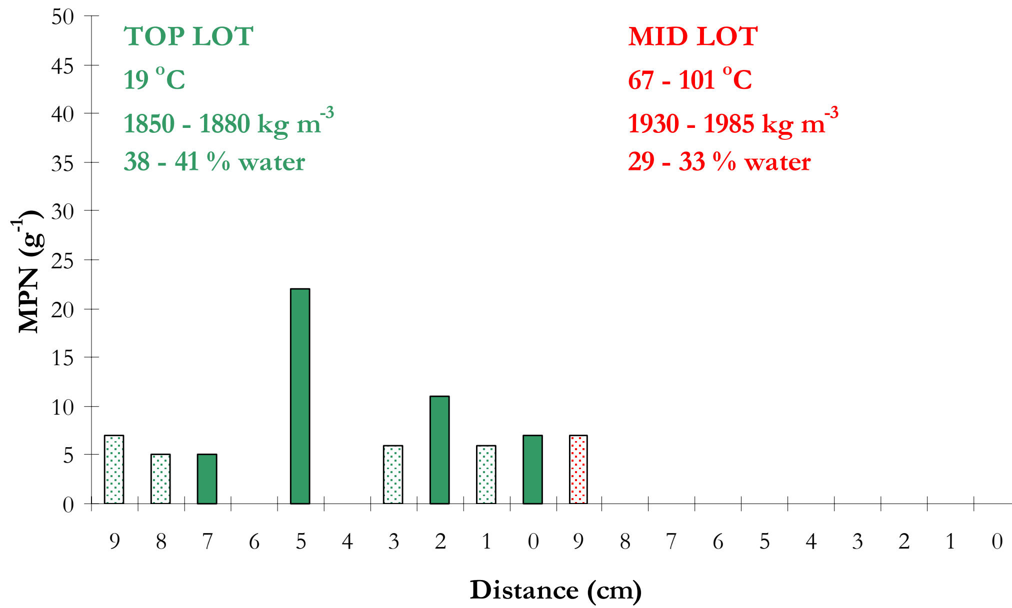

Figure 9. *MPN of sulphate-reducing bacteria in the TOP LOT and MID LOT bentonite originating 0-9 cm from the copper in the A2 parcel. The dotted staples show samples where sulphate-reducing bacteria were present in the enrichment culture used as inoculum but not in the MPN tubes and represents the highest theoretical number of sulphate-reducing bacteria in the bentonite sample.*

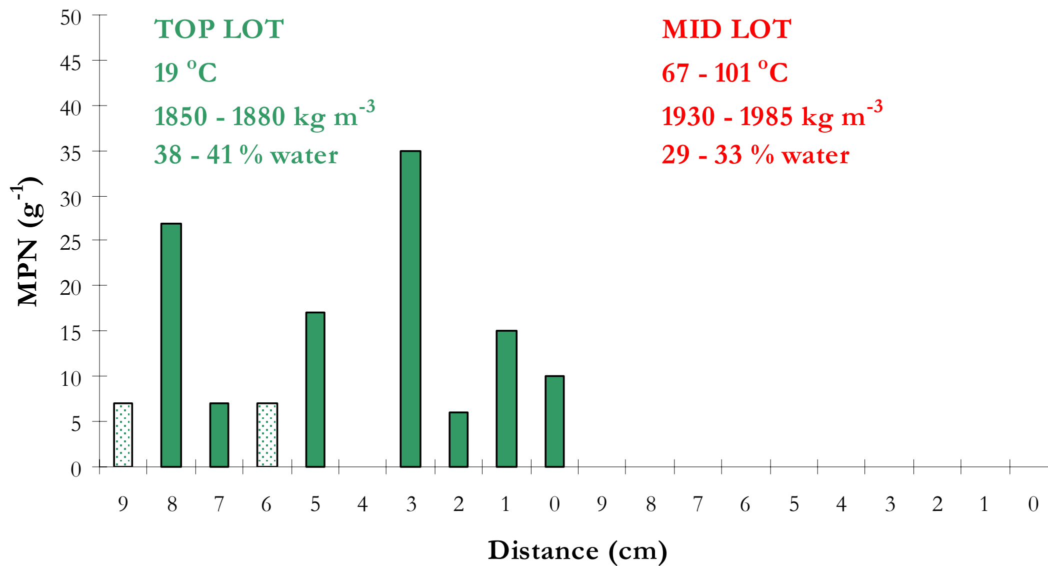

Figure 10. *MPN of acetogens in the TOP LOT and MID LOT bentonite originating 0-9 cm from the copper in the A2 parcel. The dotted staples show samples where acetogens were present in the enrichment culture used as inoculum but not in the MPN tubes and represents the highest theoretical number of acetogens in the bentonite sample.*

4.4 ATP in the bentonite

ATP measurements showed the living bio-volume in a sample. All samples with bentonite from the A2 parcel except one from MID LOT contained less than 5×10^4 amol ATP g^{-1} (Table 2). This corresponds to 10^4-10^6 cells g^{-1}, if the ATP content in the cells in the bentonite is regarded to be the same as in anaerobic bacteria in deep granitic groundwater. These bacteria have been shown to contain 0.1-1 amoles ATP $cell^{-1}$ (Eydal & Pedersen 2006). The MID LOT sample with a detectable content of ATP also showed a high number of CHAB (49×10^3). Deep granitic environments are anaerobic, and such high levels of aerobic bacteria (CHAB) could imply contamination when the A2 parcel was put free. However, this sample was situated in the middle a bentonite block, which basically rules out the risk for such contamination.

Table 2. *Enumeration of cultative heterotrophic aerobic bacteria (CHAB), sulphate-reducing bacteria (SRB), autotrophic acetogens (AA) and determination of the ATP content in the TOP LOT and MID LOT bentonite from the LOT A2 parcel.*

		Distance from Cu (cm)									
Analysis	**Position**	**9**	**8**	**7**	**6**	**5**	**4**	**3**	**2**	**1**	**0**
CHAB (CFU[a] × 10^3 g^{-1} ± stdev)	TOP LOT	8 ± 4	13 ± 9	9 ± 5	3 ± 2	13 ± 5	0.7 ± 0.3	22 ± 14	58 ± 7	0.6 ± 0.3	4 ± 0.4
MPN SRB (g^{-1} with 99% confidence limits[b])	TOP LOT	-	-	5 (2-30)	-	22 (3-53)	-	-	11 (2-39)	-	7 (3-41)
MPN AA (g^{-1} with 99% confidence limits[b])	TOP LOT	-	27 (3-64)	7 (3-42)	-	17 (4-64)	-	35 (2-54)	6 (3-37)	15 (3-54)	10 (2-36)
ATP (amole × 10^5 g^{-1} ± stdev)	TOP LOT	-	-	-	-	-	-	-	-	-	-
CHAB (CFU[a] × 10^3 g^{-1} ± stdev)	MID LOT	-	-	0.12 ± 0.08	-	-	49 ± 34	-	-	0.09 ± 0.08	-
MPN SRB (g^{-1} with 99% confidence limits[b])	MID LOT	-	-	-	-	-	-	-	-	-	-
MPN AA (g^{-1} with 99% confidence limits[b])	MID LOT	-	-	-	-	-	-	-	-	-	-
ATP (amole × 10^5 g^{-1} ± stdev)	MID LOT	-	-	-	-	-	1 ± 0.2	-	-	-	-

[e] CFU – colony forming units, [b]determined using the method of Cornish and Fischer described in Klee, 1993

5. CONCLUDING REMARKS

The sampling procedures and analysis protocols in this experiment worked properly. The only cultured bacteria in the samples were the ones intrinsic in the bentonite during sampling. The origin of the bacteria in the bentonite is still to be determined but probably do they originate both from the surrounding groundwater and from the bentonite itself.

The numbers of AA and SRB were low compared to the surrounding groundwater, which can be expected because of the harsh conditions in the bentonite. Nevertheless, they could be found repeatedly in many of the TOP LOT bentonite samples. The results show that the bacteria inside the TOP LOT bentonite are viable and have a potential to produce both sulphide and organic carbon in form of acetate. The elevated density, temperature or/and the lower water content in the MID LOT bentonite compared to the TOP LOT bentonite obviously made it difficult for the bacteria to survive. However, in a few MID LOT samples bacteria were still viable.

6. REFERENCES

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