

Results report

Sampling and analyses of gases and microorganisms in the water from MINICAN in 2007, 2008 and 2010

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April 2011

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This report concerns a study which was conducted for SKB. The conclusions and viewpoints presented in the report are those of the authors. SKB may draw modified conclusions, based on additional literature sources and/or expert opinions.

Data in SKB's database can be changed for different reasons. Minor changes in SKB's database will not necessarily result in a revised report. Data revisions may also be presented as supplements, available at www.skb.se.

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Abstract

The MINICAN project is located at the depth of 450 m in the Äspö Hard Rock Laboratory and was initiated to study how corrosion of the cast iron insert inside a perforated copper canister would evolve with time. Miniature canisters with different perforations and with and without bentonite buffer in steel cages were installed and monitored. Samples for microbiological and gas composition together with samples for groundwater chemistry have been analysed at three occasions in 2007, 2008 and 2010. The results show how the microbial populations outside the canisters have evolved from a mixture of microorganism able to grow on organic material, like heterotrophic organisms, and acetogens that grow on hydrogen gas and carbon dioxide in 2007, to populations with a large proportion of sulphate-reducing bacteria in 2010. The highest number of sulphate-reducing bacteria was found in MINICAN experiment A02C, canister with one hole at the top of the copper canister, in 2010 with $2.4 \times 10^4 \text{ mL}^{-1}$ followed by $8 \times 10^3 \text{ mL}^{-1}$ in A03 (hole in the bottom of the canister) and $7 \times 10^3 \text{ mL}^{-1}$ in A06 (two holes at the top and no bentonite). The numbers of culturable heterotrophic bacteria were between 200 and 530 mL^{-1} in the experiments with bentonite in 2007 but below detection in all experiments in 2010. The same trend was shown for acetogenes. Measurable amounts of hydrogen gas were found in all experiments at all sampling occasions. There was no general trend for the amounts of hydrogen but there was an increase in three of the experiments and in the groundwater outside MINICAN. It was found that the water chemistry differed between A06 and A02–A04 experiment by higher sulphate and chloride concentrations in A06 compared to the others. By plotting the concentrations of chloride, sulphate against time, a decrease in sulphate concentration was found in all canister experiments. The chloride concentrations were stable during the same period. On the other hand, an increase in sulphate concentration was found in the groundwater outside the MINICAN project. In summary, the results from the three microbiology investigations of MINICAN show that the microbial population has developed from a heterotrophic community using organic material, presumably from the bentonite, in the first years to a population with increased numbers of sulphate-reducing bacteria in the last year. This progress is reflected in the water chemistry by a decrease in sulphate concentration in the canister experiments compared to stable chloride concentration. A possible energy source for the sulphate-reduction is hydrogen gas produced by corrosion of the steel cage surrounding the bentonite. Increased ferrous iron concentrations over the time in the canister experiments support this assumption.

Sammanfattning

MINICAN projektet är placerat på 450 m djup i Äspö tunneln. Syftet med projektet är att studera hur korrosionen av gjutjärnsinsatsen i en genomborrad kopparkapsel påverkas över tid. Miniatyrmodeller av kopparkapslar med hål på olika platser och med och utan bentonit i en stålbur, installerades och har övervakats. Prover för mikrobiologi och gas analyser samt grundvatten kemi har analyserat vid tre tillfällen, 2007, 2008 och 2010. Resultaten visar hur den mikrobiologiska populationen utanför kapseln utvecklats från att bestå av mikroorganismer som lever av organiska föreningar, såsom heterotrofa mikroorganismer, och acetogener vilka lever på vätgas och koldioxid vid provtagningen 2007 till populationer som till stor del består av sulfatreducerande bakterier vid provtagningen 2010. Det högsta antalet sulfatreducerare, $2.4 \times 10^4 \text{ mL}^{-1}$, uppmättes i MINICAN experimentet A02, kapsel med ett hål högst upp, följt av $8 \times 10^3 \text{ mL}^{-1}$ i A03, kapsel med ett hål i botten samt $7 \times 10^3 \text{ mL}^{-1}$ i A06, kapsel utan bentonit med två hål högst upp. Antalet odlingsbara heterotrofa mikroorganismer 2007, låg mellan 200 och 530 mL^{-1} i experimenten med bentonit men antalen hade sjunkit till under detektionsnivån för metoden vid provtagningen 2010. Antalet acetogener uppvisade samma trend. Mätbara mängder av vätgas kunde uppmätas i alla experiment vid alla provtagningarna. Vätgaskoncentrationen uppvisade ingen trend över tiden men en ökning uppmättes i tre av experimenten och i grundvattnet. Vattenkemidata visade att koncentrationen av klorid och sulfat i A06 är högre än i experimenten A02–A04 och i grundvattnet. Sulfatkoncentrationen sjönk över tid i jämförelse med kloridkoncentrationen i alla kapsexperimenten. I grundvatten ökade sulfatkoncentrationen däremot under samma tid. Sammanfattningsvis visar resultaten från de mikrobiologiska undersökningarna att mikrobpopulationens sammansättning har förändrats över tiden från en med heterotrofa och acetogena mikroorganismer som utnyttjar organiska föreningar till en population bestående av en stor del sulfatreducerande bakterier. Förändringen i mikrobpopulationen kan även följas i en minskning av sulfatkoncentrationen över tid. En möjlig energikälla för de sulfatreducerande bakterierna är vätgas som producerats vid korrosionen av stålburarna runt bentoniten. En ökande koncentration av tvåvärt järn utanför kopparkapslarna stödjer denna teori.

Contents

1	Introduction	7
1.1	The MINICAN experiment	7
1.2	Microbial corrosion and hydrogen gas metabolism	7
2	Material & Methods	9
2.1	Sampling occasions and analyses performed	9
2.2	Sampling procedures	9
2.3	Analyses and data treatment	10
3	Results	11
3.1	Microbial composition in MINICAN	11
3.1.1	ATP	11
3.1.2	TNC	12
3.1.3	CHAB	12
3.1.4	MPN AA	12
3.1.5	MPN SRB	13
3.2	Gas composition in MINICAN	14
3.2.1	Total gas volume	14
3.2.2	Hydrogen gas	16
3.2.3	Methane	16
3.2.4	Carbon dioxide	17
3.2.5	Carbon monoxide	17
3.2.6	Argon	17
3.2.7	Helium	17
3.2.8	Nitrogen	18
3.2.9	Ethane, ethylene, ethane, propane and propene	18
3.2.10	Oxygen	18
3.3	Water chemistry	19
4	Discussion	23
4.1	The microbial populations in the MINICAN experiments	23
4.1.1	Number and activity	23
4.1.2	The composition of the microbial populations	23
4.1.3	Sulphate	24
4.1.4	Ferrous iron	24
4.1.5	Hydrogen gas	25
4.2	Conclusions	25
4.3	Future research	25
5	References	27

1 Introduction

1.1 The MINICAN experiment

The MINICAN project is located at the depth of 450 m in the Äspö Hard Rock Laboratory (HRL) research tunnel. MINICAN consists of five different experiment canisters (Table 1-1), denoted experiment A02–A06. Four of the MINICAN test copper canisters are surrounded by bentonite in a support steel cage, of which the bentonite in experiment A05 is fully compacted according to the KBS-3 approach (dry density 1,600 kg m⁻³) and experiments A02–A04 are compacted with bentonite to a lower density than will be used (dry density 1,300 kg m⁻³). Experiment A06 lacks bentonite. In all the MINICAN copper canisters, holes with a diameter of 1 mm have been introduced to allow Äspö groundwater to come in contact with the interior cast iron inserts.

1.2 Microbial corrosion and hydrogen gas metabolism

The experimental part of the MINICAN project started in 2007 and aimed at, during a five year period, examine how the corrosion of the cast iron insert develops inside the perforated copper canisters. In the real waste repository, corrosion of the cast iron will in a worst-case scenario expose the spent nuclear fuel to groundwater and release radionuclides into the surroundings. Another potential risk with corrosion is that hydrogen gas can be produced because when iron comes in contact with anaerobic water, cathodic hydrogen is formed at the iron surface (King and Miller 1971). Hydrogen gas is unwanted in the KBS-3 storage for two reasons. The first is that an increase in gas volume will build up the pressure inside the system. The second is that development of hydrogen gas is closely linked to activity of sulphate-reducing bacteria (SRB). Hydrogen gas may be used as an excellent energy source for many of the microbes in the deep granitic subsurface (Pedersen 1999), in particular the SRB.

SRB utilize both inorganic and organic energy sources, of which the major subsurface sources are hydrogen gas and acetate, respectively. Cathodic hydrogen is believed to be directly scavenged by SRB if they carry the enzyme hydrogenase (Cord-Ruwisch and Widdel 1986). In addition, there are indications that SRB (and methanogens) can use metallic iron directly in their metabolism as their electron donor (Dinh et al. 2004).

Table 1-1. The MINICAN experiments installed at the Äspö Hard Rock Laboratory.

Experiment name	Sampling point	Filling of the cage around the test canister	Introduced hole in the copper canister to the cast iron
A02	KA3386 A02	Highly permeable, low density bentonite (1,300 kg m ⁻³)	Hole (1 mm in diameter) located at the top of the test canister
A03	KA3386 A03	Highly permeable, low density bentonite (1,300 kg m ⁻³)	Hole (1 mm in diameter) located at the bottom of the test canister
A04	KA3386 A04	Highly permeable, low density bentonite (1,300 kg m ⁻³)	Holes (1 mm in diameter) located both at the bottom and top of the test canister
A05	KA3386 A05	Fully compacted bentonite (1,600 kg m ⁻³) from blocks	Hole (1 mm in diameter) located at the top of the test canister
A06	KA3386 A06	Only groundwater in contact with the test canister	Two holes (1 mm in diameter) located at the top of the test canister

The **direct** corrosion net formula is given as equation 1:



(Dinh et al. 2004)

SRB living on organic compounds such as acetate can produce sulphide, which indirect can corrode the iron chemically and form FeS.

The **indirect** reaction net formula is given as equation 2:



(Dinh et al. 2004)

As a result of equations 1 and 2, there may be a boost corrosion effect from hydrogen driven sulphide production around a small penetration of a copper canister. Copper sulphide will form rapidly and the penetration will grow much more rapidly with, than without microbial sulphide production. A first appreciation of the potential for this boost corrosion effect will be obtained when MINICAN A04 is retrieved during 2011.

Consequently, the main question to answer in this microbiology part of the MINICAN project was whether microbial sulphate reducing activity could be coupled with high (or low) levels of hydrogen gas from iron corrosion in the MINICAN experiments. This question was approached by determination of the composition and amounts of microbial populations in the MINICAN environment, the composition and amount of gases and the groundwater chemistry in the surrounding groundwater and inside the support cages.

Analyses of microbial presence and activity, chemistry and dissolved gas in groundwater from the cages around the copper canisters were performed after each other in May, August and September 2007, and in October 2008 and December 2010. Sampling and analysis of gases comprised hydrogen, carbon monoxide, argon, carbon dioxide, methane, ethane, ethane, ethylene, propane, propene, propyn, helium, oxygen and nitrogen. Sampling and analysis microorganisms comprised total number of cells (TNC), quantitative most probable number (MPN) of SRB and autotrophic acetogens (AA), culturable heterotrophic aerobic bacteria (CHAB) and analysis of adenosine triphosphate (ATP) measurement were performed.

2 Material & Methods

2.1 Sampling occasions and analyses performed

Samplings of water for gas-, microbe- and chemical analyses from the MINICAN experiments were performed in 2007, 2008 and in 2010. Table 2-1 shows information on experiment names, sampling points, dates for sampling of dissolved gas, microbiology and water chemistry. Table 2-2 lists the performed analyses .

2.2 Sampling procedures

A sterile tube and junctions with a mounted valve, stopcock and a needle was attached to the connection of each experimental sampling point listed in Table 2-1. An anaerobic sampling vessel was attached to the sterile tube and the needle was penetrated through the septa of the vessel. During sampling, the stopcock and the outflow from the SKB MINICAN connections were opened and the water filled the sampling vessels. An additional needle was penetrated through the septum of the sampling vessel shortly after the sampling begun, to eliminate dangerous pressure build-up.

Table 2-1. Analyses performed in May, August and September 2007, in October 2008 and in December 2010 in the groundwater (G) and in water from inside the cage (C) surrounding the canisters in the MINICAN experiments.

Experiment name	Sampling point	Sampling date dissolved gas	Sampling date microbes	Sampling date water chemistry
A02C	KA3386 A02Canister	2007-09-28,	2007-08-21,	2007-05-22,
		2008-10-15,	2008-10-15,	2008-10-15,
		2010-12-08	2010-12-08	2010-12-08
A03C	KA3386 A03Canister	2007-09-28,	2007-08-21,	2007-05-22,
		2008-10-15,	2008-10-15,	2008-10-15,
		2010-12-08	2010-12-08	2010-12-08
A03G	KA3386 A03Ground-water	2007-09-28,	2007-08-21,	2007-05-22,
		2008-10-15,	2008-10-15,	2008-10-15,
		2010-12-08	2010-12-08	2010-12-08
A04C	KA3386 A04Canister	2007-09-28,	2007-08-21,	2007-05-22,
		2008-10-15,	2008-10-1,	2008-10-15,
		2010-12-08	2010-12-08	2010-12-08
A05-C	KA3386 A05-canister	— ^a	—	—
A06C	KA3386 A06Canister	2007-09-28,	2007-08-21,	2007-05-22,
		2008-10-15,	2008-10-15,	2008-10-15,
		2010-12-08	2010-12-08	2010-12-08

^a Water could not be extracted from experiment A05-C.

Table 2-2. Parameters analysed in the groundwater in the groundwater and in water from inside the cage surrounding the canisters in the MINICAN experiments.

Gas analyses		Microbial analyses		Chemical analyses	
Sampling vessel	Analyses	Sampling vessel	Analyses	Sampling vessel	Analyses
PVB sampler	H ₂ , CO ₂ , CO, CH ₄ , C ₂₋₃ H ₂₋₈ , O ₂ , He, Ar, N ₂	10–100 mL anaerobic tube or bottle	TNC, ATP, CHAB, MPN SRB and AA	According to SKB standard	SO ₄ ²⁻ , HCO ₃ ⁻ , Fe ²⁺ , S ²⁻ , Cl ⁻ , pH, acetate

In 2007, the first 50 mL of water sampled from the MINICAN experiments flushed the sample equipment and were discarded. Thereafter, the following volumes of water were sampled for analyses according to Table 2-2: ATP and TNC sampling (30 mL), MPN SRB (15 mL), CHAB (10 mL), acetate (10 mL), MPN AA (10 mL) and dissolved gas (200 mL). The chemistry sampling was performed by SKB in 2007-05-22.

In 2008, the first 30 mL of water sampled from the MINICAN experiments flushed the sample equipment and were discarded. Thereafter, the following volumes of water were sampled for analyses according to Table 2-2: acetate (10 mL), MPN SRB (10 mL), MPN AA (10 mL), ATP (30 mL), TNC (30 mL), CHAB (30 mL), dissolved gas (250 mL) and chemistry (2,500 mL).

In, 2010, the first 50 mL of water sampled from the MINICAN experiment flushed the sample equipment and were discarded. acetate (10 mL), Fe^{2+} (10 mL), MPN SRB (10 mL), MPN AA (10 mL), TNC (30 mL), ATP (50mL), CHAB (30 mL), dissolved gas (250 mL) and chemistry (2,500 mL), were used for respective analysis.

2.3 Analyses and data treatment

Gas analysis was performed as described previously by Pedersen et al. (2008ab) and TNC, MPN SRB, MPN AA, CHAB and ATP analyses were performed according to Hallbeck and Pedersen (2008). ATP was determined using the ATP Biomass Kit HS for determining total ATP in living cells (no. 266–311; BioThema, Handen, Sweden).

Data treatment and graphics were performed using STATISTICA software, version 10.0 (Statsoft, Tulsa, OK, USA).

3 Results

3.1 Microbial composition in MINICAN

The microbial compositions inside and outside the support cages of the MINICAN experiments in 2007, 2008 and 2010 are shown in Table 3-1, Table 3-2 and Table 3-3. Each microbiological parameter will be treated in the following sections.

3.1.1 ATP

The amount of ATP inside the cages of the MINICAN experiments A02C, A03C, A04C and A06C in 2007 ranged from 1,700 to 3,000 amoles mL⁻¹. The groundwater in A03G contained 3,300 amoles ATP mL⁻¹. The ATP content in this groundwater in 2008 was in the same order of magnitude, 4,400 amoles mL⁻¹. Interestingly, the sampling in 2008 from inside the cages of the MINICAN experiments A02C, A03C, A04C and A06C consistently showed an increase in ATP content compared to 2007 (between 1.7 and 7.3 times). The largest increase in ATP was found in MINICAN experiment A02C (17,400 amole mL⁻¹, 7.3 times the content in 2007). On the other hand, all ATP samples from inside the cages were lower in 2010 than in 2008. In the groundwater from A03G, the ATP was 27,700 amol L⁻¹ in 2010.

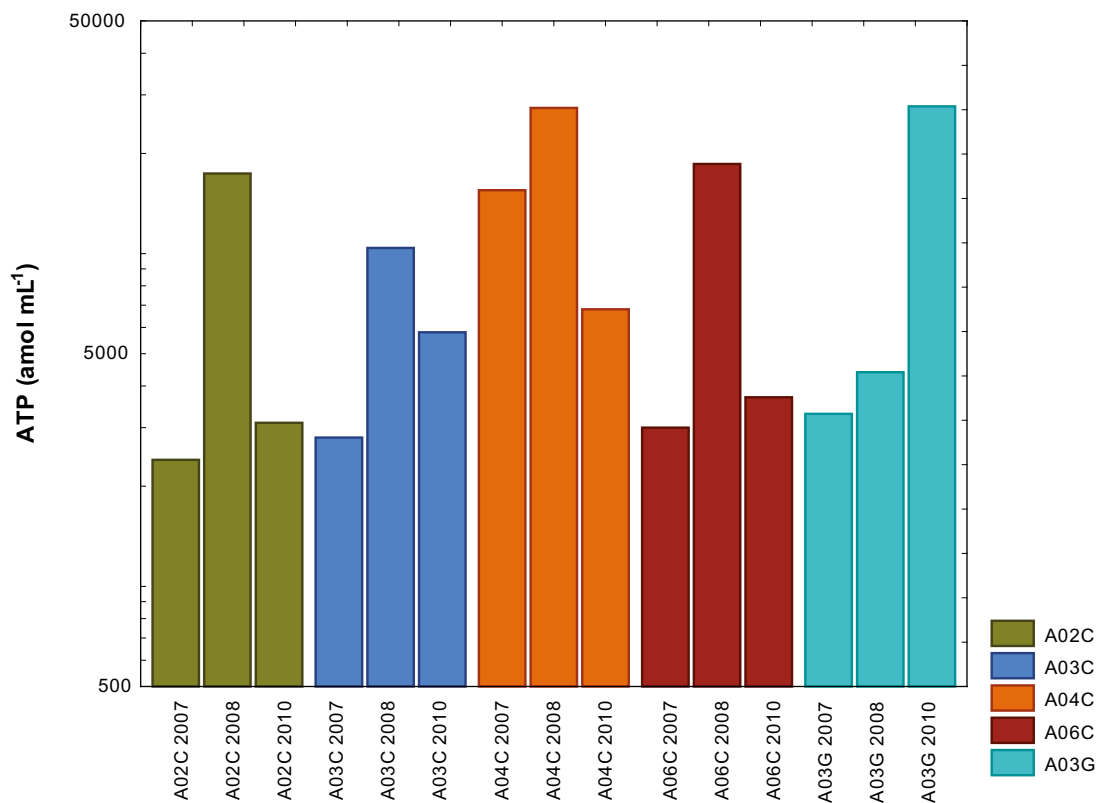


Figure 3-1. The amount of adenosine triphosphate, ATP, in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010.

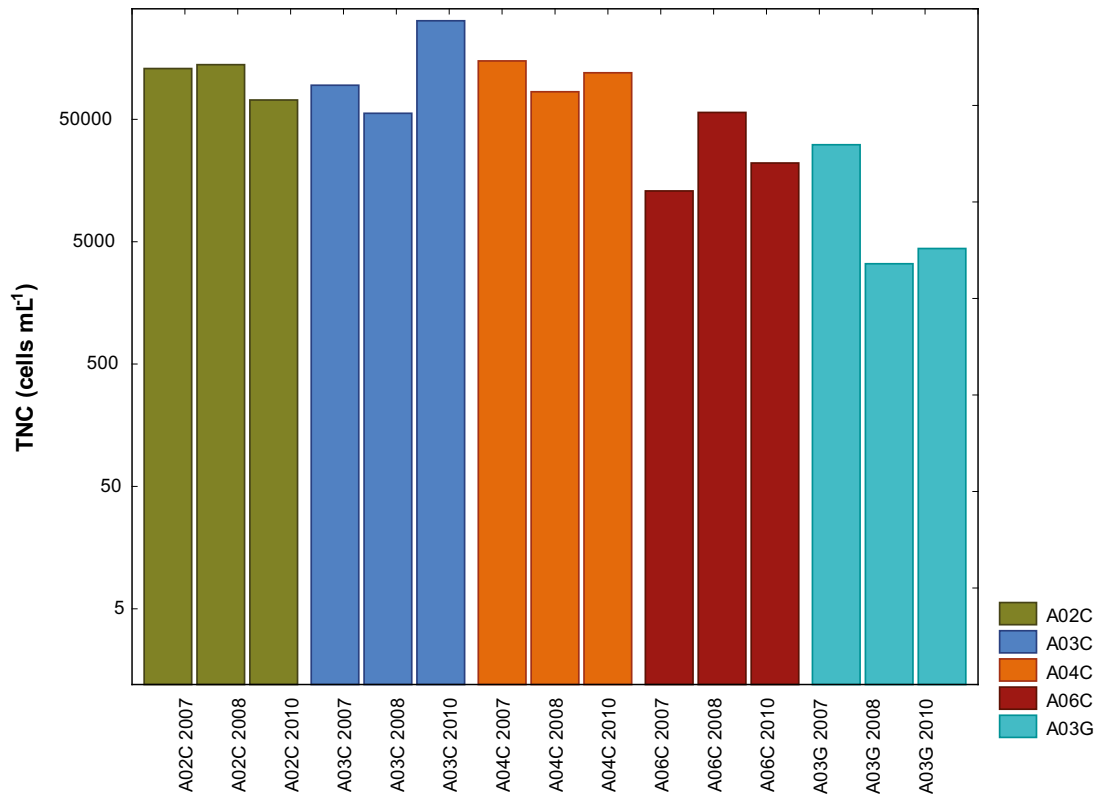


Figure 3-2. The total number of cells, TNC, in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010.

3.1.2 TNC

The TNC data from the three sampling occasions are found in Figure 3-2. The TNC analyses of water from inside the cages of the MINICAN experiments in 2007 and 2008 showed approximately the same numbers (between 10^4 and 10^5 cells mL^{-1}). There was a minor increase in cell numbers in MINICAN experiment A02C and A06C and a minor decrease in MINICAN experiments A03C and A04C. However, the TNC in A03G, a 10-fold decrease from 2007 to 2008 was observed (from 3.0×10^4 to 3.0×10^3 cells mL^{-1}). In 2010 the TNC in A03G was similar to the number measured in 2008. The highest increase in TNC 2010 compared to 2008 was found in A03C, from 5.6×10^4 to 3.2×10^5 mL^{-1} .

3.1.3 CHAB

The numbers of CHAB decreased from 2007 to 2008 (Table 3-1, Table 3-2). In 2007, the number of CHAB inside MINICAN experiments A02C, A03C and A04C ranged from 200 to 530 cells mL^{-1} and in 2008 the same ranged from below detection to 20 cells mL^{-1} . In A03G and A06C very few (3 cells mL^{-1} and below detection) CHAB were detected in 2008 compared to 20 and 17 cells mL^{-1} the year before. In 2010, the numbers of CHAB for all samples were below detection (Table 3-3).

3.1.4 MPN AA

The numbers of AA in the MINICAN experiments determined by MPN in 2007 ranged from 8 to 50,000 mL^{-1} (Table 3-1). The highest amount of AA was found in MINICAN experiment A02C, A03C and A04C (7,000–50,000 cells mL^{-1}). In A03G and in A06C the numbers of AA were low (below detection limit and 8 cells mL^{-1} , respectively). However, in 2008 the high numbers of AA inside the cages of the MINICAN experiments A02C, A03C and A04C had disappeared (Table 3-2). The numbers decreased during the year to 1.7–90 cells mL^{-1} . The numbers in A03G and in A06C were 0.4 cells mL^{-1} and 2,700 cells mL^{-1} , respectively. The acetate concentrations in 2007 ranged from 6.9–11 mg L^{-1} . In 2008, the concentrations ranged from 1.6–2.9 mg L^{-1} , reflecting the lower numbers of AA this year. The numbers of AA in 2010 were below detection in all experiments and the acetate concentration varied between 1.7 and 3.5 mg L^{-1} (Table 3-3).

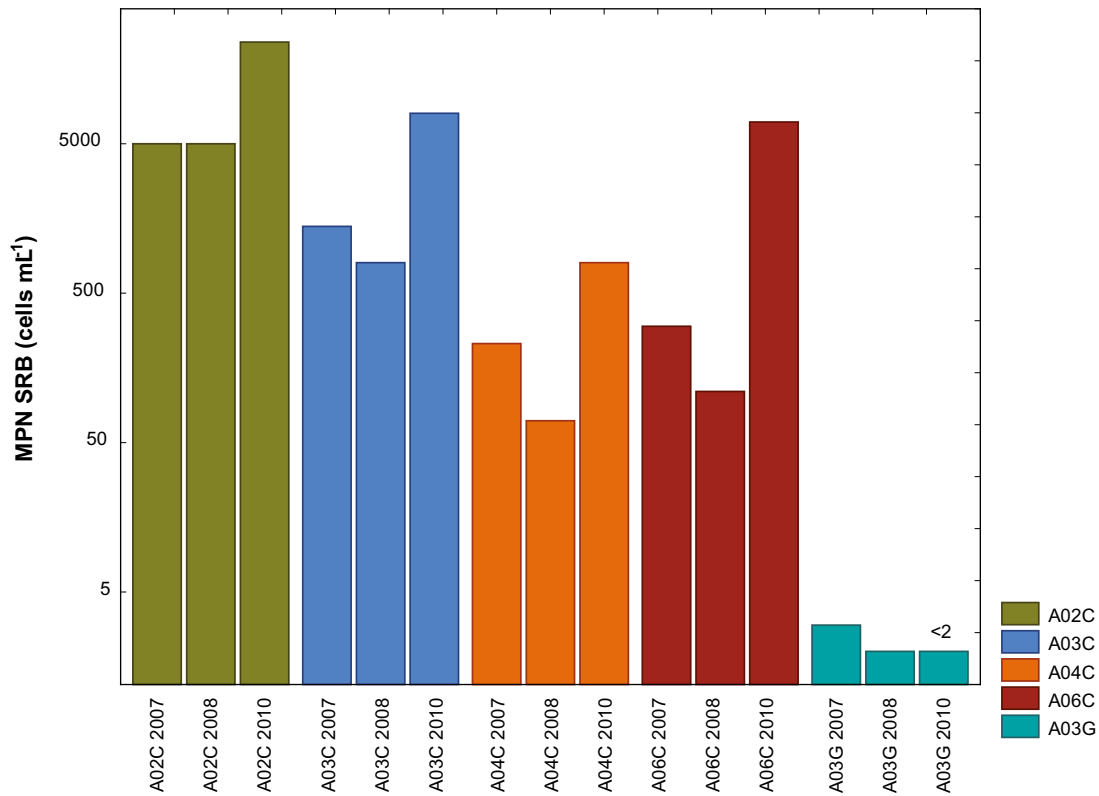


Figure 3-3. The most probable number (MPN) of sulphate-reducing bacteria, SRB, in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010.

3.1.5 MPN SRB

The numbers of SRB in the MINICAN experiments determined by MPN in 2007 ranged from 230 to 5,000 mL⁻¹ (Table 3-1) (Figure 3-3). In difference to the CHAB and AA numbers which decreased during the following year, the SRB numbers inside the cages of the MINICAN experiments remained in the same order of magnitude in 2008 (ranging from 70 to 5,000 mL⁻¹) (Table 3-2) (Figure 3-3). The highest amount of SRB was found in MINICAN experiment A02C (5,000 mL⁻¹) both in 2007 and 2008 but also in 2010. In 2010 the SRB numbers ranged from 800 to 24,000 mL⁻¹. The amount of SRB in A03G were low in 2007, 2008 and 2010 (2, 3 and <2 mL⁻¹), respectively.

Table 3-1. Microbial composition in samples from the four experiment cages (C) and groundwater (G) collected in 2007-08-21.

Experiment name	ATP (amol mL ⁻¹)	TNC (mL ⁻¹)	CHAB (mL ⁻¹) ^a	MPN SRB (mL ⁻¹)	MPN AA (mL ⁻¹) ^c	Acetate (mg L ⁻¹)
A02C	2,400 ± 450 ^a	130,000 ± 16,000 ^a	227 ± 42	5,000 (2,000–17,000) ^c	30,000 (2,000–17,000)	8.5
A03C	2,800 ± 240	95,000 ± 39,000	200 ± 57	1,400 (600–3,600)	50,000 (20,000–200,000)	8
A03G	3,300 ± 710	31,000 ± 11,000	20 ± 35	3 (1–12)	3 (1–12)	6.9
A04C	15,500 ± 810	150,000 ± 61,000	530 ± 28	230 (90–860)	7,000 (3,000–21,000)	6.9
A06C	3,000 ± 680	13,000 ± 4,900	17 ± 12	300 (100–1,100)	bd	11

^a Standar deviation.

^b Below detection.

^c 95% confidence interval.

Table 3-2. Microbial composition in samples from the four experiment cages (C) and groundwater (G) collected in 2008-10-15.

Experiment name	ATP (amol mL ⁻¹)	TNC (mL ⁻¹)	CHAB (mL ⁻¹) ^a	MPN SRB (mL ⁻¹)	MPN AA (mL ⁻¹)	Acetate (mg L ⁻¹)
A02C	17,400 ± 2,500 ^a	180,000 ± 4,600 ^a	bd ^b	5,000 (2,000–17,000) ^c	1.7 (0.7–4) ^c	1.7
A03C	10,400 ± 690	180,000 ± 18,000	3	800 (300–2,500)	17 (7–48)	1.6
A03G	4,400 ± 570	2,500 ± 880	bd	2.3 (0.9–8.6)	0.4 (0.1–1.7)	2.9
A04C	27,400 ± 1,820	200,000 ± 22,000	bd	70 (30–210)	90 (30–290)	1.8
A06C	18,600 ± 1,270	71,000 ± 11,000	bd ^b	110 (40–300)	2,700 (1,200–6,700)	2.3

^a Standar deviation.

^b Below detection.

^c 95% confidence interval.

Table 3-3. Microbial composition in samples from the four experiment cages (C) and groundwater (G) collected 2010-12-08.

Experiment name	ATP (amol mL ⁻¹) ^a	TNC (mL ⁻¹) ^a	CHAB (mL ⁻¹)	MPN SRB (mL ⁻¹)	MPN AA (mL ⁻¹) ^c	Acetate (mg L ⁻¹)
A02C	3,100 ± 1,710 ^a	72,000 ± 10,000	bd ^b	24,000 (10,000–94,000) ^c	bd	3.5
A03C	5,800 ± 1,220	320,000 ± 52,000	bd	8,000 (3,000–25,000)	bd	2.9
A03G	27,700 ± 3,770	4,400 ± 1,100	bd	<2	bd	1.8
A04C	6,800 ± 2,330	120,000 ± 7,800	bd	800 (300–2,500)	bd	1.8
A06C	3,700 ± 840	22,000 ± 1,500	bd	7,000 (3,000–21,000)	bd	2.8

^a Standar deviation.

^b Below detection.

^c 95% confidence interval.

3.2 Gas composition in MINICAN

The data for the complete gas composition inside the cages of the four experiments and outside A03 in MINICAN from the samples from 2007, 2008 and 2010 are shown in Table 3-4, Table 3-5 and Table 3-6.

3.2.1 Total gas volume

Figure 3-4 shows the total gas volumes dissolved in the groundwater inside the cages of the four MINICAN experiments and outside A03, where water was extractable. The gas volume increased from 2007 to 2008 (with 32%) in one of the experiments, A02C. Experiments A03C, A04C and A06C instead showed a decrease in gas volume per water volume (with 15–25%). The gas volume had decreased for all sampling points except for experiment A04C, at the 2010 sampling compared to 2008. The decrease was between 20 and 30%.

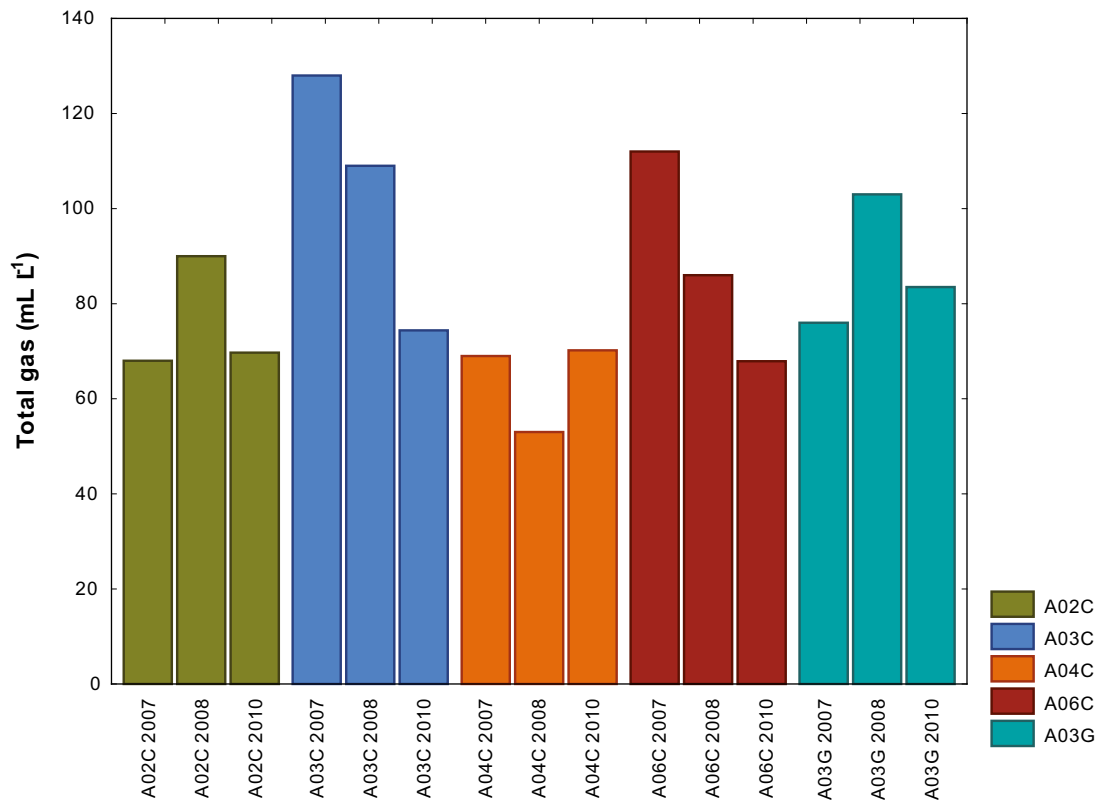


Figure 3-4. The total amount of gas in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010.

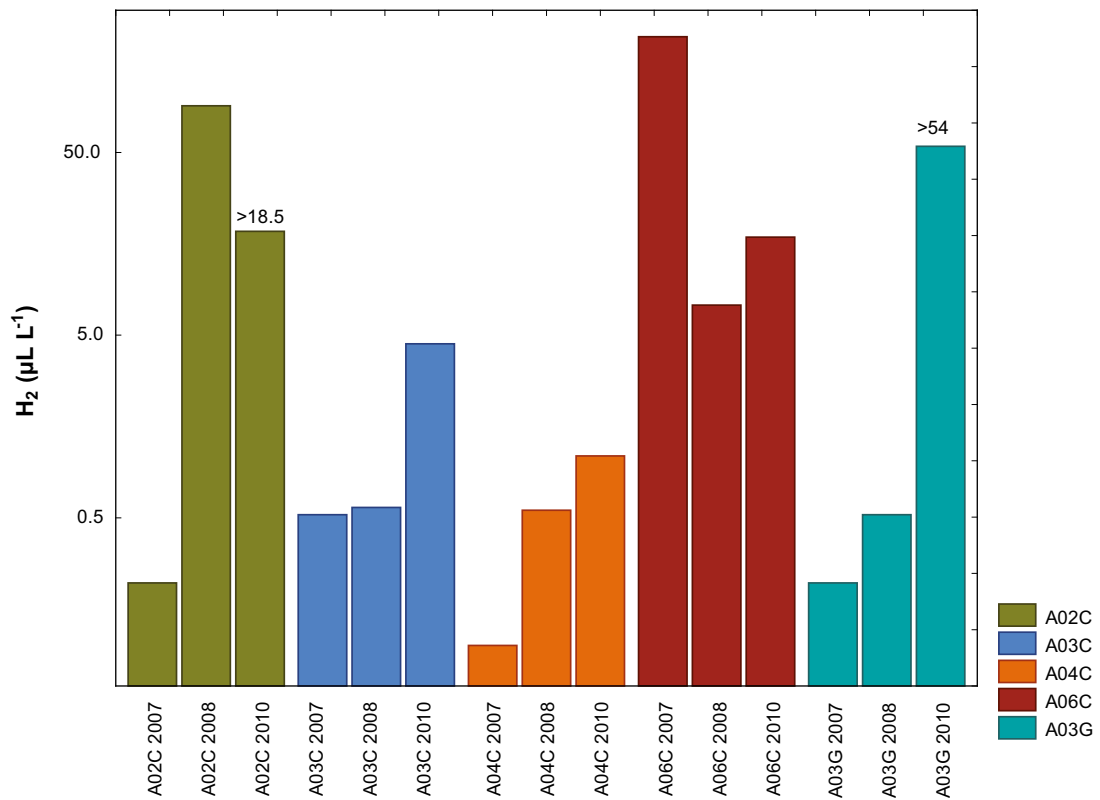


Figure 3-5. The amount of hydrogen gas, H₂, in in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010.

3.2.2 Hydrogen gas

Generally in 2007, the water both outside and inside the cages of the experiments contained fairly low concentrations of hydrogen gas ($0.10\text{--}0.52\ \mu\text{L L}^{-1}$), with the exception of the water in experiment A06–C, which contained $215\ \mu\text{L L}^{-1}$, approximately 130–2,200 times more than in the other samples at the time (Figure 3-5). Interestingly, in 2008 the hydrogen content in experiment A02C had increased to $90\ \mu\text{L L}^{-1}$. This was approximately 160–170 times more than in experiments A03C and A04C (which still contained 0.57 and $0.55\ \mu\text{L L}^{-1}$), and 12 times more than in experiment A06C in which the hydrogen content had decreased to $7\ \mu\text{L L}^{-1}$. The results from 2010 show that the hydrogen gas amounts had increased approximately 10 times in all four experiments. Two samples, from A02C and A03G both 2010, the absolute volume of hydrogen could not be measured because of difficulties to separate hydrogen and helium on the gas chromatograph. These volumes are therefore reported as higher than values.

3.2.3 Methane

The methane data are presented in Figure 3-6. In 2007, the methane concentrations in water from the experiments ranged from 104 to $326\ \mu\text{L L}^{-1}$. The methane contents were slightly higher in MINICAN experiments A02C, A03C and A04C than the groundwater outside experiment A03G (1.2–1.6 times), and on the opposite lower inside MINICAN experiment A06C compared to the groundwater outside experiment A03G (0.6 times). In 2008, the methane content had increased in experiment A02C and A03C ($500\text{--}593\ \mu\text{L L}^{-1}$) compared to the year before and in experiment A04C and A06C (215 and $216\ \mu\text{L L}^{-1}$). The methane content in A03G was $344\ \mu\text{L L}^{-1}$. The 2010 methane data show that the amounts were lower in A02C, A03C, 323 and $387\ \mu\text{L L}^{-1}$ than in 2008. Also in the groundwater A03G the methane had decreased. In the experiments A04C and A06C there were only small changes compared to 2008. In 2010, the methane concentrations were lower at all sampling points except in A04C which showed a minor increase compared to 2008.

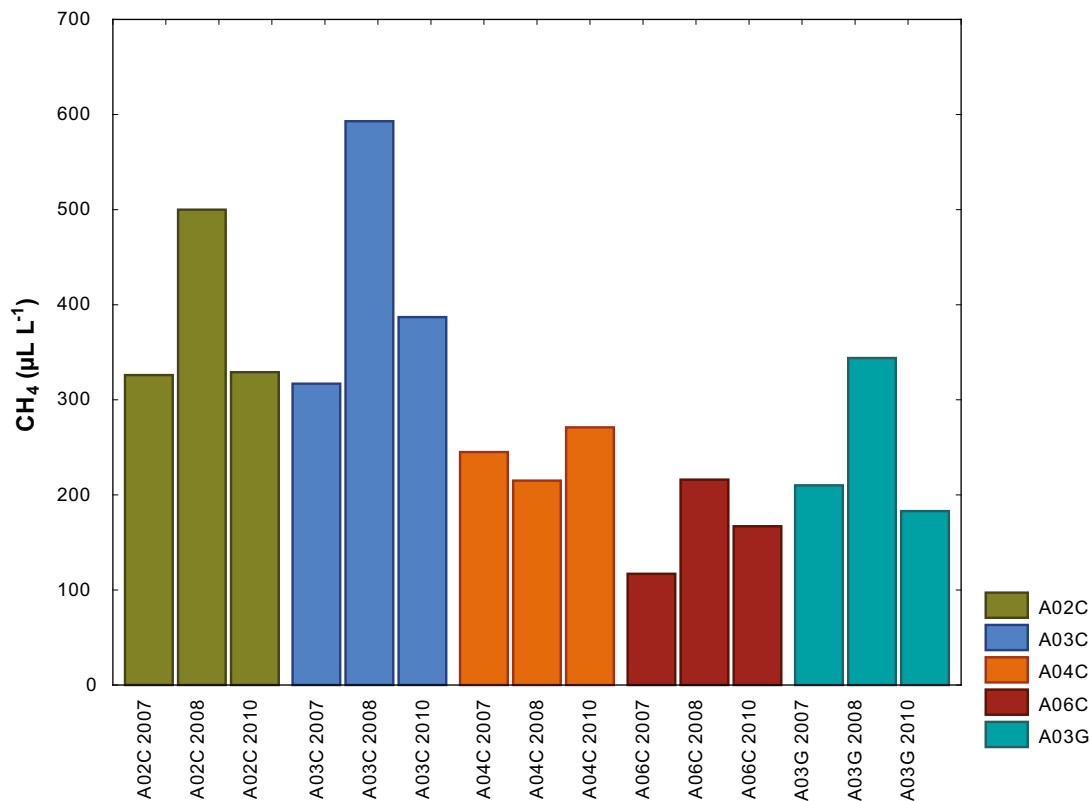


Figure 3-6. The amount of methane, CH₄, in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010.

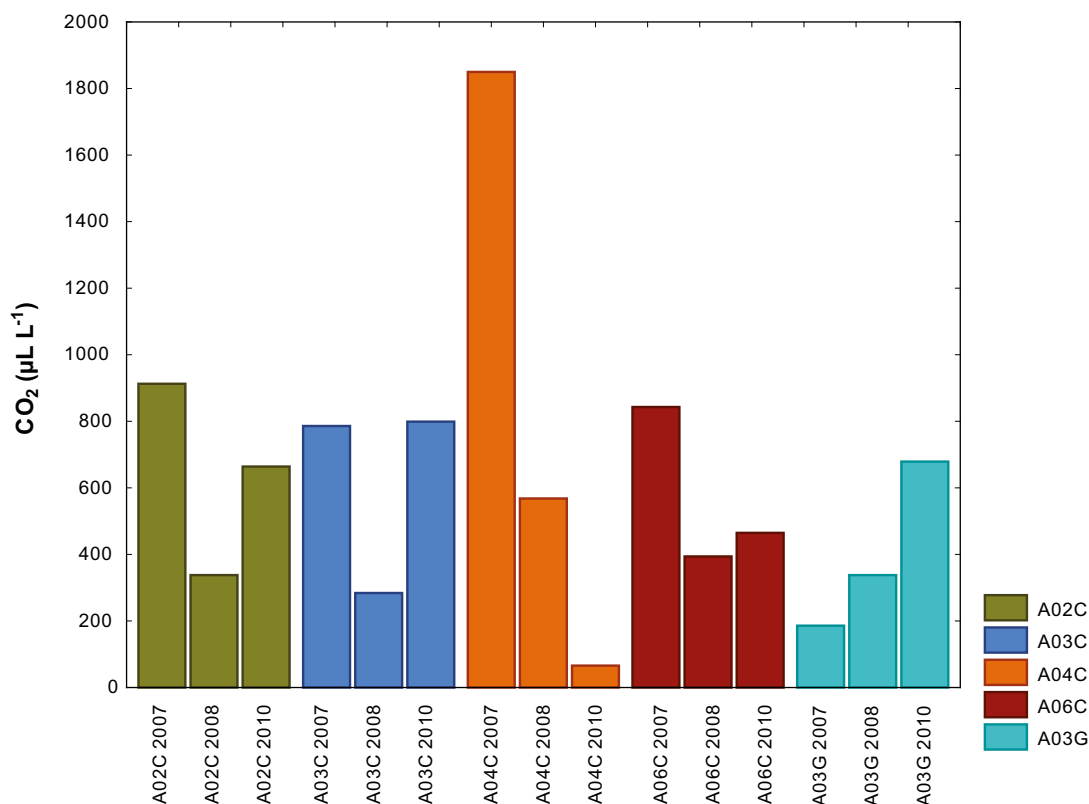


Figure 3-7. The amount of carbon dioxide in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010.

3.2.4 Carbon dioxide

Carbon dioxide content in the MINICAN experiments in 2007 was approximately four to ten times higher (786–1,850 $\mu\text{L L}^{-1}$) inside the cages of the experiments A02C, A03C, A04C and A06C than in A03G (186 $\mu\text{L L}^{-1}$). In 2008, the carbon dioxide contents were generally lower than in 2007. Experiments A02C, A03C and A06C contained 284–394 $\mu\text{L L}^{-1}$. Experiment A04C contained 568 $\mu\text{L L}^{-1}$, slightly more than in the other experiments but less than the previous year. A03G contained more carbon dioxide than previous year, 338 $\mu\text{L L}^{-1}$. The results from 2010 showed, an increasing trend in all samples but A04C. A continuously increasing trend over 2007 to 2010 was recorded only in the groundwater sample A03G.

3.2.5 Carbon monoxide

In 2007, the content of carbon monoxide in the MINICAN experiments was two to six times higher in A03G (3.4 $\mu\text{L L}^{-1}$) than inside the cages of all the MINICAN experiments (0.6–1.1 $\mu\text{L L}^{-1}$). The contents of carbon monoxide were similar inside the cages of the MINICAN experiments A02C, A03C and A04C (0.5–0.7 $\mu\text{L L}^{-1}$) in 2008 compared to the previous year. In experiment A06C, the carbon monoxide content was higher in 2008 compared to 2007 (6.8 $\mu\text{L L}^{-1}$). In 2010, the changes in carbon monoxide content were small compared to previous results. A02C, A03C, A04C and A06C had 0.28, 0.26, 0.26 and 0.25 $\mu\text{L L}^{-1}$, respectively and the groundwater sample A03G had 0.43 $\mu\text{L L}^{-1}$.

3.2.6 Argon

Argon was detected inside the cages of the MINICAN experiments from below detection limit to 1,190 $\mu\text{L L}^{-1}$. No specific trends could be detected from 2007 to 2010.

3.2.7 Helium

Helium was apart from nitrogen the most abundant gas in the MINICAN experiment, ranging from 4,920–14,900 $\mu\text{L L}^{-1}$. The content of helium in each specific sampling point was fairly constant from 2007 to 2010. The highest amounts of helium were found in A06C at all sampling occasions.

3.2.8 Nitrogen

The most abundant gas in the MINICAN experiment was nitrogen, ranging from 44,900–118,000 $\mu\text{L L}^{-1}$. The content of nitrogen decreased slightly (with 25%) in most of the samples from 2007 to 2008, except in MINICAN experiment A02C where the nitrogen content instead increased (with 34%). Nitrogen in samples from 2010 varied between 59.4 mL L^{-1} and 68.9 mL L^{-1} with the highest amount in the groundwater A03G.

3.2.9 Ethane, ethylene, ethane, propane and propene

The gases ethane, ethylene, ethane and propane were detected in trace amounts (in the order of 0.1–1.1 $\mu\text{L L}^{-1}$) in some of the samples while the concentration of propene were below detection limits in all samples.

3.2.10 Oxygen

The oxygen concentration was below detection in all samples.

Table 3-4. Dissolved gas in samples from the four experiment cages (C) and groundwater (G) collected in 2007-09-28.*

Experiment name	Gas/water (mL L^{-1})	H ₂ ($\mu\text{L L}^{-1}$)	CO ($\mu\text{L L}^{-1}$)	CH ₄ ($\mu\text{L L}^{-1}$)	CO ₂ ($\mu\text{L L}^{-1}$)	C ₂ H ₆ ($\mu\text{L L}^{-1}$)	C ₂ H ₂₋₄ ($\mu\text{L L}^{-1}$)	C ₃ H ₈ ($\mu\text{L L}^{-1}$)	C ₃ H ₆ ($\mu\text{L L}^{-1}$)	Ar ($\mu\text{L L}^{-1}$)	He ($\mu\text{L L}^{-1}$)	N ₂ ($\mu\text{L L}^{-1}$)
A02-C	68	0.22	0.58	326	913	0.33	0.14	<0.1	<0.1	838	7,980	57,800
A03C	128	0.52	1.08	317	786	0.20	0.07	<0.1	<0.1	1,060	8,420	118,000
A03G	76	0.22	3.39	210	186	0.09	<0.1	<0.1	<0.1	649	8,110	67,100
A04C	69	0.10	0.72	245	1,850	0.12	0.02	<0.1	<0.1	842	4,920	61,100
A06C	112	215	1.14	117	843	0.65	<0.1	<0.1	<0.1	710	14,200	95,600

*O₂ was below detection in all samples.

Table 3-5. Dissolved gas in samples from the four experiment cages (C) and groundwater (G) collected in 2008-10-15.

Experiment name	Gas/water (mL L^{-1})	H ₂ ($\mu\text{L L}^{-1}$)	CO ($\mu\text{L L}^{-1}$)	CH ₄ ($\mu\text{L L}^{-1}$)	CO ₂ ($\mu\text{L L}^{-1}$)	C ₂ H ₆ ($\mu\text{L L}^{-1}$)	C ₂ H ₂₋₄ ($\mu\text{L L}^{-1}$)	C ₃ H ₈ ($\mu\text{L L}^{-1}$)	C ₃ H ₆ ($\mu\text{L L}^{-1}$)	Ar ($\mu\text{L L}^{-1}$)	He ($\mu\text{L L}^{-1}$)	N ₂ ($\mu\text{L L}^{-1}$)
A02-C	90	90.1	0.52	500	338	<0.1	<0.1	<0.1	<0.1	1,110	9,990	77,400
A03C	109	0.57	0.53	593	284	0.18	<0.1	<0.1	<0.1	1,190	9,100	91,900
A03G	103	0.52	0.70	344	338	<0.1	<0.1	<0.1	<0.1	740	9,070	98,200
A04C	53	0.55	0.65	215	568	0.28	<0.1	0.05	<0.1	185	6,600	44,900
A06C	86	7.30	6.80	216	394	1.08	<0.1	0.05	<0.1	<0.1	14,900	71,200

*O₂ was below detection in all samples.

Table 3-6. Dissolved gas in samples from the four experiment cages (C) and groundwater (G) collected in 2010-12-08.

Experiment name	Gas/water (mL L^{-1})	H ₂ ($\mu\text{L L}^{-1}$)	CO ($\mu\text{L L}^{-1}$)	CH ₄ ($\mu\text{L L}^{-1}$)	CO ₂ ($\mu\text{L L}^{-1}$)	C ₂ H ₆ ($\mu\text{L L}^{-1}$)	C ₂ H ₂₋₄ ($\mu\text{L L}^{-1}$)	C ₃ H ₈ ($\mu\text{L L}^{-1}$)	C ₃ H ₆ ($\mu\text{L L}^{-1}$)	Ar ($\mu\text{L L}^{-1}$)	He ($\mu\text{L L}^{-1}$)	N ₂ ($\mu\text{L L}^{-1}$)
A02C	69.7	>18.5	0.28	329	664	0.24	<0.1	<0.1	<0.1	626	8,670	59,400
A03C	74.4	4.5	0.26	387	799	0.32	<0.1	<0.1	<0.1	425	9,180	63,600
A03G	70.2	1.1	0.26	271	66.1	0.04	<0.1	<0.1	<0.1	589	8,800	60,400
A04C	67.9	17.2	0.25	167	465	0.1	<0.1	<0.1	<0.1	773	7,030	59,500
A06C	83.5	>54.1	0.43	183	679	0.44	<0.1	<0.1	<0.1	1,120	12,500	68,900

*O₂ was below detection in all samples.

3.3 Water chemistry

The results from the water chemistry analyses in the MINICAN experiments in 2007, 2008 and 2010 are shown in Table 3-7, Table 3-8 and Table 3-9. The sulphate concentrations inside the cages of the MINICAN experiments A02C, A03C, A04C and A06C decreased during the 2007 to 2010 except for a slight increase in A03C from 2008 to 2010, see Figure 3-8. The sulphate concentration in A03G increased from 2007 to 2008 but decreased again in 2010. The highest concentration of sulphate was found in A06C, approximately 100 mg L⁻¹ higher compared to the other experiments and the groundwater in A03G. The ferrous iron concentration increased from 2007 to 2010 for all experiments except in A04C where the concentration decreased from 2008 to 2010. The highest ferrous iron concentration was found in A02C with 55 mg L⁻¹. Ferrous iron was low in the groundwater outside A03C, (Figure 3-10). pH values are shown in Figure 3-9 and it shows that the tendency for all experiments and the groundwater is decreasing pH expect for A04C where there was a slight increase 2010 compared to 2008. Sulphide was low all three years (0.02–0.08 mg L⁻¹). Given the high concentrations of ferrous iron in the experiments, any sulphide produced would precipitate as iron sulphide. There were differences in the chloride concentrations both between the different experiments but there was also tendency to an increase along the timeline. The highest chloride concentration was found in A06C with values between 11,310 and 11,420 mg L⁻¹. The lowest concentrations were found in A04C with values from 6,671 to 7,968 mg L⁻¹ (Figure 3-4).

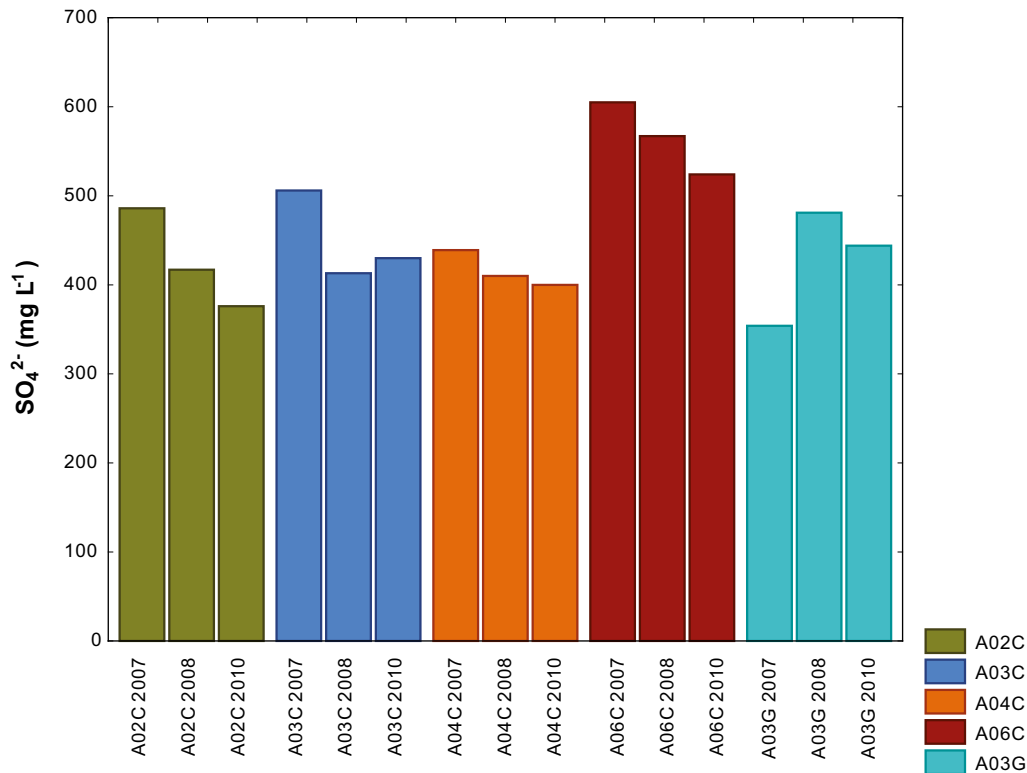


Figure 3-8. The amount of sulphate, SO_4^{2-} , in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010.

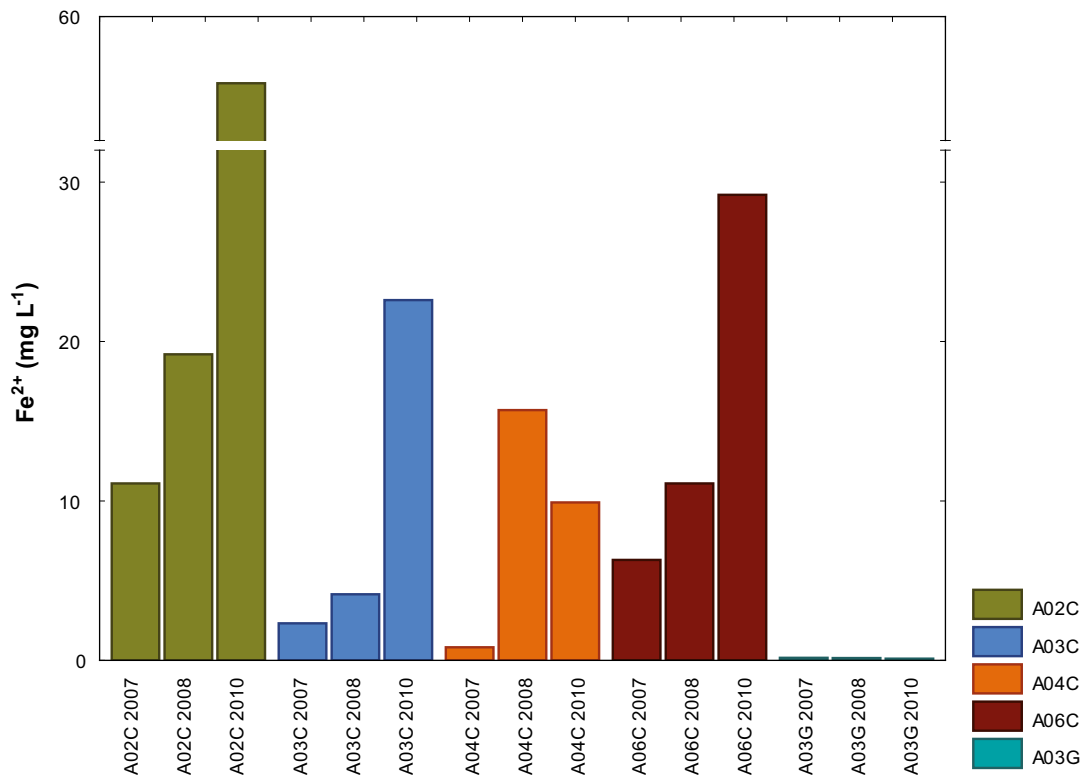


Figure 3-9. The amount of ferrous iron, Fe²⁺, in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010. Please note the scale brake in the y-axis.

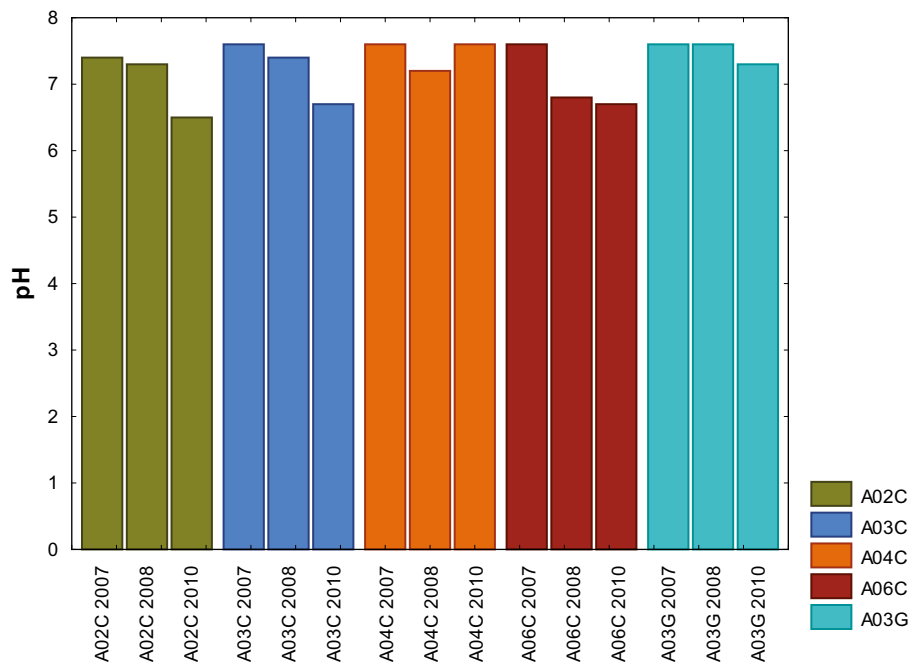


Figure 3-10. pH in groundwater samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010.

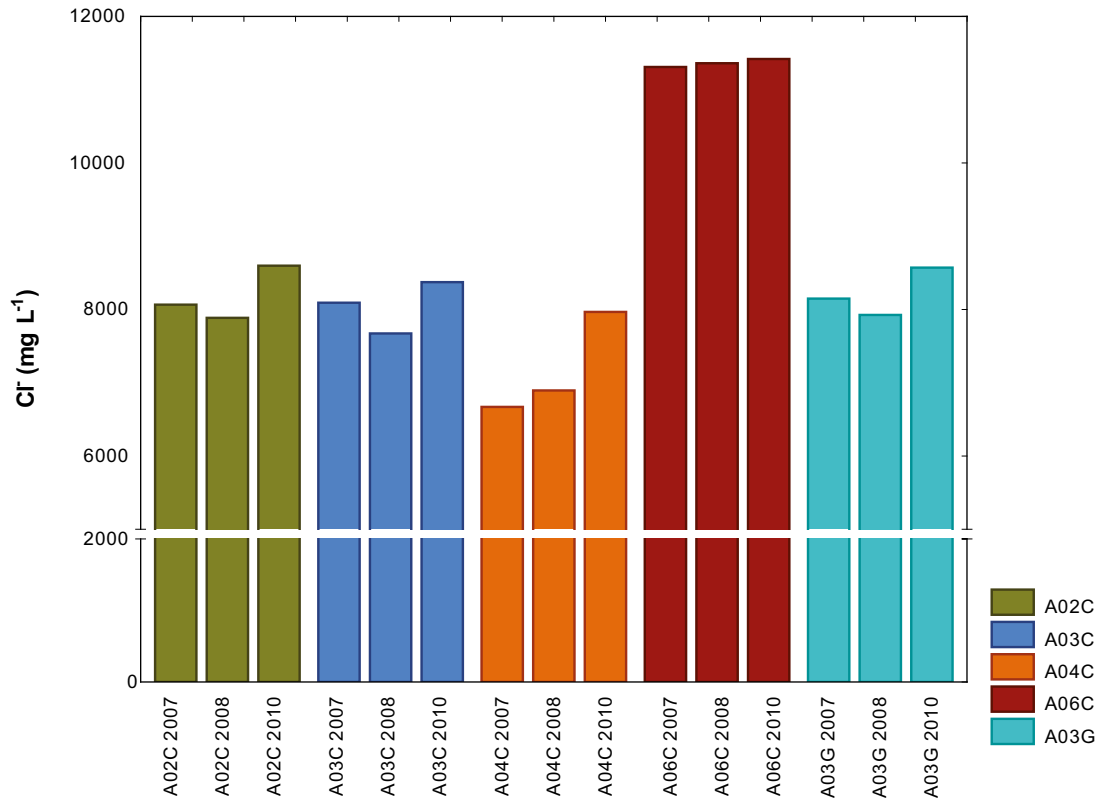


Figure 3-11. Chloride, Cl^- , in samples from the four experiment cages (C) and groundwater (G) in MINICAN from 2007, 2008 and 2010. Note the axis-break in the y-axis.

Table 3-7. Chemical composition of samples from the four experiment cages (C) and groundwater (G) collected in 2007-05-22.

Experiment name	SKB sample number	SO ₄ ²⁻ (mg L ⁻¹)	HCO ₃ ⁻ (mg L ⁻¹)	S ²⁻ (mg L ⁻¹)	Fe ²⁺ (mg L ⁻¹)	pH	Cl ⁻ (mg L ⁻¹)
A02C	14287	486	28	0.036	11.1	7.4	8,066
A03C	14288	506	27	0.057	2.33	7.6	8,093
A03G	14283	354	28	0.062	0.16	7.6	8,149
A04C	14289	439	51	0.037	0.82	7.6	6,671
A06C	14290	605	14	0.051	6.30	7.6	11,310

Table 3-8. Chemical of samples from the four experiment cages (C) and groundwater (G) collected in 2008-10-15.

Experiment name	SKB sample number	SO ₄ ²⁻ (mg L ⁻¹)	HCO ₃ ⁻ (mg L ⁻¹)	S ²⁻ (mg L ⁻¹)	Fe ²⁺ (mg L ⁻¹)	pH	Cl ⁻ (mg L ⁻¹)
A02-C	14642	417	19	0.021	19.2	7.3	7,887
A03-C	14643	413	20	0.020	4.15	7.4	7,674
A03-G	14645	481	23	0.023	0.15	7.6	7,926
A04-C	14644	410	38	0.022	15.7	7.2	6,895
A06-C	14641	567	8	0.030	11.1	6.8	11,361

Table 3-9. Chemical of samples from the four experiment cages (C) and groundwater (G) collected in 2010-12-08.

Experiment name	SKB sample number	SO ₄ ²⁻ (mg L ⁻¹)	HCO ₃ ⁻ (mg L ⁻¹)	S ²⁻ (mg L ⁻¹)	Fe ²⁺ (mg L ⁻¹)	pH	Cl ⁻ (mg L ⁻¹)
A02-C	20548	376	14	0.037	55.7	6.5	8,599
A03C	20550	430	14	0.044	22.6	6.7	8,373
A03G	20549	444	17	0.055	0.11	7.6	8,572
A04C	20552	400	32	0.059	9.91	7.3	7,968
A06C	20555	524	6	0.084	29.2	6.7	11,420

4 Discussion

4.1 The microbial populations in the MINICAN experiments

4.1.1 Number and activity

TNC in the four cages of the MINICAN experiments that could be sampled, A02C, A03C, A04C and A06C did not change significantly during the time of the experiments (Figure 3-2). The most pronounced change was a decrease in TNC in the groundwater sampled, A03G, from 3×10^4 to 3×10^3 mL⁻¹. The TNC values plotted in the range that has been reported from Fennoscandian groundwater systems (Hallbeck and Pedersen 2008, Pedersen et al. 2008).

The ATP values were rather scattered and are therefore difficult to interpret (Figure 3-1). For example were the ATP values from the cages 2010 rather low compared to 2008. On the other hand did the groundwater sample from A03G show an increase in ATP from the earlier samples from around 4,000 amol mL⁻¹ to around 28,000 amol mL⁻¹. It appears as if there were components in the water sampled from the canisters that might have interfered with the ATP measurements as was indicated by varying internal standard readings in the analysis. One possible component was the high concentrations of ferrous iron, especially in 2010. ATP was measured with an enzymatic kit and high concentrations of iron are known to disturb many enzymatic reactions. There might also have been interfering components from the bentonite present.

4.1.2 The composition of the microbial populations

The culturable heterotrophic bacteria (CHAB) can grow on organic compounds with oxygen as electron acceptor. These organisms can be facultative anaerobes and by that be able survive in anaerobic environments. In the samples taken in 2007, there were CHAB present in all experiments and the sampled groundwater with the highest number in A04C and the lowest in A03G and A06C, the ones without bentonite. This suggests a possible growth-stimulating effect of the bentonite. The MX-80 bentonite (used in the MINICAN experiments) contains about 0.20–0.25% organic carbon (Svensson D 2008, pers. comm.), which potentially can serve as a carbon and energy source for subsurface bacteria able to utilise organic carbon. The CHAB values have since then decreased in all samples and were below detection in 2010. The result shows that in 2010, the prerequisites for CHAB to survive had changed and CHAB had disappeared from the MINICAN experiments.

The autotrophic acetogens (AA) grow with hydrogen as energy source and electron donor and with carbon dioxide as carbon source and electron acceptor. These bacteria were high in numbers when analysed 2007 in A02C, A03C and A04C but low in numbers in A06C and A03G. The counts were lower in 2008 except in A06C with 2.7×10^4 mL⁻¹. In 2010, the numbers of AA were at or below detection indicating that the AA had disappeared from the MINICAN experiment.

Sulphate-reducing bacteria (SRB) have been present in the MINICAN experiments from the start in 2007. The highest numbers have been found in A02C with 2.4×10^4 in 2010. In A03C and A06C the numbers in 2010 were higher than in the previous samplings with 8.0×10^3 and 7.0×10^3 , respectively. The number of SRB in A04C had increased in 2010 compared to the previous samplings but this experiment had lower numbers than the others. The percentage SRB of TNC for the MINICAN experiments in 2010 was 33% in A02C, 2.5% in A03C, 0.6% in A04C and 32% in A06C. Ferrous iron concentrations may have increased due to corrosion of the support cages and this process generated hydrogen gas. Hydrogen support growth of SRB as discussed in the introduction. As ferrous iron concentration in A04C was lower than in the other MINICAN cages, less corrosion had probably occurred and less hydrogen was produced compared to several of the other cages. Less hydrogen production can then explain the lower number of SRB in the A04C experiment. Figure 3-5 shows that there was hydrogen in all MINICAN experiments and in the groundwater outside A03C, A03G. The high hydrogen concentration in A02C and A06C could be a result of iron corrosion, which is supported by the high ferrous iron concentrations in these experiments (Figure 3-9). Correlations between microbial sulphate reduction and water chemistry would then be expected.

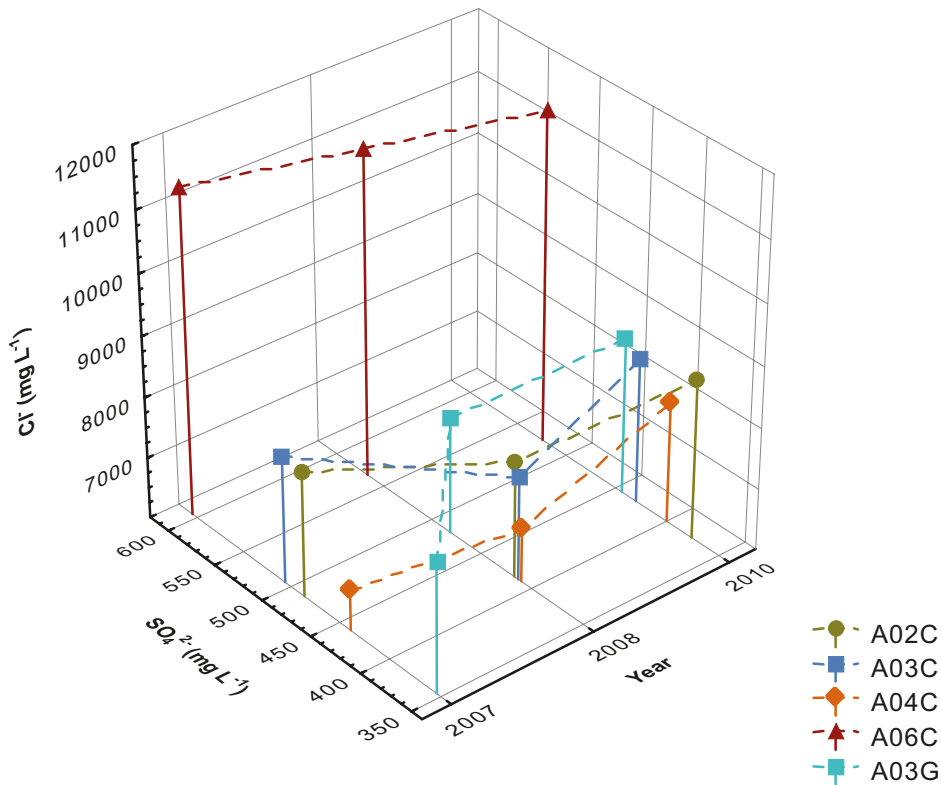


Figure 4-1. The correlation between sulphate (SO_4^{2-}), chloride (Cl^-) and year in samples from the four experiment cages (C) and groundwater (G) in MINICAN.

4.1.3 Sulphate

There were low concentrations of sulphide in the water sampled in 2010 even though the numbers of SRB were the highest measured in the MINICAN experiments so far. Is it possible to access the sulphate reduction from the sulphate data? This has to be done wisely since the sulphate concentration is linked to the origin of the groundwater and by that often also to the salinity. To elucidate if the sulphate concentration was influenced by sulphate reduction a plot with sulphate versus chloride and year was made (Figure 4-1). Figure 4-1 shows that in all experiments and groundwater but A04C the sulphate concentration vary more than the variation in chloride. If the variation only depended on dilution with another groundwater the variation should be equal for sulphate and chloride. From this it can be concluded that sulphate-reduction has lowered the sulphate concentration in the MINICAN-experiments. Figure 4-1 shows that the water in A06C has much higher salinity than the other experiments, with concentrations around 11,000 mg L⁻¹ compared to the others that had a chloride concentrations between 7,000 up to 8,000 mg L⁻¹. The chloride concentrations in groundwater outside A06C on the other hand had chloride concentration in the same range as the other experiments and the groundwater outside the experiments, A03G. It appears as if the A06 experiment was fed with a more saline groundwater than the other experiments in MINICAN. The high helium concentration in A06C supports this assumption because deep groundwater generally has both higher salinity and helium concentrations. Chloride concentration in A04C changed more, toward higher chloride concentration, than the other during the experiment also pointing towards supply of water with another origin even though the sulphate concentration decreased.

4.1.4 Ferrous iron

The highest concentration of ferrous iron was found in A02C, A06C and A03C, which indicated that the corrosion of the support cages in these three experiments might have been the most pronounced of the MINICAN experiments. The ferrous iron in A04C-experiment was lower in 2010 than in 2008. The A04C-experiment had the lowest hydrogen concentration and number of SRB in 2010 compared to the other experiments, which could be connected to less intensive iron corrosion.

4.1.5 Hydrogen gas

There were measurable amounts of hydrogen gas in all samples from MINICAN in 2010. Interestingly the highest concentration was found in the groundwater outside A03C, in A03G with $>54 \mu\text{L L}^{-1}$. The origin of the hydrogen here is unknown. It could originate from the iron corrosion process inside the MINICAN copper canisters or from corrosion of the stainless steel cages, or from the geosphere. The reason for why the hydrogen concentrations in the cages (C) were lower than in the groundwater (G) could be because SRB consumed hydrogen in the vicinity to the positions of iron corrosion of the bentonite holding cages. A02C and A03C were the experiments with the highest concentrations of hydrogen. These were also the experiments with the highest concentrations of ferrous iron and large SRB populations.

It is important to understand that compounds readily consumed by microorganisms are less likely to build up in high concentrations compared to compounds ignored by microorganisms.

4.2 Conclusions

The following conclusions can be drawn from the microbial examinations and groundwater chemistry in the MINICAN experiments.

- There has been a succession in the microbial populations in the MINICAN experiments from a more complex population with CHAB, AA and SRB in 2007 towards populations with higher numbers of SRB and low or no CHAB and AA.
- There seemed to be a growth-stimulating effect of bentonite in the MINICAN experiments, possibly bentonite served as a good carbon and energy source for subsurface bacteria able to utilise organic carbon. This phenomenon has been observed before in the Prototype Repository (Eriksson 2008). In 2007, during this putative bentonite degradation carbon dioxide levels were high. The high carbon dioxide levels correlated with high abundance of AA at that time.
- Intensive iron corrosion appeared to correlate with large populations of SRB. The ferrous iron concentration in the MINICAN experiments had increased in 2010 compared to 2008 in all experiments but A04C. The experiments, A02C and A06C with the highest ferrous iron concentrations also had the highest numbers of SRB, 33 and 32%, respectively, of the total number of cells, suggesting a clear connection between iron corrosion and SRB activity.
- The microbial sulphate reduction has decreased the sulphate concentration in MINICAN. Due to different and varying groundwater chemistry, as attested by the varying chloride concentrations, it was not possible to quantify the sulphide generating process or to calculate the sulphide production rate based on disappearance of sulphate only.
- The data discussed in this report come from one sampling occasion per year, 2007, 2008 and 2010, where a limited amount of water was available. The variation in the measured parameters was difficult to assess, because there was not water enough for repeated samples. The way forward here is to repeat sampling with shorter intervals than presently applied.

4.3 Future research

- The MINICAN experiments have offered a unique opportunity to examine what microbial processes will operate *in situ* if the copper canisters in a KBS-3 repository are penetrated. However, the assumptions and theories in the report are based on results from three sampling occasions over four years. It is necessary to continue the monitoring programme to be able to further confirm the findings.
- The different groundwater chemistry in the MINICAN experiments provide great opportunities to investigate the influence of the varying chemical groundwater compositions on the activity and composition of microbial sulphide producing populations composition and their effect on sulphide corrosion of copper canisters.

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