Report
R-16-17
April 2017

Microbial sulphide production during consumption of H_2 and organic compounds released from stationary borehole equipment

Alexandra Chukharkina
Anders Blom
Karsten Pedersen

SVENSK KÄRNBRÄNSLEHANTERING AB

SWEDISH NUCLEAR FUEL
AND WASTE MANAGEMENT CO

Box 3091, SE-169 03 Solna
Phone +46 8 459 84 00
skb.se

SVENSK KÄRNBRÄNSLEHANTERING

ISSN 1402-3091
SKB R-16-17
ID 1560979
April 2017

Microbial sulphide production during consumption of H_2 and organic compounds released from stationary borehole equipment

Alexandra Chukharkina, Anders Blom, Karsten Pedersen
Microbial Analytics Sweden AB

Keywords: Bacteria, Borehole equipment, Corrosion, Extraction, Groundwater, H_2, Leaching, Metallic materials, Organic materials, Sulphide, KBP4000.

Abstract

High sulphide concentrations have been found in groundwater from boreholes with stationary equipment. The only possible process that produces sulphide in groundwater is the reduction of sulphate by species of sulphate reducing bacteria (SRB) using available sources of electron donors and carbon sources. Materials used in borehole equipment may provide electron donors to SRB, in the form of H_2 or organic compounds, for sulphide production. To understand if material in stationary borehole equipment releases such compounds to the groundwater, extraction and leaching experiments were performed. The metallic materials used in borehole equipment are steel and aluminium. These metals were exposed to sterilized filtered groundwater at 30 °C and 70 °C and to natural groundwater at 30 °C. Experiments were carried out in closed vessels under O_2-free conditions during several months, and gas analyses were performed. Five kinds of polymeric materials in borehole equipment were selected and extracted with hexane to get a comprehensive knowledge about compounds that can be released to groundwater. Thereafter leaching experiments were performed in both sterile-filtered and natural groundwater under O_2-free conditions during 6 months. Solid-phase extraction (SPE) from the groundwater followed by compound analysis using gas chromatography with mass spectroscopic detection of released compounds was carried out. Both steel and aluminium released H_2, and this release was continuous under sterile conditions. In the study higher release rates were observed for aluminium samples compared to steel samples. Release of H_2 varied from sample to sample and was higher at 70 °C compared to 30 °C. Local corrosion of one aluminium rod was connected with a very large amount of H_2 released over a relatively short time. Under natural conditions the concentrations of H_2 levelled out rapidly, after just two weeks of leaching. That confirmed the assumption that H_2 could be consumed by bacteria present in the natural water. An array of organic compounds was found in the hexane extracts of the polymeric material. Several of these compounds were also found in the SPE extracts of groundwater leachates and the concentrations of the compounds increased with leaching time under sterile conditions. The extractions after six mounths were influenced by unintentional bacterial growth after extended time. Under natural conditions the concentrations of the compounds generally decreased with time. Concentrations of diethylhexylphthalate (DEHP) and toluene in the PVC samples on the contrary, increased in comparison with the results under sterile conditions. Under natural conditions elemental sulphur was found in the leachates from PVC and PU Slitan 90A-05. Leachates from both these samples showed presence of hydrogen sulphide, the concentrations increased with leaching time. Microbiological analyses showed high level of cells including sulphate reducing bacteria in the water samples. A flow cell filled with the investigated materials was installed in a borehole for on-site growth of biofilms, and the highest ATP results were received for polyurethane (PU) Slitan 90A-05, High density polyethene and polyvinyl chloride PV samples. Combined result of the droplet digital polymerase chain reaction (ddPCR) analysis of SRB tracers in DNA, microbiological and chemical analyses showed that PVC and PU Slitan 90A-05 are more attractive for bacteria than the other organic materials. The results of the experiment showed that both metallic and organic materials used to construct borehole equipment can provide enough electron donors to explain the observed sulphide production by SRB. However, the exact processes present in the described system are complicated and need further investigations.

Contents

Abbreviations used in the report

Abbreviation	Meaning
AGW	Analytical grade water
ADP	Adenosine diphosphate
AMP	Adenosine monophosphate
AODC	Acridine orange direct count
ATP	Adenosine-5’-triphosphate
APS	Adenosine-5’-phosphosulphate
ddPCR	Droplet digital polymerase chain reaction
DEHP	Di-ethylhexyl phthalate
DIDP	Di-iso-decyl phthalate
DINP	Di-iso-nonyl phthalate
DNA	Deoxyribonucleic acid
GC-MS	Gas chromatography-mass spectrometry
GC	Gas chromatography
HRP	Hydrophilic reversed phase
MIC	Microbially induced corrosion
MPN	Most probable number
PA	Polyamide
PDHID	Pulsed discharge helium ionization detector
PEEK	Polyether ether keton
PEHD	High density polyethylene
PU	Polyurethane
PVC	Polyvinyl chloride
qPCR	Quantitative polymerase chain reaction
SF-groundwater	Sterile-filtered groundwater
SRB	Sulphate reducing bacteria
SPE	Solid phase extraction
TCD	Thermal conductivity detector
TNC	Total number of cells
UV	Ultra violet

1 Introduction

While the presence, numbers and diversity of sulphate reducing bacteria (SRB) in deep groundwater have been well documented, their activity is less well studied. There are Scandinavian cases when sulphide concentrations in groundwater exceed the safety case value of 5 μM sulphide used for Forsmark, Sweden (see SKB 2011, Section 12.6.2) almost 1000 times. Extreme values of 3 mM sulphide were observed in drillholes at Äspö Hard Rock laboratory (HRL) (Rosdahl et al. 2011) and the underlying reasons for this accumulation are not yet fully understood (Drake et al. 2014). Hence, a remaining key issue for the safety case is to identify the factors controlling the rate of sulphide production in the geosphere, including man-made artefacts. Availability of electron donors, such as the H_2 and CH_4 from deep geological sources, and electron acceptors such as sulphate and ferric iron is hypothesized to be one of several controlling factors. The extremely high sulphide concentrations have been observed in borehole sections left unattended for a while, i. e. when no samples have been withdrawn for several months or years (Rosdahl et al. 2011). Chemical reduction of sulphate to hydrogen sulphide at temperatures and pressures prevailing in deep groundwater environment is shown to be extremely slow (Cross et al. 2004), and the only possible explanation to sulphide production is sulphate reduction by strains of SRB. The SRB use the S atom in the sulphate molecule as an electron-acceptor and the reduced product is hydrogen sulphide. For this microbial process, an electron donor is needed, for example, organic compounds or H_2. One possible source of these compounds can be the materials used to construct borehole equipment. The outcome of previous investigations was ambiguous with no clear indication of the origin of the electron donors needed for microbial sulphide production from sulphate. A systematic study in the laboratory was, therefore, requested to investigate the possibility that materials in stationary borehole equipment can trigger sulphide production by supplying SRB with electron donors.

The general objective of the study was to determine if any material used to construct borehole equipment can provide enough electron donors to explain the observed sulphide production by SRB. The study was performed by laboratory experiments apart from an *in situ* collection of biofilms on the materials using a flow cell packed with the different materials anaerobically connected to a pumped flow of borehole water.

In a first experimental series the release of organic compounds and H_2 from borehole equipment under sterile conditions at 30 °C and 70 °C was investigated (Chukharkina et al. 2016). In these sterile experiments it was shown that metallic parts of the borehole equipment, aluminium and steel, released H_2 when immersed in O_2-free groundwater. H_2 release was larger with aluminium rods than with steel rods and increased with increased temperature. Besides, it was observed that local corrosion of aluminium rods can release very large amounts of H_2 over a relatively short time. The polymeric materials of stationary borehole equipment contained many different organic compounds of which several were released to groundwater. The release was generally continuous over time. Due to the difficulty to create entirely sterile conditions without destroying the polymeric material composition, bacteria were observed in sample vessels containing polymeric materials after 6 months of leaching in groundwater. It was accompanied by a decrease in amounts of organic compounds detected in the water, which suggests that microbiological activity interfered with the experiments via consumption of the released compounds. The distinct influence of the bacteria in the system was unclear and warranted additional investigation in a second experimental series.

The second experimental series was carried out under natural conditions therefore, involving the presence of the bacteria. All the procedures were similar to what was applied during the sterile experiments, but the natural groundwater used for leaching in the second experiment was not sterilised. Metallic compounds were immersed in natural water at 30 °C and the waters were analysed subsequently using gas chromatography (GC). Experiments were not performed at 70 °C. Sulphate analyses of the natural groundwater were performed using spectrophotometric detection. Organic components were leached in natural groundwater and analysed using gas chromatography with mass spectroscopy (GC-MS). At the same time as extractions from groundwater in contact with organic components were carried out, microbiological and sulphide analyses were performed. Water samples from the leachates from the two last extraction occasions, were cultured for SRB and analysed eight weeks later.

A flow cell was constructed during the second experimental series. It contained parts of borehole equipment and was placed at a borehole in Forsmark and groundwater was pumped through the flow cell for biofilm formation. After 12 weeks sampling of biofilm material was performed in the laboratory and analysed using ATP and droplet digital polymerase chain reaction (ddPCR) techniques.

2 Material and Methods

Two experiments were done during this project that aimed to investigate microbial sulphide production during consumption of released H_2 and organic compounds from metallic and polymeric materials used to construct stationary borehole equipment. In the first experimental series leaching of the investigated borehole components under sterile conditions was performed. These results was reported in March 2016 (Chukharkina et al. 2016). We refer to this report for details on methods and results. In the second experimental series leaching of the borehole components was performed under natural conditions, using natural groundwater from Forsmark containing bacteria.

Detailed descriptions of typical stationary borehole instrumentation can be found in Rosdahl et al. (2011, Chapter 3). All polymeric and metallic materials were provided by Geosigma AB.

2.1 Source and preparation of the groundwater for the laboratory experiments

The groundwater was collected on-site from the borehole KFM03A:4 in Forsmark in sterile polycarbonate containers and immediately sent over night, refrigerated to Microbial Analytics Sweden AB in Mölnlycke. The chemical composition of the groundwater for the experiments under sterile and natural conditions are presented in Appendix 1 Table A1-1. Data of the chemical compositions were provided by SKB. The sampled section was between 633.5 and 650 m borehole length and at a depth of −631 m above sea level (masl). This section was analysed for bacteria in 2004 and SRB were found (Pedersen and Kalmus 2004). For the experiments under sterile conditions water was collected 2014-10-08 and for the experiments under natural conditions the sampling was made 2015-10-19. Both samplings were performed by SKB personnel.

Preparation of the sterile-filtered groundwater

To mimic the conditions on location in the drilled holes it was necessary to use water of similar ionic strength, alkalinity, pH and content of dissolved organic carbon. At the beginning of the project it was briefly suggested that the water should be made up artificially from high-purity chemicals and laboratory grade water. However, this idea was abandoned in favour of using sterile-filtered (SF) groundwater collected from boreholes in Forsmark known to experience problems with elevated sulphide concentrations. By using sterile-filtered groundwater all dissolved components, including the minor ones, would be present in correct ratios. It was decided to sterile-filter instead of heat-treating the water (autoclaving) since changes in temperature might cause precipitation in high-ionic strength groundwater.

The SF-groundwater (0.2 µm pore size) was prepared using a screw-on sterile filter unit (Sarstedt Filtropur BT50 83.1823.101) connected to a sterile Duran 1 000 ml glass bottle. The unit was connected to a water aspirator and the water was suction-filtrated into the sterile bottle.

Preparation of natural groundwater

As natural groundwater the groundwater collected from the borehole 2015-10-19 was used. The raw water, without any further preparation (filtration) was used for the experiment under natural conditions.

2.2 Release of H_2 from metallic materials

2.2.1 Sources of metallic materials from stationary borehole equipment

Two metallic materials used in borehole equipment were investigated. Aluminium rods with 16 mm diameter are used as anchor rods for the packers. For the tests only the mid parts of the rods were

used since the attached threaded connectors in the ends of the rods were reported to be made of stainless steel. Stainless steel (316L) rods with 16 mm diameter are alternatively used as anchor rod for packers. The metallic rods had several different suppliers and specific identification information was not provided by Geosigma.

2.2.2 Preparation of experiments with metallic materials

The preparation procedures adopted a methodology developed for analysis of H_2 release from copper under O_2-free conditions (Bengtsson et al. 2013, Johansson et al. 2015).

Preparation of the metallic rod samples

The supplied steel and aluminium rods were too large in diameter to fit the 26 mL gas tight, anaerobic borosilicate experimental tubes (Product #2048-18150, Bellco Glass Inc., NJ, USA) with matching impermeable butyl rubber stoppers (Product #2048-117800) used for the experiments. They were, therefore, machined to a diameter of 12 mm and a length of approximately 100 mm and the surface was polished. This treatment removed surface oxides and after machining, the samples were kept dry to avoid formation of new oxides. Five replicates of aluminium and stainless steel rods, respectively, were prepared (Figure 2-1). Parameters of the rod samples are presented in Table 2-1. Five blank samples without metallic rods were prepared as well using the same groundwater, vessels and stoppers as was used for metallic rods. Blank samples were treated in exactly the same way as samples containing metal parts.

Before placing the metallic rods in the sterile glass vessels the samples were sterilized by immersion in 95 % ethanol (Solveco art.nr 1394, Rosersberg, Sweden). The preparation was done inside an anaerobic glovebox (COY Laboratory Products, Grass Lake, MI, USA) and the metallic rods as well as the glass vessels and the rubber stoppers were kept in this environment for days to minimize the amount of O_2 possibly trapped on surfaces. Inside the box the metallic rods were removed from the ethanol bath with plastic tweezers, briefly dried and carefully placed inside the glass vessels. The vessels were closed with Bellco butyl rubber stoppers and removed from the anaerobic glovebox. Outside the box the stopper was secured in place with a crimped-on aluminium ring (Cromacol 20-ACB, Thermo Scientific, Langerwehe, Germany). The closed vessels were evacuated and refilled with pure N_2 (Alphagaz1 N_2, klass 2, Air Liquide) to a total pressure of 1.30 bar using a gas bench.

Experiments under sterile conditions

For the experiment under sterile conditions the SF-groundwater was poured into a sterile glass Erlenmeyer flask and closed with a sterilized lid fitted with PEEK tubes, one of them extending into the fluid. Pure 0.2 µm filtrated N_2 was added to this tube to purge the water from dissolved O_2 and to fill the flask volume above the fluid surface with an inert atmosphere. Purging was performed for half an hour and then the flask was sealed, creating an overpressure inside. After that the stoppered glass vessels with metallic rods were filled with O_2-free SF-groundwater via a syringe through the stopper to a level leaving the metal rod completely submerged. The exact volumes of water are shown in Table 2-2. After filling, the vessels were evacuated and refilled with N_2 to a total pressure of 2 bars in the gas bench.

All vessels were analysed for content of O_2 and H_2 at the start of the experiments. Thereafter, they were enclosed in bubble plastic wrap and placed in an anaerobic jar that was evacuated and refilled with pure N_2 to a total pressure of 1.2 bar yielding an O_2-free atmosphere. The samples were stored at elevated temperature in a heated cabinet (Binder FD53, Skafte Medlab, Onsala, Sweden) at 30 °C. Tests using a higher temperature (70 °C) revealed reactions between saline water and aluminium that were primarily caused by the elevated temperature and therefore these tests are excluded from results and discussion.

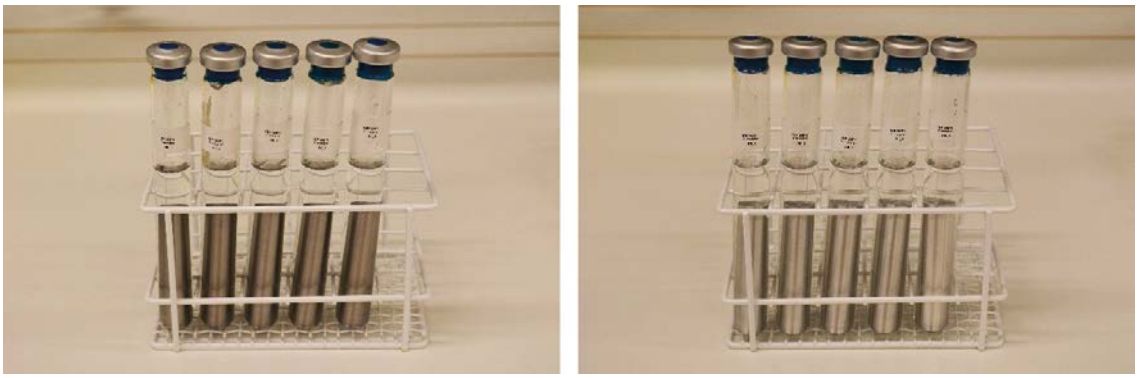

Figure 2-1. *Metallic rods contained in glass vessel with O_2-free sterile filtered groundwater. Left, stainless steel; right, aluminium.*

Experiments under natural conditions

For experiments with natural groundwater containing bacteria the procedure was exactly the same. The exact volumes of water poured in the vessels were 9.6 mL and 9.5 mL for aluminium and steel samples, respectively (Table 2-3). After filling, the vessels were evacuated and refilled with N_2 to a total pressure of 2 bars in the gas bench.

During the experiments with natural groundwater containing bacteria the samples were incubated at 30 °C.

Table 2-1 Physical parameters of the metallic rod samples used in the experiments, T = 30 °C.

Material	Sample	Mass (g)	Length (cm)	Diameter (cm)	Surface area (cm^2)
Sterile conditions					
Aluminium	1	24	7.7	1.2	31.3
	2	24	7.7	1.2	31.3
	3	24	7.7	1.2	31.2
	4	24	7.6	1.2	31.1
	5	24	7.6	1.2	31.1
Stainless steel	1	87	9.6	1.2	38.7
	2	87	9.6	1.2	38.7
	3	87	9.6	1.2	38.7
	4	87	9.6	1.2	38.7
	5	87	9.6	1.2	38.7
Natural conditions					
Aluminium	1	21	7.4	1.2	28.7
	2	22	7.3	1.2	28.7
	3	22	7.3	1.2	28.6
	4	21	7.0	1.2	28.6
	5	22	7.3	1.2	28.7
Stainless steel	1	63	7.0	1.2	28.8
	2	63	7.1	1.2	28.9
	3	63	7.0	1.2	28.8
	4	63	7.0	1.2	28.8
	5	62	7.0	1.2	28.8

Table 2-2 Volumes of water and gas used in the experiment with the metallic rods samples under sterile conditions.

Material	Sample	V H_2O (mL)	V gas (mL)
Aluminium	1	9.6	7.7
	2	9.8	7.5
	3	10.3	7.0
	4	9.6	7.8
	5	9.6	7.8
Stainless steel	1	9.6	5.4
	2	9.6	5.4
	3	9.5	5.5
	4	9.0	6.0
	5	9.5	5.5

Table 2-3 Volumes of water and gas used in the experiment with the metallic rods samples under natural conditions.

Material	Sample	V H_2O (mL)	V gas (mL)
Aluminium	1	9.6	8.5
	2	9.6	8.5
	3	9.6	8.5
	4	9.6	8.5
	5	9.6	8.5
Stainless steel	1	9.5	8.5
	2	9.5	8.5
	3	9.5	8.5
	4	9.5	8.5
	5	9.5	8.5

2.2.3 Analysis of H_2 and dissolved sulphide

Analysis of H_2 during the sterile experiment was performed using two different gas chromatograph systems. One system was based on a Bruker 450 gas chromatograph (Bruker Daltonics, Fremont, California) equipped with an PDHID detector (Valco Instruments Company, Inc. (VICI) Houston, TX 77055, USA), a carrier gas purifier system using a heated getter and a parallel two-column setup of CP7355 PoraBOND Q (50 m × 0.53 mm, ID) and a CP7536 MOLSIEVE 5A PLOT (25 m × 0.32 mm, ID). H_2 was also analysed on DANI Master GC using MXT-Molsieve 5A Plot 30 m × 0.53 mm × 50 µm and OPT 270M-MICRO thermal conductivity detector (TCD) system, using He as carrier gas. All chromatographs were calibrated using certified gas mixes (Air Liquide, Specialty gases, Krefeldt, Germany) that mimic the gas composition of the analysed samples. The certified accuracy of the gas mixtures was stated to be ± 2 % relative deviation. During the experiment under natural conditions gas analyses were performed only using the Bruker 450 gas chromatograph, the system with the lowest detection limit (2 ppb), the uncertainty of the method was 10 %.

For the experiment with natural groundwater, hydrogen sulphide was measured in the groundwater samples. After 108 days of the experiment 1 mL of each water sample from the vessels with metallic rods was transferred to a clean centrifuge tube (Sarstedt, 15ml, sterile with mounted lid) and preserved by addition of 10 µL 1M NaOH and 10 µL $Zn(CH_3COO)_2$. A blank sample was collected at the same time. Analyses of hydrogen sulphide were performed using a UV/visible spectrophotometer (Genesys10UV, VWR, Stockholm, Sweden) according to the Swedish standard SIS 028115.

2.2.4 Calculations of released gas

The gas chromatographs were calibrated with varying volumes of H_2 and O_2 and the output from the GC consequently were volumes of the respective analysed gas per injected volume of sample. This report shows gas data as mbar of the analysed H_2 as in the vial gas phase and as nmol per metal surface. The combined gas law was used for calculating these values as described previously by Chukharkina et al. (2016).

2.3 Release of organic compounds from polymeric materials

2.3.1 Sources of polymeric materials from stationary borehole equipment

Four different polymeric materials used to construct five different parts of the borehole equipment were selected for this study:

- Polyamide (PA) tubes are used for water sampling, circulation and inflation of the packers. This tubing is produced by Munkplast AB without any specified identification.
- Black polyvinyl chloride (PVC) tape, Nitto 225; normally used for insulation of electrical conduits, but here used in the borehole installations for general attachment purposes and fixation of tubing.
- High density polyethylene (PEHD) constitute the polymeric dummy used to reduce the internal volume of borehole sections, a white polyethylene HD-1000 produced by PEGES, article nr. GS2147.
- Polyurethane is used in two parts of the equipment.
  - Rubber gasket for packers made of yellow tinted polyurethane, produced by UW Elast with identification name Slitan 80A-71.
  - Rubber gasket for packers made of slightly yellowish polyurethane, produced by UW Elast with identification Slitan 90A-05.

2.3.2 Preparation of experiments with polymeric materials

Information about leachable compounds from polymeric materials is usually not obtainable from the producers, unless the product is intended for medical use or food production and storage. For the materials used in stationary borehole equipment, specifications of composition were not available and in some cases (e.g. the PVC tape) the final product consists of multiple polymeric materials such as resins and wax as well as glue and plastic film. To narrow down the spectra of possible leachable compounds the tests were started with a fingerprinting of compounds that could be extracted from the polymeric materials using the strong organic solvent hexane. Hexane was chosen based upon its interaction with the polymeric mainframe of the organic components.

Extraction with hexane and component analysis

The polymeric materials were cut into small pieces with scissors and a knife, apart from the PE dummy that was ground to achieve small flakes. The materials were extracted using a Soxhlet setup using 80 mL hexane (VWR Prolabo Pestinorm for GC-MS analysis). Hexane was refluxed for four hours with a cycle time of approximately 15 minutes. The obtained extracts were analysed on GC-MS (Varian CP 3800 GC and Varian Saturn 2000 MS, Varian Inc. Palo Alto, California USA) using EI-ionization and splitless injection. The analytical column used was an OPTIMA MN-5 phase 30 m × 0.25 × 0.25 (Macherey-Nagel GmbH & Co, Düren, Germany) and the temperature was programmed from 35 to 300 °C using a constant He carrier gas flow of 1 ml/min and a linear temperature gradient of 8 °C/min. Spectra were obtained in the range 35–400 amu and were background corrected before identification using a NIST/EPA/NIH Mass Spectral Library (NIST 98) and NIST Mass Spectral Search Program version 1.6. The whole process was controlled using Star Chromatography Software version 6 for GC-MS (Varian AB). Samples were injected using a Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and the injected volume was 1 µl.

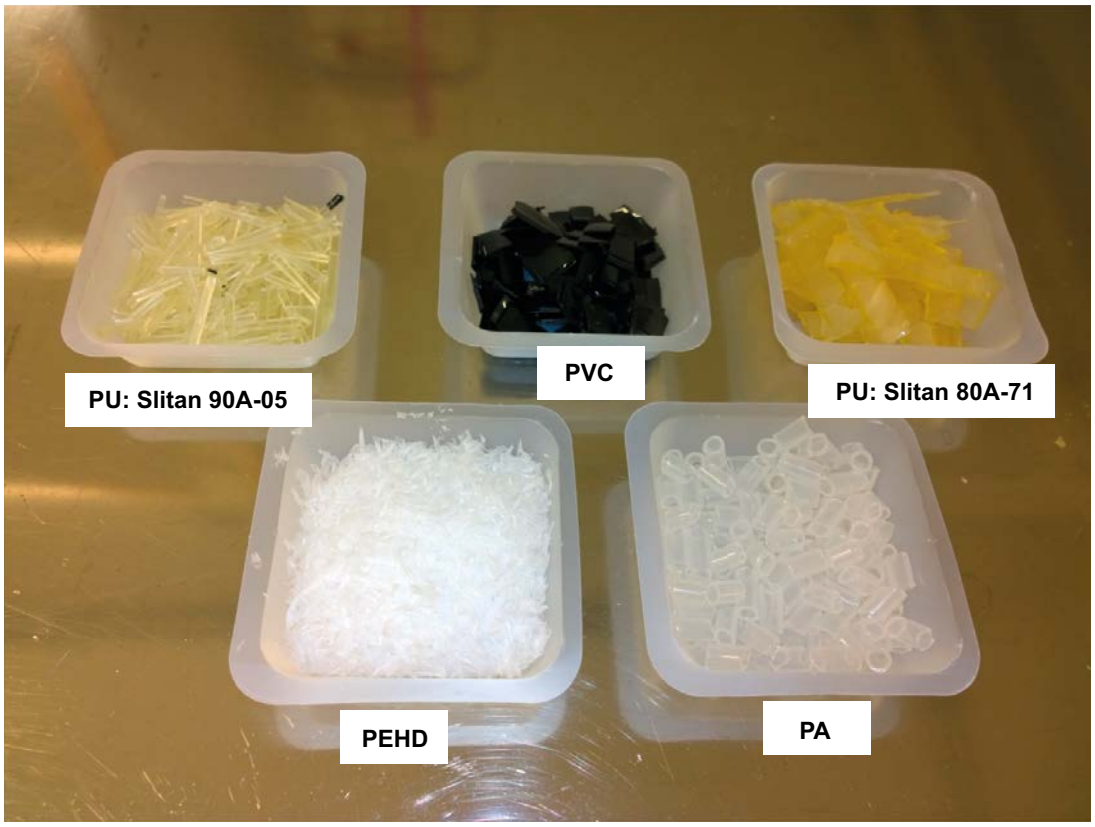

Figure 2-2. *Disintegrated polymeric materials used to construct five different parts of the borehole equipment that were selected for this study. PU, polyurethane; PVC, polyvinyl chloride; PEHD, High density polyethylene; PA, polyamide.*

Table 2-4 Masses of the samples of polymeric material used in the leaching experiments.

Sample	Subsample	Analysis day		Mass (g)
		Sterile conditions	Natural conditions	
PA	1	28	31	6.1
	2	98	94	6.1
	3	182	186	6.1
PVC	1	28	31	5.0
	2	98	94	5.0
	3	182	186	5.0
PEHD	1	28	31	2.5
	2	98	94	2.5
	3	182	186	2.5
PU: Slitan 90A-05	1	28	31	10.0
	2	98	94	10.0
	3	182	186	10.0
PU: Slitan 80A-71	1	28	31	10.0
	2	98	94	10.0
	3	182	186	9.5

Leaching with sterile filtered groundwater

For the leaching experiment into groundwater under sterile conditions the polymeric materials were sterilized by rinsing in 70 % ethanol (Solveco, Rosersberg, Sweden). After that the materials were weighed and added to a 300 mL sterile serum bottle that was closed by a sterile rubber lid and a threaded aluminium ring. The closed bottles were evacuated using a gas bench to remove O_2 inside the bottles and they were refilled with pure N_2. Control bottles containing SF-groundwater without polymeric material were prepared in the same manner and subsequently treated in exactly the same way as for the polymeric material samples. The parameters of the final samples are presented in Table 2-4. SF-groundwater was prepared and added from a sterile Erlenmeyer flask in the same way as was done for the metallic materials. The serum flasks were filled with 150 ml O_2-free SF-groundwater. After filling, the vessels were evacuated and refilled with N_2 to a total pressure of 1.3 bar in the gas bench.

Great care was taken throughout the experiment to avoid contact between the rubber lid of the flasks and the SF-groundwater inside. The flasks were stored standing at room temperature in a dark room. They were swirled from time to time since some of the polymeric materials were floating causing a limited contact with the water.

Leaching with natural groundwater

For the leaching under natural conditions the polymeric materials were not sterilized and the bottles were filled with non-sterilized groundwater. Otherwise the experiment was carried out the same way as for the experiment under sterile conditions.

Figure 2-3. *Release experiments with polymeric materials used to construct stationary borehole equipment in groundwater. Upper left, high density polyethylene; lower left, polyurethane Slitan 80A-71; upper right polyamide tubing; lower right, PVC tape.*

2.3.3 Extraction of leachates and component analysis

When sampling the water for released organic compounds an approach using SPE was chosen to improve detection limits. Two different SPE-cartridges were used for extraction: HR-P, a wide range more polar polymer and C18-hydra, a non-polar phase (Macherey-Nagel GmbH & Co, Düren, Germany). SPE cartridges were activated by rinsing with 5 ml methanol (Labsolute for HPLC, TH Geyer, Germany) followed by 5 ml distilled water to remove the methanol. Samples with a volume of 50 ml were collected anaerobically from the serum flasks using a 60 ml syringe (HSW, Norm-Ject without rubber seal) and a syringe pushed through the rubber stopper. The total volume of the sample was forced through the cartridge using a combination of applied pressure on the syringe and sucction on the collecting side generated with the help of a hand pump or water aspiration. The flow rate was kept at around one drop per second. To dry the packing material air was forced through the cartridge using the same 60 ml-syringe used for collecting the sample. Between 4 and 5 syringe volumes of air were used causing a spray of water out of the SPE-cartridge. After drying the cartridge was eluted with 2 ml of hexane or methanol down into a GC-vial, in the case of C18 hydra and HRP cartridges respectively. For elution all-glass syringes (HSW, Germany) were used to minimize leaching from the syringe to the solvent. Blank samples of analytical grade water (AGW) were extracted and eluted in exactly the same way as for samples. Vials were kept refrigerated until analysis. Samples of pure solvent (hexane and methanol respectively) were also analysed to trace possible contaminations and the sequence of analysis on the GC-MS was chosen keeping expected levels of analytes in mind. In general, the sequence went from lower to higher concentrations with multiple blank runs on pure solvent throughout the sequence. Repeated analysis of the pure SF-water extraction on SPE was used to monitor the GC-MS system integrity through the analytical run that usually was in the magnitude of 18–24 hours for each sampling occasion. Collected spectra from the chromatograms were compared with a NIST library for identification. In some cases, no good library match could be obtained and in those cases a qualitative judgement made on the basis of the most characteristic ions, the qualifier ions, was made indicating the general compound class or similarities between different unidentified peaks. Qualifier ions usually originate from functional groups in the organics structure.

The obtained results for the blank samples were subtracted from the results from the samples with polymeric materials. In practice results of blank extractions were in the form of new peaks in the chromatogram not overlapping with analytes of interest so these peaks could be completely omitted without working out concentration ratios. One of these peaks was chosen as internal standard to correct for varying volumes of elution solvent. Since the origin presumably was the packing material this approach could not take into account varying volumes of sample water, but accuracy achieved from the scale on the syringes was judged as sufficient.

Analytical procedures for samples under sterile conditions

Analyses of the leachates prepared under sterile conditions were performed using GC-MS (Varian CP 3800 GC and Varian Saturn 2000 MS, Varian Inc. Palo Alto, California USA) using EI-ionization and splitless injection. The analytical column used was an OPTIMA MN-5 phase 30 m × 0.25 × 0.25 (Macherey-Nagel GmbH & Co, Düren, Germany) and the temperature was programmed from 35 to 300 °C using a constant He carrier gas flow of 1 ml/min and a linear temperature gradient of 8 °C/min. Spectra were obtained in the range 35–400 amu and were background corrected before identification using a NIST/EPA/NIH Mass Spectral Library (NIST 98) and NIST Mass Spectral Search Program version 1.6. The whole process was controlled using Star Chromatography Software version 6 for GC-MS (Varian AB). Samples were injected using a Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and the injected volume was 1 µl.

Analytical procedures for samples under natural conditions

For analyses of the leachates prepared under natural conditions two GC-MS system were used: Varian 3800 GC Saturn MS, described earlier, and Agilent 7890B/240MS, using EI-ionization and splitless injection. The analytical column used in the Agilent GCMS was an VF-5ms 5 % diphenyl/95 % dimethyl polysiloxane, 30 m × 0.25 × 0.25. Spectra were obtained in the range 35–400 amu and were background corrected before identification using a NIST 2014 MS Library Bundle. The whole process was controlled using MS Workstation 7.0.1 for GC-MS (Agilent AB). Samples were injected using an Agilent Autoinjektor 7693 and the injected volume was 1 µl.

Sulphide analysis of the water samples were performed at day 31, 94 and 186 the same time as the extraction procedures. Sulphide analyses carried out at day 31 were performed according to a procedure described by Widdel and Bak (1992). The analyses at days 98 and 186 were performed according to the Swedish standard SIS 028115, using a UV/visible spectrophotometer (Genesys10UV, VWR, Stockholm, Sweden).

2.4 Flow cell for biofilm collection

2.4.1 Construction and set up of the flow cell

The flow cell for biofilm collection was constructed by Micans as a hollow tube of stainless steel with PEEK-tabs on both sides. The materials were arranged in order to pack the flow cell, to provide a sufficient surface for bacteria to interact with the material and to make the sampling of biofilms itself easy. Figure 2-4 shows a scheme of the packed flow cell.

In cases of PEHD and PA samples, the small parts of material were placed on the PVC tape and rolled like a sushi roll in order to keep them on place and prevent them floating into the cell.

The flow cell itself was installed on top the borehole KFM03A:4 in Forsmark. The sampled section was between 633.5 and 650 m borehole length and at a depth of ~631 m above sea level (masl). The flow cell was attached to a pump in the borehole section that was pumping groundwater through the flow cell at rate of approximately 200 mL/min during 12 weeks.

2.4.2 Sampling of biofilms

After 12 weeks the flow cell was removed and immediately sent refrigerated to Microbial Analytics Sweden AB in Mölnlycke. Sampling of biofilms from the materials in the flow cell was performed at the laboratory 2016-05-24.

PEHD and PA samples were collected in plastic vials and Eppendorf tubes. In cases of all others samples the material surfaces were swabbed to collect sample material and thereafter the tip of swab was detached from the handle and placed in an Eppendorf tube to be stored and analysed for ATP as described below. Several tips of the swabs were placed in an Eppendorf tube and stored at −20 °C.

When sampling for ddPCR analyses, the surfaces were swabbed with sterile DNA-free swabs (COPAN4N6FLOQSwabs™, art.no 4479438, Life Technologies), and sampling for ATP-analyses, were performed using sterile ATP-free swabs (Hy-Lite swabs, art.no. 1.30103.0001 1, Merck, Th. Geyer). See Eriksson et al. (2016) for details about these methods.

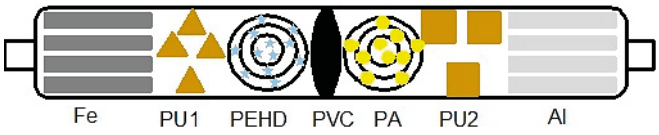

Figure 2-4. *Scheme of the constructed flow cell. Fe – steel rods (4 pieces), PU1 – Slitan 90A-05, PEHD – flakes of high density polyethylene dummy packed in the PVC band, PVC – a lump of twisted PVC, PA – bits of polyamide tube packed in the PVC band, PU2 – Slitan 80A-71, Al – aluminium rods (4 pieces).*

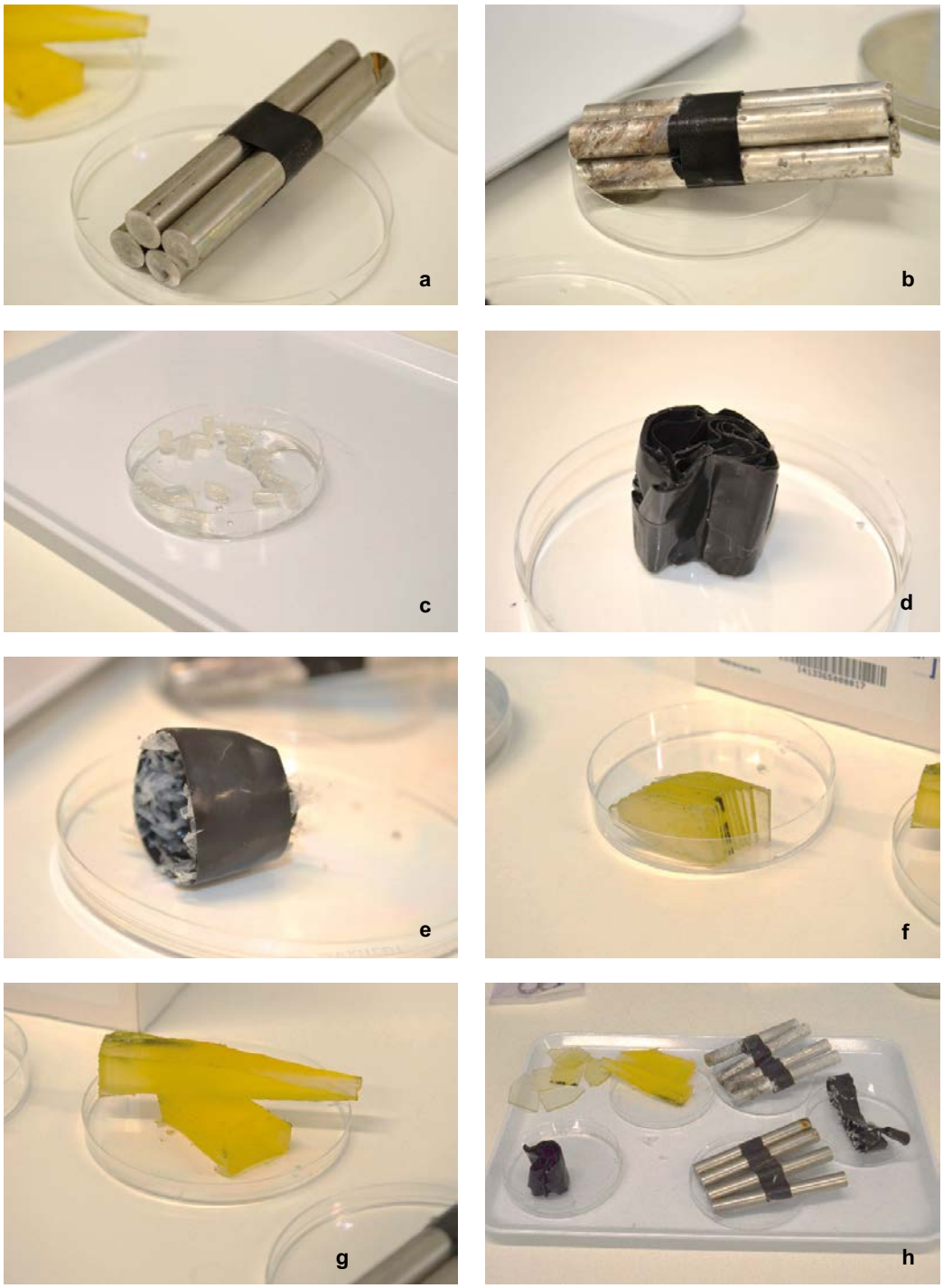

Figure 2-5. *Parts of the flow cell used for the molecular analyses: a – steel rods, b – aluminium rods, c – cuttings of the polyamide tube, d – polyvinylchloride tape, e – flakes of high density polyethylene dummy in PVC band, f – polyurethane Slitan 90A-05, g – polyurethane Slitan 80A-71, h – all components after exposure to groundwater for 12 weeks.*

2.5 Microbiological analyses

2.5.1 Total number of cells

The total number of cells (TNC) was determined using the acridine orange direct count method as devised by Hobbie et al. (1977) and modified by Pedersen and Ekendahl (1990). The acridine orange dye binds to nucleic acids and is fluorescent in blue light. The acridine orange method is described in detail in several papers (e.g. Pedersen et al. 2008). Briefly described, water samples were filtered

(−20 kPa) onto 0.22-µm-pore-size Sudan black-stained polycarbonate filters, 13 mm in diameter, mounted in stainless steel analytical filter holders. The filtered cells were stained for 5–7 min with 200 µL of a 10 mg L^{-1} acridine orange solution, dried, and mounted between microscope slides and cover slips using fluorescence-free immersion oil. The number of cells was counted under blue light (390–490 nm), using a band-pass filter for orange light (530 nm), in an epifluorescence microscope at 1 000 × magnification. At least 600 cells and 15–30 microscopic fields (1 field = 0.01 mm^2) were counted on each filter. The expected distribution of cells on the filters should follow a normal distribution. Usually, three subsamples filtered on three filters were counted, and the average of these three results is reported together with the standard deviation of the mean. Finally, the personnel conducting microscope counting must be inter-calibrated; otherwise, there may be different interpretations of what should be counted.

2.5.2 Adenosine-5'-triphosphate (ATP) from material surfaces

Adenosine-5'-triphosphate (ATP) transports chemical energy within cells for metabolism. ATP is a multifunctional nucleotide used in cells as a coenzyme. It is often called the "molecular unit of currency" in intracellular energy transfer. It is produced by cellular respiration, photosynthesis, or fermentation and used by enzymes and structural proteins in many cellular processes, including biosynthetic reactions, motility, and cell division. One molecule of ATP contains three phosphate groups, and it is produced by ATP synthase from inorganic phosphate and adenosine diphosphate (ADP) or adenosine monophosphate (AMP). Metabolic processes that use ATP as an energy source convert it back into its precursors, so ATP is continuously recycled in organisms. The average bacterium contains 1×10^{-18} moles (amol) of ATP, but this concentration varies significantly with cell size and metabolic status. Active cells have more ATP than inactive, non-metabolizing cells do. The analysis of ATP thus captures both biomass and activity.

The ATP Biomass Kit (HS no. 266-311; BioThema, Handen, Stockholm) was used to determine total ATP in the biofilms. The ATP biomass method used here has been described and tested in detail, and evaluated for use with Fennoscandian Shield groundwater (Eydal and Pedersen 2007). Typically, three subsamples were extracted and analysed and the average of these three results was reported together with the standard deviation of the mean. The method reports ATP, which correlates with the activity and size of the cells in a sample. A sample containing large, active cells will consequently contain more ATP than will a sample containing the same number of small, inactive cells.

Surfaces to be analysed were swabbed with ATP-free polyester swabs from COPAN. Each swab sample was placed in an Eppendorf tube containing 1 mL of B/S extractant from the ATP Biomass Kit, vortexed for 2 minutes and stored in the dark for a minimum of 30 minutes before analysis. Tests were performed previously to optimize sample storage procedures for laboratory and field conditions (Eriksson et al. 2016).

2.5.3 Most probable numbers of cultivable anaerobic bacteria

Preparation of media

Media for the most probable number (MPN) of bacteria in groundwater from Forsmark was composed based on previously measured chemical data from the site. This allowed artificial media to be composed that most closely resembled *in situ* groundwater chemistry for optimal microbial cultivation (Hallbeck and Pedersen 2008, Haveman and Pedersen 2002). Medium for the metabolic group of SRB were autoclaved and dispensed anaerobically into 27 mL anaerobic sealed tubes (Bellco Glass Inc. (www.bellcoglass.com), product number 2048-00150) with butyl rubber stoppers (product number 2048-117800) crimped with aluminium seals (product number 2048-11020). All culture tubes were flushed with 80/20 % N_2/CO_2 gas and then filled with 9 mL of SRB media. The final pH was adjusted to between 6.5 to 7.5 with 1 M HCl or 1 M NaOH.

Inoculations and analysis of SRB

Inoculations for SRB were performed at the same day as extraction 2 and 3, after 94 and 186 days respectively. Sampling was performed by using sterile anaerobic syringes to withdraw 1 mL sample of groundwater incubated with material and then inoculated to MPN tubes containing SRB cultivating

medium. After inoculating all MPN tubes were incubated in the dark at 18 °C for 8 weeks. SRB were detected by measuring sulphide production using the $CuSO_4$ method according to Widdel and Bak (1992) on a UV visible spectrophotometer (Genesys10UV, VWR, Stockholm, Sweden). Product formation at a concentration three times or more above that of the not inoculated control tubes was taken as positive for MPN analyses. The MPN procedures resulted in protocols with tubes that scored positive or negative for growth. The results of the analyses were rated positive or negative compared with control levels. Three dilutions with five parallel tubes were used to calculate the MPN of SRB, according to the calculations found in Greenberg et al. (1992). The detection limit was 0.2 cells mL^{-1}.

2.5.4 Extraction and quantification of extracted double stranded DNA

Collected DNA on the swabs, from material surfaces of metal and polymeric materials in the flow cells, was extracted with PowerWater® DNA Isolation Kit, (order no. 14900-100-NF, MO BIO Laboratories, Immuno diagnostics, Hämeenlinna, Finland). Extracted nucleotides were first quantified on a ND-1000 UV-vis spectrophotometer (Nanodrop Technologies, Wilmington, DE, USA), for quality control of the extraction efficiency and purity. Double stranded (ds) DNA concentrations were quantified fluorometrically with a Stratagene MX3005p fluorometer with MXPro software (Agilent Technologies Inc., Santa Clara, CA, USA) and the Quant-it™ dsDNA high sensitivity assay kit reagent from Molecular Probes (cat. no. Q33120, Life Technologies Ltd, UK, Thermo Fisher Scientific) according to the manufacturer's specifications. Extracted DNA was stored at −20 °C until ddPCR.

2.5.5 Droplet digital polymerase chain reaction

Primers for the detection of SRB were designed for qPCR and ddPCR (Table S1). Sulphate-reducing bacteria were detected with the primers apsAF304 and apsAR416 which are specific for the functional gene for the adenosine-5'-phosphosulfate (APS) reductase (Ben-Dov et al. 2007). Protocols for ddPCR were optimized by running primers on an annealing temperature gradient at 55–68 °C.

The extracted DNA was quantified with ddPCR by using the QX200™ Droplet Digital™ PCR system (Bio-Rad, Temse, Belgium). This method enables the precise and absolute quantification of target nucleic acids in a sample without the use of a standard curve. A pre-sample solution was prepared containing 12.5 µL of 2× QX200 ddPCR EvaGreen Supermix (Bio-Rad, cat no. 186-3010), 0.5 µL forward and reverse primers and 7.5 µL molecular grade water. A volume of 4 µL was pipetted from each sample vial and mixed with 21 µL pre-sample solution. Twenty microliters of this solution was pipetted in eight compartments of the Droplet Generator Cartridge (Bio-Rad, cat no. 186-3008) and droplets were generated. The entire droplet emulsion volume of 40 µL was further loaded in a semi-skirted and PCR-clean 96-well PCR plate (Eppendorf, Leuven, Belgium). The loaded 96-well PCR plate was then heat sealed with pierce-able foil in the PX1™ PCR Plate Sealer and placed in a C1000 Touch™ Thermo Cycler (both from Bio-Rad). The same thermal cycling conditions were applied as those used for qPCR except that the extension step was omitted, annealing temperature decreased to 58 °C and only 40 PCR cycles were run. After PCR amplification, the droplets were analysed in a QX200™ droplet reader (Bio-Rad), and the absolute quantification of PCR targets was analysed using QuantaSoft™software version 1.7.4 with a threshold placed at an amplitude as low as possible for each sample. These data were analysed with Poisson distribution to determine the target DNA template concentration in the original sample.

3 Results

3.1 Release of H_2 from metallic materials

3.1.1 Release of H_2 from steel rods

Release of H_2 from stainless steel rods under sterile conditions

The experiments at 30 °C under sterile conditions were carried out for 140 days. Gas analyses were performed after 0, 8, 22, 43, 71, 113 and 140 days of the experiment. After each analysis one rod was removed for water analysis, causing the gas series to be truncated. Results for water analysis are presented by Chukharkina et al. (2016). Exposure of the steel samples showed an increase in the partial pressure of H_2 in the gas phase (Figure 3-1). The gas release was more intensive during the first 30–40 days but the values varied from sample vessel to sample vessel. Figure 3-3 shows the result obtained for the steel rods per cm^2 metal surface.

Release of H_2 from stainless steel rods under natural conditions

The experiments at 30 °C under natural conditions were carried out for 108 days and gas analyses were performed after 0, 7, 21, 42, 73 and 108 days accordingly.

Figure 3-2 and Figure 3-4 show release of H_2 from the steel rods immersed in groundwater containing bacteria, as gas pressure and nmol per rods surface respectively. H_2 levels in the steel samples in the present experiment increased by day 7 and in the case of S1, increased further by day 21. After day 7 for most of the samples, H_2 concentration in the gas phase decreased to 0. For sample S1 such a decrease was observed after day 21. After decreasing, concentrations of H_2 in the samples remained the same during all 108 days of the experiment, while under sterile conditions concentration of H_2 in gas phase continued to grow. During this experiment a black precipitate was observed on the glass in all tubes contained steel rods (see Figure 3-5).

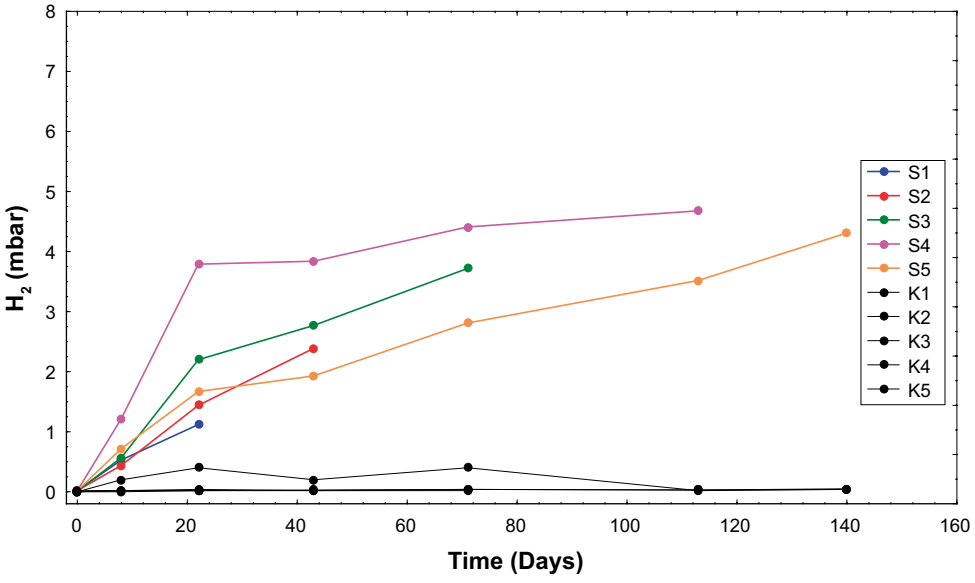

Figure 3-1. *H_2 release from the steel rods immersed in sterile filtered groundwater to the gas phase. S1 to S5 represent discrete steel samples according to Table 2-1. K1 to K5 represent control vessels without steel rods.*

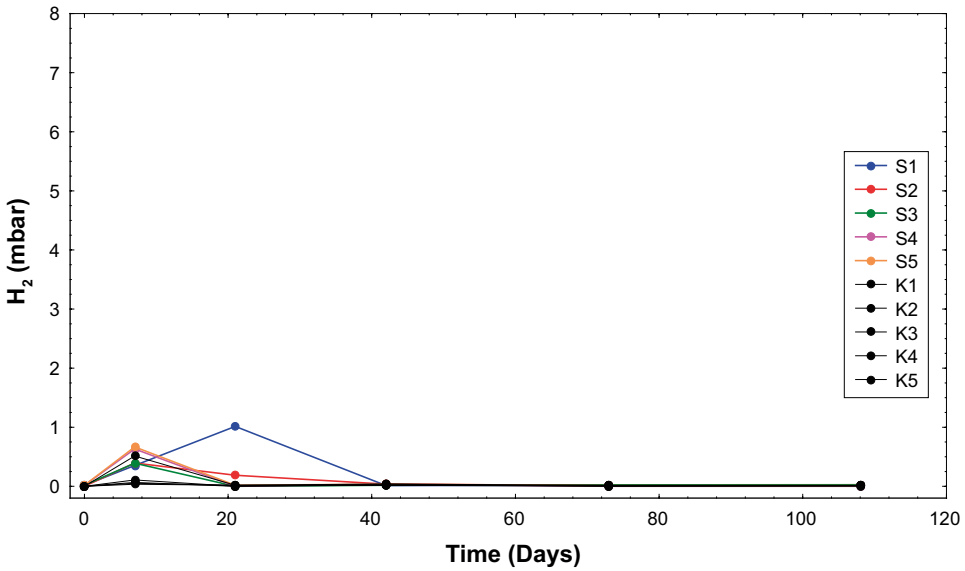

Figure 3-2. *H_2 measured from the steel rods immersed in natural groundwater to the gas phase. S1 to S5 represent discrete steel samples according to Table 2-1. K1 to K5 represent control vessels without steel rods.*

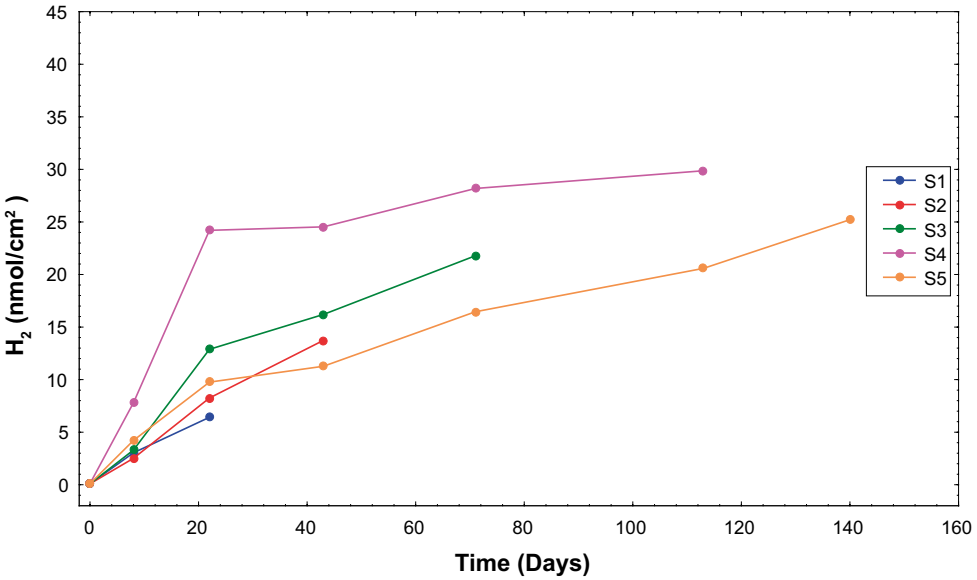

Figure 3-3. *H_2 release from the steel rods immersed in sterile filtered groundwater at 30 °C per cm^2 of rod surface. S1 to S5 represent discrete steel samples according to Table 2-1.*

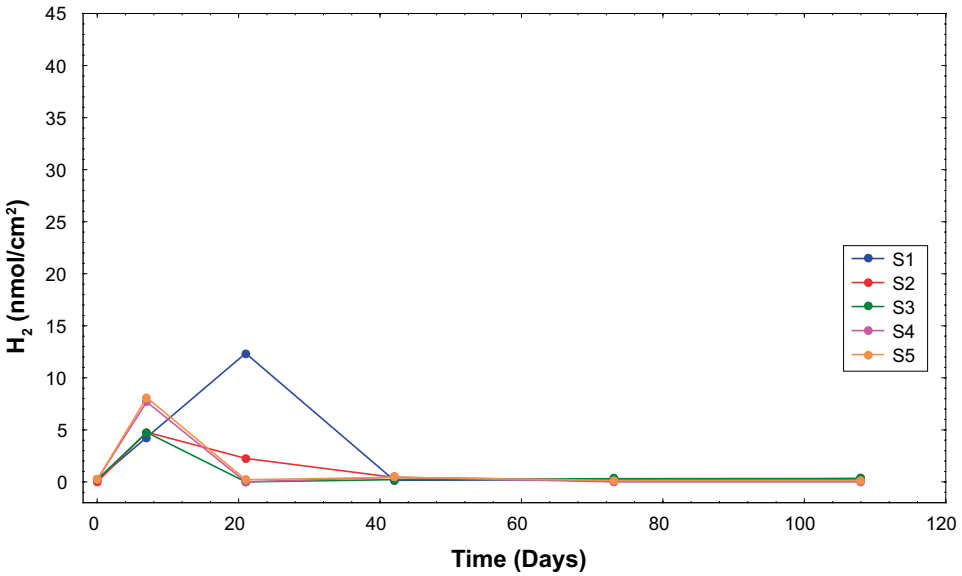

Figure 3-4. *H_2 measured from the steel rods immersed in natural groundwater at 30 °C per cm^2 of rod surface. S1 to S5 represent discrete steel samples according to Table 2-1.*

Figure 3-5. *Black coloured precipitate on the tube glass in a tube containing a steel rod immersed in natural groundwater.*

3.1.2 Release of H_2 from aluminium rods

Release of H_2 from aluminium rods under sterile conditions

Aluminium rods in SF-groundwater at 30 °C showed a significant release of H_2 (Figure 3-6) and the partial pressures varied between the samples. The release of H_2 was intensive in the first 20 days of the experiment after which the release rate decreased but it did not level out as observed for the steel vessels. Sample vessel A3 and particularly sample vessel A5 showed a clear increasing trend in partial pressure of H_2 throughout the experimental time. The highest value was observed in sample A5 at day 140 (9 mbar). The results are recalculated to nmol H_2 per cm^2 of aluminium rod surface as shown in Figure 3-8. The sample A4 showed the lowest values during the experiment and showed no H_2 at all by day 113. When the tube was opened at day 113, a distinct smell of hydrogen sulphide was noticed. The water from the sample tube was analysed for TNC and bacteria were observed.

Release of H_2 from aluminium rods under natural conditions

The experiment under natural conditions was carried out for 108 days with gas analyses performed after 0, 7, 21, 42, 73 and 108 days accordingly. Concentration of H_2 in the gas phase of the aluminium samples increased by day 7 to 1–3 mbar, like in the experiment under sterile conditions. In the samples A3, A4 and A5 concentration of H_2 declined after that to the background level and remained on the same level during the whole experiment, by day 108. For sample A2 and A1 partial pressure of H_2 fall off after day 21 and 42 and decreased to the background level after day 42 and day 73 respectively. Figure 3-7 and Figure 3-9 shows the partial pressure and concentration of H_2 per aluminium rod surface respectively.

3.1.3 Sulphide analyses of the water leachates from metallic components

Natural water leachates from metallic components were analysed for sulphide content after 108 days of the experiment. The used method did not show any positive results, and concentration of the sulphide in all samples was below detection.

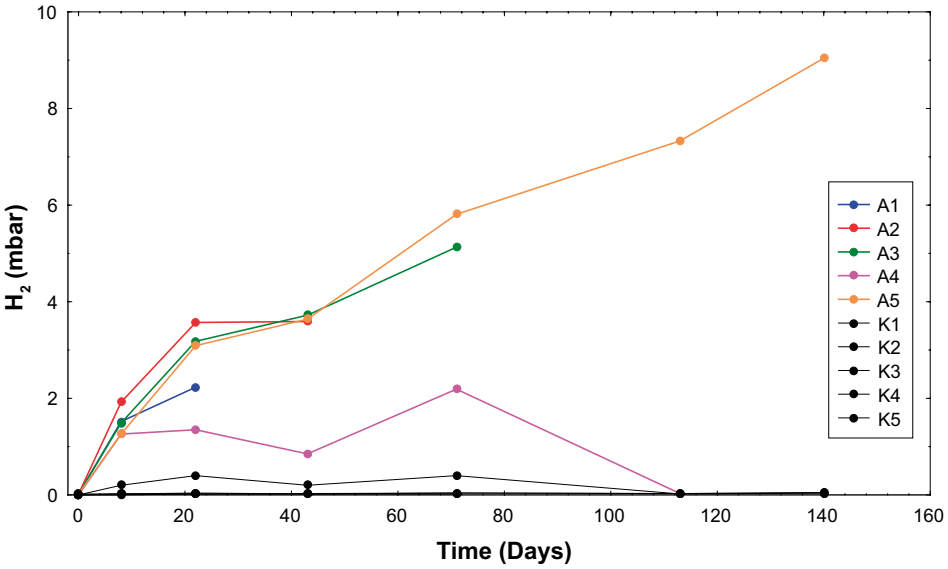

Figure 3-6. *H_2 release from the aluminium rods immersed in sterile filtered groundwater to the gas phase of the vessels. A1 to A5 represent discrete aluminium samples according to Table 2-1. K1 to K5 represent control vessels without aluminium rods.*

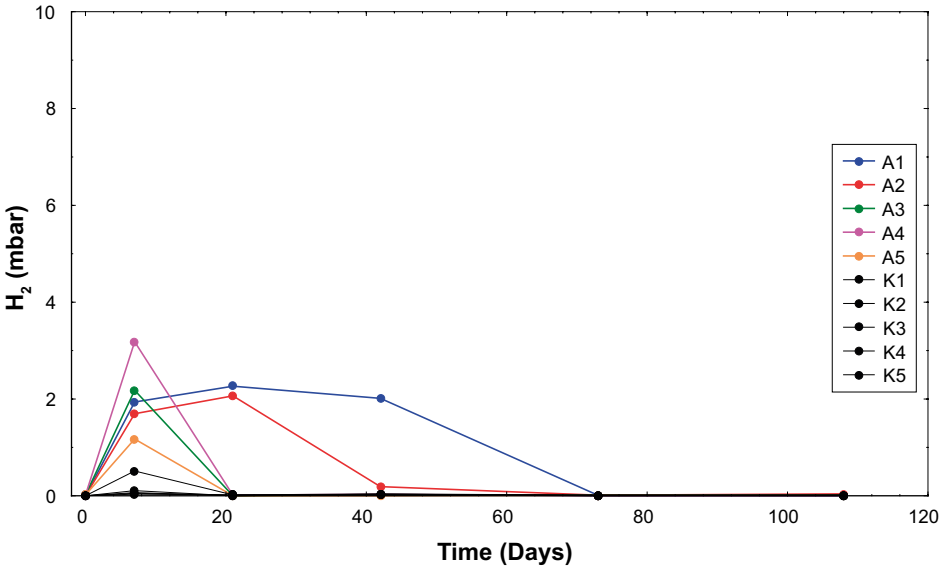

Figure 3-7. *H_2 measured from the aluminium rods immersed in natural groundwater to the gas phase of the vessels. A1 to A5 represent discrete aluminium samples according to Table 2-1. K1 to K5 represent control vessels without aluminium rods.*

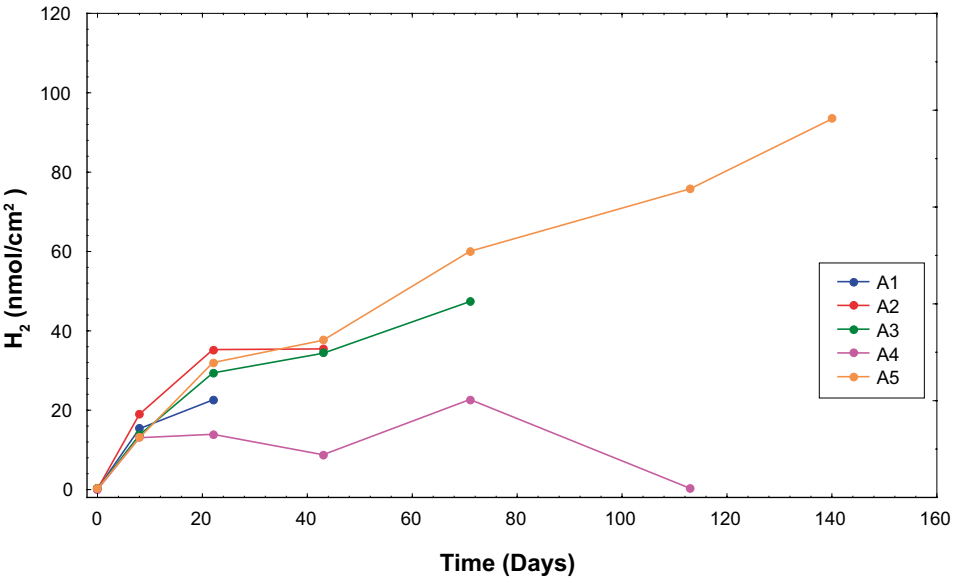

Figure 3-8. *H_2 release from the aluminium rods immersed in sterile filtered groundwater per cm^2 of rod surface. A1 to A5 represent discrete aluminium samples according to Table 2-1.*

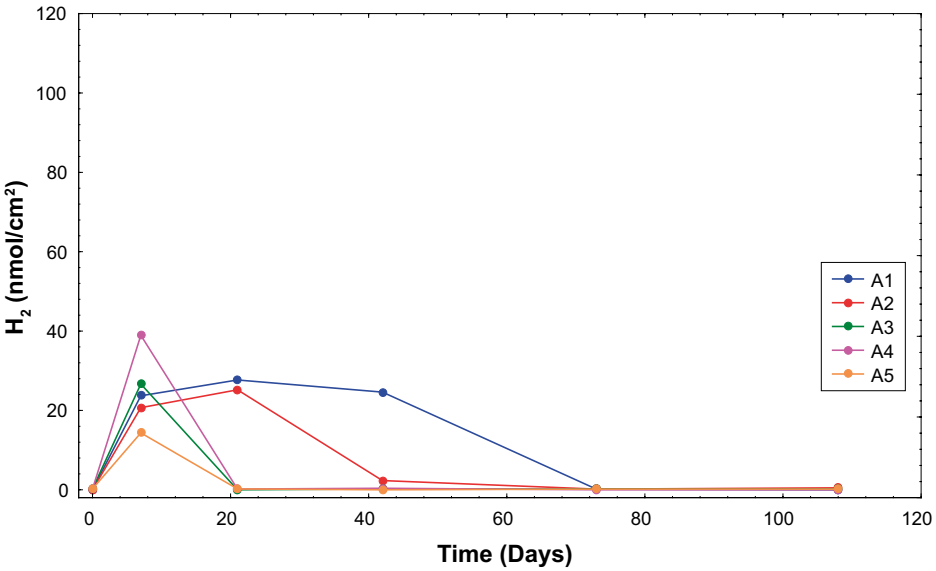

Figure 3-9. *H_2 measured from the aluminium rods immersed in natural groundwater per cm^2 of rod surface. A1 to A5 represent discrete aluminium samples according to Table 2-1.*

3.2 Release of organic compounds from polymeric materials

All results are non-quantitative since calibration could not be made for individual, unknown compounds. Instead the normalized and blank corrected area for the peak of interest at the sampling occasion after 28 days was chosen as base for relative calculation. This area was assigned to be 100 relative units and used as starting value for calculations of the further results. Since the compounds were expected to be absent in water at the starting time at day 0, origo was included in the graph bars as well, in order to give a theoretical indication of the release rate during the first 28 days. All peak areas were within the linear range on the instrument and since peak area is directly proportional to amount of each compound, this relative approach is valid for comparison over time.

3.2.1 Polyamide tubes

Component analysis of hexane extract

Hexane extraction of the polyamide tubes released a small number of compounds, mostly aliphatic hydrocarbons. Two other compounds were identified: N-butylbenzenesulfonamide and azacyclotridecan-2-one (Table 3-1). The peaks for these two compounds had higher intensity than those from the aliphatic hydrocarbons.

Table 3-1. Results for component analysis of the polyamide tubes extracted with hexane.

t_R (retention time)	Compounds	CAS
20.45	N-butylbenzenesulfonamide	3622-84-2
20.64	Azacyclotridecan-2-one	947-04-6
0–29	aliphatic hydrocarbons	–

Component analysis of leachate under sterile conditions

Both compounds found with hexane extraction were also found in water after the SF-groundwater leaching. The leaching experiment lasted for 182 days and three extractions were made, at days 28, 98 and 182. Concentration of N-butylbenzenesulfonamide in the water increased three times by day 98 compared to day 28 after which the concentration decreased by 25 % (Figure 3-10). Concentration of azacyclotridecan-2-one increased almost 4.5 times by day 98 compared to day 28 after which the concentration remained roughly the same until day 182. At the last component analysis occasion, day 182, particles were observed in the water sample. The sample was analysed for TNC and 1.1×10^5 cells mL^{-1} were detected.

Component analysis of leachate under natural conditions

The same compounds as in the experiments with SF-groundwater were also found in water during the experiment under natural conditions after the leaching into groundwater containing bacteria. This experiment lasted for 186 days and three extractions were made, at days 31, 94 and 186. Figure 3-11 shows that concentration of both N-butylbenzenesulfonamide and azacyclotridecan-2-one in the natural groundwater containing bacteria decreased almost 2.5 times by day 94, after which it increased a little by day 186. It showed a trend quite opposite to the one in the sterile experiment where concentrations of the compounds in the water increased. At the day 186, during the extraction, also the distinct smell of hydrogen sulphide was noticed. Concentration of the sulphide in the water sample and microbial data are shown in Figure 3-21 and respective tables.

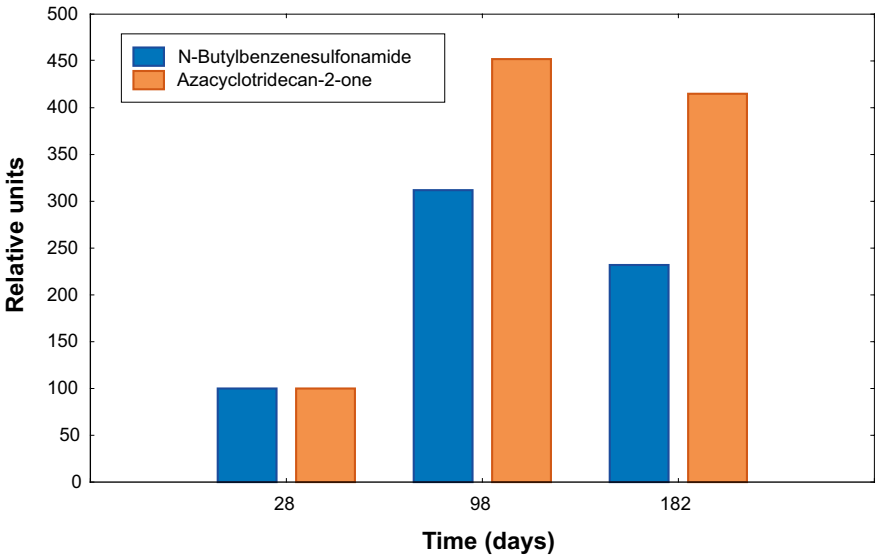

Figure 3-10. *Relative amounts of organic compounds leached from the polyamide tube in sterile filtered groundwater over leaching time.*

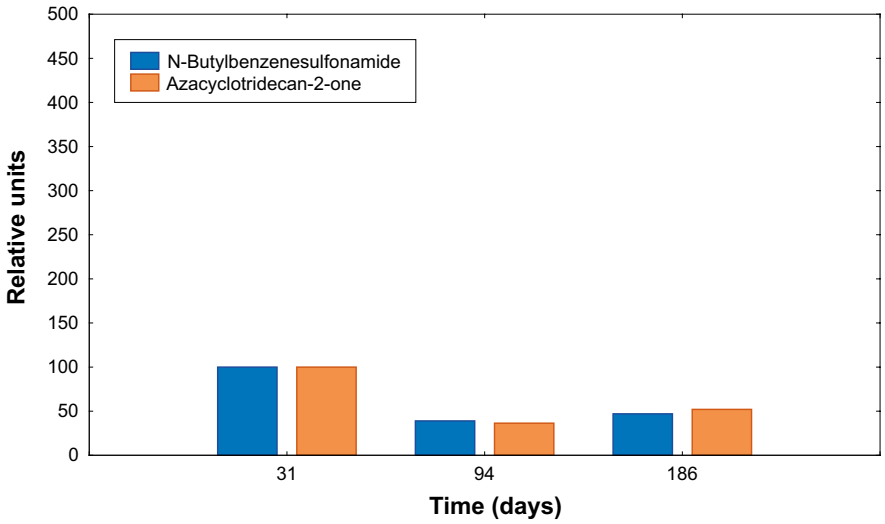

Figure 3-11. *Relative amounts of organic compounds leached from the polyamide tube in natural groundwater over leaching time.*

3.2.2 PVC tape

Component analysis of hexane extract

The results from the component analysis of PVC tape are shown in Table 3-2.

Table 3-2. Results for component analysis of the PVC tape.

t_R (retention time)	Compounds	CAS
5.60	Toluene	108-88-3
7.50	o-Xylene	95-47-6
7.94	*Aromatic hydrocarbon*	–
9.46	Propylbutylether	3073-92-5
10.30	Hexanol	111-27-3
10.94	*Benzole derivative*	–
14.95	*Alcohol*	–
17.37	Butylated hydroxytoluene	128-37-0
18.50	Tetraethylbutylphenol	–
18.99	*Phthalate*	–
19.31	Dimethylphthalate	131-11-3
19.67	Octylbenzoate	94-50-8
19.82	Phenylethylphenol	4237-44-9
20.03	*Phenol derivative*	–
23.51	2-Benzoylacetophenone	120-46-7
24.99	Dibutylphthalate	84-74-2
26.95	*Phthalate*	–
27.27	DEHP	117-81-7
+28.80	DIDP	26761-40-0
+28.80	DINP	68515-48-0

Compounds in italic has less of 80 % library matching in the NIST library.
DIDP and DINP give several peaks in the chromatogram from 28.80 minutes and onwards.

Component analysis of leachates under sterile conditions

Two compounds, toluene and di-ethylhexyl phthalate (DEHP), were found in the SF-groundwater after leaching and these compounds had the highest intensity of all detected peaks. Therefore, they were chosen as compounds of interest to illustrate leaching results. Relative amounts of toluene and DEHP in water after three extraction occasions are shown in Figure 3-12. The amount of toluene

decreased after 98 days compared to 28 days, the decrease was about 28 % from the initial value obtained at the first extraction. The relative amount of toluene increased slightly again after 182, but it did not reach the initial value. Concentration of DEHP dropped considerably during the same time, the amount of DEHP after 98 days was 27 % of the amount observed at day 28. DEHP was not found in the water sample at the third extraction at day 182. During sampling it was observed that the water was cloudy and contained visible particulates that did not exist in the samples at start of the experiment. TNC analysis of the sample was performed and 0.84×10^5 cells mL^{-1} were detected.

Component analysis of leachate under natural conditions

In the water samples at the experiment under natural conditions toluene and benzoic acid were found. DEHP (di-ethylhexyl phthalate), found at the previous experiment, was not detected in water samples containing bacteria neither at the first nor the second extraction occasion. It was however found at the third extraction by day 186 in noticeable amount (se Figure 3-13). Toluene, found as well at the previous experiment under sterile conditions, showed another trend now: its concentration remained on the same level at days 31 and 94 after the sampling, but increased three times by day 186. Benzoic acid was observed first at the present conditions and its concentration in water decreased rapidly from day 31 by day 186. At the microbial experiment octasulfur (S_8) was found in water samples from PVC. S_8 was first detected at the second extraction at day 94 and its concentration increased more than 50 times by day 186. At day 186 also a strong smell of hydrogen sulphide was detected, and the water sample was cloudy.

3.2.3 High density polyethylene dummy material

Component analysis of hexane extract

Component analysis of the PEHD dummy showed mostly presence of aliphatic hydrocarbons, alcohols and some esters (Table 3-3). In addition, N-butylbenzenesulfonamide was detected in the hexane extract.

Table 3-3 Results for component analysis of the PEHD dummy.

t_R (retention time)	Compounds	CAS
0–18.5	Aliphatic hydrocarbons	–
18.63	Dodecanoic acid ester	
20.44	N-butylbenzenesulfonamide	3622-84-2

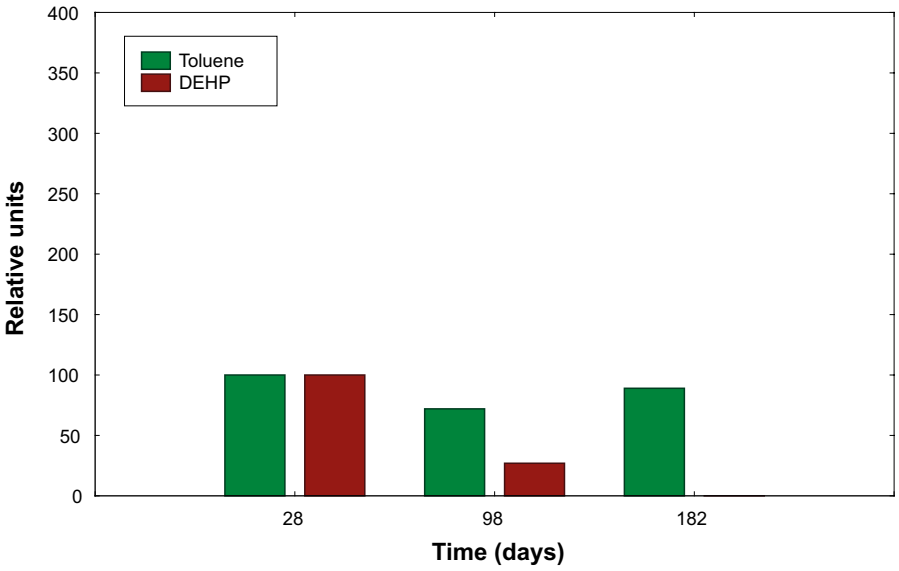

Figure 3-12. *Relative amounts of organic compounds leached from the PVC tape in sterile filtered groundwater over leaching time.*

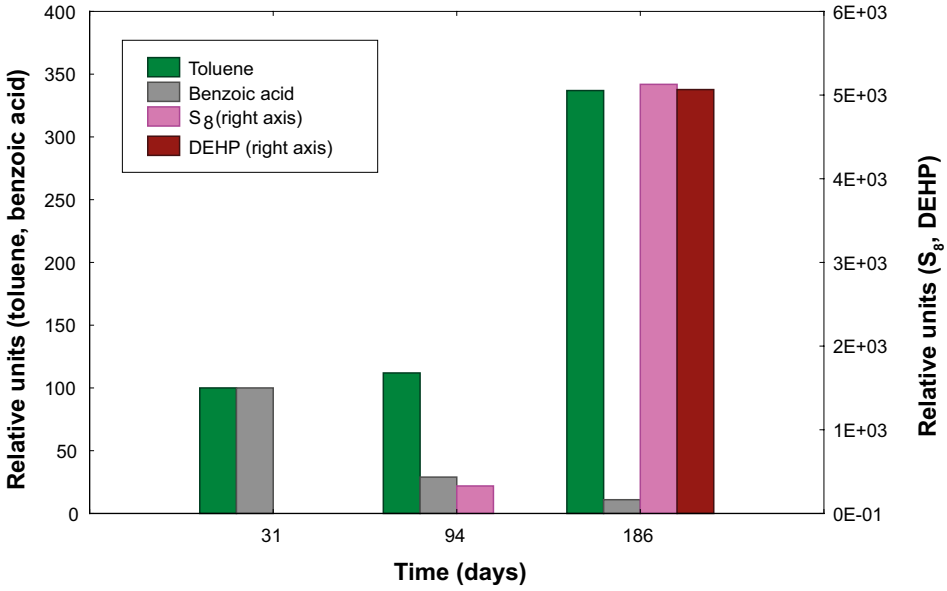

Figure 3-13. *Relative amounts of organic compounds leached from the PVC tape in natural groundwater over leaching time.*

Component analysis of leachate under sterile conditions

The amount of N-butylbenzenesulfonamide remained the same for 98 days and was doubled by day 182 (Figure 3-14). Some more compounds were found at the third leaching occasion, but were absent at two first leaching occasions. Therefore, it was not possible to show the leaching progress in these cases. The water sample from 182 days was tested for TNC and 2.1×10^5 cells mL^{-1} were detected.

Component analysis of leachate under natural conditions

N- butylbenzenesulfonamide was detected in water samples under natural conditions as well and its concentration remained the same for 94 days. However, it decreased by half by day 186 (se Figure 3-15). No other compounds were found in noticeable amount in the samples at the second experiment. No sulphide smell was noticed as well at any extraction occasion.

3.2.4 Polyurethane packer Slitan 90A-05

Component analysis of hexane extract

Results of the component analysis for this sample revealed various esters, alcohols, amines, aromatic compounds and three organic compounds which could not be identified by the NIST 98 library (Table 3-4). These three compounds were obviously the main compounds extractable from this type of polymeric material, as the intensities of their peaks were much higher than for any other compound found in this sample. These compounds were regarded to belong to the same group, because they have the same qualifier ions (71 and 73), but different retention times. In the present work they were defined as compounds 1a, 1b and 1c.

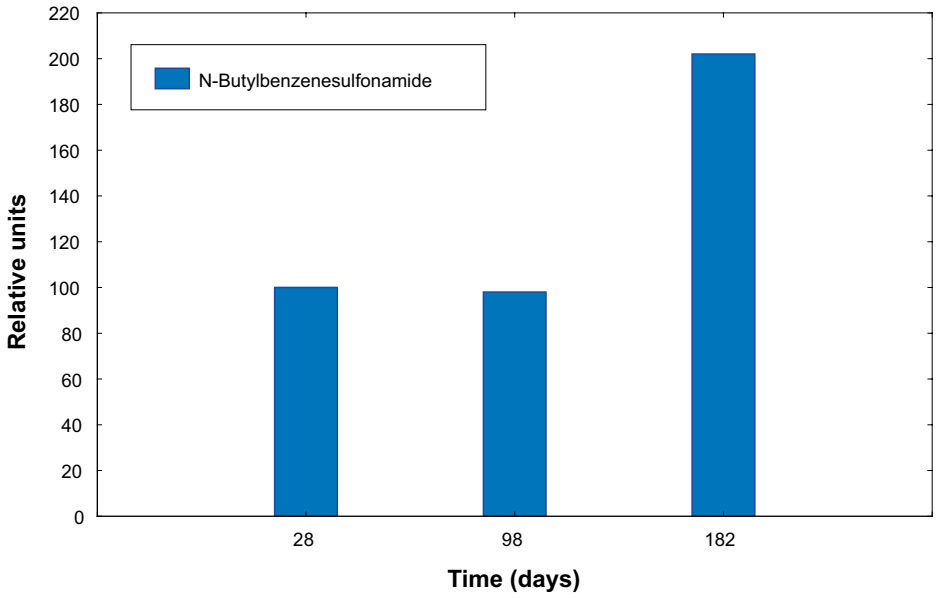

Figure 3-14. *Relative amounts of organic compounds leached from the PEHD dummy material in sterile filtered groundwater over leaching time.*

The unknown compounds with qualifier ions 71 and 73 found with hexane extraction were found in SF-groundwater leached samples under sterile conditions at all leaching occasions (Figure 3-16). The relative amount of all three compounds in water increased by day 28 and decreased by day 182. In the water sample day 182 large particles were observed and two different types of bacteria were observed in the microscope. A TNC of 0.71×10^5 cells mL^{-1} was detected.

Table 3-4. Results for component analysis of the PU Slitan 90A-05.

t_R (retention time)	Compounds	CAS
10.29	Butanedioic acid dimethylester	106-65-0
10.39	*Bicycloaldehyde*	–
13.34	Fenoxyetanol	122-99-6
13.58	Hexanedioic acid dimetylester	
15.29	Benzenediisocyanate	–
17.38	Butylated hydroxytoluene	204-881-4
18.37	Unknown compound: compound 1a	–
18.62	Dodecanoic acid ester	–
23.39	*Bicyclic benzene ring*	–
23.51	Unknown compound: compound 1b	–
26.69	Hexanoic acid ester	–
26.92	*Bensenamine dimer, chlorinated*	–
27.51	Unknown compound: compound 1c	–
28.17	Triphenylphosphinesulfide	3878-45-3

Compounds in italic has less than 80 % library matching in the NIST library.
Component analysis of leachate under sterile conditions.

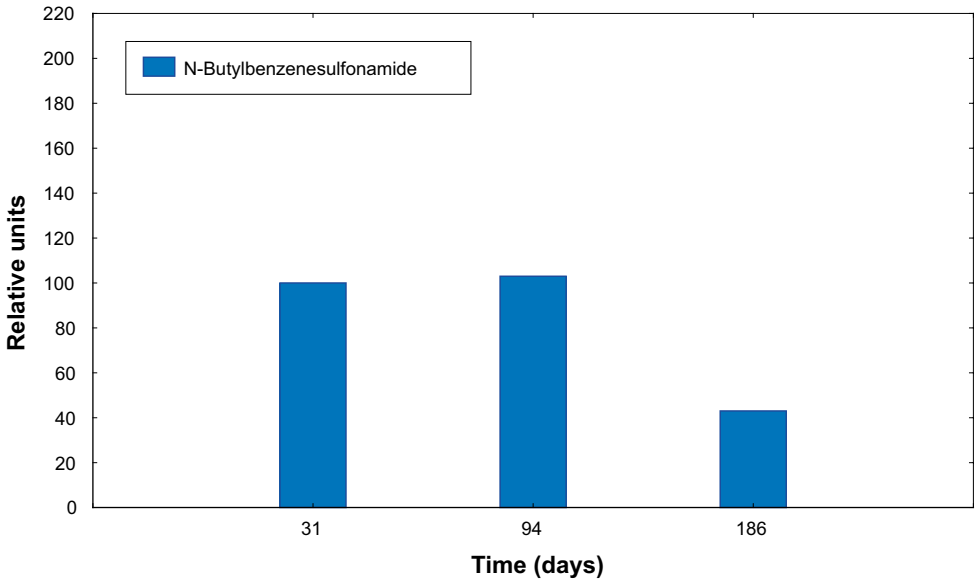

Figure 3-15. *Relative amounts of organic compounds leached from the PEHD dummy material in natural groundwater over leaching time.*

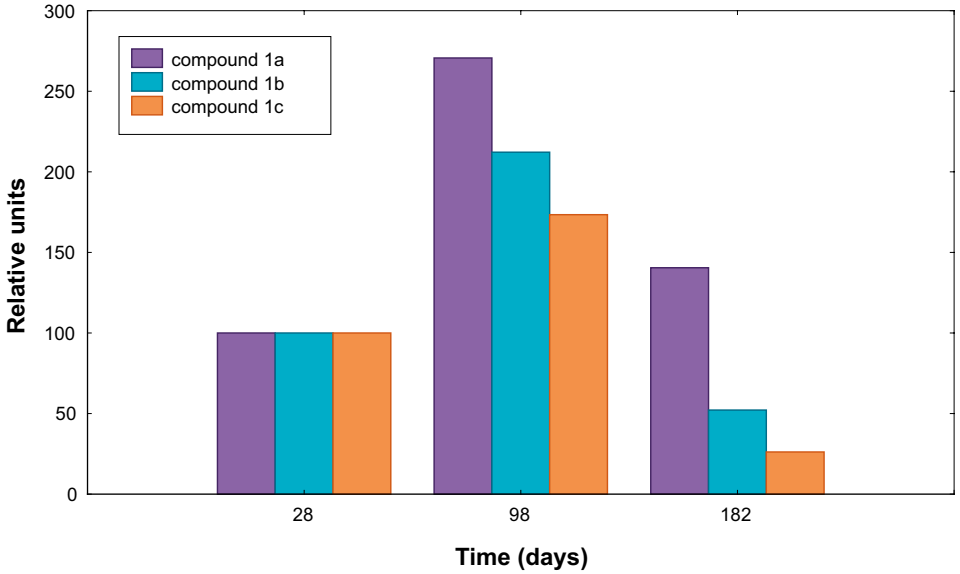

Figure 3-16. *Relative amounts of organic compounds leached from the PU packer material Slitan 90A-05 in sterile filtered groundwater over leaching time.*

Component analysis of leachate under natural conditions

All three compounds found in the previous experiment under sterile conditions were found in the groundwater samples at natural conditions as well. The present version of the NIST library determined them as compounds containing a 3-methyl-1-penten-3-ol fragment. All three isomers showed different trends during the duration of the second experiment (se Figure 3-17). Concentration of the first isomer decreased slightly by day 186, while concentration of the second decreased by day 94 and increased again by day 186. The third isomer showed increase of concentration almost 2 times by day 94, but it decreased after that by 30 %. In all three analyses under natural conditions octasulfur (S_8) was detected in the samples. Its concentration increased almost 7.5 times from day 31 to day 186. Distinct smell of hydrogen sulphide was noticed during the extraction.

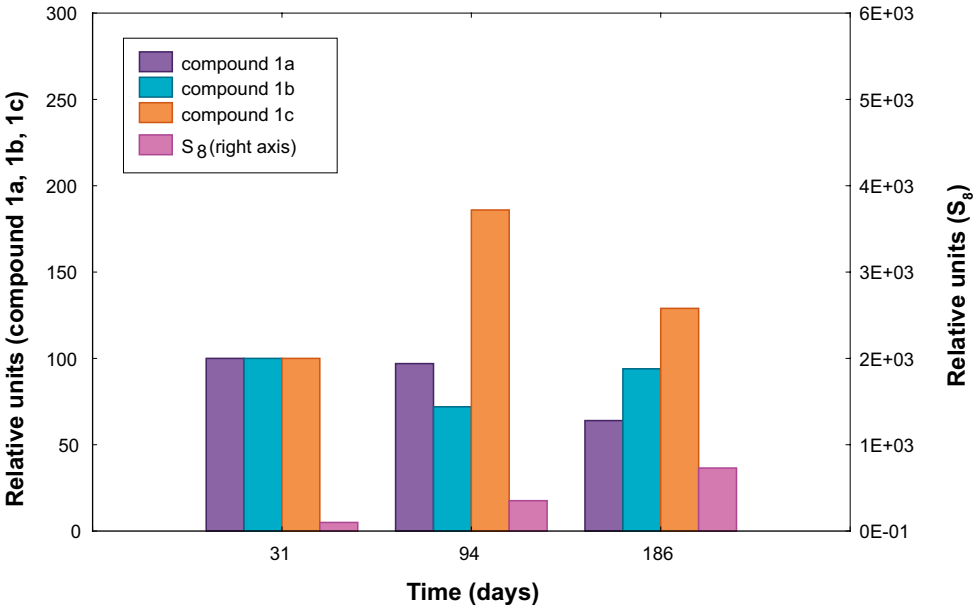

Figure 3-17. *Relative amounts of organic compounds leached from the PU packer material Slitan 90A-05 in natural groundwater over leaching time.*

3.2.5 Polyurethane packer Slitan 80A-71

Component analysis of hexane extract

Component analysis of PU packer Slitan 80A-71 showed the presence of many aromatic compounds and alcohols (Table 3-5). Two of the compounds, at retention times 26.9 and 28.6 min., could not be identified by the NIST 98 library, but had a high intensity of the peaks in the chromatogram. These organic compounds were assumed to belong to the same group, because they have the same qualifier ion m/z 173, though they differed slightly in the other detected ions. They were defined as compound 4a and 4b respectively, in the present work.

Table 3-5. Results for component analysis of the PU Slitan 80A-71.

t_R (retention time)	Compounds	CAS
13.52	*Benzothiazol derivative*	
13.73	Phenoxypropanol	
14.80	Unknown compound: compound 2	
15.87	*Benzaldehyde derivative*	
16.31	Benzofuran	
17.37	Butylated hydroxytoluene	
17.70	Unknown compound: compound 3	
18.97	*Phthalate*	
27.15	Unknown compound: compound 4a	
28.85	Unknown compound: compound 4b	

Compounds in italic has less of 80 % library matching in the NIST library.
Component analysis of leachate under sterile conditions.

The unknown compounds with qualifier ion 173 were also found in groundwater leachates under sterile conditions. In the water samples two other compounds were found at all leaching occasions, having retention times 14.8 and 17.7 min. These compounds had high intensities of their peaks as well and were also chosen as compounds of interest, being defined as compound 2 and 3 respectively. Compound 2 had qualifier ions 55, 112 and 142 and compound 3 had the main qualifier ion 201. Analysis of the leaching data showed that relative amount of all the compounds increased by day 98 compared to day 28. The relative amounts of compounds 3, 4a and 4b augmented two – three

times, whereas the amount of compound 2 increased dramatically from 100 to 641 relative units. The amounts of the compounds decreased by day 182. Results are presented on Figure 3-18. Large particles were observed in the water sample by day 182. The TNC analysis detected 0.44×10^5 cells mL^{-1}.

Component analysis of leachate under natural conditions

All the compounds detected in sterile groundwater leachates were found in the leachates under natural conditions. Compound 2 was identified by NIST 2014 library as glycerol-1,2-diacetat. Its concentration decreased constantly from day 31 and was just 13 % from the initial by day 186 (Figure 3-19). Compound 3 was identified as a linalyl acetate derivative and it showed the same trend. Concentration of all the detected compounds at the experiment under natural conditions decreased from day 31 to day 94, unlike the sterile experiment, when concentrations on the contrary increased during the same period. During the third extraction occasion on day 186 small particles were observed in the water sample and a faint sulphide smell was noticed as well.

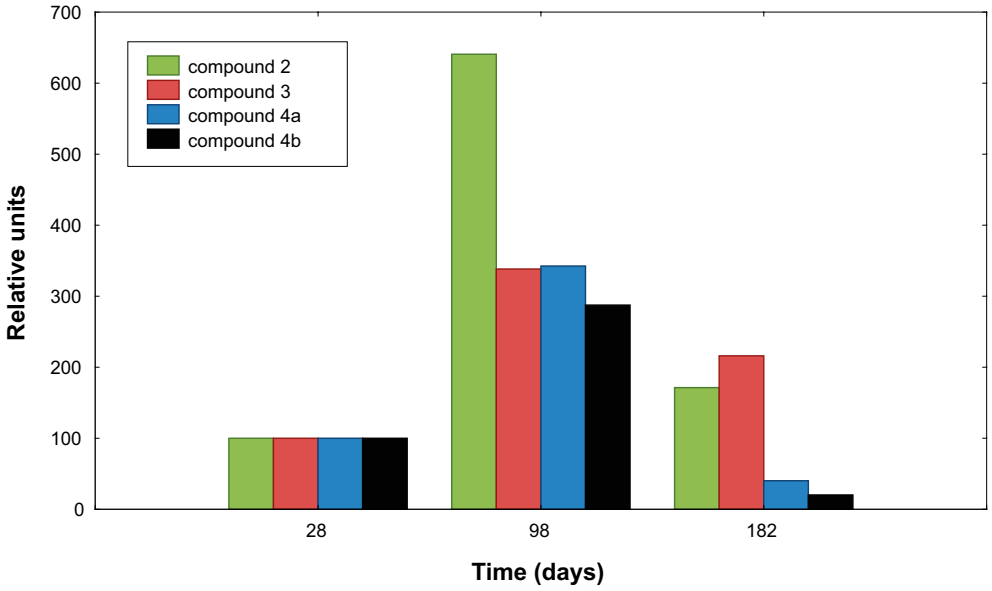

Figure 3-18. *Relative amounts of organic compounds leached from the PU packer material Slitan 80A-71 in sterile filtered groundwater over leaching time.*

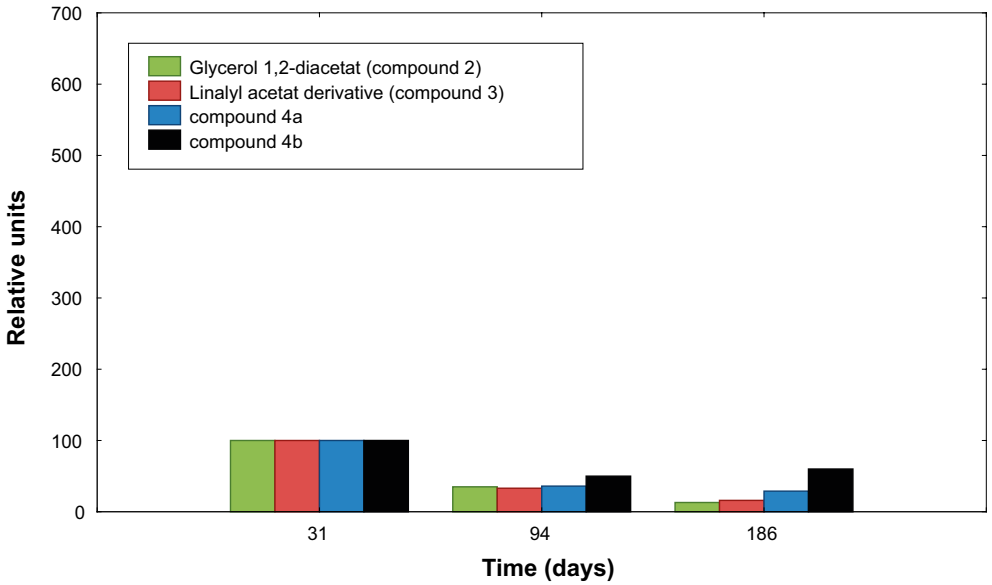

Figure 3-19. *Relative amounts of organic compounds leached from the PU packer material Slitan 80A-71 in natural groundwater over leaching time.*

3.3 Microbiology and sulphide analyses of the leachates from the polymeric material

3.3.1 TNC analyses

The groundwater used for the experiment under natural conditions was analysed for cell content at the first sample preparation, at day 0. The TNC analysis detected 6.4×10^4 cells mL^{-1}. All leachates were analysed for cell and sulphide content the same days as SPE-extraction for organic compounds was performed, at 31, 94 and 186 days of the experiment.

In the control sample of natural groundwater (Figure 3-20) total number of cells in the sample analysed at day 31 was 1.7×10^5 cells mL^{-1} and in samples analysed at day 94 and 186 it showed 8.1×10^4 and 5.7×10^5 cells mL^{-1} respectively. Results for the leachates from PA samples showed that amounts of cells decreased from 2.3×10^5 to 1.5×10^5 cells mL^{-1} by day 186 (Figure 3-21). Amounts of cells in the leachates from PVC samples, on the contrary, increased by day 186 to 1.2×10^6, as shown at Figure 3-22. Leachates from other samples showed the same tendency: amount of cells increased by day 186 in leachate from PEHD to 7.1×10^5, in leachate from PU Slitan 90A-05 to 1.0×10^6 and in leachate from PU Slitan 80A-71 to 1.1×10^6 (Figure 3-23, Figure 3-24 and Figure 3-25 respectively). The whole results for TNC analyses are shown in Table 3-6.

Table 3-6. Results of TNC analyses performed at the experiment under natural conditions.

Sample	Days			
	0	31	98	186
	TNC ± SD (cell mL^{-1})			
Control	$6.4 \times 10^4 \pm 7.6 \times 10^3$	$1.7 \times 10^5 \pm 3.7 \times 10^4$	$8.1 \times 10^4 \pm 1.0 \times 10^4$	$5.7 \times 10^5 \pm 2.3 \times 10^4$
PA	–	$2.5 \times 10^5 \pm 2.0 \times 10^4$	$1.9 \times 10^5 \pm 1.2 \times 10^4$	$1.5 \times 10^5 \pm 1.1 \times 10^4$
PVC	–	$5.0 \times 10^5 \pm 1.9 \times 10^5$	$9.5 \times 10^5 \pm 2.4 \times 10^5$	$1.2 \times 10^6 \pm 2.4 \times 10^5$
PEHD	–	$1.9 \times 10^5 \pm 4.5 \times 10^4$	$2.3 \times 10^5 \pm 2.5 \times 10^4$	$7.1 \times 10^5 \pm 5.2 \times 10^4$
PU 90A-05	–	$1.9 \times 10^5 \pm 6.0 \times 10^4$	$2.6 \times 10^5 \pm 1.2 \times 10^5$	$1.0 \times 10^6 \pm 1.3 \times 10^4$
PU 80A-71	–	$5.0 \times 10^5 \pm 1.9 \times 10^5$	$7.4 \times 10^5 \pm 3.1 \times 10^5$	$1.1 \times 10^6 \pm 1.1 \times 10^5$

3.3.2 SRB analyses

SRB analyses of water from the leachates were performed about 8 weeks after the inoculation of SRB at days 98 and 186. The results are presented in Figure 3-20 to Figure 3-25 and Table 3-7.

Table 3-7. Results of SRB analyses of the leachates performed at experiment under natural conditions.

Sample	SRB (cell mL^{-1}) (low and upper limits for 95 % confidence interval)					
	98 days			186 days		
	MPN index	Lower	Upper	MPN index	Lower	Upper
Control	≥ 16000	–	–	8.0	3.0	25.0
PA	≥1600	–	–	70000	30000	210000
PVC	90000	30000	290000	17000	7000	48000
PEHD	≥1600	–	–	11.0	4.0	29.0
PU 90A-05	≥ 16000	–	–	300000	100000	1300000
PU 80A-71	1.7	0.7	4.0	160000	60000	530000

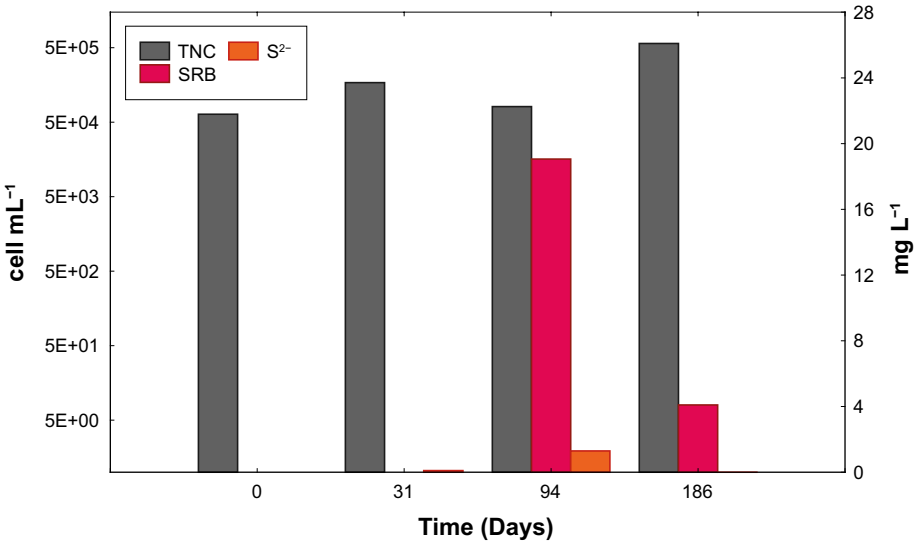

Figure 3-20. *Results for TNC, SRB and sulphide analyses of the control sample of groundwater.*

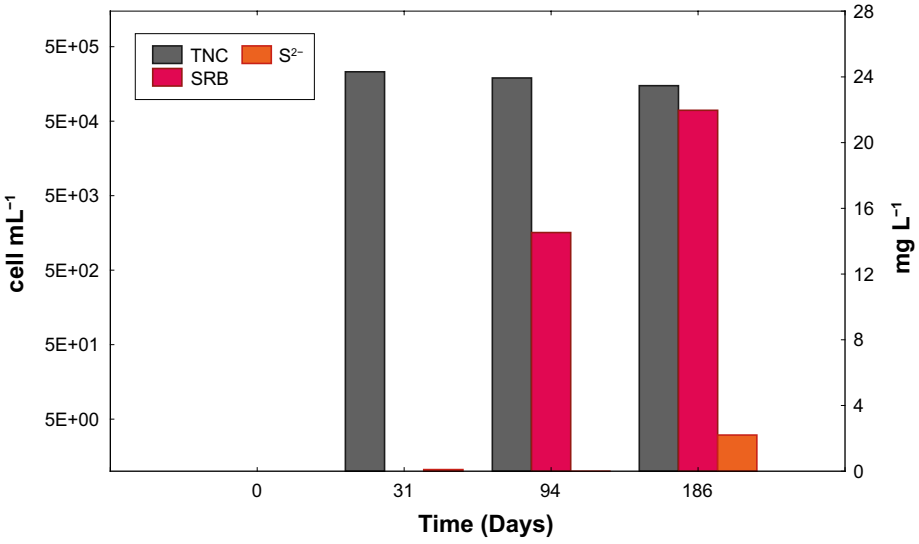

Figure 3-21. *Results for TNC, SRB and sulphide analyses of the leachate from PA sample.*

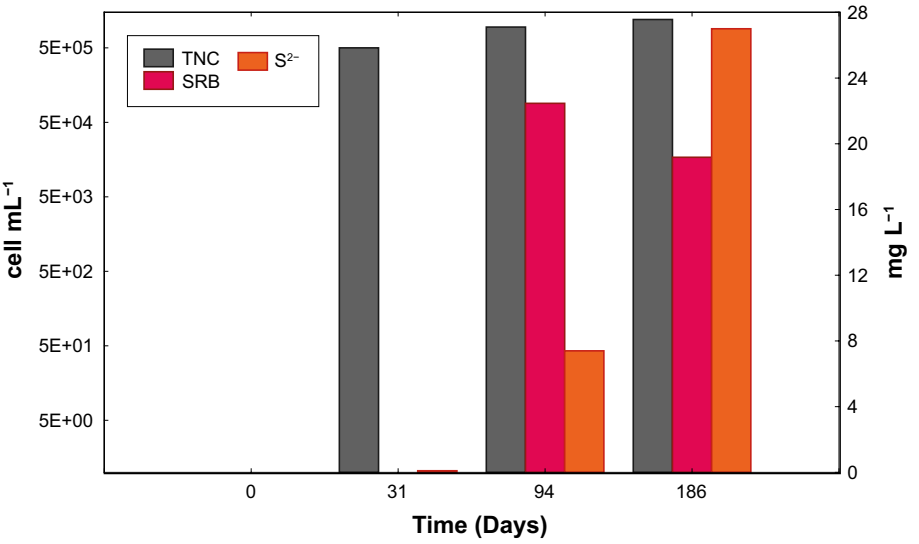

Figure 3-22. *Results for TNC, SRB and sulphide analyses of the leachate from PVC sample.*

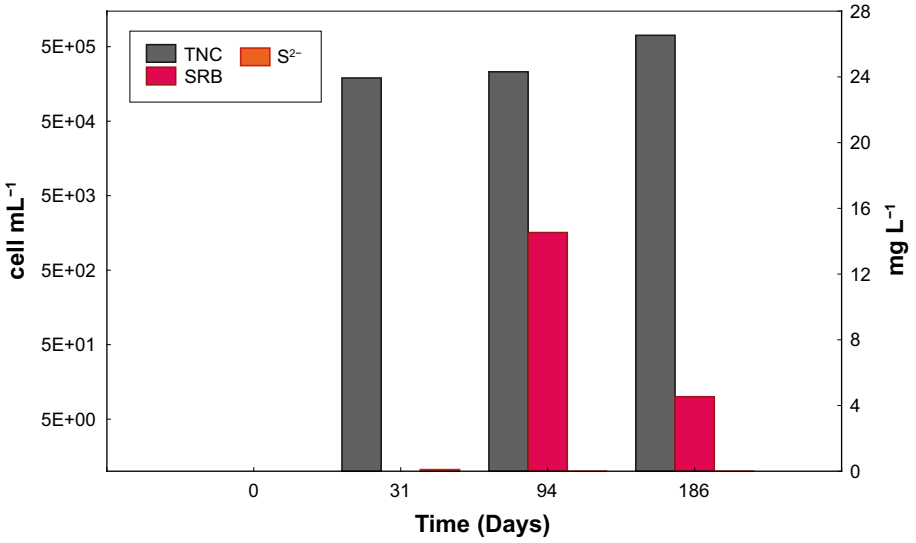

Figure 3-23. *Results for TNC, SRB and sulphide analyses of the leachate from PEHD sample.*

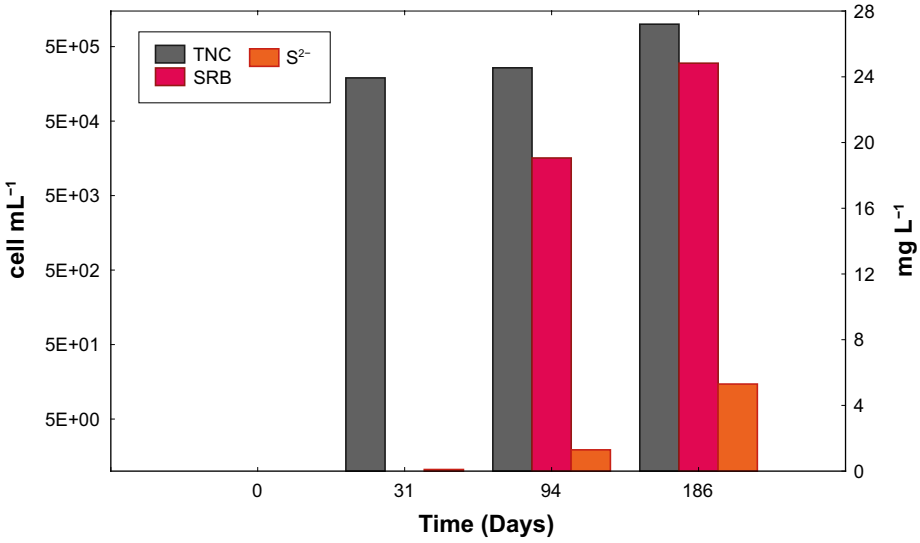

Figure 3-24. *Results for TNC, SRB and sulphide analyses of the leachate from PU Slitan 90A-05 sample.*

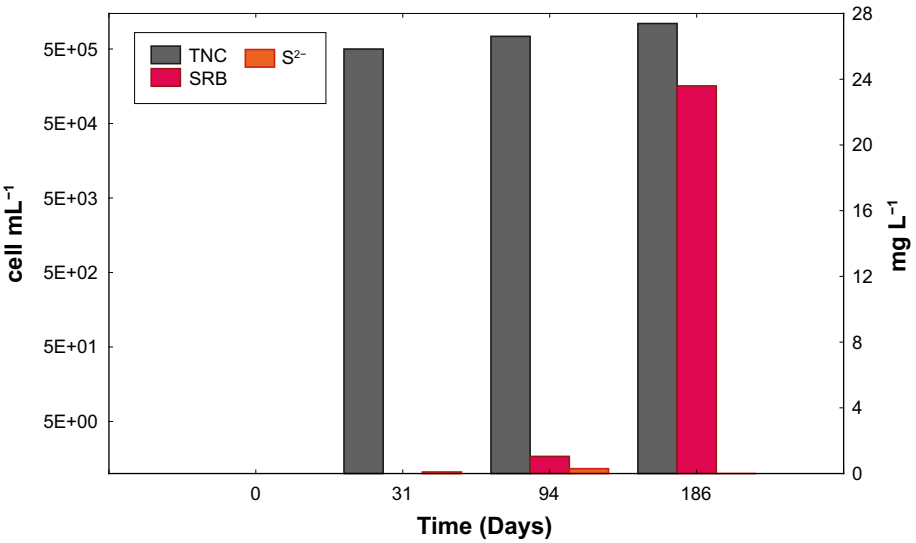

Figure 3-25. *Results for TNC, SRB and sulphide analyses of the leachate from PU Slitan 80A-71 sample.*

At the first analysis occasion after 98 days since sampling, the water samples showed high level of cells per mL, except the sample containing PU 80A-71, where MPN index was 1.7 cell mL^{-1}. In this sample, as well as in sample contained PVC, a rusty coloured precipitate was observed, which could affect the absorbance and therefore the results. It was difficult to evaluate the most probable numbers of cells for these samples and results were estimated approximately. By day 186 number of cells in samples containing PA, PU 90A-05 and PU 80A-71 increased whereas number of cells in control samples and samples contained PVC and PEHD decreased in comparison with day 98.

3.3.3 Sulphide analyses of leachates

Sulphide analyses were performed at days 31, 98 and 186 of the experiment, the same time as the SPE-extraction. In all samples sulphide concentration in the leachate at day 31 was detected at 0.1 mg L^{-1}. However, at the first analysis occasion a method with lower sensibility was chosen, and it is possible that sulphide concentration in the samples at day 31 was below detection nevertheless. No sulphide smell was observed at this occasion. Sulphide concentration in the control sample was 1.3 mg L^{-1} at day 98 and was detected as below detection at day 186 (see Figure 3-20). In the leachate from PA sample sulphide was observed only at day 186 and was 2.2 mg L^{-1} (Figure 3-21). The weak smell of sulphide was detected during the extraction. The highest amounts of sulphide were found in the leachates from PVC samples (shown at Figure 3-22): the sulphide concentration was 7.4 mg L^{-1} at day 98 and increased to 27 mg L^{-1} by day 186. The water had a strong smell of sulphide at both occasions. No sulphide was observed in water leachates from PEHD sample (Figure 3-23). The results for sulphide analysis of leachates from PU Slitan 90A-05 are shown at Figure 3-24. Sulphide concentration in the water was 1.3 mg L^{-1} at day 98 and increased about 5 times by day 186. Results of the analysis from the leachates of PU Slitan 80A-71 showed decreasing sulphide concentrations from 0.3 mg L^{-1} at day 98 to 0 mg L^{-1} by day 186 (Figure 3-25). Results for sulphide analyses of leachates are shown in Table 3-8.

Table 3-8. Results for sulphide analyses of leachates of the organic components performed under natural conditions.

Sample	S^{2-} (mg L^{-1})		
	31 days	98 days	186 days
Control	0.1	1.3	< 0.1
PA	0.1	< 0.1	2.2
PVC	0.1	7.4	27.0
PEHD	0.1	< 0.1	< 0.1
PU Slitan 90A-05	0.1	1.3	5.3
PU Slitan 80A-71	0.1	0.3	< 0.1

3.4 Flow cell results

After being immersed in the groundwater during 12 weeks both aluminium and steel rods had visible signs of corrosion, and the aluminium rods were covered with hydroxide-oxide film (see Figure 2-5).

3.4.1 ATP results

ATP results for flow cell parts are presented in Figure 3-26 and Table 3-9. The results show that PEHD and PU samples contained more ATP than other samples and, therefore, larger and more active cells as well. Swabs from metallic surfaces contained on the contrary less ATP than the organic components. Results for PVC subsamples 1 and 2 varies due to the sampling when swabbed surfaces of the sample were quite different.

Table 3-9. Results of ATP analysis for flow cell components.

Sample	ATP (amol mL^{-1})	
	Subsample 1	Subsample 2
PA	53900	46400
PVC	85400	267000
PEHD	366000	307000
PU Slitan 90A-05	392000	354000
PU Slitan 80A-71	142000	115000
Al	30700	34800
Fe	37100	47400

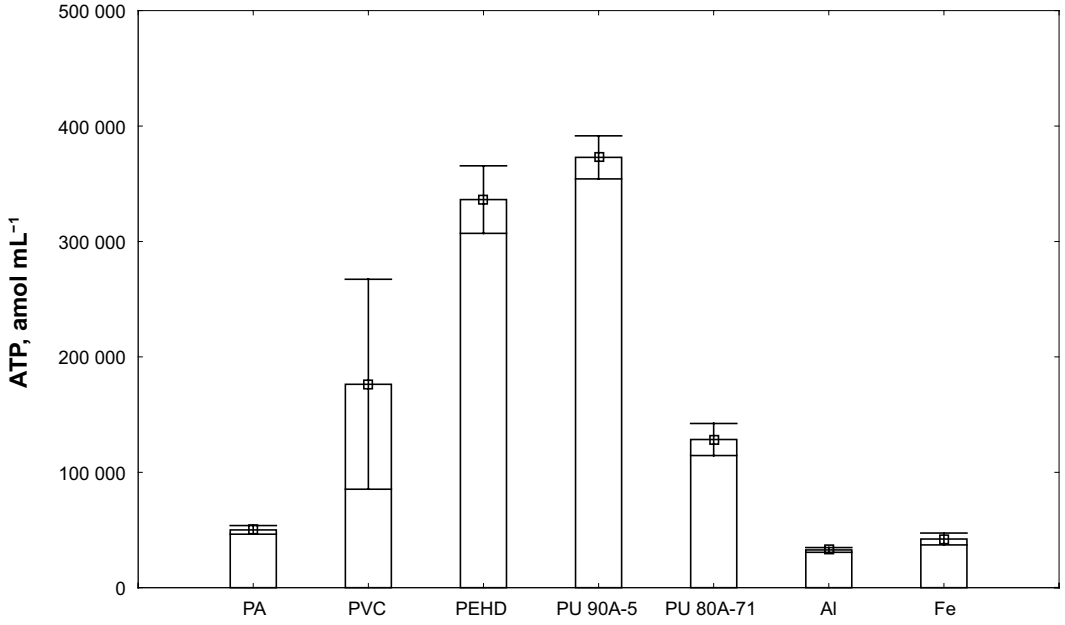

Figure 3-26. *Results for ATP analysis of flow cell components. Bars indicate standard deviation for 3 analyses.*

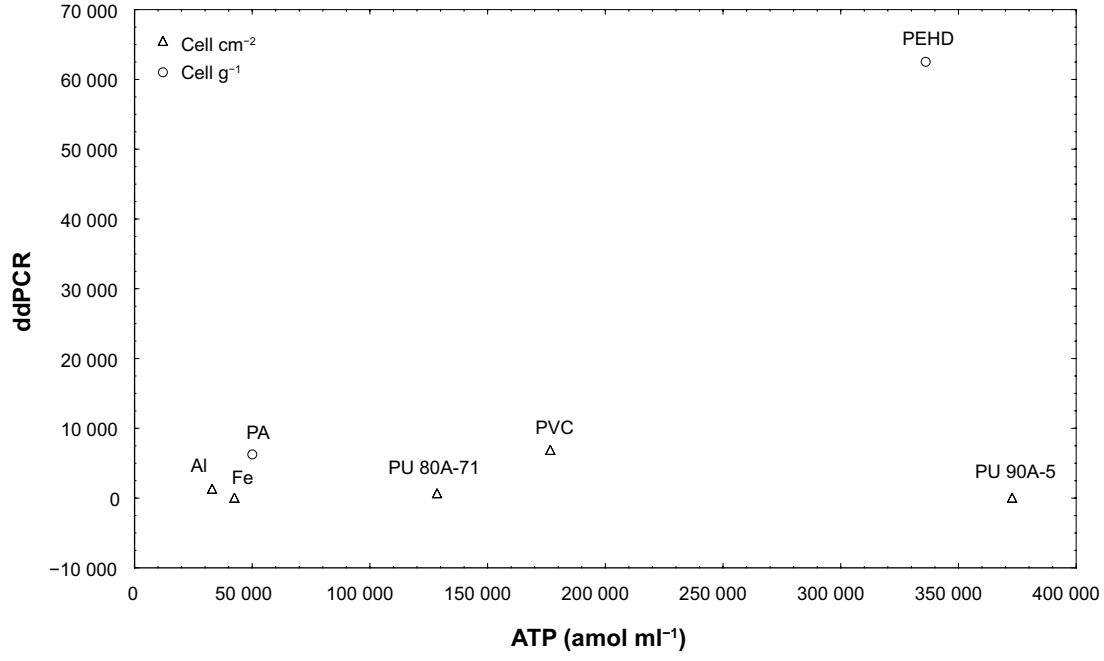

Figure 3-27. *ATP against ddPCR for SRB results for flow cell components.*

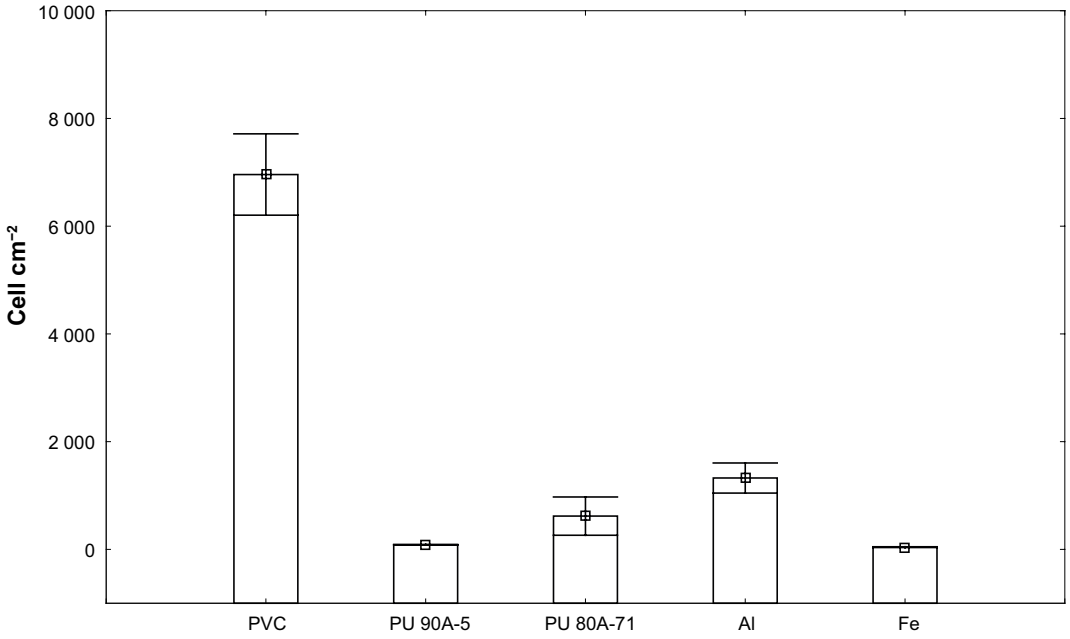

Figure 3-28. *Results of ddPCR for SRB analyses for PVC, PU Slitan 90A-05, PU Slitan 80A-71, Al and Fe samples. Bars indicate standard devia tion for 3 analyses.*

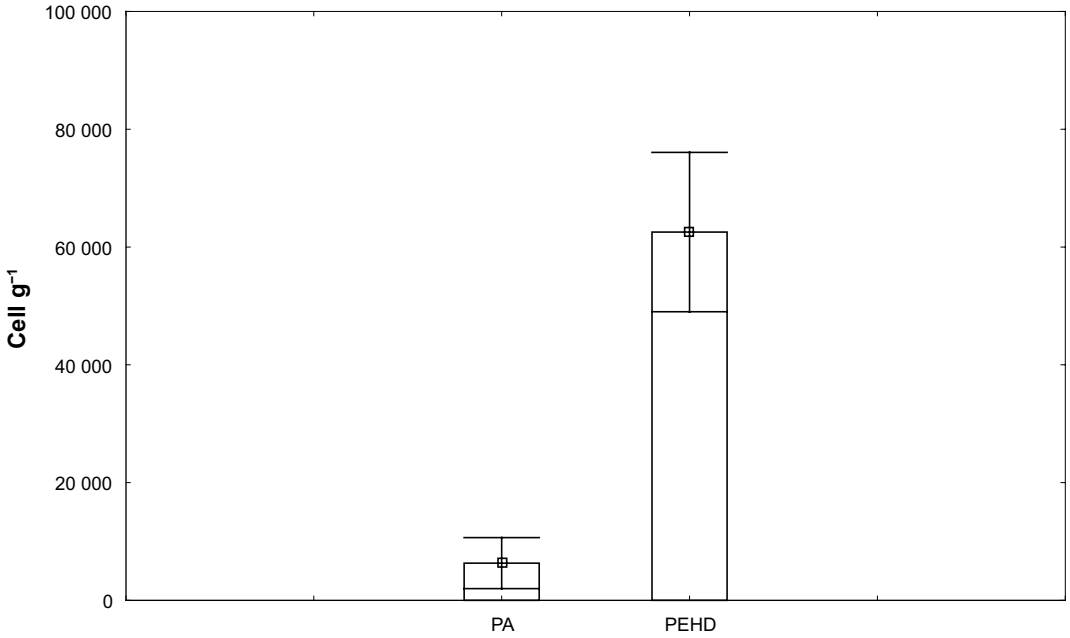

Figure 3-29. *Results of ddPCR for SRB analyses for PA and PEHD samples. Bars indicate standard deviation for 3 analyses.*

4 Discussion

The general purpose of this study was to investigate if material used to construct stationary borehole equipment can provide enough electron donors to explain the observed sulphide production by SRB. The project plan outlined a laboratory study in two parts, where the first part investigated release of H_2 and organic compounds from borehole equipment material under sterile conditions (Chukharkina et al. 2016). The second part, reported here, involved the presence of the bacteria. Here, the release H_2 from metallic parts of stationary borehole equipment immersed in both sterile filtered and natural groundwater was analysed. The release of organic compounds from polymeric materials of stationary borehole equipment to both kinds of groundwater was also investigated.

4.1 H_2 release from metallic materials

4.1.1 H_2 release under sterile conditions

In the first experimental series of the work (Chukharkina et al. 2016) the experiments were set up under sterile conditions to exclude the activity of bacteria that could possibly influence the results by consumption of H_2. These experiments were carried out at two temperatures, 70 °C and 30 °C, where incubation at 70 °C was performed in order to accelerate possible H_2 release, and incubation at 30 °C to keep reactions closer to a rate relevant for the natural conditions in the borehole.

Two possible mechanisms than can explain the observed H_2 are described in the previous work. The tests of H_2 release from steel showed an initial evolution of H_2 in vessels incubated at 30 °C and 70 °C that levelled out at 4 and 6 mbar, respectively, after approximately 2 months. Release and rates at 70 °C were generally higher than at 30 °C. In the report by Chukharkina et al. (2016) it was modelled how the observed H_2 release could induce sulphide production in the borehole in regard to all parameters in the borehole.

One of the 70 °C vessels with an aluminium rod had a very large build-up of pressure mostly explained by an increase in the partial pressure of H_2, which likely originated from a local corrosion attack of the rod. The experiment showed that aluminium in contact with warm saline groundwater produced much larger amounts of H_2 compared to what was observed for stainless steel at the same temperature. The decrease in H_2 release at 30 °C compared to 70 °C was very large. At the lower temperature the partial pressures of H_2 were in the same magnitude as those observed with steel rods. However, the release was continuous over the experimental time for tubes not infested by SRB. That would indicate a corrosion process to dominate the H_2 release. Observations from the field attest that aluminium rods and tubes may corrode severely in saline groundwater (Rosdahl et al. 2011). Some of the vessels with aluminium rods showed a loss of H_2 during the experimental time, both at high and low temperature. In the case of sample A4 at 30 °C with an almost total loss of H_2 a simple test of microbiological sulphate-reducing activity revealed that the sample was indeed not sterile. There was even an odour of hydrogen sulphide which can only be explained by activity of SRB.

This results revealed microbial growth in several of the samples, which showed that it was difficult to obtain sterile conditions due to the complicated nature of the borehole materials provided for the experiment. The fact that the groundwater was sterile-filtered and the materials disinfected with ethanol reduced microbial activity of bacteria at least during the earlier stages of the tests.

The analysis results of dissolved metals were inconclusive. They were not explained by the content of these metals in the used groundwater and a correlation between amounts of H_2 and metals was not found (Chukharkina et al. 2016).

4.1.2 H_2 release under natural conditions

The situation in the vessels changed when natural non-filtered groundwater was used for the second experimental part of the project. It has been shown that in the presence of bacteria there is risk for microbially induced corrosion (MIC) where SRB produce sulphide with H_2 as electron donor (Cord-Ruwisch and Widdel 1986, Enning and Garrelfs 2014). Production of the sulphide in such cases can be described by the following reaction:

$$4H_2 + SO_4^{2-} + 2H^+ \rightarrow H_2S + 4H_2O \quad (1)$$

If this situation is present, H_2 released early in the experiments would be consumed by bacteria and its concentration would be levelled out, which was observed in the samples incubated at natural conditions. The results of the second experiment showed that under natural conditions H_2 was produced initially with the same rates as under sterile conditions, but after approximately seven days it disappeared from the gas phase and observed concentrations were near background values, as shown in the Figure 3-1 and Figure 3-2.

In the previous report by Chukharkina et al. (2016) a possible mechanism describing the decrease of H_2 in the samples was discussed. The butyl rubber stoppers are not totally impermeable to H_2 diffusion and with increasing partial pressure of H_2 the mass transfer of H_2 via the stopper by diffusion will increase until a steady state between release of H_2 and out-diffusion is reached. However, such an explanation is unlikely at the present experiment because the partial pressure of H_2 was quite low when the decrease of the concentrations was observed. The other possible explanation is the microbially induced corrosion. Groundwater used in the experiment contained SRB and in the presence of H_2 the SRB should produce hydrogen sulphide according to the equation 1. However, the sulphide analysis of water samples from analysed tubes did not give a conclusive result (Table A1-13). For the samples containing steel rods concentration of the sulphide in water was below detection. At the same time, some black precipitate formed on the glass, at the interface between water and gas phase in all tubes with steel rods (see Figure 3-5).

It is known that in nature, under low-oxygen conditions, when sulphate-reducing bacteria uses the sulphates present in the water to oxidize the organic matter, some of the produced hydrogen sulphide react with metal ions in the water to produce metal sulphides, which are not water-soluble. These metal sulphides, such as iron (II) sulphide, are often black or brown, contributing to the colour of sludge formed under such conditions.

The ferrous sulphide in such cases can be formed according to one of the equations below:

$$Fe^{2+} + H_2S \rightarrow FeS + 2H^+ \quad (2)$$

$$Fe + H_2S \rightarrow FeS + H_2 \quad (3)$$

and FeS presence is usually indicated by a visible black precipitate.

If ferrous ions react with hydrogen sulphide as it is shown in the equation (2), ferrous sulphide will be produced and protons will be released as the reaction product, causing decrease of the pH in the system. In the environment at low pH, ferrous sulphide will be dissolved giving hydrogen sulphide as a product of the dissolution, which would increase the hydrogen sulphide concentration in the system and shift the chemical equilibrium to the left in equation 2. In such case more reaction products would be produced, and, taking into account low pH conditions, more hydrogen sulphide would be released. The whole system in such cases would be in equilibrium as long as the conditions are unchanged.

If the reaction (3) take place, free iron present in the water will react with hydrogen sulphide, yielding ferrous sulphide and H_2 as reaction products. In the system containing bacteria H_2 will be rapidly consumed by the bacteria releasing hydrogen sulphide (in the case of SRB). Hydrogen sulphide will once again react with remaining atoms of free ferrous iron, according to the reaction (3) above. Thus, we get a kind of continuous process in which both free iron and bacteria are involved, until one of the compounds is fully consumed and cannot further provide a reagent for the reaction. This explanation of the processes occurring in the system seems more likely, considering all the circumstances and taking into account results from the experiment under natural conditions. Rapid consumption of the H_2, black precipitate, release of the iron into the water and presence of the bacteria in the same system indicates that there may have been microbially induced corrosion on-going under the natural condition. However, if this assumption is correct, it still needs further investigation including a prototype of the system, more parallel samples, detailed gas and water analyses as well as microbiological analyses for the bacterial content to prove the conclusion. The processes taking place in the system apparently involves small concentrations and occur in the interface of the different phase states.

Considering the scheme discussed above, aluminium in the water would react with hydrogen sulphide according to a similar scenario, yielding aluminium sulphide as a product of the reaction. Aluminium sulphide, however, is not so easy to detect. It is a colourless species which is sensitive to moisture and will hydrolyse to hydrated aluminium oxides or hydroxides in water.

$$2Al + 3H_2S \rightarrow Al_2S_3 + 6H_2 \quad (4)$$

$$Al_2S_3 + 6H_2O \rightarrow 2Al(OH)_3 + 3H_2S \quad (5a)$$

$$Al_2S_3 + 3H_2O \rightarrow Al_2O_3 + 3H_2S \quad (5b)$$

In both cases (5a) and (5b) the production of hydrogen sulphide will increase its concentration in the system which will eventually keep the system at equilibrium as it is described earlier, as expressed in the equation (3). At the same time, H_2 obtained in equation (4), would possibly be consumed by bacteria producing more hydrogen sulphide. Henceforth it becomes difficult for us to assume what can possibly happen in the system, more experiments are needed to give a definite answer about the exact mechanisms. However, in this case two types of corrosion can occur: as a result of the reaction proceeding in the system, there is a risk for microbially induced corrosion. Besides, being immersed into the saline water, aluminium can undergo chemical corrosion as well.

The questions about how these two metals behave in the same system, how they influence each other, which of them is more preferable for the bacteria and which of them contributes mostly to the possible corrosion are open questions as well.

4.2 Release of organic compounds from polymeric materials

4.2.1 Release of organic compounds under sterile conditions

The organic compounds were analysed for leachability since compounds leaching into groundwater from borehole equipment may act as a source of electron donors and carbon for bacteria. Leaching in pure solvent was performed on finely divided polymeric materials using hexane to give a worst case scenario. The results from the component analysis of extracts in hexane were used to identify possible compounds leachable to groundwater. In the following analysis of leaching to groundwater exactly the same analytical conditions were used on the GC-MS system as used for the hexane extracts. Because of this retention times remained constant and direct comparison with the spectra from solvent extraction could be made when evaluating the results from leaching into water. The analysis was performed without calibration and because of this the results are qualitative and not quantitative.

All compounds detected in the sterile filtered groundwater could be traced back to the polymer hexane extraction results, indicating that groundwater in contact with these materials may contain significantly higher levels of organic compounds than the groundwater itself. In some case it was not possible to identify the compound detected in the component analysis. When the matching quality for the compounds in the NIST library was not good enough for a certain assessment it was in some cases still possible to identify the compound family (e.g phthalates). This assessment was based on manual evaluation of characteristic fragments in the ionization pattern. There was also a group of compounds that could not be identified at all. For polymeric materials that situation is not uncommon since the only compounds normally identifiable via library search are free pure compounds. Reactive monomers and shorter oligomers are not available as pure compound standards and therefore they do not occur in the search libraries. Still they can make up a large fraction of the leachable compounds from polymers, because reaction monomers and oligomers usually contain polar groups that facilitate transfer to the water phase. Especially PU materials are known to contain these unidentifiable compounds. Another source of uncertainty when dealing with oligomers is that they might decompose thermally in the hot vaporizing inlet of the GC yielding reformulated compounds. This decomposition makes it impossible to estimate the original length of the oligomers.

As in the case with the metal rods the results should be interpreted keeping in mind that there probably was an increasing microbiological activity over time in the vessels, starting at a low level because of the disinfection actions taken during sample setup. Theoretically this would imply that

an increasing level of an organic substance in the water over time indicates leachability but also that the substance is not used by present bacteria. On the other hand, if the substance shows an increasing concentration during the early stages of leaching followed by decreasing concentrations later in the test, this could indicate leachability and also the accelerated degradation of the substance caused by the higher microbiological activity at the later sampling time. Analysis shows the steady-state concentrations of individual compounds but does not give information about rates of conversion. The latter being more important as assessment for microbiological activity.

4.2.2 Release of organic compounds under natural conditions

All the compounds found in the leachates under sterile conditions were also found under natural, non-sterile conditions. In the experiment under natural conditions a new release of the mass-spectrum library (NIST 14) was used that made it possible to recognise fragments of some compounds unidentified in the previous investigation. Generally, the steady-state concentrations of the compounds detected under natural conditions, decreased over time. That is different from the results obtained under sterile conditions, when almost all concentrations increased up to extraction occasion number two. At the same time, sulphide concentrations in the water samples increased generally until day 186, and during the third extraction sulphide smell was noticed in four samples out of five. The most interesting results were observed in the leachates from PVC samples and from PU samples marked as Slitan 90A-05.

Polyvinylchloride samples released toluene in the water as was found in the previous experiment under sterile conditions. Toluene concentration remained at approximately the same level in the sterile filtered groundwater during 182 days. In the natural groundwater toluene concentration was also on the same level by day 31 and 94. However, it increased almost 3.5 times by day 186. A different situation was observed in the case of DEHP. During the sterile experiment its concentration decreased with time and was below detection by the third extraction at day 182, when bacteria were detected. Under natural conditions, having high level of the bacteria in the samples initially, there was no DEHP found in the leachates until day 186. Numerous studies have demonstrated that bacteria play the major role in phthalates degradation in the environment under various conditions. It was shown that in river water under aerobic conditions DEHP with concentration about 20 mg/L degrades by 20 % in 5 days (Hashizume et al. 2002). Under anaerobic conditions in river sediment during 28 days remaining DEHP was 8.3 % of the initial concentration (Chang et al. 2005). It was also shown that under anaerobic conditions phthalates degrade to benzoic acid as an intermediate product (Liang et al. 2008). In the water sample under natural conditions benzoic acid was detected, and its steady-state concentration decreased from day 31 until day 186, while the concentration of DEHP increased. Taking into account the information above, the possible explanation can be that DEHP, existing initially in the polymeric material, degrades under natural conditions to benzoic acid from the beginning of the experiment. However, rates of the degradation become lower by day 186 and degradation possibly stops, while leaching of DEHP in the water continues, which results in a higher DEHP concentration found in late samplings. It also explains the lower concentration of benzoic acid found at day 186. The decline of degradation in the microbiologically active PVC sample at the final day, indicated by a higher presence of DEHP, could also possibly indicate that the sample reached concentrations of DEHP exceeding the toxic level for these bacteria. If that was the case growth could have continued if water was exchanged or the total volume was larger. Toluene is also known to degrade to benzoic acid and can potentially undergo the same process as DEHP, differing by the initial rate. Degradation of toluene, if it took place, was also lower by day 186, then under sterile conditions, resulting in higher toluene concentration and lower amounts of benzoic acid. At this stage it is difficult to say which of the components degrades more and contributed most to the formation of benzoic acid and as fuel for the microbial processes.

At the same time, concentration of sulphide in the water increased noticeably from day 31 to day 186 under natural conditions. Also an elemental form of sulphur, a ring structure detectable by GC-MS, appears in the samples and its concentration in the water increased dramatically by day 186. Sulphate reducing bacteria (SRB) reduces sulphate to sulphide using organic molecules as electron source. This process is described by following equation:

$$2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2CO_2 + 2H_2O + S^{2-} \quad (6)$$

Some bacteria are known to produce elemental sulphur under anaerobic conditions using sulphide, and this process is used for the sulphide utilization:

$$NO_3^- + 2.5HS^- + 3.5H^+ \rightarrow \frac{1}{2}N_2 + 2.5S + 3H_2O \quad (7)$$

Suggesting that SRB metabolise in the system according to equation (6), hydrogen sulphide would be produced and its concentration would increase in time, which is shown in the present experiment (Figure 3-22). At the same time, if the produced sulphide is used by other bacteria according to equation (7), it would result in production of elemental sulphur which is also observed in the samples (Figure 3-13). In that case SRB should start the process, and the other bacteria should accelerate their input in the process after a sufficient amount of hydrogen sulphide was produced. Assuming that these two processes proceed parallel, we would get both hydrogen sulphide and sulphur in concentrations increasing over time.

It is however unclear which organic compound in the PVC the SRB consumed and used as an electron donor. It is also unclear which bacteria in this system utilized hydrogen sulphide in producing elemental sulphur. Detailed mechanisms and rates of these two processes in the present system are vague as well and need more detailed investigation of the chemistry and microbiology of the system PVC – borehole groundwater.

The similar production of hydrogen sulphide and elemental sulphur was observed in the leachates from the polyurethane sample marked as Slitan 90A-05. In this case sulphur was detected at the first analysis and its concentration increased by day 186. The same tendency was observed for hydrogen sulphide as well. However, the changes in steady-state concentrations of isomers of compound 1, containing a 3-methyl-1-penten-3-ol fragment, are rather unclear to assert that its degradation occurs resulting in sulphide production.

It is clear that bacteria under the natural conditions in the investigated leachates from polymeric materials of the borehole equipment launch processes causing sulphide and sulphur production. It is however difficult to conclude if the consumption of DEHP and toluene and formation of hydrogen sulphide and sulphur occurred during the same process, or if these processes are simultaneous, or even competitive. It is possible that some bacteria start one process, but become ousted by another type at the latest stages, and that another microbiological process then dominates the system. In some sample tubes an increased turbidity was noticed and the nature of this aggregated phase was not further investigated. Without thorough investigations of the microbial environment in the system and its influence on the chemical composition it is difficult to point out the detailed mechanism of the hydrogen sulphide and sulphur formation in the system.

4.3 Flow cell

The results of ATP analysis showed that the largest and most active cells were present on PEHD and PU slitan 90A-05 samples in the flow cell. The PVC sample also had a high ATP value, though the scattering between the first and second PVC subsamples was big (Figure 3-26). ddPCR analysis for SRB revealed that all the investigated materials contributed to the growth of SRB in natural groundwater, to different extents. High cell numbers were found in the extracts from PVC and PEHD samples (Figure 3-28, Figure 3-29). Both PVC and PU Slitan 90A-05 had large release of hydrogen sulphide and octasulphur in the experiment under natural conditions, while PEHD sample was not releasing significant amounts of organic substances and showed low numbers of bacteria compared with the other materials on an individual basis. Results indicate that PEHD contributes to the total microbiological system as a surface for biofilms to be formed on, it is however difficult to estimate its contribution due to the tight contact with PVC tape during the experiment. The PEHD sample for the flow cell collection was designed as a kind of sushi-roll where flakes of the PEHD were pressed to the adhesive side of the tape. It is therefore difficult to estimate to which extent PEHD and PVC tape itself contributed to the bacteria collection. Unfortunately, it is impossible to compare results from ddPCR analysis for PVC and PEHD samples due to the differences in scales (volume and surface), and the question if PEHD only contributes to its own results, or it is mutual results for both PEHD and PVC, remains open. Considering that PEHD samples showed no significant influence regarding the formation of hydrogen sulphide, it is realistic to suggest that the adhesive side of the PVC tape was the very source of energy for the bacteria.

On the other hand, this result could also be an additional indication that the substances released from the different materials do travel over a distance before being consumed as substrate by bacteria. In the analysis of water in the laboratory tests only substances free in the water phase were analysed, something that also indicates such mobility. So, in the case of PEHD sample in the flow cell, bacteria could consume organic compounds released by PVC tape, but preferred to settle on the flakes of PEHD due to better surface properties for attachment and growth.

Metallic components placed into the flow cell did show low numbers of living cells. Numbers of SRB cells per surface in both cases were comparable with number of cells for PU samples, but much lower than for the PVC tape. Besides, after being immersed in water during three month, both aluminium and steel rods had visible signs of corrosion and the aluminium rods were covered with hydroxide-oxide film. It can be suggested that SRB might be involved in the process on the metal surfaces which leads to local corrosion of the metal. It is therefore clear that metals contribute to the SRB formation in the whole system, and this contribution gives not more than 30 % of all SRB cells, according to the ddPCR results.

Assuming that metallic components contribute to the SRB formation less than polymeric materials ones, it is however difficult to estimate more precisely which component is more attractive for the bacteria. It is clear that PEHD, PVC and PU Slitan 90A-05 provide more active bacteria, where SRB is more characteristic for PVC and, possibly, PEHD samples. However, the experiment was too restricted to make a certain assessment to what component that was most preferred for the SRB.

5 Conclusions

The results showed that there is a clear difference in the measured amount of H_2 under sterile and natural conditions. In the latter case released H_2 was consumed rapidly and ferrous sulphide formed in the case of the steel rods.

Metal by itself does act as an energy source for the SRB, probably because of the H_2 evolved from the sample rods. It is unclear if this H_2 originates from H_2 dissolved in the metal or as a result of a corrosion process. In first case the metal would be exhausted of H_2 after a finite released amount, stopping the SRB activity. If H_2 on the other hand originates from ongoing corrosion reactions the total amount of H_2 produced over time would be much higher. H_2 as dissolved gas moves freely in the water and the flow cell experiment shows that the metal surfaces themselves were not preferred for biofilm growth when organic substrates are present.

It is clear that in the first experimental series, intended to be sterile, microbiological activity did develop anyway. It is very difficult to keep the material samples completely sterile without destroying them by heat sterilisation and the incubation time was extended. Still it is assumed that the initial activity of bacteria was low compared with the situation in natural water. The early increase in concentration of leachable substances from the polymeric material indicates that the release itself does not depend on microbiological activity. Over time dissolved substances would accumulate in the water also without the presence of bacteria. In no case did substances detected in the hexane leaching become present in the water only after microbiological activity. When new substances were detected in the late stages of incubation they could be identified as degradation products originating from previously released substances. As a consequence, organic substances can be leached from a polymeric material into water over a long time under sterile conditions yielding an intense burst of microbiological activity if the sterile conditions are interrupted by presence of SRB.

PVC and PU Slitan 90A-05 materials immersed in the natural water produce hydrogen sulphide and elemental sulphur, and concentrations of these compounds increase with time. These materials also showed a high ATP level in the settled biofilms. Amounts of the released sulphide and sulphur, as well as consumption of two important components and results from the flow cell make the PVC sample most possible the preferred component for the activity of bacteria.

The content of possible substrate in the polymeric material is a finite amount. On the other hand the mobility of for instance plasticisers as DEHP in PVC in the polymeric materials is significant and the total amount than can be leached could be high. It is for instance not uncommon to find a plasticiser content of 10–20 % by weight in soft and flexible PVC. As a result, the total amount of released substance could be high and the corresponding bacterial growth could be large.

References

SKB's (Svensk Kärnbränslehantering AB) publications can be found at www.skb.com/publications.

Ben-Dov E, Brenner A, Kushmaro A, 2007. Quantification of sulfate-reducing bacteria in industrial wastewater, by real-time polymerase chain reaction (PCR) using dsrA and apsA genes. Microbial Ecology 54, 439–451.

Bengtsson A, Chukharkina A, Eriksson L, Hallbeck B, Hallbeck L, Johansson J, Johansson L, Pedersen K, 2013. Development of a method for the study of H_2 gas emission in sealed compartments containing canister copper immersed in O_2-free water. SKB TR-13-13, Svensk Kärnbränslehantering AB.

Chang B V, Liao C S, Yuan S Y, 2005. Anaerobic degradation of diethyl phthalate, di-*n*-butyl phthalate, and di-(2-ethylhexyl) phthalate from river sediment in Taiwan. Chemosphere 58, 1601–1607.

Chukharkina A, Blom A, Pedersen K, 2016. Release of H_2 and organic compounds from metallic and polymeric materials used to construct stationary borehole equipment. SKB R-16-01, Svensk Kärnbränslehantering AB.

Cord-Ruwisch R, Widdel F, 1986. Corroding iron as a hydrogen source for sulphate reduction in growing cultures of sulphate-reducing bacteria. Applied Microbiology and Biotechnology 25, 169–174.

Cross M M, Manning D A C, Bottrell S H, Worden R H, 2004. Thermochemical sulphate reduction (TSR): experimental determination of reaction kinetics and implications of the observed reaction rates for petroleum reservoirs. Organic Geochemistry 35, 393–404.

Drake H, Hallbeck L, Pedersen K, Rosdahl A, Tullborg E-L, Wallin B, Sandberg B, Blomfeldt T, 2014. Investigation of sulphide production in core-drilled boreholes in Äspö Hard Rock Laboratory. Boreholes KA3110A, KA3385A and KA3105A. SKB TR-13-12, Svensk Kärnbränslehantering AB.

Enning D, Garrelfs J, 2014. Corrosion of iron by sulfate-reducing bacteria: new views of an old problem. Applied and Environmental Microbiology 80, 1226–1236.

Eriksson L, Edlund J, Johansson L, Rabe L, Bengtsson A, Pedersen K, 2016. Methods for sampling and analysis of attached and planktonic microorganisms in deep granitic rock aquifers. SKB R-16-09, Svensk Kärnbränslehantering AB.

Eydal H S C, Pedersen K, 2007. Use of an ATP assay to determine viable microbial biomass in Fennoscandian Shield groundwater from depths of 3–1000 m. Journal of Microbiological Methods 70, 363–373.

Greenberg A E, Clesceri L S, Eaton A D, 1992. Estimation of bacterial density. In Standard methods for the examination of water and wastewater. 18th ed. Washington, DC: American Public Health Association, 9-45–9-52.

Hallbeck L, Pedersen K, 2008. Characterization of microbial processes in deep aquifers of the Fennoscandian Shield. Applied Geochemistry 23, 1796–1819.

Hashizume K, Nanya J, Toda C, Yasui T, Nagano H, Kojima N, 2002. Phthalate esters detected in various water samples and biodegradation of the phthalates by microbes isolated from river water. Biological and Pharmaceutical Bulletin 25, 209–214.

Haveman S A, Pedersen K, 2002. Distribution of culturable microorganisms in Fennoscandian Shield groundwater. FEMS Microbiology Ecology 39, 129–137.

Hobbie J E, Daley R J, Jasper S, 1977. Use of nucleopore filters for counting bacteria by fluorescence microscopy. Applied and Environmental Microbiology 33, 1225–1228.

Johansson J, Blom A, Chukharkina A, Pedersen K, 2015. Study of H_2 gas emission in sealed compartments containing copper immersed in O_2-free water. SKB TR-15-03, Svensk Kärnbränslehantering AB.

Liang D-W, Zhang T, Fang H H, He J, 2008. Phthalates biodegradation in the environment. Applied Microbiology and Biotechnology 80, 183–198.

Pedersen K, Arlinger J, Eriksson S, Hallbeck A, Hallbeck L, Johansson J, 2008. Numbers, biomass and cultivable diversity of microbial populations relate to depth and borehole-specific conditions in groundwater from depths of 4–450 m in Olkiluoto, Finland. The ISME Journal 2, 760–775.

Pedersen K, Ekendahl S, 1990. Distribution and activity of bacteria in deep granitic groundwaters of southeastern Sweden. Microbial Ecology 20, 37–52.

Pedersen K, Kalmus A, 2004. Forsmark site investigation. Total numbers and metabolic diversity of microorganisms in borehole KFM03A Results from three investigated borehole sections, 639.0–646.1 m, 939.5–946.6 m and 980.0–1001.2 m. SKB P-04-89, Svensk Kärnbränslehantering AB.

Rosdahl A, Pedersen K, Hallbeck L, Wallin B, 2011. Investigation of sulphide in core drilled boreholes KLX06, KAS03 and KAS09 at Laxemar and Äspö. Chemical-, microbiological- and dissolved gas data from groundwater in four borehole sections. SKB P-10-18, Svensk Kärnbränslehantering AB.

SKB, 2011. Long-term safety for the final repository for spent nuclear fuel at Forsmark Main report of the SR-Site project. SKB TR-11-01, Svensk Kärnbränslehantering AB.

Widdel F, Bak F, 1992. Gram-negative, mesophilic sulphate-reducing bacteria. In Balows A, Trüper H G, Dworkin M, Harder W, Schleifer K-H (eds). The prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, applications. New York: Springer, 3352–3378.

Appendix 1

Table A1-1. Characteristics of the ground water from the borehole KFM03A:4, section 633.5–650 m, −631 masl in October 2014 and 2015 respectively.

Analysis	Unit	Results	
		October 2014	October 2015
pH		7.76	7.71
Conductivity (25 °C)	mSm^{-1}	1593	1589
Flushing water	%	2.9	1.8
Sodium fluorescein	$\mu g\ L^{-1}$	5.7	3.6
Bromide (Br^-), IC	$mg\ L^{-1}$	33.3	34.7
Chloride (Cl^-), titration	$mg\ L^{-1}$	5673	5716
Fluoride (F^-), potentiometry	$mg\ L^{-1}$	1.47	1.55
Sulfide (HS^-)	$mg\ L^{-1}$	0.515	0.268
Sulphate (SO_4^{2-}), IC	$mg\ L^{-1}$	187.1	191.5
Carbonate (HCO_3^-), alkalinity	$mg\ L^{-1}$	21.1	19.6
Nitrogen (NH_4^+)	$mg\ L^{-1}$	0.229	0.197
Nitrogen (NO_2^-)	$mg\ L^{-1}$	0.0011	< 0.0002
Nitrogen ($NO_3^- + NO_2^-$)	$mg\ L^{-1}$	0.0011	< 0.0003
Nitrogen (NO_3^-)	$mg\ L^{-1}$	< 0.0003	< 0.0003
Phosphorus (PO_4^{3-})	$mg\ L^{-1}$	0.0008	< 0.0005
Total organic carbon	$mg\ L^{-1}$	0.9	1.0
Dissolved organic carbon	$mg\ L^{-1}$	1.0	1.0
Iron (Fe^{2+})	$mg\ L^{-1}$	0.519	0.589
Aluminium (Al^{3+})	$\mu g\ L^{-1}$	14.9	1.33

Table A1-2. Results of the ICP-MS analysis of water sample with metallic rods, leaching at 70 °C, sterile conditions (presented in the previous report, Chukharkina et al. 2016).

Material	Sample	Days	Fe, mg/L	accuracy ±	Al, mg/L	accuracy ±
Steel	1	17	2.49	0.17	0.397	0.071
	2	35	4.13	0.28	0.573	0.146
	3	67	3.33	0.23	0.505	0.147
	4	99	3.60	0.25	0.563	0.129
	5	104	3.19	0.22	0.481	0.205
Aluminium	1	17	0.349	0.025	0.635	0.086
	2	67	0.424	0.029	0.680	0.094
	3	35	0.324	0.025	2.74	0.335
	4	99	0.460	0.033	1.60	0.241
	5	104	0.329	0.03	0.998	0.142
K (control ground water)	1	17	< 0.1	–	< 300	–
	2	67	< 0.1	–	< 300	–
	3	104	< 0.1	–	< 300	–
AGW	1	17	< 0.02	–	< 60	–
	2	99	< 0.02	–	< 60	–
	3	104	< 0.02	–	< 60	–

Table A1-3. Results of the ICP-MS analysis of water sample with metallic rods, leaching at 30 °C, sterile conditions (presented in the previous report, Chukharkina et al. 2016).

Material	Sample	Days	Fe, mg/L	accuracy ±	Al, mg/L	accuracy ±
Steel	1	22	2.98	0.20	0.145	0.085
	2	43	3.61	0.25	<60	–
	3	71	3.70	0.25	0.178	0.056
	4	113	5.26	0.37	0.198	0.117
	5	140	5.18	0.36	0.351	0.186
Aluminium	1	22	1.15	0.08	0.738	0.128
	2	43	2.48	0.17	0.188	0.028
	3	71	2.91	0.20	0.250	0.036
	4	113	2.16	0.15	0.328	0.088
	5	140	1.58	0.11	1.53	0.184
K (control ground water)	1	22	<0.04	–	<60	–
	2	71	<0.04	–	<60	–
	3	140	<0.04	–	<60	–
AGW	1	22	<0.02	–	<60	–

The accuracy stated was according to the original reports calculated as a 95 % confidence interval not taking into account any gross errors as mistakes in dilutions and water sample treatment. The estimation was based on guidelines from Eurachem on the topic uncertainty of measurement.

Table A1-4. Partial pressure of analysed H_2 in vial (mbar), experiment at 70 °C, sterile conditions.

Day	P_5 (mbar)				
	S1	**S2**	**S3**	**S4**	**S5**
0	0.0	0.0	0.0	0.0	0.0
17	3.6	3.0	3.5	1.6	3.3
35		4.5	4.7	2.5	4.8
67			6.4	3.8	7.0
99				3.9	4.9
154					5.5
	A1	**A2**	**A3**	**A4**	**A5**
0	0.1	0.0	0.0	0.0	0.0
17	36.0	24.5	6.6	57.0	13.2
35	32.5		8.3	994.4	23.0
67			52.7	395.9	186.8
99				307.3	32.7
154					18.0
	K1	**K2**	**K3**	**K4**	**K5**
0	0.0	0.0	0.0	0.0	0.0
17	0.0	0.0	0.0	0.0	0.0
35		0.1	0.1	0.0	0.1
67		0.0	0.0	0.1	0.0
99			0.1	0.1	0.1
154			0.1	0.1	0.1

Table A1-5. Partial pressure of analysed H_2 in vial (mbar), experiment at 30 °C, sterile conditions.

Day	P_5 (mbar)				
	S1	**S2**	**S3**	**S4**	**S5**
0	0.02	0.02	0.01	0.02	0.02
8	0.53	0.44	0.57	1.22	0.72
22	1.12	1.44	2.20	3.79	1.67
43		2.39	2.77	3.84	1.93
71			3.72	4.41	2.81
113				4.68	3.52
140					4.31
	A1	**A2**	**A3**	**A4**	**A5**
0	0.02	0.02	0.02	0.00	0.02
8	1.52	1.92	1.49	1.26	1.26
22	2.23	3.57	3.18	1.35	3.09
43		3.59	3.72	0.85	3.64
71			5.13	2.19	5.81
113				0.03	7.33
140					9.04
	K1	**K2**	**K3**	**K4**	**K5**
0	0.00	0.02	0.00	0.00	0.00
8	0.00	0.02	0.02	0.02	0.02
22	0.02	0.02	0.04	0.02	0.04
43		0.02	0.02	0.03	0.02
71		0.02	0.04	0.04	0.04
113			0.03	0.03	0.02
140			0.04	0.05	0.04

Table A1-6. Partial pressure of analysed H_2 in vial (mbar), experiment at 30 °C, natural conditions.

Day	P_5 (mbar)				
	S1	**S2**	**S3**	**S4**	**S5**
0	0.02	0.00	0.02	0.02	0.02
7	0.35	0.39	0.39	0.63	0.67
21	1.01	0.19	0.00	0.00	0.02
42	0.01	0.04	0.02	0.04	0.04
73	0.02	0.01	0.03	0.00	0.01
108	0.02	0.03	0.03	0.00	0.01
	A1	**A2**	**A3**	**A4**	**A5**
0	0.00	0.00	0.02	0.02	0.02
7	1.94	1.70	2.18	3.18	1.17
21	2.26	2.06	0.00	0.01	0.00
42	2.01	0.19	0.02	0.03	0.00
73	0.01	0.01	0.01	0.00	0.01
108	0.00	0.04	0.01	0.00	0.01
	K1	**K2**	**K3**	**K4**	**K5**
0	0.00	0.00	0.00	0.00	0.00
7	0.05	0.07	0.11	0.04	0.51
21	0.02	0.00	0.00	0.02	0.02
42	0.02	0.04	0.02	0.04	0.02
73	0.00	0.00	0.01	0.01	0.01
108	0.01	0.00	0.02	0.02	0.02

Table A1-7. Amount of H_2 in vial (nmol), experiment at 70 °C, sterile conditions.

Day	n_1				
	S1	**S2**	**S3**	**S4**	**S5**
0	0	0	0	0	5
17	720	613	707	314	650
35		903	956	502	955
67			1290	744	1390
99				765	966
154					1090
	A1	**A2**	**A3**	**A4**	**A5**
0	13	0	0	0	0
17	7300	3940	1240	8900	2770
35	6600		1560	155000	4900
67			9900	61600	39200
99				478200	6900
154					3800
	K1	**K2**	**K3**	**K4**	**K5**
0	0	0	0	0	0
17	0	33	33	11	0
35		37	49	0	59
67		0	0	62	0
99			45	53	52
154			51	50	49

Table A1-8. Amount of H_2 in vial (nmol), experiment at 30 °C, sterile conditions.

Day	n_1				
	S1	**S2**	**S3**	**S4**	**S5**
0	4	4	3	4	4
8	116	96	127	299	162
22	246	316	493	927	374
43		528	623	943	433
71			835	1080	631
113				1150	791
140					970
	A1	**A2**	**A3**	**A4**	**A5**
0	5	5	5	0	5
8	476	587	425	402	402
22	701	1090	909	429	984
43		1100	1070	271	1160
71			1460	698	1850
113				9	2340
140					2890
	K1	**K2**	**K3**	**K4**	**K5**
0	0	12	0	0	0
8	0	12	12	12	12
22	13	13	26	13	26
43		11	11	23	11
71		12	24	24	24
113			22	23	11
140			24	36	23

Table A1-9. Amount of H_2 in vial (nmol), experiment at 30 °C, natural conditions.

Day	n_1				
	S1	**S2**	**S3**	**S4**	**S5**
0	6	0	6	7	7
7	123	138	137	221	234
21	355	65	0	0	6
42	4	13	6	13	13
73	7	5	9	0	4
108	8	10	9	0	4
	A1	**A2**	**A3**	**A4**	**A5**
0	0	0	6	6	6
7	679	594	764	1 100	411
21	793	723	0	5	6
42	706	67	6	11	0
73	4	4	5	0	5
108	0	14	5	0	5
	K1	**K2**	**K3**	**K4**	**K5**
0	0	0	0	0	0
7	35	44	72	23	344
21	11	0	0	12	12
42	12	24	13	26	13
73	0	0	10	10	10
108	9	0	10	10	10

Table A1-10. Amount of H_2 per cm^2 surface, experiment at 70 °C, sterile conditions.

Day	A				
	S1	**S2**	**S3**	**S4**	**S5**
0	0	0	0	0	0
17	18	15	18	8	16
35		23	24	12	24
67			32	18	34
99				19	24
154					27
	A1	**A2**	**A3**	**A4**	**A5**
0	0	0	0	0	0
17	183	91	30	204	70
35	165		38	3 550	123
67			241	1 410	989
99				1 100	173
154					96

Table A1-11. Amount of H_2 per cm^2 surface, experiment at 30 °C, sterile conditions.

Day	A				
	S1	**S2**	**S3**	**S4**	**S5**
0	0	0	0	0	0
8	3	3	3	8	4
22	6	8	13	24	10
43		14	16	24	11
71			22	28	16
113				30	21
140					25
	A1	**A2**	**A3**	**A4**	**A5**
0	0	0	0	0	0
8	15	19	14	13	13
22	22	35	29	14	32
43		35	34	9	37
71			47	23	60
113				0	75
140					93

Table A1-12. Amount of H_2 per cm^2 surface, experiment at 30 °C, natural conditions.

Day	n_1				
	S1	**S2**	**S3**	**S4**	**S5**
0	0.22	0.00	0.22	0.23	0.23
7	4.29	4.78	4.76	7.69	8.11
21	12.36	2.26	0.00	0.00	0.21
42	0.16	0.44	0.22	0.46	0.44
73	0.23	0.16	0.31	0.00	0.15
108	0.26	0.33	0.32	0.00	0.16
	A1	**A2**	**A3**	**A4**	**A5**
0	0.00	0.00	0.23	0.20	0.22
7	23.69	20.70	26.66	38.94	14.33
21	27.67	25.20	0.00	0.18	0.21
42	24.61	2.32	0.22	0.37	0.00
73	0.15	0.15	0.16	0.00	0.16
108	0.00	0.48	0.17	0.00	0.17

Table A1-13. Results for sulphide analyses of water samples contained metallic rods.

Sample	S^{2-}, mg L^{-1}
S1	< 0.1
S2	< 0.1
S3	< 0.1
S4	< 0.1
S5	< 0.1
A1	0.2
A2	0.2
A3	0.3
A4	0.7
A5	2.8
K1	0.6
K2	0.8
K3	0.4
K4	1.2
K5	0.9

Arkitektkopia AB, Bromma, 2017

SKB is responsible for managing spent nuclear fuel and radioactive waste produced by the Swedish nuclear power plants such that man and the environment are protected in the near and distant future.

skb.se