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Stephanie Lerm [a], Dr. Mashal Alawi [a], Dr. Rona Miethling-Graff [d], Dr. Markus Wolfgramm [b], Kerstin Rauppach [b], Dr. Andrea Seibt [c], Dr. Hilke Würdemann [a]

Influence of microbial processes on the operation of a cold store in a shallow aquifer: impact on well injectivity and filter lifetime

Bedeutung mikrobieller Prozesse für den Betrieb eines Kältespeichers in einem oberflächennahen Aquifer: Einfluss auf Brunnen-Injektivität und Filterstandzeit

[a] Helmholtz-Zentrum Potsdam GFZ Deutsches GeoForschungsZentrum, Internationales Geothermiezentrum, Telegrafenberg, 14473 Potsdam, Germany

[b] Geothermie Neubrandenburg GmbH (GTN), Seestr. 7A, 17033 Neubrandenburg, Germany

[c] BWG Geochemische Beratung GbR, Seestr. 7A, 17033 Neubrandenburg, Germany

[d] Johann Heinrich von Thünen Institut, Bundesforschungsinstitut für Ländliche Räume, Wald und Fischerei Institut für Biodiversität, 38116 Braunschweig, Germany. Current address: a

contact:
Dr. Hilke Würdemann
Internationales Geothermiezentrum, Deutsches GeoForschungsZentrum
Telegrafenberg, 14473 Potsdam, Germany
Tel. +331 288 1516
Fax +331 288 1529
email: wuerdemann@gfz-potsdam.de

ABSTRACT

In this study, the operation of a cold store, located in 30-60 m depth in the North German Basin, was investigated by direct counting of bacteria and genetic fingerprinting analysis. Quantification of microbes accounted for 1 to 10 x 10^5 cells per ml fluid with minor differences in the microbial community composition between well and process fluids. The detected microorganisms belong to versatile phyla *Proteobacteria* and *Flavobacteria*. In addition to routine plant operation, a phase of plant malfunction caused by filter clogging was monitored. Increased abundance of sulphur-oxidizing bacteria indicated a change in the supply of electron acceptors, however, no changes in the availability of electron acceptors like nitrate or oxygen were detected. Sulphur- and iron-oxidizing bacteria played essential roles for the filter lifetimes at the topside facility and the injectivity of the wells due to the formation of biofilms and induced mineral precipitations. In particular, sulphur-oxidizing *Thiothrix* generated filamentous biofilms were involved in the filter clogging.

KURZFASSUNG

Im Rahmen dieser Studie wurde der Betrieb eines in 30-60 m Tiefe gelegenen Kältespeichers des Norddeutschen Beckens durch Bestimmung der Bakterien-Zellzahlen und genetischer Fingerprinting Analysen untersucht. Eine Zellzahlbestimmung ergab 1 bis 10 x 10^5 Zellen pro ml Fluid, wobei geringe Unterschiede in der mikrobiellen Zusammensetzung zwischen Brunnenproben und Prozessfluiden nachgewiesen wurden. Die identifizierten Mikroorganismen wurden den Phyla *Proteobacteria* und *Flavobacteria* zugeordnet. Neben routinemäßigem Anlagenbetrieb wurde eine Phase mit technischen Störungen durch zugesetzte Filter dokumentiert. Die Zunahme an Schwefel-oxidierenden Bakterien zeigte eine erhöhte Verfügbarkeit von Elektronenakzeptoren an, obwohl keine Änderungen in der Verfügbarkeit von Elektronenakzeptoren, wie Nitrat oder Sauerstoff, nachgewiesen werden konnte. Schwefel- und Eisen-oxidierende Bakterien spielten durch die Bildung von Biofilmen und induzierter mineralischer Ausfällungen eine essentielle Rolle für die Filterstandzeiten in der obertägigen Anlage und die Injektivität der Bohrungen. Vor allem Schwefel-oxidierende *Thiothrix* bildeten filamentöse Biofilme und trugen wesentlich zum Zusetzen der Filter bei.

Keywords: geothermal aquifer storage, groundwater, microbial community, 16S rRNA, clogging, *Thiothrix* sp.

1. Introduction

Shallow aquifers are increasingly used for aquifer thermal energy storage (ATES), e.g. storage of winter's natural cold for several months by the installation of wells and corresponding topside facilities. This technology represents an environmentally friendly alternative to conventional greenhouse gas-emitting fossil fuel supplied systems for building heating and cooling. In Germany, three different regions, the North German Basin, the Upper Rhine Graben and the Molasse Basin provide suitable conditions, like high temperatures, productivities or covering layers, for geothermal applications. In the North German Basin, ATES in the near subsurface serves for air-conditioning in buildings or housing-complexes. For efficient utilization of this energy source, failure due to scaling, biofouling or corrosion must be avoided. These phenomena are documented for many water-bearing systems and microorganisms are often involved or even responsible for its formation (Honegger et al. 1989, Flemming 2002, Beech and Sunner 2004, Coetser and Cloete 2005, Little and Lee 2007, Valdez et al. 2009). Few studies have examined biological and chemical processes in geothermal power plants (Honegger et al. 1989, Inagaki et al. 1997, Takai and Horikoshi 1999).

Based on increasing technical accessibility of groundwater horizons and aseptic sampling techniques, developed in the late 1970s for the shallow and deep subsurface, several studies were conducted to characterize the diversity of indigenous microorganisms in aquifers in different geological formations (e.g. Dunlap et al. 1977, Wilson et al. 1983, Phelps et al. 1989, Baker et al. 2000, Roden and Wetzel 2003, Goldscheider et al. 2006, Briée et al. 2007, Griebler and Lueders 2009, Brielmann et al. 2009, Pronk et al. 2009). As aquifers are heterogeneously structured, they represent a variety of habitats with different physical and chemical conditions, created by host rock types, fluid temperature, pH, and salinity (Griebler and Lueders 2009). The nature of each subsurface environment controls the type of microbial community that can develop and the rate at which it can grow. Microbes are either free floating (planktonic) in groundwater or attached to mineral grains and reservoir rock surface, partly via special cellular attachment structures, necessitating sampling of fluid and solid samples. It is assumed that the attached way of living is favourable for bacteria in aquatic sediment systems, poor in organic carbon and nutrients (Harvey et al. 1984, Alfreider et al. 1997, Griebler et al. 2002). Microbes attached to surfaces form structured biofilms holding together by secreted slimy adhesive substances, termed extracellular polymeric substances (EPS) (e.g. Costerton et al. 1995, van Lossdrecht et al. 1995, Flemming et al. 2007). Due to internal zones of varying nutrients and physical conditions biofilms often contain different types of metabolically interacting organisms (synergism) and build complex biochemical networks that are balanced for an efficient exploitation of resources in the chemical energy depleted ecosystem. Parts of the biofilm may be dispersed either by cell division processes, due to nutrient levels or quorum sensing, or shearing because of flow effects (Hall-Stoodley et al. 2004). These detached parts are passively transported as suspended particles with the flow and are the basis for further microbial settlement processes to surfaces, e.g. at inside walls of geothermal plant casing.

In general, groundwater aquifers are depleted in easy availble organic energy sources due to degradation processes in the upper subsurface (Gibert 1994, Chapelle 2001). Aquifer microorganisms gain their energy by two different pathways for growth and reproduction. The heterotrophic pathway bases on the complete and incomplete oxidation of organic carbon to carbon dioxide or simple organic molecules like organic acids or ethanol. Oxidizing agents are molecular oxygen or oxidized inorganic compounds, like nitrate and sulphate. However, lithoautotrophic organisms incorporate carbon dioxide and use inorganic compounds for energy generation.

For microbial analysis of the complex subsurface environment, different nucleic acid techniques based on 16S ribosomal ribonucleic acid (rRNA) genes are available and enable identification and classification of isolated 16S rRNA gene sequences without the limitation of time-consuming culturing methods (Amann et al. 1997). The submission of 16S rRNA sequences to the public database GenBank of the National Centre for Biotechnology Information (NCBI) comprising 16S rRNA sequences of up to 677,000 bacterial species allows a phylogenetic affiliation by similarity analyses as well as metabolic attribution from references in the database (Cole et al. 2009).

In this study, we present microbial monitoring of a shallow aquifer used for cold storage near the Berliner Reichstag (German Parliament). Genetic fingerprinting was used to characterize the microbial diversity in fluids of three wells reaching the aquifer as well as fluid and filter samples taken at the corresponding topside facility. In particular, our investigations focused on the detection of dominant metabolic processes by following changes in microbial community structure in plant deriving fluid and filter samples that are caused by fluid recharge- and discharge processes over a period of 21 months. In addition, the total cell numbers in fluid samples were determined using epifluorescence microscopy with DAPI staining. DAPI (4',6-diamidino-2-phenylindole) is a chemical agent that passes through an intact cell membrane and forms fluorescent complexes with natural double-stranded DNA. Thus, DAPI is used extensively in fluorescence microscopy. The microbiological results were correlated with results of chemical and mineralogical analyses to determine the dominant microbial processes at this cold store and the potential influence of microbes on plant operation.

Site description:
The aquifer is located in Quaternary sands at 30-60 m depth in the area of the Mittelbrandenburg plates and glacial valleys. It reaches the surface of the North German Basin and is characterized by the glacio-fluvial deposits of the last ice age (Fig. 1).

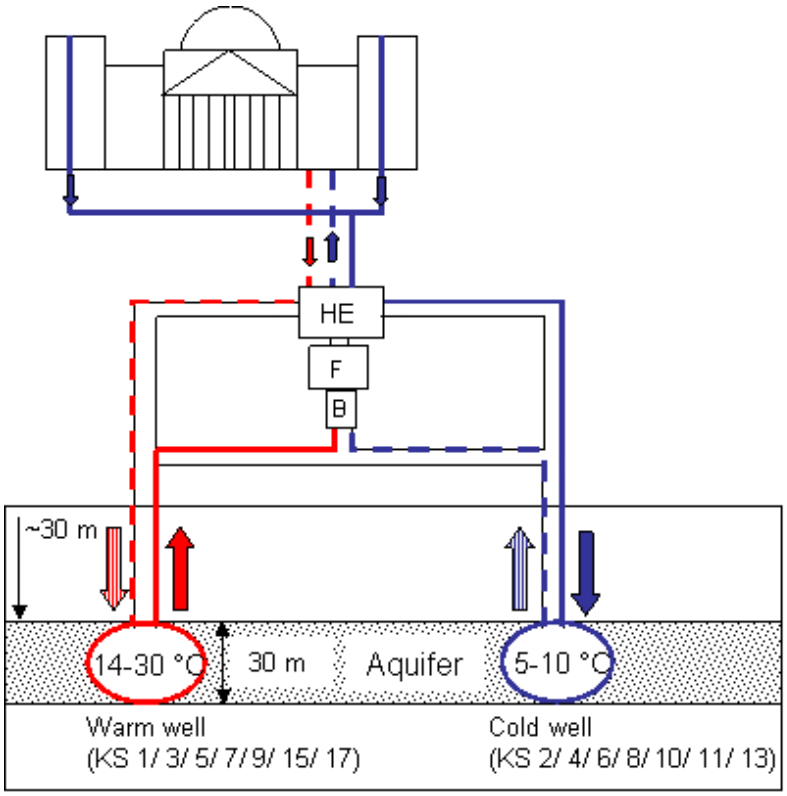

Fig. 1. Principle scheme of the cold store with the location of sampling devices for fluid (B, bypass) and filter (F) at the topside facility. For simplification only one well from the warm and the cold side and one building (Reichstag) that is connected to the topside facility are presented. The arrows indicate the fluid flow direction during recharge (winter, plain line) and discharge (summer, dashed line) mode. HE Heat exchanger.

The aquifer is developed by wells and the associated underground infrastructure is connected with different buildings of the German Parliament through a complex pipeline system. The investigated ATES system is a seasonal cold store and has served as air-conditioning in the Reichstag since 2003 (Kabus and Seibt 2000, Sanner et al. 2005). Due to cold storage and fluid recharge and discharge processes the geothermal plant has a cold (south field) and warm (north field) side. Both sides are exploited by seven wells developing the aquifer (Fig. 2).

Dependent on the demand for cooling, certain wells from the cold and the warm side are connected and used as production- and injection-wells, respectively. In winter, fluid with 22 to 14 °C is pumped up from the warm side of the aquifer, cooled down to approximately 5 °C by cooling towers and reinjected in the cold side. In summer, fluid from the cold side is produced with temperatures ranging from 6 to 10 °C, providing the cooling of the buildings via heat exchanger. Afterwards, the warmed-up fluid is pumped back into the warm side of the aquifer with temperatures ranging between 15 and 30 °C. Because of the different flow directions the wells are equipped with pumps, production- and injection pipes. For retention of solid particles, transported with the production flow, filter systems are installed preceding the heat exchanger at the Reichstags building and the Paul Löbe

Haus, each containing 8 and 16 filter bags, respectively. At the Reichstag building Eaton filter bags (LOFCLEAR-128-To2A-30l, pore size 25-40 µm) are used, whereas at Paul Löbe Haus Ama filter bags (AP50/25/10P2SS, pore size 10-50 µm) were used until autumn 2008. Since autumn 2008, Eaton filter bags were also used at Paul Löbe Haus. To prevent the intrusion of oxygen into the groundwater system the piping is under nitrogen pressure of about 0.4 to 0.9 bar.

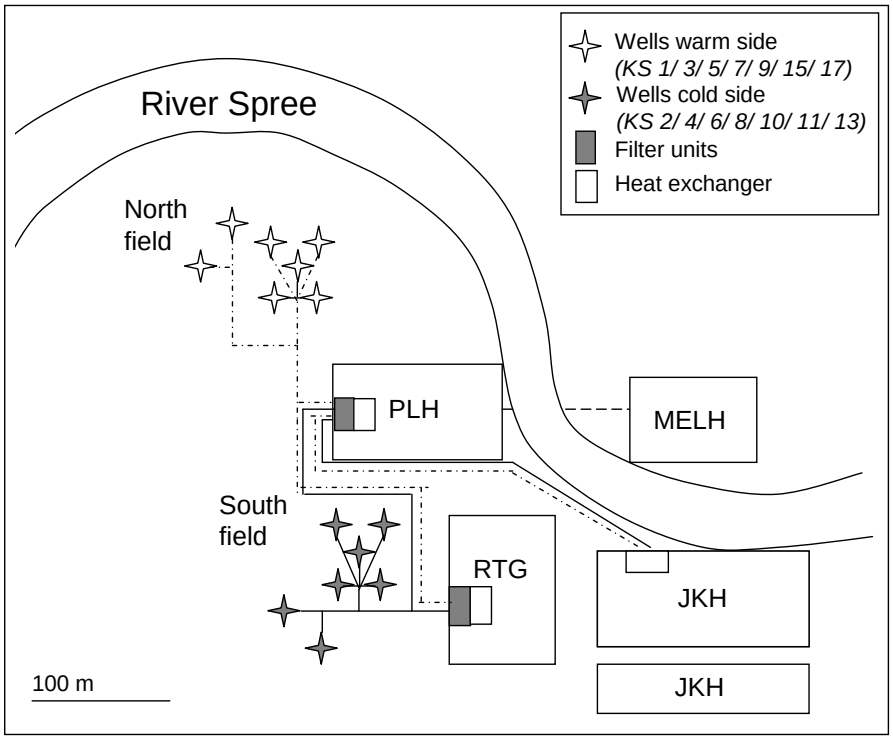

Fig. 2. Schematic illustration of the cold store. PLH Paul Löbe Haus, RTG Reichstag, JKH Jakob Kaiser Haus, MELH Marie Elisabeth Lüders Haus.

The aquifer fluid is characterized as a shallow groundwater with a low salinity (< 1 g l^{-1}), a pH of 7.4 and redox potential of 40-80 mV (Tab. 1). The concentration of molecular oxygen is below the detection limit. Sulphate and dissolved organic carbon (DOC) -concentrations, both relevant for microbial processes, ranged between 164-221 mg l^{-1} and 3.6-6.2 mg l^{-1}, respectively. Nitrate was only rarely detected with 0.3 mg l^{-1} on average (Opel et al. 2008). The concentration of the low weight molecular organic acids formate and acetate amounted to circa 0.1 mg l^{-1} (Vetter et al. 2011).

Tab. 1: Operational and physico-chemical site characteristics

Reservoir rock	Complete start-up	Depth [m]	Temp. [°C]	Max. flow rate [m^3 h^{-1}]	pH	Salinity [g l^{-1}]	Redox potential SHE [mV]	DOC [mg l^{-1}]	Sulphate [mg l^{-1}]	Nitrate [mg l^{-1}]	Ferrous iron [mg l^{-1}]	Flow reversion
Sand	2003	30 - 60	6/30	300	7.4	< 1	40 - 80	3.6 - 6.2	164 - 221	1.3	2.1 - 2.4	March/ November

SHE standard hydrogen electrode, DOC dissolved organic carbon

Sampling and sample preparation

In the course of routine well regeneration, fluids deriving from the wells KS 3, KS 5, KS 7, developing the warm side of the aquifer (north field), were sampled once in 2006 using a manual downhole sample, that was disinfected with ethanol before use. In addition, fluid and filters were sampled at the topside facility in the Reichstag and the Paul Löbe Haus from the operating geothermal plant during the recharge and discharge mode once in June 2006 and in 2007 till 2009 over the course of 21 months, including a phase of decreased filter lifetimes at the topside facility in summer/autumn 2008 (Fig. 3, Tab. 2).

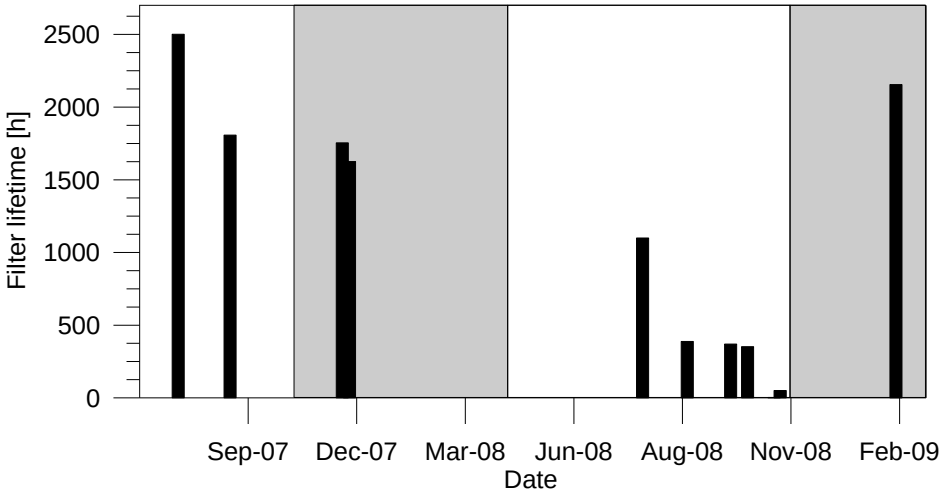

Fig. 3. Filter lifetimes at the topside facility before and after disinfection. grey (recharge mode), white (discharge mode)

Fluid was taken by a bypass system preceding the filter system at the topside facility and collected in sterile 1 litre Schott Duran glass bottles. The sampling equipment was purged before use with the produced fluid from which the sample was taken in order to minimize the risk of contamination. For genetic profiling, bacteria in fluids were concentrated by filtration on 0.22 µm cellulose acetate filters (Sartorius, Goettingen, Germany) and stored at -80 °C until processed. Single filter bags taken from the filter systems were stored in autoclaved aluminium foil at -80 °C until processed.

Tab. 2: Overview of fluid and filter samples

Date	Type	Sample origin	Operation Mode	Temperature $_{out}$ [°C]	Temperature $_{in}$ [°C]
June 2006	Fluid	wells	-	-	-
May 2007	Fluid	PLH	discharge	7.7	19.7
March 2008	Fluid	PLH	recharge	16.2	7.3
July 2008	Filter	PLH	discharge	8.9	23.7
August 2008	Fluid	PLH	discharge	10.3	15.9
September 2008	Fluid	PLH	discharge	10.4	15.4
October 2008	Fluid	RTG	discharge	9.9	14.5
November 2008	Filter	RTG	recharge	14.5	14.5
December 2008	Fluid	PLH	recharge	14.0	7.8
January 2009	Fluid	PLH	recharge	15.0	7.2
February 2009	Filter	PLH	recharge	13.8	6.2

wells (KS 3, KS 5, KS 7), PLH (Paul Löbe Haus), RTG (Reichstag)

Total cell counts

For DAPI-staining, fluid samples taken at the topside facility were used. Fresh fluid samples were fixed with formaldehyde to a final formaldehyde concentration of 1 % for 4 h at 4 °C. To concentrate the cells, fluid volumes ranging from 1 to 30 ml were filtered on 0.2 µm black Nuclepore Polycarbonate Membrane Filters (Whatman) and stored at -20 °C. In addition, 0.5 g filter material was incubated with detergent mix at weak mixing conditions for 30 minutes. The buffer was prepared with DI-H_2O, containing disodium EDTA dihydrate (100 mM), sodium pyrophosphate decahydrate (100 mM) and TWEEN 80 (1 % vol/vol) (Kallmeyer et al. 2008). Afterwards, the liquid phase was pipetted off with exhausting the filter material and collected in appropriate tubes. This procedure was repeated twice for optimal detachment of cell assemblages from filter material. Slurries were centrifuged at 8,000 g for 20 min (Eppendorf 5804). Sample fixation was carried out according to Pernthaler et al. (2001). Pellets were fixed with 4 % formaldehyde (3 vol/vol) for 4 h at 4 °C. The fixed samples were washed with phosphate-buffered saline (PBS) solution (1x, pH 7.2-7.4) and stored in Ethanol-PBS (1:1) at -20 °C. All samples were diluted 1:10 with 1x PBS solution. The cells were detached from solid particles by mild sonication for 10 s with a MS73 probe (Sonoplus HD70; Bandelin, Berlin, Germany).

A volume of 10 µl of each prepared filter sample was spotted on gelatine-coated Teflon-laminated slides (Zarda et al. 1997). The slides were dried at 45 °C for 15 min. The membrane filters were cut in small pieces (3x3 mm) and also placed on slides. Subsequently, 10 µl of DAPI (4´,6-diaminodino-2-phenylindole, 1 mg ml^{-1} working solution) was dropped onto each well and incubated in the dark at room temperature for 10 min, as described by Morozova et al. (2010). Afterwards, the slides were washed in ice-cold double-distilled water and allowed to air-dry. Finally, the slides were embedded in Citifluor AF1 antifading solution (Plano, Wetzlar, Germany), covered with a coverslip and were examined microscopically using Axio Imager M2 (Zeiss, Germany).

DNA extraction and SSCP- and DGGE- fingerprinting

To investigate microbial community structure in fluid and filter samples, the genetic fingerprinting techniques single strand conformation polymorphism (SSCP, Schwieger and Tebbe 1998) and denaturing gradient gel electrophoresis (DGGE, Muyzer et al. 1996) were used to characterize the dominant species. These cultivation-independent approaches based on polymerase chain reaction (PCR) -amplified 16S rRNA genes from bacterial DNA, extracted from the samples allow the determination of differences in a variety of samples and the detection of changes in community structure over time. In these PCR reactions specific starter molecules called "primers" were used to detect different bacterial groups with genes encoding for specific metabolic enzymes. For generation of genetic profiles, PCR products are separated electrophoretically and stained for visualization. Different band intensities are reflecting the relative abundance of species in the community. By DNA sequencing of dominant bands in the profiles the dominant organisms become phylogenetically classified and subsequent metabolically characterized.
In detail, for DNA extraction cellulose acetate filters (Whatman) and filters bags were cut with sterile scalpel into 3x3 mm pieces. DNA was extracted using the MoBio Ultra Clean Soil Kit (Carlsbad, USA) according to the standard operating procedure. To reduce shearing of DNA, cells of the cellulose acetate filter were lysed alternatively by heating (2x 5 min at 80 °C) and vortexing for 5 seconds.
Partial sequences of the 16S rRNA genes were amplified by PCR using universal primers that hybridized to all bacteria (com1/com2-ph, Schwieger and Tebbe 1998) and primers encoding the dissimilatory sulphite reductase β-subunit in sulphate reducing communities (DSR2060F-GC/DSR4R, Geets et al. 2006). Products of universal PCR were analyzed by SSCP-analysis according to Schwieger and Tebbe (1998) and Dohrmann and Tebbe (2004). DNA in polyacrylamide gels was visualized by silver-staining (Bassam et al. 1991). Products of SRB (sulphate reducing bacteria) -specific PCR were analyzed by DGGE-analysis according to Muyzer et al. (1996) and with a denaturant gradient ranging from 40 % to 85 %. DNA in polyacrylamide gels was visualized by ethidium bromide staining (1 %) and gel images were obtained using GeneFlash (Syngene). Dominant bands of SSCP- and DGGE-profiles were cut from the gel, reamplified and directly sequenced using the corresponding 16S rDNA primers. Nucleotide sequences were aligned by the use of the software package ARB (http://www.arb-home.de) and were compared with the Basic Local Alignment Search Tool (BLAST) function of the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/). Sequences analyzed in this study have been deposited in the EMBL database of the European Bioinformatics Institute (www.ebi.ac.uk/embl) under the accession numbers HQ690775 to HQ690806.

Mineralogical and geochemical analyses

The solid particles in the filters were analyzed using scanning electron microscopy (Cambridge S200) with energy dispersive X-ray spectroscopy (SEM-EDX). The concentrations of inorganic anions (e.g. nitrate, sulphate) and the dissolved low molecular weight organic acids (e.g. acetate) in fluids were quantified by ion chromatography (ICS 3000, Dionex Corp.) as previously described by Vieth et al. (2008). The redox potential, pH and fluid temperature were determined during the sampling procedures using a pH/mV/temperature meter (WTW). The oxygen concentration was determined by an electrode, installed in a flow-through chamber in order to measure in a continuous flow environment and to improve the detection limit (0.01 ml l^{-1}). The iron content was quantified by ion chromatography according to DIN EN ISO 10304-2.

2. Results and Discussion

Microbial community structure in well fluid

SSCP-fingerprintings of well fluids (KS 3, KS 5, KS 7), sampled once in 2006, showed similar profiles, comprising seven dominant bands each. The profiles are shown in Fig. 4A. Results of DNA sequencing of dominant bands are presented in Tab. 3 and revealed affiliations to *Nitrospirae* (band 5) and Beta-*Proteobacteria*, in particular to the metabolic versatile *Rhodocyclaceae* (band 1) and iron-oxidizing *Gallionella* species (bands 3 and 4). In addition, Epsilon-*Proteobacteria* (band 7) and sulphur-oxidizing organisms of the genus *Thiothrix* (band 6) were identified. Strains of *Thiothrix* have been reported to be mixotrophic, requiring several small organic compounds as well as a reduced inorganic sulphur source. Under anaerobic conditions, they are able to oxidize thiosulphate or intracellular sulphur globules with nitrate as terminal electron acceptor (Larkin and Shinabarger 1983, Nielsen et al. 2000, Rosetti et al. 2003). Even though the oxygen concentration was below the detection limit and nitrate was rarely detected with 0.3 mg l^{-1} on average, the mass flow rate has to be considered because it represents a continuous nitrate supply.
In addition, until recently, it was thought that iron-oxidation was limited to oxic or microaerobic environments. However, the characterization of microorganisms, capable of coupling nitrate-reduction to ferrous iron-oxidation, indicate that microorganisms can play a role in iron-oxidation also in anoxic habitats (Emerson 2010). Even as no molecular oxygen was detected in fluids similar processes might be considered. Iron-oxidizing *Gallionella* sp. is probably responsible for the formation of iron hydroxides, using fluid present ferrous iron that was in a range of 2.1 to 2.4 mg l^{-1}. Iron hydroxide deposits were detected by the detailed analysis of solid particles using scanning electron microscopy (SEM-EDX) (Wolfgramm et al. 2010) (Fig. 7B). In 2007, filter slots in the well casing were clogged with iron hydroxides, necessitating well regeneration to re-establish sufficient well productivity. The well regeneration was done by cleaning the well casings using brushes, a chemical reagent (Aixtractor 2.0) and a fluid-pulse procedure. In addition, iron hydroxide deposits were the main mineral components formed in filter bags of the topside facility. In some cases the iron hydroxides formed thick crusts.

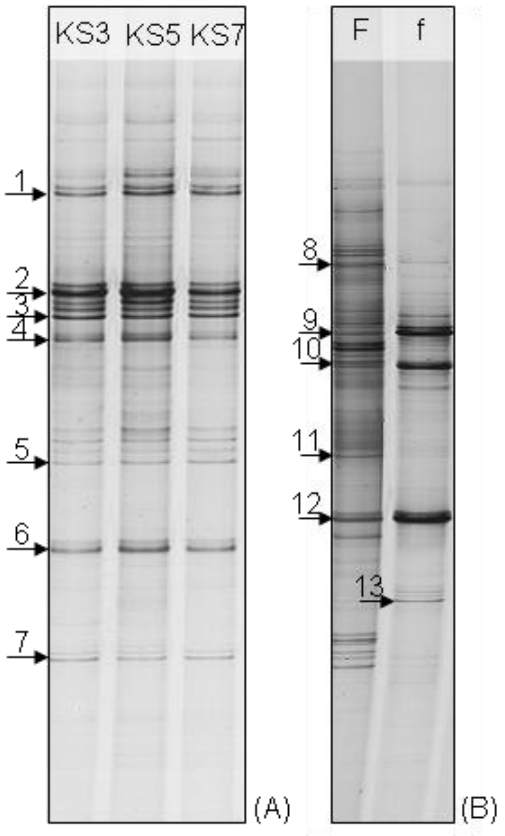

Fig. 4. SSCP-analysis of 16S rRNA gene fragments using bacterial DNA from fluid samples taken directly from wells KS 3, KS 5, KS 7 (north field, warm side) in march 2006 (A) and fluid (F) and filter (f) samples taken at the topside facility in June 2006 (B). Arrows indicate the positions of bands that were sequenced to identify the species of microorganisms and to conclude its metabolic capabilities.

Tab. 3: Phylogenetic affiliation of partial bacterial 16S ribosomal RNA gene sequences from SSCP-profiles of well fluids (bands 1-7) and fluid and filter sampes from the topside facility (bands 8-13)

Band	Phylum/Class	Closest relative, (Genbank accession number)	Similarity [%]	Genbank accession number
1, 8	Beta-*Proteobacteria*	Uncultured *Rhodocyclaceae* bacterium, clone 127, (FM207957)	98	HQ690775 HQ690782
2, 9		Uncultured bacterium, clone LKC3_198.29, (AY510251)	98	HQ690776 HQ690783
3		*Gallionella* sp., clone MWE_N10, (FJ391503)	99	HQ690777
4		Uncultured *Gallionella* sp., clone HC16, (FJ391516)	99	HQ690778
10		Uncultured *Curvibacter* sp., clone 469C11, (EU127421)	97	HQ690784
11		Uncultured *Gallionella* sp., clone Hc1, (FJ391520)	99	HQ690785
6	Gamma-*Proteobacteria*	*Thiothrix unzii* , (L79961)	97	HQ690780
12		Uncultured *Thiotrichales* bacterium, clone MS4-42, (GQ354932)	98	HQ690786
7, 13	Epsilon-*Proteobacteria*	Uncultured bacterium, clone AA_25_UNI, (AB473796)	98	HQ690781 HQ690787
5	*Nitrospirae*	Uncultured *Nitrospirae* bacterium, clone 356, (AB252945)	100	HQ690779

RNA ribonucleic acid, SSCP single strand conformation polymorphism

Microbial community in fluid and filter of the topside facility

The SSCP-profiles gained from fluid and filter of the topside facility, sampled in June 2006, differed in band intensities, but not in the relative abundance of bands (Fig. 4B). Results of DNA sequencing of the dominant bands are also presented in Table 3 and revealed same microbes as predominating in well fluids, like the *Rhodocyclaceae* bacterium (band 8), *Gallionella* sp. (band 11), *Thiothrichales* (band 12) and Epsilon-*Proteobacteria* (band 13), with the exception of *Nitrospirae* relatives. However, betaproteobacterial *Curvibacter* species were detected additionally in fluids of the topside facility. These minor differences in microbial composition could probably lead back to the fact that the fluid, sampled at the topside facility, is a mixture of the currently producing wells.

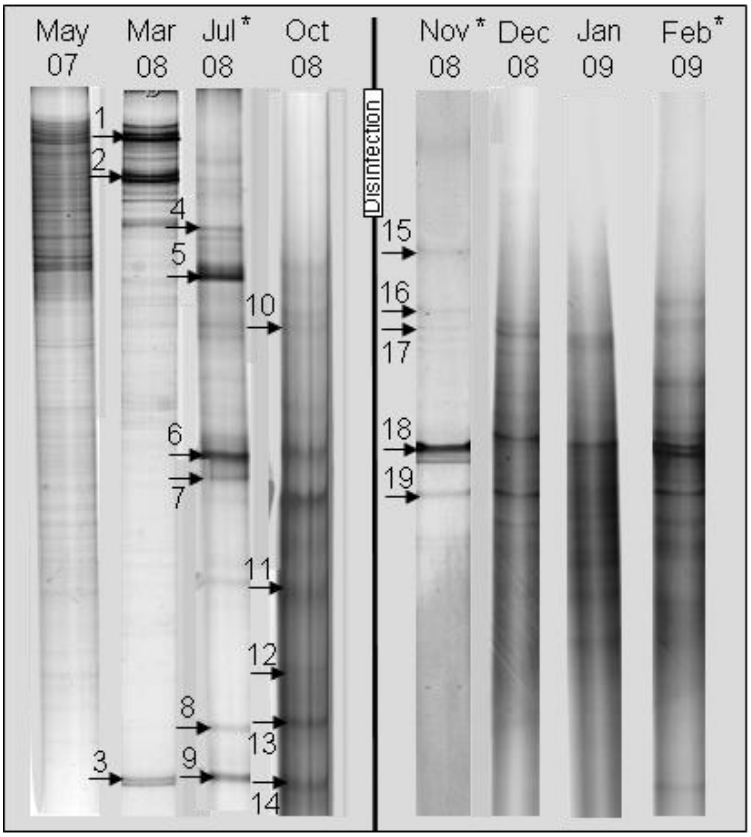

Fig. 5. SSCP-analysis of PCR-amplified 16S rRNA gene fragments of bacterial community DNA extracted from fluid and filter (*) samples taken at the topside facility from May 2007 till February 2009. Arrows indicate the positions of bands that were sequenced to identify the species of microorganisms and to conclude its metabolic capabilities.

The period, monitored from May 2007 till February 2009, was characterized by two distinct phases: undisturbed plant operation and a period with a drastically reduced injectivity and reduction of filter lifetime. During normal filter lifetimes of 1,850 hours on average, the total cell counts in fluid samples taken at the topside facility amounted circa 1 x 10^5 cells per ml. This is in the same magnitude of cell counts obtained with DAPI-staining for groundwater from different types of aquifers (Ghiorse and Wilson 1988, Goldscheider et al. 2006). SSCP-profiles gained from fluid and filter samples of the topside facility in this period, comprised up to eight dominant bands that differed significantly in abundance and intensity over time (Fig. 5). DNA sequencing of the dominant bands revealed the presence of Beta-, Delta-, Epsilon-, Gamma-*Proteobacteria*, and *Flavobacteria* with 92 to 100 % similarity to sequences in the GenBank database (Tab. 4).

Tab. 4: Phylogenetic affiliation of partial bacterial 16S ribosomal RNA gene sequences from SSCP-profiles of fluid and filter samples from the topside facility

Band	Class	Closest relative, (Genbank accession number)	Similarity [%]	GenBank Accession Number
1	Beta-*Proteobacteria*	Uncultured *Aquaspirillum* sp., clone T5-4, (AF526926)	99	HQ690788
2		*Ferribacterium* sp., clone 24-19 (HM124374)	97	HQ690789
4		*Rhodocyclaceae* bacterium, clone FTL11, (DQ451827)	99	HQ690791
5		Uncultured bacterium, clone LKC3_198.29, (AY510251)	98	HQ690792
7		*Bacteroidetes* bacterium, clone VNs52, (FJ168485)	93	HQ690794
10		*Nitrosospira* sp., clone 17SS, (EF015571)	97	HQ690797
17		Uncultured *Propionivibrio* sp., clone RUGL1-577, (GQ420984)	97	HQ690804
3	Epsilon-*Proteobacteria*	*Sulfuricurvum kujiense* , strain YK-2, (AB080643)	98	HQ690790
8		Uncultured bacterium, clone AA_25_UNI, (AB473796)	93	HQ690795
9		Uncultured bacterium, clone MVP-6, (DQ676341)	98	HQ690796
6	Gamma-*Proteobacteria*	Sulphur-oxidizing bacterium, clone NBRC 105220, (AB506456)	98	HQ690793
11		Uncultured *Thiothrix* sp., clone U3-AlvEE, (AB425225)	92	HQ690798
12, 13		*Thiothrix unzii* , (L79961)	94, 95	HQ690799 HQ690800
14		Uncultured *Thiothrix* sp., clone U3-AlvEE, (AB425225)	97	HQ690801
15	*Flavobacteria*	*Flavobacterium psychrolimnae* , (AB455260)	99	HQ690802
16		Uncultured bacterium, clone HY7, (AB263622)	98	HQ690803
18		*Flavobacterium* sp., clone NMC26, (GU321361)	100	HQ690805
19		*Flavobacterium* sp., clone HMD1068, (GU291855)	99	HQ690806

RNA ribonucleic acid, SSCP single strand conformation polymorphism

The predominance of *Proteobacteria* in the microbial community of the process fluid is consistent with previous observations within several freshwater ecosystems (López-Archilla et al. 2007, Blöthe and Roden 2009, Griebler and Lueders 2009) showing *Proteobacteria* particularly involved in cycling of iron- and sulphur-compounds (López-Archilla et al. 2007, Haaijer et al. 2008, Weber et al. 2008, Lerm et al. 2011). In addition, sulphate reducing bacteria were detected by specific PCR-DGGE-profiling (Fig. 6, Tab. 5). However, the redox potential measured in the fluid ranged between 40 and 80 mV and sulphate reduction is typically characterized by lower redox potential of less than -150 mV different redox-zones which may have existed in biofilms could have provided the conditions favorable for such strict anaerobic processes.

Heterotrophic Beta-*Proteobacteria* of denitrifying *Aquaspirillum* species (band 1), iron (III)-reducing *Ferribacterium* species (band 2) and sulphate reducing bacteria dominated in March 2008, operation with normal filter lifetimes. These organisms are classically identified within freshwater environments and aquifers that can be stagnant or brackish, containing organic matter (Cummings et al. 1999, Pot et al. 2002, Dann et al. 2009, Wu and Yang 2009). In addition, relatives of the epsilonproteobacterial species *Sulfuricurvum kujiense* (band 3), which is associated with oxidation of reduced sulphur compounds and nitrate-reduction in sulphidic environments (Kodama and Watanabe 2003), were detected in this period, too. *S. kujiense* was recently also observed in other shallow aquifers, used for geothermal heat storage (Lerm et al. 2011). The simultaneous presence of oxidizing and reducing microorganisms indicates an internal syntrophic iron- and sulphur-cycle that has been described previously for aquatic systems (Holmer and Storkholm 2001, Weber et al. 2006, Blöthe and Roden 2009), partly rich in their sulphide content (Macalady et al. 2006, Satoh et al. 2009, Engel et al. 2010), hydrothermal vents, microbial mats, marine sediments, and wastewater biofilms (Celis-García et al. 2008). The reduction in filter lifetime, related to the total amount of fluid volume passing the filters, indicated problems in plant operation due to filter clogging. This was preceded by an increase in the DOC-content in fluids from 3.7 mg l^{-1} in March up to 6.2 mg l^{-1} in August. In addition, this corresponded with a shift in the bacterial community structure. During the period from July till November 2008, showing decreasing filter lifetimes, Beta-

Proteobacteria of metabolic versatile *Rhodocyclaceae* (band 4), fermentative *Bacteroidetes* (band 7) and Epsilon-*Proteobacteria* (bands 8 and 9) became predominant. Members of the phylum *Bacteroidetes* are frequently abundant in freshwater and marine ecosystems and may have a specialized role in the uptake and degradation of organic matter in aquatic environments (Kirchman 2002). In addition, the abundance of sulphate reducing bacteria probably increased, as indicated by strong DGGE band patterns and a slight decrease in the fluid sulphate concentration from 200 mg l^{-1} down to 164 mg l^{-1} from August till November.

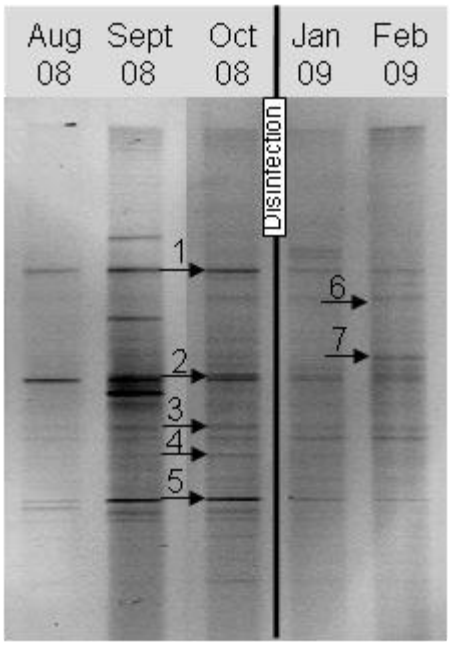

Fig. 6. DGGE-analysis of PCR-amplified *dsrB* fragments of sulphate reducing bacterial community from fluid samples taken at the topside facility from August 2008 till February 2009. Arrows indicate the positions of bands that were sequenced to identify the species of microorganisms and to conclude its metabolic capabilities.

Tab. 5: Phylogenetic affiliation of partial bacterial 16S ribosomal RNA gene sequences from SRB-specific DGGE-profiles of fluid samples from the topside facility

Band	Domain	Closest relative, (Genbank accession number)	Similarity [%]	Genbank accession number
1	Bacteria	Uncultured sulphate-reducing bacterium clone LGWI06, (EF065053)	82	HQ690807
2		Uncultured sulphate-reducing bacterium clone LGWN07, (EF065094)	85	HQ690808
3		Uncultured sulphate-reducing bacterium clone UMTRAdsr826-5, (AY015614)	92	HQ690809
4		Uncultured sulphate-reducing bacterium clone LGWK18, (EF065071)	88	HQ690810
5		Uncultured sulphate-reducing bacterium clone LGWK18, (EF065071)	94	HQ690811
6		Uncultured sulphate-reducing bacterium clone LGWG25, (EF065022)	80	HQ690812
7		Uncultured sulphate-reducing bacterium clone Dikom_E8, (FJ648430)	87	HQ690813

RNA ribonucleic acid, SRB sulphate reducing bacteria, DGGE denaturing gradient gel electrophoresis

The following period of minimal filter lifetimes with finally 350 h compared to maximal 2,500 h corresponded with increasing cell numbers up to 9.8 x 10^5 cells per ml. The microbial community was characterized by the predominance of gammaproteobacterial sulphur-oxidizing Gamma-*Proteobacteria* of the genus *Thiothrix* (bands 6, 11-14) and ammonia-oxidizing *Nitrosospira* species (band 10). Relatives of the genus *Nitrosospira* appear very abundant in anoxic marine sediments (Freitag and Prosser 2003), are often related to nitrogen contamination in groundwater environments and use nitrite as electron acceptor (Ivanova et al. 2000, Kampschreur et al. 2006, Miller and Smith 2009, Reed et al. 2010). Strains of *Thiothrix* are characterized by ensheathed filaments that may attach to substrates with slimelike holdfasts and form rosettes (Brigmon and de Ridder 1998). McGlannan and Makemson (1990) revealed a predominance of *Thiothrix* species in a habitat under favorable conditions, being nearly a monoculture. The capability of outcompete other bacteria probably allowed the predominance of *Thiothrix* in the studied cold store. In addition, *Thiothrix* sp. were detected in flowing water, containing sulphide concentration of at least 0.1 mg l^{-1}, less than 10 % oxygen and with neutral pH, like cave water (Macalady et al. 2008), wastewater treatment systems (Farquhar and Boyle 1972, Nielsen et al. 2000, Eikelboom and Geurkink 2002), and in a natural spring and municipal water storage tank (Brigmon et al. 2003). In all these systems *Thiothrix* caused problems due to sludge bulking by the formation of white

filamentous biofilms. Biofilms containing *Thiothrix* induced biofouling and led to physical blockage of water pipes and other groundwater processing equipment like agricultural irrigation systems and spring water bottling plant filters (Ford and Tucker 1975, Brigmon et al. 1997). Consistently, this predominance of *Thiothrix* may have led to the drastically reduced filter lifetimes of the investigated cold store. Large quantities of long filaments (> 100 µm), known from *Thiothrix* sp., forming radial rosettes, were visualized by DAPI-staining and SEM-analysis, especially in the filter samples, anticipating any quantification (Fig. 7A).

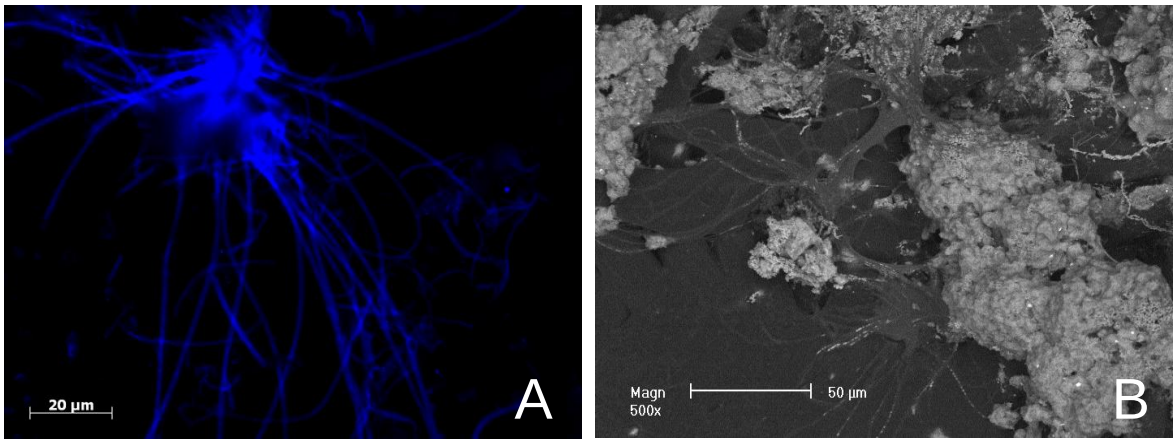

Fig. 7. Fluorescence microscopic image of DAPI-stained filamentous cells in a filter bag from September 2008 (A), SEM-image of iron hydroxide crusts (light grey), wrapped with filaments (mid grey) in a filter bag (dark grey) from August 2008 (B).

The reasons for changing conditions favoring the growth of *Thiothrix* are not clear because no relation to plant operation, fluid recharge and discharge processes in particular, could be ruled out. However, since temperatures in winter 2007/2008 were relatively mild with 8.7 °C on average, elevated temperatures in the wells might have influenced the microbial interactions, resulting in changed microbial composition and enhanced growth of *Thiothrix* sp. that has been detected already in well fluids at this site in 2006. In addition, the shift to oxidative processes indicates changes in the chemical fluid composition, although significant changes in e.g. concentrations of electron acceptors like nitrate or oxygen were not observed by chemical monitoring of the process water. If the chemical compounds are present only for a short time in the fluid they can be hardly detected by a 1-month interval sampling. However, biological systems may reflect temporary changed conditions. This is may be enhanced due to intracellular storage of metabolic relevant compounds.
In contrast, the composition of sulphate reducing bacteria did not change significantly during reduced filter lifetimes. However, metabolism of the sulphate reducing bacteria probably contributed to the filter clogging by formation of iron sulphide deposits, detected in filter bags of the topside facility by SEM-analyses (Wolfgramm et al. 2010). In addition, the produced sulphids were probably substrates for the filamentous *Thiothrix*. Beyond that, Wolfgramm et al. (2010) reported an increase in the relative percentage of sulphidic precipitations in filter materials since plant start-up.
To re-establish the injectivity of the wells and to recover filter lifetime as in time before failure, a disinfection treatment with the reactive oxidative hydrogen peroxide (H_2O_2) was conducted. After this chemical plant treatment heterotrophic bacteria, in particular *Flavobacteria* (*Flavobacterium* sp., bands 15, 18-19) and Beta-*Proteobacteria* (*Propionivibrio* sp., band 17) became predominant, whereas Epsilon-*Proteobacteria* and *Thiothrix* sp. were not detected any longer. Again, the banding pattern of sulphate reducing bacteria was not influenced by the disinfection treatment. The treatment with hydrogen peroxide probably disturbed the biofilms of *Thiothrix* sp. in plant processing equipment and recovered normal filter lifetimes. In addition, reduced sulphide minerals became oxidized, preventing any subsequent precipitation and withdrawing the substrate for sulphur-oxidizing *Thiothrix* sp.. The slight decrease in total bacterial counts to 1.7 x 10^5 cells per ml fluid demonstrated the efficiency of the disinfectant and the die-off of microorganisms. In drinking water installations the disinfection with chlorine, ozone, and UV have been also successfully tested for the control of microbes and biofouling (Smith 2002). However, the detection of microorganisms after a disinfection treatment, especially species of *Flavobacteria*, is documented in several studies. Kim et al. (2000) demonstrated a hydrogen peroxide

resistance of *Flavobacterium* sp. in a high-purity water system. This insensitivity against the strong oxidative agent is probably based on the enzyme apparatus of this genus. *Flavobacterium* sp. is catalase positive and thus able to degrade hydrogen peroxide to water and oxygen (Holmes et al. 1984). In addition, *Flavobacterium* sp. was tolerant to other disinfectant chemicals, like chlorine and chloramines, that caused the die-off of microorganisms in a drinking water reservoir (Wolfe et al. 1985). In this context, *Thiothrix* sp. is catalase negative and was not detected after the treatment with hydrogen peroxide anymore. Furthermore, *Propionivibrio* sp. was detected in chlorine treated drinking water (Williams et al. 2004). To our knowledge, up to now, there are no studies presenting resistance of *Propionivibrio* sp. against hydrogen peroxide treatment, but based on our results and the resistance against the oxidative disinfectant chlorine one can assume this. In accordance with the unaffected abundance of SRB, catalase activity was also detected in sulphate reducing bacteria, allowing hydrogen peroxide elimination and survival at oxygen exposure (Cypionka et al. 1985, Dolla et al. 2006). Further investigations focusing the effectiveness of disinfectants and certain microbial insensitivities in groundwater systems are required.

3. Conclusions

This study demonstrates that microbes may have a considerable influence on the reliability and operating lifetime of a geothermal plant that is used for energy cold storage. Molecular biological monitoring was able to identify the dominant microorganisms, especially involved in metabolic iron- and sulphur-cycling. The interpretation of shifts in the microbial community composition, correlated with precipitated minerals, supported the plant operation under the aspect of problem identification and countermeasures. However, changes in chemical parameters could not be indicated by monitoring of process water. *Thiothrix* served as an as indicator organisms for increased filter clogging in the topside facility as well as in the injection wells, probably due to slight changes in the availability of electron acceptors and more favourable temperature conditions. More detailed monitoring of environmental conditions during process failure and quantification of active cells is planned for the future to further increase process understanding.

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