Lerm, S., Westphal, A., Miethling-Graff, R., Alawi, M., Seibt, A., Wolfgramm, M., Würdemann, H. (2013): Thermal effects on microbial composition and microbiologically induced corrosion and mineral precipitation affecting operation of a geothermal plant in a deep saline aquifer. - Extremophiles, 17, 2, 311-327

DOI: 10.1007/s00792-013-0518-8

Stephanie Lerm[1], Anke Westphal[1], Rona Miethling-Graff[1], Mashal Alawi[1], Andrea Seibt[2], Markus Wolfgramm[3], Hilke Würdemann[1#]

Thermal effects on microbial composition and microbiologically induced corrosion and mineral precipitation affecting operation of a geothermal plant in a deep saline aquifer

[1] Helmholtz Centre Potsdam GFZ German Research Centre for Geosciences, International Centre for Geothermal Research ICGR, Telegrafenberg, D-14473 Potsdam, Germany
[2] Boden Wasser Gesundheit GbR. (BWG), D-17041 Neubrandenburg, Germany
[3] Geothermie Neubrandenburg (GTN), D-17041 Neubrandenburg, Germany

Corresponding author. Mailing address: Helmholtz Centre Potsdam GFZ German Research Centre for Geosciences, International Centre for Geothermal Research ICGR, Telegrafenberg, D-14473 Potsdam, Germany. Phone: 0331/288-1516. Fax: 0331/2300-662. E-mail: wuerdemann@gfz-potsdam.de.

ABSTRACT

The microbial diversity of a deep saline aquifer used for geothermal heat storage in the North German Basin was investigated. Genetic fingerprinting analyses revealed distinct microbial communities in fluids produced from the cold and warm side of the aquifer. Direct cell counting and quantification of 16S rRNA genes and dissimilatory sulfite reductase (*dsrA*) genes by real-time PCR proved different population sizes in fluids, showing higher abundance of bacteria and sulfate reducing bacteria (SRB) in cold fluids compared with warm fluids. The operation-dependent temperature increase at the warm well probably enhanced organic matter availability, favoring the growth of fermentative bacteria and SRB in the topside facility after the reduction of fluid temperature. In the cold well, SRB predominated and probably accounted for corrosion damage to the submersible well pump, and iron sulfide precipitates in the near wellbore area and topside facility filters. This corresponded to lower sulfate content in fluids produced from the cold well as well as higher content of hydrogen gas that was probably released from corrosion, and maybe favored growth of hydrogenotrophic SRB. This study reflects the high influence of microbial populations for geothermal plant operation, because microbiologically induced precipitative and corrosive processes adversely affect plant reliability.

Introduction

As subsurface structures have been increasingly investigated, it has become evident that phylogenetically and physiologically diverse microorganisms are widely distributed throughout deep terrestrial and marine environments and catalyze a broad range of geochemical reactions. These reactions are related to the degradation of organic matter (Parkes et al. 1994, 2000; Whitman et al. 1998; Wellsbury et al. 2002), rock and mineral weathering and alteration (Rogers et al. 1998; Bennett et al. 2001; Shock 2009; Gadd 2010), quality of groundwater (Goldscheider et al. 2006; Wilson et al. 2006; Griebler and Lueders 2009), and failures in engineered subsurface systems caused by corrosion and scaling (Van Hamme et al. 2003; Valdez et al. 2009; Javaherdashti 2011). Geothermal gradients in the terrestrial crust range from approximately 10°C km^{-1} to 60°C km^{-1} (Philpotts 1990). In addition, in certain regions, such as the Mesozoic deep waters developed in northeast Germany, aquifers are classified as high-salinity Na-Ca-Cl-waters (Wolfgramm et al. 2011). Elevated temperature and high salinity create harsh living conditions for microbes. Only specific microorganisms subsist at these elevated temperatures and high salinity due to modifications in proteins, DNA, and cell membrane composition as well as intracellular accumulation of low molecular compounds (Aerts et al. 1985; Kempf and Bremer 1998; Kumar and Nussinov 2001; Roberts 2005). Moreover, some microorganisms are able to get into a dormancy stage by forming spores, cysts or other types of resting cells and survive starvation, exposure to extreme temperatures, and elevated background radiation (Burke and Wiley 1937; Amy 1997; Suzina et al. 2004; Johnson et al. 2007).

The exploitation of sedimentary geologic formations in the North German Basin (NGB) has provided suitable conditions for temporary aquifer heat and cold storage (ATES aquifer thermal energy storage) (Schmidt et al. 2004). The regional groundwater system in the NGB can be subdivided into upper quaternary and tertiary fresh water and several deep Mesozoic salt water systems that are frequently separated by different aquitards. In recent years, several geothermal facilities have been installed and are currently used for ATES, developing the freshwater and saline groundwater zone (Seibt and Kabus 2006).

Highly mineralized geothermal fluids with an excess of sulfate tend to scale formation (sulfide, carbonate, silica) in the reservoir, pipelines and topside structures when brine is cooled in the course of fluid production and energy extraction (Skinner et al. 1967; Dalas and Koutsoukos 1989; Gallup 2002). Furthermore, chemically and microbially induced corrosion additionally occurs in plant infrastructure and adversely affects plant operations and commercial benefits (Gallup 2009; Valdez et al. 2009; Miranda-Herrera et al. 2010). Therefore, the detrimental effect of microbes on power plant components has increasingly garnered attention. Indeed, several studies have been conducted to identify the organisms responsible for phenomena such as scaling, biofouling, and corrosion of installed iron or steel in groundwater wells and geothermal plants (Taylor et al. 1997; Sand 2003; Cullimore 2007; Little and Lee 2007; Valdez et al. 2009) to reduce these sources of failure, plant downtime, and the cost-intensive replacement of plant components. For example, the effectiveness of groundwater wells can be influenced by scaling, as iron and other metallic cations are enriched by precipitation that leads to the formation of amorphous or crystalline structures. Biofouling refers to the accumulation of microorganisms that form complex biofilms that include mineral deposits and adversely affect the hydraulic characteristics of water flow (Howsam 1988; Cullimore 1999). Microbiologically influenced corrosion (MIC) usually occurs with various types of corrosion and with scaling (Little et al. 1996; Valdez et al. 2009). Studies focusing on biofilms in several industrial water systems reported extensive biofouling and associated mineral precipitation leading to reduced efficiency of heat exchangers and reduced flow rates in the piping (Characklis 1990; Flemming 2002; Demadis 2003; Coetser and Cloete 2005).

Marine scientists and the petroleum industry rapidly recognized that phylogenetically diverse anaerobic sulfate reducing bacteria (SRB) are crucial for the degradation of organic matter in terrestrial and aquatic subsurface environments (Bastin et al. 1926; Jørgensen 1982; Magot et al. 2000; D´Hondt et al. 2002; Sass and Cypionka 2004) by using sulfate as a terminal electron acceptor, thereby producing hydrogen sulfide (H_2S). Hydrogen sulfide is a toxic and corrosive gas that leads to a variety of environmental and economic problems, including reservoir souring, corrosion of metal surfaces, and the plugging of reservoirs due to the precipitation of metal sulfides (Magot et al. 2000). Therefore, SRB are considered important biocatalysts for MIC. Besides mackinawite and pyrrhotite, one of the most common metal sulfides is pyrite (FeS_2), which is produced when microbially generated sulfide reacts with ferrous iron (Fe^{2+}) that is present in natural water or released from corroded steel (Hamilton 1985; Morse et al. 1987). Based on research into these phenomena, specific chemicals are recommended for well regeneration and are used as inhibitors when continuously injected into geothermal installations (Videla 2002; Akpabio et al. 2011). Furthermore, new casing materials are used. Although biocorrosion is a common problem, it is site-specific and depends on fluid temperature as well as on the chemical conditions such as salinity and dissolved organic carbon (DOC)-content, and the abundant microbial community (Valdez et al. 2009). An understanding of the underlying mechanisms requires knowledge of the carbon and energy sources that support biofilm microorganisms and catalyze such activities. Thus far, only few studies focused on the microbial processes that occur in geothermal plants in the context of microbiologically mediated operational failure (Sand 2003; Pryfogle 2005; Alawi et al. 2011; Lerm et al. 2011a, b). As microorganisms are directly influenced by their environment, they can be used as bioindicators (Avidano et al. 2005; Steube et al. 2009) while reflecting changes in reservoir and topside conditions caused by aquifer utilization.

In this study, the microbial community structure in a deep saline aquifer in the NGB that is used for temporary aquifer heat storage was investigated. The operation of the geothermal plant which comprises of seasonal heat discharge and recharge processes is expected to be affected by microbial activities as well as by chemical reactions of the aquifer's minerals and organic matter. Therefore, we focused on the microbial and chemical processes that occur in the aquifer with respect to the operation of the geothermal plant. Direct cell counting, genetic fingerprinting, and real-time PCR based on 16S rRNA genes and genes encoding the dissimilatory sulfite reductase β-subunit (*dsrB*) of SRB were used for the detection and quantification of microorganisms abundant in fluids, to gain information on the conditions and processes occurring downhole. We were particularly interested in monitoring the presence, biodiversity and dynamics of SRB at this site, because they are known to be involved in sulfide precipitation and corrosion and, therefore, may significantly influence plant operation. Besides gas chromatographic determination of the gases dissolved in aquifer fluid, geochemical analyses were used to identify inorganic and organic substrates that are relevant as energy and carbon sources for microorganisms and products of microbial metabolism. Scanning electron microscopy (SEM) analyses of mineral scales from topside facility filters provided information on aquifer minerals and were useful for detecting microbiologically induced mineral scales.

Materials and methods

Site description and plant design

The investigated aquifer was located in Neubrandenburg (North German Basin, Germany) and has been used for seasonal heat storage since 2005. The water-bearing sandstone formation is situated at a

depth of 1,228 m - 1,268 m and is developed by a geothermal doublet with an internal distance of 1,300 m between wells GtN 1/86 and GtN 4/86.

The original aquifer fluid temperature amounted to 54°C. During geothermal plant operation fluid temperature around well GtN 1/86 increased, because during the summer (April till November) fluid was produced from well 4/86, charged with surplus heat from the local gas and steam power station, and then injected into the warm well in the aquifer (recharge mode). This area is termed the "warm side". Until September 2009, fluids at 80°C were injected to the warm well; subsequently the injection temperature was raised to 85°C. In addition, the operation of the plant caused a temperature decrease at the other well, GtN 4/86. This side is termed the "cold side". A temperature decrease in the aquifer fluid was caused, because during the winter (November till April), fluid with a temperature from 65°C to 80°C was produced from the warm well, used for district heat supply, and was then injected into the cold well with a temperature of 45°C to 54°C (discharge mode).

Thus, during plant operation, the direction of fluid flow changed seasonally in April and November. In one season, between 200,000 m³ and 400,000 m³ of fluid was circulated, with an average flow rate of 80 $m^3 h^{-1}$. The distribution of the quantities of the charged and discharged heat was not balanced; since plant startup the recharging period has lasted approximately 7.5 months per year and the discharging period has lasted approximately 4.5 months per year. Filter systems are installed upstream of the heat exchanger at the topside facility to retain solid particles transported with the production flow from the aquifer. Due to the different flow directions both wells are equipped with pumps, production and injection pipes, and a filter system (Fig. 1). The wells and the topside facility were kept under nitrogen pressure (~ 10 bar) to prevent precipitation of iron oxides or hydroxides, and carbonate minerals due to oxygen intrusion and degassing processes. Further information concerning plant operations and energetic aspects are given in Kabus and Wolfgramm (2009) and Obst and Wolfgramm (2010). Since the plant startup in 2005, three cycles of operations, including fluid discharge and recharge were completed. In 2008, plant operation was impaired due to corrosion damage to a submersible pump in the cold well that caused eight month of plant downtime.

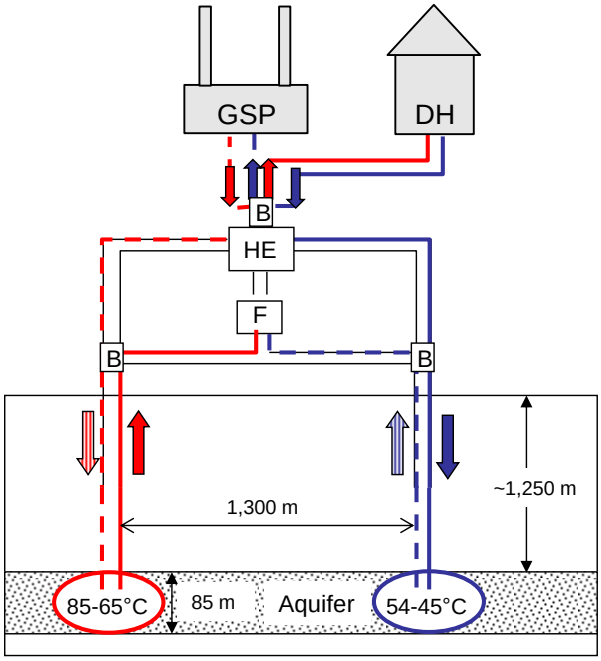

Fig. 1 Principle scheme of the ATES used for heat storage with the location of sampling devices for fluid (B, bypass) and filter (F) at the topside facility. The *arrows* indicate the fluid flow direction during recharge (summer, dashed line) and discharge (winter, plane line) mode. GSP Gas and steam plant, DH District heating, HE Heat exchanger

Sample collection

Fluid and filter samples were collected over a period of two years at the topside facilities of the two wells via a bypass and from filter devices. It should be noted that the plant is managed by the public utilities of the city Neubrandenburg and is not operating for research purposes. Thus, the access to samples, particularly filter samples, was partly restricted and samples were taken every few months and not in general at the production and injection well at the same time.
By investigating fluid and filter samples we minimized the risk of detecting only the suspended free cells because filters represent an appropriated surface on which microbes form biofilms. The bypasses are located upstream of the filter devices for each well. Each filter device contains 2 x 4 filters (EATON DURAGAF POXL 1 P02E 20l) with a 1-micron rating that are regularly replaced after a definite volume of fluid which has passed through the filter (termed filter lifetime). Filters were replaced more often and independent from the fluid volume in case of increased injection pressure caused by high particle loading rate, e.g., after plant restart. In addition to fluid sampling, filter samples were taken. Fluid samples were collected in sterile 1 liter Schott Duran glass bottles.

Genetic fingerprinting

Characterization of bacterial communities in fluid and filter samples was done by AMODIA Bioservice GmbH (Braunschweig, Germany), including filtration of 1 liter fluid on a 0.22 µm cellulose acetate filter (Sartorius, Goettingen, Germany), and using single strand conformation polymorphism (SSCP) fingerprinting of PCR-amplified 16S rRNA gene fragments according to Schwieger and Tebbe (1998) and Dohrmann and Tebbe (2004). Portions of the filter sample taken at the topside facility were processed in parallel with the fluid samples. Due to long-term monitoring, genetic profiles were generated individually for each sample and subsequently arranged. Partial sequences of the 16S rRNA genes were amplified by PCR using the universal bacterial primers F519 and R926-ph (Schwieger and Tebbe 1998). DNA concentrations of samples have not been adjusted during PCR procedures to approximately equalize the DNA yields in the samples in order to use SSCP-profiling as a semi-quantitative approach.
For the specific analysis of the diversity of SRB denaturing gradient gel electrophoresis (DGGE) fingerprinting was performed (Muyzer and Smalla 1998). Total genomic DNA was extracted from filters using the FastDNA™ Spin Kit for Soil (MP Biomedicals, Santa Ana, USA) with protocol modifications, including gentle shaking of samples in lysis buffer to dissolve cells from cellulose acetate filters, longer period of time for DNA elution from matrix, followed by a higher final centrifugation step. Concentration of extracted DNA was determined fluorometrically (FLUOstar OPTIMA, BMG Labtech, Ortenberg, Germany). Genes encoding the dissimilatory sulfite reductase β-subunit (*dsrB*) of SRB were amplified using the primer set DSR2060F-GC/DSR4R (Geets et al. 2006). DGGE was performed at 59°C with a denaturant gradient from 40 % to 75 %. DNA in polyacrylamide gels was visualized by silver staining (Bassam et al. 1991). Nucleotide sequences obtained by DNA sequencing were aligned using the ARB package ARB (Ludwig et al. 2004) and were compared using the Basic Local Alignment Search Tool (BLAST) of the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/). The sequences analyzed in this study have been deposited in the EMBL database of the European Bioinformatics Institute (www.ebi.ac.uk/embl) under the GenBank accession numbers JQ291307 - JQ291353 (16S rRNA gene sequences) and JQ411234 - JQ411236 (*dsr* gene sequences).

Quantification of total bacterial 16S rRNA genes and dsrA genes by real-time PCR

Abundances of bacteria and SRB were determined in six samples taken from the warm and cold well in January 2009, September 2009, and October 2009 by quantitative real-time PCR (qPCR) analysis of 16S rRNA genes and *dsrA* genes, respectively, using a StepOnePlus™ real-time PCR System (Applied Biosystems, Carlsbad, CA, USA). Real-time qPCR was performed using Power SYBR® Green PCR Master Mix (Life Technologies). For the total Eubacteria quantification the primers 338F and 805R (Yu et al. 2005) were used for the 16S rRNA gene fragment, and cloned 16S rRNA gene from *Escherichia coli* (JM109) as a standard. Total SRB were quantified using the primers DSR1F (Wagner et al. 1998) and DSR500R (Wilms et al. 2007) for amplifying the *dsrA* operon, and cloned *dsrAB* genes from *Desulfotomaculum geothermicum* (DSMZ 3669) as a standard. Each 20 µL PCR reaction contained 10 µL Life Technologies Power SYBR Green, 0.2 µM of each primer, 10 µg BSA and 1 µL of template DNA. Thermal cycling included an initial denaturation step for 10 min at 95°C followed by 40-50 cycles of amplification with following parameters 10 s at 95°C, 20 s at 58°C, and 30 s at 72°C. After the run, a melting curve was recorded between 58°C and 95°C to discriminate between specific amplicons and unspecific fluorescence signals. Real-time PCR was performed three times for each sample to verify the results.
For standard curves, total DNA of *E.coli* (JM 109) was extracted using the manufacturer´s protocol of the FastDNA™ Spin Kit for Soil (MP Biomedicals, Santa Ana, USA). Total genomic DNA of *Desulfotomaculum geothermicum* (DSMZ 3669) was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). Nearly full-length 16S rRNA genes and *dsrAB* genes were PCR amplified from extracted DNA with the primers 27F/1492R (Lane 1991), and DSR1F/DSR4R (Wagner et al. 1998), respectively. After purification of PCR products using the Fermentas GeneJET™ PCR Purification Kit (THERMO Fisher Scientific, Waltham, USA), PCR products were subsequently ligated into the pGEM-T vector and transformed into competent *E. coli* (JM109) cells (pGEM-T Cloning Kit, Promega, Mannheim, Germany) according to the manufacturer`s instructions. Colonies with inserts were selected by blue-white screening. The plasmids containing the target DNA fragments were checked by sequencing. DNA concentration of plasmid and plasmid dilutions ranging from 10^{-1} to 10^{-8} served as template for qPCR standard curves. The detection limit of the method was 3×10^{1} genes l^{-1} for the bacterial 16S rRNA genes and 2×10^{2} genes l^{-1} for the *dsrA* genes.

Direct cell counting

Alternatively to qPCR, the total number of bacteria in the fluids produced from the warm and cold side of the aquifer was determined in random samples taken in January 2010 and February 2011, respectively, using direct microscopic cell counting. The commissioned laboratory MicroPro GmbH (Gommern, Germany) used an improved Neubauer counting chamber (depth 0.1 mm). Due to the high salinity in fluids, the detection limit amounted to 10^{4} cells ml^{-1}.

Geochemical and mineralogical analyses

The residues in the filters were analyzed using a scanning electron microscope (Cambridge S200) with energy dispersive X-ray spectroscopy (SEM-EDX). The redox potential, pH, and fluid temperature were determined during the sampling procedures using a pH/mV/temperature meter (WTW, Weilheim, Germany). Vetter et al. (2012) measured the concentrations of the dissolved low-molecular-weight organic acids (e.g., acetate) in the fluids using ion chromatography (ICS 3000, Dionex Corp.) as previously described by Vieth et al. (2008). In particular, we focused on the ions participating in

processes related to the detected microorganisms. The sulfate and iron content were quantified by inductively coupled plasma mass spectrometry (ICP-MS) according to DIN EN ISO 17294-2 and by ion chromatography according to DIN EN ISO 10304-2, respectively. Fluid-soluble anions and cations were determined including ion balance calculations. The oxygen concentration was determined using an electrode installed in a flow-through chamber for measurements in a continuous-flow environment and to improve the detection limit (0.01 ml l^{-1}). Gases such as nitrogen, carbon dioxide, methane, hydrogen, hydrogen sulfide, and helium were released from the fluid on-site using a mobile degasser, and the gas volume was subsequently quantified using a drum-type gas meter. The gas compositions were determined in the lab using a gas chromatograph. Further details concerning gas measurements in geothermal fluids are given in Seibt and Thorwart (2011).

Results

Geochemical characterization of fluids

The hydrochemical parameters of the investigated process fluids are summarized in Table 1. The fluid with a slight acidic pH had a mineralization of 130 - 134 g l^{-1}, with sodium and chloride concentrations of 50 (± 1) g l^{-1} and 80 (± 2) g l^{-1}, respectively. Additional components were calcium, magnesium, potassium, and hydrogen carbonate in significant lower proportions. Aquifer fluid was characterized by a high sulfate concentration (900 - 1,000 mg l^{-1}); whereas the sulfate content of fluids originating from the cold side of the aquifer was approximately 100 mg l^{-1} lower than the fluids from the warm side of the aquifer. Remarkably, at the beginning of the discharge period the sulfate content of fluids produced from the warm well was also approximately 100 mg l^{-1} lower (Fig. 2). The DOC content was approximately 3.5 mg C l^{-1}. Small amounts of the short-chain organic acids formate and acetate were detected, ranging between 0.1 mg l^{-1} and 1.9 mg l^{-1} (Vetter et al. 2012). The redox potential of less than -50 mV of the fluid represented anoxic, reduced conditions. No oxygen was detected in the fluids. The total gas content of the fluids amounted to 7 %, while carbon dioxide (86.2 vol.-%) and nitrogen (13.8 vol.-%) were the predominant gases. The hydrogen content of the fluids produced from the warm and cold well of the aquifer was approximately 0.03 vol.-% and 0.21 vol.-%, respectively, and trace quantities of methane were only detected in fluids produced from the cold well. In addition, hydrogen sulfide was only detected in fluids produced from the cold well, with average values of 0.2 vol.-%.

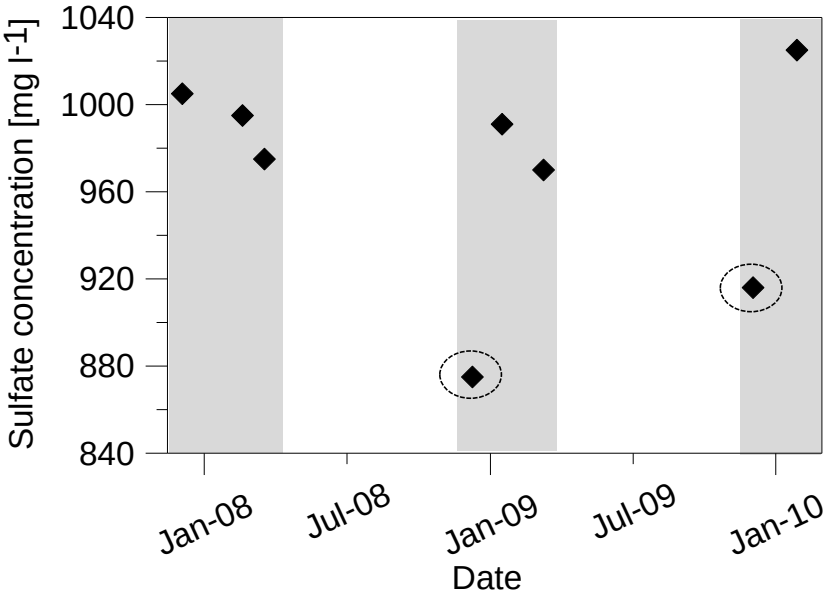

Fig. 2 Seasonal course of sulfate concentration in fluids produced from the warm well. *Circles* indicate artifact sulfate values at the beginning of the discharge mode (as marked by the grey-shaded area) caused by previous injection of fluids produced from the cold well

Table 1 Physico-chemical fluid characteristics

Reservoir rock	Depth [m]	Temperature [°C]	pH	Mineralisation [g l^{-1}]	Redox potential SHE [mV]	DOC [mg C l^{-1}]	Sulfate [mg l^{-1}]	Ferrous iron [mg l^{-1}]	Gases [vol.-%]
sandstone	1,250 - 1,335	45-54^{c} 65-90^{w}	6.0	131	< -50	~3.5	900^{c} 1,000^{w}	15 (±2)	CO_2 ~86 $^{c, w}$ N_2 ~14 $^{c, w}$ H_2 ~0.2 ^{c}/ 0.03 ^{w} H_2S ~0.2 ^{c}/ b.d.l.^{w} CH_4 0.09 ^{c}/ b.d.l.^{w}

SHE standard hydrogen electrode, DOC dissolved organic carbon, well-specific values indicated with ^{c} cold well, ^{w} warm well, b.d.l. below detection limit

Mineral precipitates

Reservoir materials mainly consisted of quartz, feldspar, and clay minerals (kaolinite). In fluid produced from the warm well, 0.001 g m^{-3} of solids was retained in filters from the topside facility. The majority (80 %) of mineral precipitates in these filters consisted of calcium carbonate crusts (Fig. 3a) and thin crusts of iron sulfides. Accessory minerals included nickel and copper sulfides, chalcopyrite (Fig. 3b), as well as the reservoir materials quartz and clay. After heat extraction at the heat exchanger and cooling of the fluid to approximately 46°C, the mineral precipitates accumulated in filters at the cold well were dominated by iron sulfide, forming crusts with more than 100 μm in size (Fig. 3c).

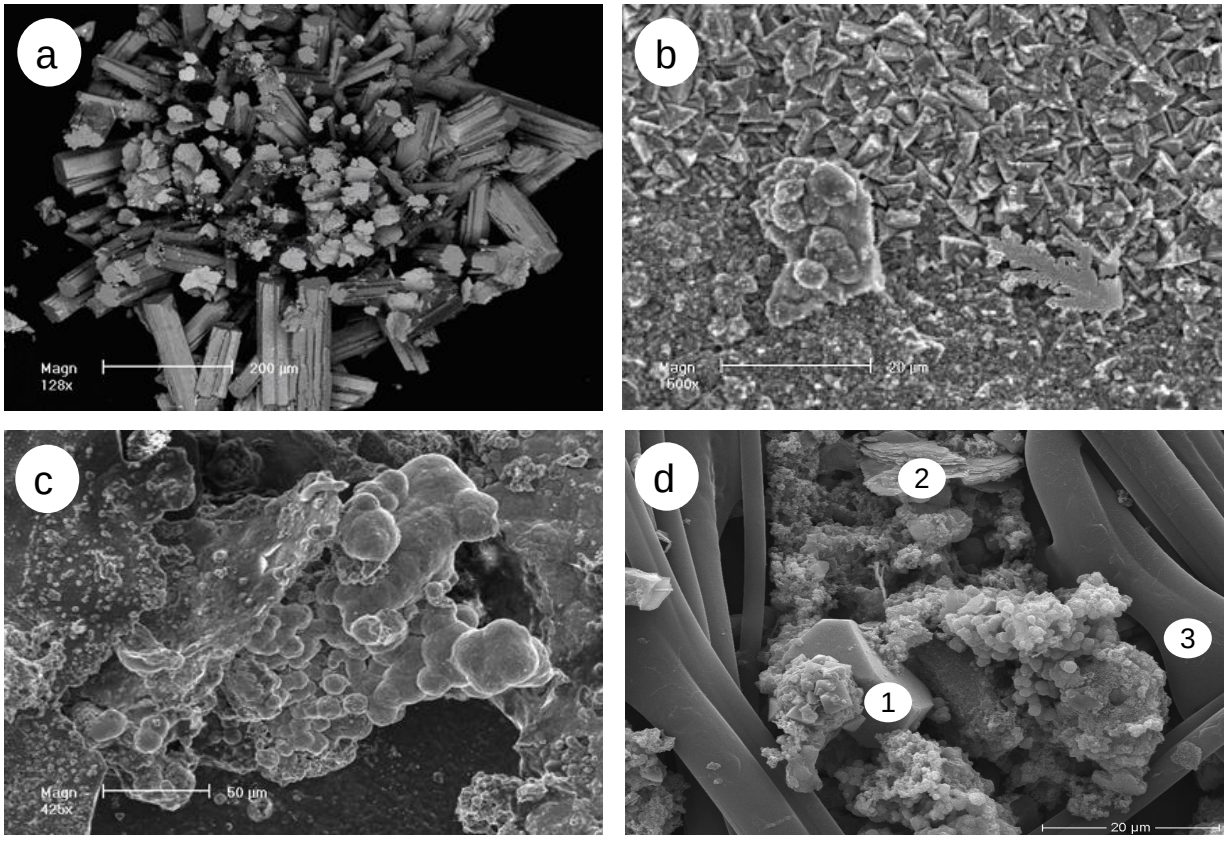

Fig. 3 SEM images of minerals in filter residues (**a)** Sticks of calcium carbonate ($CaCO_3$) and (**b)** surface of a chalcopyrite ($CuFeS_2$) crust found in filter residues before heat extraction. (**c)** Iron sulfide crusts found in filter residues after heat extraction. (**d)** Idiomorphic iron sulfides (1) with detrital clay minerals (2) found in filter residues before fluid heating, filter fibers (3).

Remarkably, 1 g m^{-3} of solids was retained in the topside facility filters from the cold well at the beginning of the recharge mode, whereas 0.01 g m^{-3} of solids was transported in the production flow at the end of this operation mode after a longer period of fluid production. The comparative high content of solids led to a reduced filter lifetime in the topside facility. The main mineral residue (~ 90 %) was iron sulfide (FeS), with a particle size of less than 1 µm (Fig. 3d). The minority of mineral residues consisted of rust, calcium carbonate, and reservoir materials. After heat supply at the heat exchanger, the mineral precipitates in the topside facility filters from the warm well were also almost exclusively composed of iron sulfide. Scale formation in the aquifer was previously described by Wolfgramm and Seibt (2006) and Obst and Wolfgramm (2010).

Abundance of bacteria and SRB in geothermal fluids

16S rRNA and *dsrA* gene copy number as a quantitative measure of bacteria and SRB abundance were determined from fluid samples originating from the warm and the cold well (Fig. 4). The total bacterial abundance in fluids produced from the warm well was in the range of detection limit (Fig. 4a); however, 1 x 10^6 gene copies $liter^{-1}$ were determined in fluids produced from the cold well (Fig. 4c). Bacterial gene copy number in fluid originating from the warm well and been cooled at the heat exchanger amounted to 1 x 10^7 copies $liter^{-1}$ (Fig. 4b). By contrast, in fluid produced from the cold well and heated up at the heat exchanger 1 x 10^4 gene copies $liter^{-1}$ were determined (Fig. 4d). The copy numbers of *dsr* genes were up to one order of magnitude lower than the copy numbers of 16S rRNA genes.

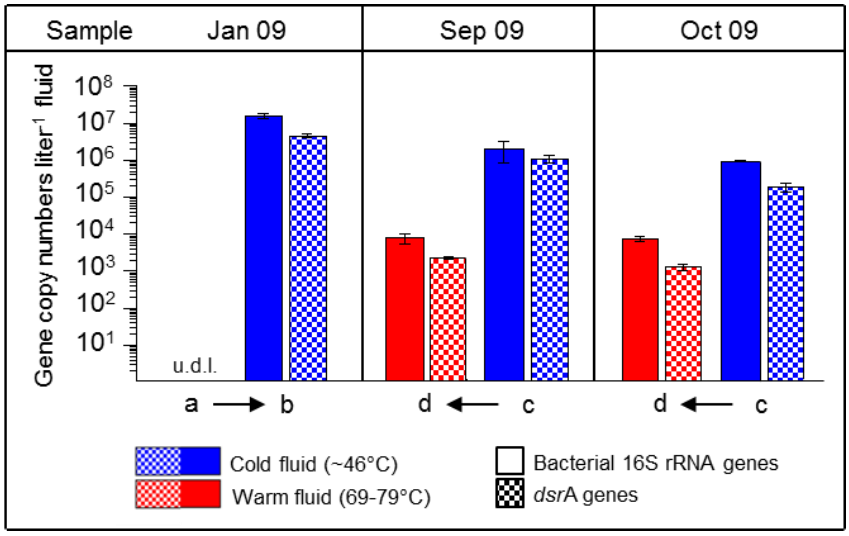

Fig. 4 Abundance of total bacteria and SRB based on the 16S rRNA gene and *dsrA* gene in fluid samples taken from the warm and the cold well of the geothermal plant in January 2009, September 2009, and October 2009 (**a)** Fluid produced from the warm well. (**b)** Cooled fluid produced from the warm well. (**c)** Fluid produced from the cold well. (**d)** Heated fluid produced from the cold well. **u.d.l.** under detection limit. → fluid flow

Direct cell counting using a Neubauer counting chamber revealed 4 x 10^6 cells ml^{-1} in fluids produced from the cold well; while, less than 10^4 cells ml^{-1} were detected per ml of fluid produced from the warm well.

Microbial communities in fluids produced from the warm and cold well

SSCP analyses revealed complex microbial communities in fluid and filter samples taken at topside facilities from the warm and cold well over a period of two years. The genetic profiles comprised up to 20 bands that differed in abundance and intensity (Fig. 5a - d). No significant differences between the fluid and filter banding patterns were observed (profiles not shown). Therefore, fluid and filter samples were used in parallel for monitoring shifts in the microbial composition.

Sequencing revealed the presence of organisms affiliated to the *Firmicutes*, *Bacteroidetes*, *Deferribacteres*, and Alpha-, Beta- and Gamma-*Proteobacteria* which were dominant in the fluid samples produced from the warm well (Fig. 5a and Table 2). The fluid temperatures ranged between 68°C and 73°C during the study period, inducing changes in the microbial composition. In detail, the SSCP genetic profile of a fluid sample taken in March 2008 showed only a few weak bands. However, one band yielded a sequence of satisfactory quality and was affiliated to *Firmicutes* of the genus *Halanaerobium* sp. (band 1). The microbial community in a fluid sample taken after eight month of plant downtime in December 2008 was of greater diversity and five sequences were affiliated to Candidatus *Desulforudis audaxviator* (band 2), *Bacteroidetes* (band 3), *Halobacteroidaceae* (band 4), *Flexistipes sinusarabici* (bands 5, 7), and *Sphingomonas* (band 6) as dominant representatives. In March 2009 the community shifted again resulting in only weak bands. Those sequences were affiliated to microorganisms of the genera *Aquabacterium* (band 8), *Pseudomonas* (bands 9, 10), *Sphingomonas* (band 11), *Acinetobacter* (band 12), and *Bacteroidetes* (band 13). *Sphingomonas* and *Bacteroidetes* were previously detected in December 2008.

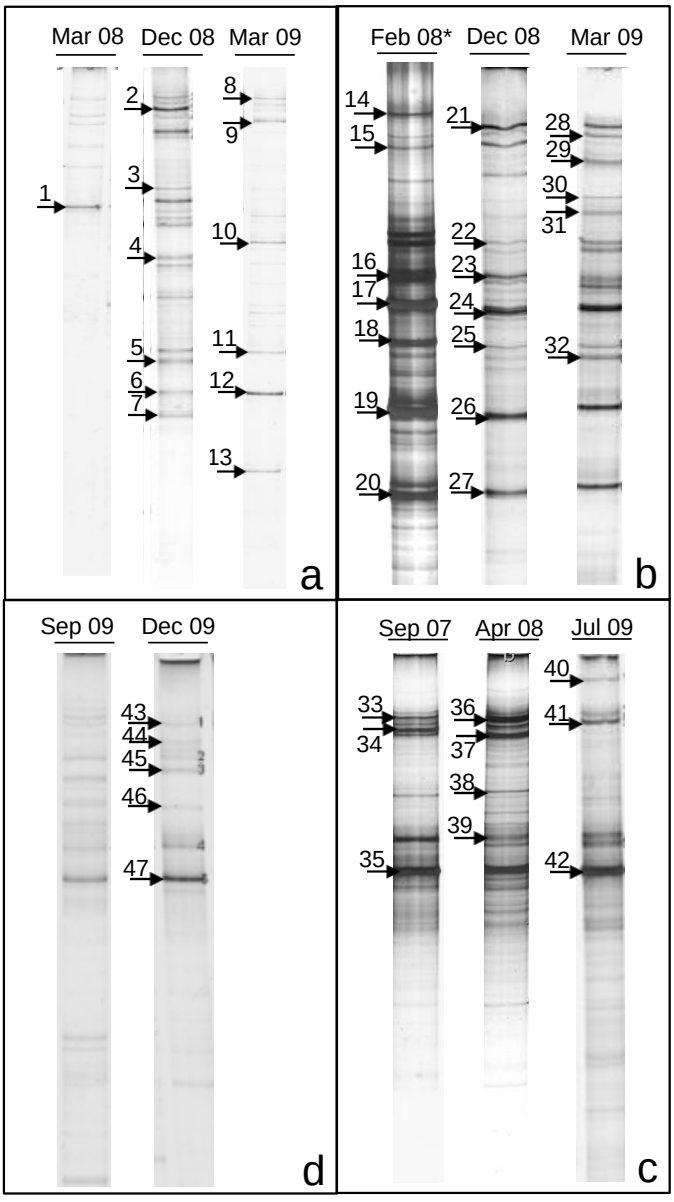

Fig. 5 Comparative SSCP-analysis of 16S rRNA gene fragments using bacterial DNA from fluid and filter (*) samples taken from sampling devices in the topside facility of the warm and the cold well from September 2007 till December 2009. Due to long-term monitoring, genetic profiles were generated individually for each sample and subsequently arranged. *Arrows* indicate the positions of bands that were sequenced. (**a)** Fluid produced from the warm well. (**b)** Cooled fluid produced from the warm well. (**c)** Fluid produced from the cold well. (**d)** Heated fluid produced from the cold well

Table 2 Phylogenetic affiliation of partial bacterial 16S ribosomal RNA gene sequences from SSCP-profiles of fluid and filter samples taken at the warm and the cold well previous and after heat supply and extraction, respectively

Sampling well	Sample	Fluid temperature [°C]	Band	Class	Closest relative, (GenBank Accession Number)	Similarity [%]	GenBank Accession Number
Fluid produced from the warm well (a)	Mar 08	72.6	1	*Firmicutes*	*Halanaerobium* sp. S191 (FJ858788)	98	JQ291307
	Dec 08	70.7	2		Candidatus *Desulforudis audaxviator* MP104C (CP000860)	95	JQ291308
			3	*Bacteroidetes*	Uncultured *Bacteroidetes* bacterium clone AS-P4-Sed-67 (FM879134)	98	JQ291309
			4	*Firmicutes*	*Halobacteroidaceae* bacterium Z7100 (HQ828140)	95	JQ291310
			5, 7	*Deferribacteres*	*Flexistipes sinusarabici* DSM 4947 (CP002858)	98, 99	JQ291311, JQ291313
			6	*Alphaproteobacteria*	*Sphingomonas* sp. T19 (HQ647266)	99	JQ291312
	Mar 09	67.6	8	*Betaproteobacteria*	Uncultured *Aquabacterium* sp. clone I-18 (AF523025)	99	JQ291314
			9	*Gammaproteobacteria*	*Pseudomonas* sp. UYSO19 (JF262574)	98	JQ291315
			10	*Gammaproteobacteria*	*Pseudomonas putida* strain HTc1 (JF703647)	98	JQ291316
			11	*Alphaproteobacteria*	*Sphingomonas* sp. T19 (HQ647266)	97	JQ291317
			12	*Gammaproteobacteria*	*Acinetobacter* sp. CmNA3 (HM352317)	99	JQ291318
			13	*Bacteroidetes*	*Bacteroidetes* bacterium WD250 (HQ341748)	98	JQ291319
Cooled fluid produced from the warm well (b)	Feb 08	46.2	14	Delta-*Proteobacteria*	*Desulfohalobium utahense* strain EtOH3 (DQ067421)	99	JQ291320
			15	*Firmicutes*	Uncultured *Halanaerobiaceae* bacterium clone TCB112x (DQ647098)	92	JQ291321
			16	*Bacteroidetes*	Uncultured *Anaerophaga* sp. clone TCB200x (DQ647171)	97	JQ291322
			17 - 20	*Bacteroidetes*	*Anaerophaga* sp. TC371 (DQ647061)	99, 98, 96, 97	JQ291323 - JQ291326
	Dec 08	46.5	21	*Deltaproteobacteria*	*Desulfohalobium utahense* strain EtOH3 (DQ067421)	98	JQ291327
			22	*Bacteroidetes*	*Anaerophaga thermohalophila* strain Fru22 (NR_028963)	99	JQ291328
			23	*Firmicutes*	*Desulfotomaculum* sp. strain NA401 (AJ866942)	89	JQ291329
			24 - 27	*Bacteroidetes*	*Anaerophaga* sp. TC371(DQ647061)	98, 98, 97, 98	JQ291330 - JQ291333
	Mar 09	48.3	28	*Deltaproteobacteria*	Uncultured *Desulfohalobiaceae* bacterium clone J2Dbac (DQ386183)	98	JQ291334
			29	*Firmicutes*	*Halothermothrix orenii* H 168	99	JQ291335
			30, 31	*Firmicutes*	Uncultured *Halanaerobiaceae* bacterium clone L5Dbac (DQ386209)	94, 90	JQ291336, JQ291337
			32	*Firmicutes*	Uncultured *Halanaerobiaceae* bacterium clone TCB112x (DQ647098)	97	JQ291338
Fluid produced from from the cold well (c)	Sep 07	45.9	33	*Deltaproteobacteria*	*Desulfohalobium utahense* strain EtOH3 (DQ067421)	98	JQ291339
			34	*Deltaproteobacteria*	Uncultured *Desulfohalobiaceae* bacterium clone J2Dbac (DQ386183)	98	JQ291340
			35	*Firmicutes*	*Desulfotomaculum* sp. strain NA401 (AJ866942)	91	JQ291341
	Apr 08	47.1	36	*Deltaproteobacteria*	*Desulfohalobium utahense* strain EtOH3 (DQ067421)	98	JQ291342
			37		Candidatus *Desulforudis audaxviator* MP104C (CP000860)	96	JQ291343
			38	*Firmicutes*	Uncultured *Halanaerobiaceae* bacterium clone L5Dbac (DQ386209)	95	JQ291344
			39	*Firmicutes*	*Desulfotomaculum* sp. strain NA401 (AJ866942)	89	JQ291345
	Jul 09	46.0	40	*Betaproteobacteria*	*Comamonas* sp. clone MZ_15 (JF690938)	100	JQ291346
			41	*Deltaproteobacteria*	*Desulfohalobium utahense* strain EtOH3 (DQ067421)	97	JQ291347
			42	*Firmicutes*	*Desulfotomaculum* sp. strain NA401 (AJ866942)	90	JQ291348
Heated fluid produced from the cold well (d)	Dec 09	74.0	43	*Betaproteobacteria*	*Comamonas* sp. clone TBA7 (FR745409)	98	JQ291349
			44	*Betaproteobacteria*	*Diaphorobacter* sp. clone QH-6 (HQ588349)	98	JQ291350
			45		Candidatus *Desulforudis audaxviator* MP104C (CP000860)	94	JQ291351
			46	*Gammaproteobacteria*	*Pseudomonas* sp. clone OPS1 (AF368760)	98	JQ291352
			47	*Firmicutes*	*Desulfotomaculum* sp. strain NA401 (AJ866942)	85	JQ291353

In contrast, fluid samples produced from the warm well with temperatures ranging between 68°C to 73°C and subsequently cooled to approximately 46°C showed a completely different microbial composition, which did not vary to the same extent as the warm fluid during monitoring (Fig. 5b). In cooled fluid samples that originated from the warm well and that were taken in February 2008, December 2008, and March 2009, relatives of *Desulfohalobium utahense* (bands 14, 21), *Desulfotomaculum* (band 23), *Desulfohalobiaceae* (band 28), *Halanaerobiaceae* (bands 15, 30-32), and *Anaerophaga* species (bands 16-20, 22, 24-27) predominated. In addition, *Halothermothrix orenii* (band 29) was detected in March 2009.
Noticeable, in fluid samples at the same temperatures (46°C) but originating from the cold well, no *Anaerophaga* species were detected. However, *Desulfohalobium utahense* (bands 33, 36, 41), *Desulfohalobiaceae* (band 34), *Desulfotomaculum* (bands 35, 39, 42), Candidatus *Desulforudis audaxviator* (band 37), *Halanaerobiaceae* (band 38), and *Comamonas* sp. (band 40) dominated in the community (Fig. 5c). The genetic fingerprints of fluid samples taken after heating in September and December 2009 and increasing the fluid temperature to 79°C and 74°C, respectively, were quite similar to fingerprinting pattern from the fluid samples taken before heating (Fig. 5c and 5d). Minor differences in profiles were mostly related to weaker bands. Furthermore, *Diaphorobacter* (band 44) and *Pseudomonas* (band 46) species were detected.
Relatives of the sulfate reducing Candidatus *Desulforudis audaxviator* were observed only once in December 2008 in a fluid sample produced from the warm well. In fluids produced from the cold well, different genera of SRB (Candidatus *Desulforudis audaxviator*, *Desulfohalobium utahense*, *Desulfotomaculum* sp.) were always detected using universal primers. The diversity of SRB in fluid samples produced from the cold well was investigated in detail by analyzing *dsrB* gene fragments. Corresponding to the analyses using universal primers, SRB-specific DGGE analysis revealed beside the presence of *Desulfohalobiaceae* and *Desulfotomaculum* relatives additional sulfate reducers related to *Desulfatibacillum* sp. in fluid produced from the cold well (profiles not shown).

Discussion

Monitoring of 16S rRNA genes using the genetic fingerprinting technique SSCP proved to be a sensitive method for detecting significantly different microbial composition in the highly saline fluids (total dissolved solids (TDS) 130 g l^{-1} - 134 g l^{-1}) produced from the warm (68°C - 73°C) and cold side (~ 46°C) of a sandstone aquifer used for geothermal heat storage. Furthermore, dynamic changes in the microbial composition in fluids following fluid heating and cooling processes at the geothermal plant were observed. Real-time PCR and direct microscopic counting were suitable for the examination of higher bacteria and SRB abundances in cold fluids compared with warm fluids. Since quantitative analyses were not done immediately after sampling, gene copy numbers are relatively low; however, data revealed a remarkable trend.
In fluid samples produced from the warm well with temperatures of 68°C - 73°C direct cell counting revealed less than 10^4 cells ml^{-1} and sequences affiliated to *Firmicutes* (*Halanaerobium praevalens*), *Bacteroidetes*, *Deferribacteres*, and the Alpha-, Beta-, and Gamma- subclasses of the *Proteobacteria* were detected. Relatives of the sulfate reducing Candidatus *Desulforudis audaxviator* were observed only once in December 2008 in a fluid sample produced from the warm well; however, those sequences probably represent artefacts since the fluid sample was taken after eight months of plant downtime, and relatives of this organism probably originated from fluids pumped from the cold well to the warm well before plant downtime. This assumption bases on the regularly detection of Candidatus *Desulforudis audaxviator* relatives in fluids produced from the cold well. Biofilm formation in piping was probably encouraged due to a gradual drop in fluid temperature during plant

downtime, and may have led to growth of species with lower temperature optimum.

Although saline fluid samples were high-tempered with temperatures above 67°C, not all of the bacterial sequences detected in fluids produced from the warm well belong to thermohalophilic microorganisms and are also common in mesophilic non-saline aerobic environments. It may be that the growth of these organisms was encouraged during sample transport or samples were contaminated during handling. However, sequences affiliated to obligate aerobic bacteria such as *Acinetobacter* sp., *Sphingomonas* sp. (Patureau et al. 2000; Ohta et al. 2004), and facultative aerobic bacteria, able to use oxygen or nitrate as electron acceptors, such as *Aquabacterium* sp. (Kalmbach et al. 1999; Straub et al. 2004), and *Pseudomonas* sp. (Cuypers and Zumft 1993; Drysdale et al. 1999; Bothe et al. 2000) were frequently detected in terrestrial subsurface environments and in the deep subseafloor biosphere (Balkwill 1997; Fry et al. 2008). Even though nitrate was not detected in fluids, the high mass flow rate of 80 m^3 h^{-1} at the geothermal plant has to be considered, because it may represent a continuous supply of small amounts of nitrate for microbes, preferable organized in biofilms that effectively retain nutrients as typically seen in the deep subsurface and any other aquatic environment (Teske and Stahl 2002).

Remarkably, *Acinetobacter* sp. has been shown to be a pioneer bacterium of biofilms in e.g. corroded gas steel pipelines (Zhu et al. 2003). Thus, *Acinetobacter*-related species probably encouraged the maturation of a biofilm in the piping of the warm well due to specific pili on their cell surface (Harshey et al. 2003) that enable the attachment of cells to the interior pipe surfaces. In addition, one should consider that the organisms identified here by partial sequence similarities are not identical to cultivated species and may have abilities not observed thus far. In addition, adaptations to environmental conditions may result in interplay between salinity and hydrostatic pressure that influences microbial growth and physiology and may partially explain the non-halophilic genera living in saline aquifers (Kaye and Baross 2004).

Heat extraction and decreasing fluid temperature had a significant influence on the microbial composition observed in fluids originating from the warm well. In addition, 16S rRNA gene and *dsrA* gene copy numbers increased 6-fold and 5-fold, respectively, indicating higher cell counts in cooled fluids. Sequences affiliated to three different fermenting organisms (*Halanaerobium, Halothermothrix, Anaerophaga*) and two genera of SRB (*Desulfohalobium* sp., *Desulfotomaculum* sp.) were detected in fluids cooled at the heat exchanger from 75°C to 46°C. Physiological characteristics of detected SRB and *Halanaerobiaceae* correspond to the subsurface environment with respect to optimum temperature, salt concentration, and pH, and are commonly found in similar anaerobic saline environments such as sediments from salt lakes (Cayol et al. 1994; Jakobsen et al. 2006), geothermal groundwater (Daumas et al. 1988), fracture water (Chivian et al. 2008), as well as in oil fields and petroleum hydrocarbon contaminated sites (Magot et al. 2000; Cayol et al. 2002; Denger et al. 2002; Bonch-Osmolovskaya et al. 2003). Halophilic *Desulfohalobium utahense* are known moderate halophilic and mesophilic sulfate and thiosulfate reducers that use a broad range of electron donors including various short-chain fatty acids and primary ($C_{1–5}$) alcohols, amino acids, H_2/acetate and H_2/yeast extract (Jakobsen et al. 2006). In addition, *Desulfotomaculum* belong to the most frequently isolated SRB in subsurface sediments, including meso- and thermophilic species using hydrogen and a number of organic compounds, such as ethanol, formate, lactate, pyruvate, malate, and succinate, for growth (Parkes and Sass 2007; Muyzer and Stams 2008).

Probably due to heat extraction activation of a dormant microbial population of fermentative bacteria and SRB was favored. Biofilms were likely established on the heat exchanger plates and the downstream piping. Microorganisms and parts of the biofilm were probably detached with the fluid flow as a result of mechanical processes, leading to the observed changes within the microbial community composition. Detected *Desulfotomaculum* are typically endospore-forming (Campbell and Postgate 1965) and also *Anaerophaga thermohalophila* form spore-like structures or spheres in ageing

cultures (Denger et al. 2002). Beside fluid temperature, substrate availability is important for stimulating dormant microorganisms. The availability of organic matter thereby depends on physical and chemical parameters, and particularly a rise in temperature increases the availability of organic matter (Kalbitz et al. 2000; Burdige 2007; Hubert et al. 2010). Thus, the temperature increase due to heat storage in the aquifer probably enabled organic matter mobilization in the warm side of the aquifer and supported the growth of fermentative bacteria downstream of the heat exchanger. Activity of fermentative bacteria is indicated by the detection of the volatile fatty acids formate and acetate in fluids, with concentrations ranging between 0.1 mg l^{-1} and 1.9 mg l^{-1} (Vetter et al. 2012). The impact of aquifer thermal energy storage on organic matter mobilization from aquifer materials was studied by Brons et al. (1991) and Jesußek et al. (2012). Even chemicals used at the topside facility or residues from well construction may serve as energy sources (Hamilton 2003; Klotzbücher et al. 2007; Struchtemeyer et al. 2011). The effect of organic compounds, introduced by drilling and well completion during plant construction that enriched a specific community of fermentative bacteria was also observed for a saline aquifer in the NGB four months after plant startup (Lerm et al. 2011b). The degradation of organic matter catalyzed by fermentative bacteria and SRB through co-operative metabolism is a well-known microbial process in deep subsurface when sulfate is abundant (Lovley and Chapelle 1995; Beech and Gaylarde 1999; Muyzer and Stams 2008).

Despite a similar fluid temperature range of approximately 46°C, different communities of SRB and fermentative bacteria were detected in cooled fluid samples originating from the warm well and in fluid samples produced from the cold well. Additionally, a higher total cell count of 4 x 10^6 cell ml^{-1} was determined in cold fluid compared to warm fluid, as well as higher abundance of bacteria and SRB indicated by 5-fold higher 16S rRNA and *dsrA* gene copy numbers. In fluid samples produced from the cold well, three different SRB (*Desulfohalobium utahense*, *Desulfotomaculum* sp., Candidatus *Desulforudis audaxviator*) and fermenting *Halanaerobiaceae* predominated in the fluid. However, no *Anaerophaga* were identified as in cooled fluids originating from the warm well. In addition, besides *Desulfohalobium* and *Desulfotomaculum*, sequences affiliated to a Candidatus *Desulforudis audaxviator* were frequently detected in fluids produced from the cold well. Sequences of Candidatus *Desulforudis audaxviator* were also found in 60°C tempered fracture water collected from a South African gold mine at a depth of 2.8 km (Chivian et al. 2008). Recently, a study by Alawi et al. (2011) identified sequences similar to Candidatus *Desulforudis audaxviator* in 61°C tempered fluid samples taken after heat extraction in the topside facility of a geothermal plant in the Molasse Basin (Germany). Candidatus *Desulforudis audaxviator* is a rarely detected microorganism so far, whereas it has the ability to subsist as a single species ecosystem that is based on hydrogen originating from radiochemical reactions in deep crustal rocks and sulfate reduction as the dominant electron-accepting process (Lin et al. 2006; Chivian et al. 2008). The repeated detection of sequences affiliated to this species in a saline aquifer of the NGB (TDS 130 g l^{-1}) as well as in a low mineralized Malm aquifer of the Molasse Basin (TDS < 1 g l^{-1}) indicates that this organism is much more distributed in the deep subsurface than previously assumed and co-existing with other SRB.

Detailed analyses of SRB with specific primers targeting genes encoding *dsrB* revealed besides *Desulfotomaculum* sp. and *Desulfohalobium* sp. only one additional genus of SRB (*Desulfatibacillum* sp.) in fluids produced from the cold well than using universal bacterial primers. Thus, the dominance of SRB in fluids from the cold side of the aquifer is emphasized.

Different microbial composition in cooled fluids produced from the warm side of the aquifer and in fluids produced from the cold well, indicated that the nutrient composition of the fluids probably differed. The concentration of organic matter did not vary significantly between the fluid of the warm and cold well during the study period. However, small differences in DOC concentration may have existed and were not detected due to the relatively low accuracy of DOC measurements caused by the high salinity. In addition, the mobilization of sedimentary organic matter is difficult to prove. Slight

changes of DOC may result in a transport of significant amounts of organic matter from the warm side of the aquifer to the near wellbore area of the colder well due to the high volume of fluids that are exchanged.

Remarkably, significant differences in the concentration of fluid soluble gases were detected. Whereas the dominant gases carbon dioxide (CO_2 appr. 86 vol.-%) and nitrogen (N_2 appr. 14 vol.-%) were likewise in fluids produced from the warm and the cold well, different amounts of hydrogen gas (H_2), hydrogen sulphide (H_2S) and methane (CH_4) were detected. The H_2 content in the gaseous phase of fluids produced from the cold well (0.21 vol.-%) compared with fluids from the warm well (0.03 vol.-%) was significantly higher and may have favored activity of hydrogenotrophic sulfate reducers closely related to *Desulfohalobium* and *Desulfotomaculum*, leading to detectable traces of H_2S only in fluids produced from the cold well (H_2S 0.2 vol.-%). Hydrogen sulfide formed by SRB contributes to well souring, corrosion of technical equipment and plugging of reservoirs (Hamilton 1985).

Detection of H_2S and higher H_2 content in fluids produced from the cold well compared with the warm well coincided with the higher corrosion damage to the submersible pump in the cold well. Thus, H_2 dissolved in fluids produced from the cold well may indicate downhole metal corrosion. Similar inferences were done for a geothermal aquifer (Criaud et al. 1987) and from laboratory corrosion experiments (Reardon 1995). Geothermal fluids with a high mineralization have a high corrosion potential, whereas the chlorine ions in the saline fluid act as catalysts causing continuous metal dissolution. Since corrosion damage was significantly higher in the cold well compared with the warm well this is probably due to ambient temperatures that were favorable for growth of the hydrogenotrophic SRB detected. It is thus likely that SRB detected in fluids produced from the cold well accelerated corrosion at the submersible pump and were most likely involved in or perhaps responsible for the operational failure. *Desulfotomaculum* species often cause corrosive damage to steel structures, such as pipeline systems and storage tanks (Hamilton 1985; Little and Lee 2007). Besides SRB, fermentative *Halanaerobium* were detected in biofilms of corroded pipelines (Neria-Gonzalez et al. 2006). Microbial fermentation, catalyzed by organisms related to *Halanaerobiaceae* may represent a further source of hydrogen. However, due to lower diversity of fermentative bacteria compared with the SRB and low DOC content in fluids, hydrogen release from fermentation processes was probably not that relevant than chemical corrosion.

Besides 16S rRNA gene sequences assigned to SRB, the isotopic signature of the residual sulfate ($\delta^{34}S(SO_4)$ > 30 ‰, Möller et al. 2008), a 10 % lower sulfate content in fluids produced from the cold well, and the detection of sulfide precipitates in topside facility filters (Fig. 4) clearly indicate metabolically active SRB. Particularly the higher sulfide content in the filter residues of the cold well compared with the filter residues of the warm well indicate enhanced activity of SRB detected in fluids due to the lower temperature. Beside iron sulfide, copper- and nickel sulfides, and carbonates were detected in filter residues of the geothermal plant indicating mineral formation processes in the fluid due to microbial induced mineralization and pressure-dependent CO_2-degassing. Commonly 80 % of the mineral residues in filters were newly created in the thermal water stream and did not represent constituents of the aquifer material (Obst and Wolfgramm 2010). The new formation of minerals was observed in different geothermal plants in Germany; whereas iron sulfides are typical for Mesozoic sandstone aquifers in depths up to 1,800 m with a fluid temperature below 90°C (Wolfgramm et al. 2011).

The activity of SRB in the well and the near wellbore area over a period of three years probably led to a 10 % lower sulfate concentration of cold well fluids compared with warm well fluids (1,000 mg l^{-1}). Therefore, the sulfate concentration of fluids produced from the warm well showed a dependence on the operational mode of the plant, corresponding to a 10 % lower sulfate content at the beginning of the discharge period with a rising trend during fluid extraction from the warm well. The increase in the sulfate content with the produced volume from the warm side of the aquifer was probably due to

mixing of fluids originating from the outer part of the subsurface heat bubble and rock materials close to the well.

Heating of fluids produced from the cold well did not caused a significant shift in the genetic fingerprinting patterns, as observed for fluids produced from the warm well and cooled due to heat extraction. However, qPCR indicated 2-fold lower abundance of bacteria and SRB in heated fluids compared to fluids upstream of the heat exchanger. Similar banding pattern before and after fluid heating, but weaker band intensities as well as lower 16S rRNA and *dsrA* gene copy numbers downstream of the heat exchanger indicated the heat-induced inactivation and destruction of cells originating from the cold side of the aquifer. Hyperthermophilic microorganisms existing in fluids downstream of the heat exchanger were probably not detected because of lower cell numbers compared to fluids upstream of the heat exchanger. Obviously, microorganisms in fluids produced from the cold well had been already inactivated due to temperature increase, but were still detected due to the high sensitivity of the genetic fingerprinting caused by PCR amplification of 16S rRNA genes.

The initial state of the microbial composition in the aquifer before well-drilling and start of plant operation could not be characterized because no fluid samples were available before and during plant startup in 2005. It is likely that in the course of plant operation two different microbial communities developed in the periphery of the two wells as a result of temperature shifts in the aquifer that changed the established growth conditions for the abundant microbes, selecting for species adapted to these modified temperature ranges. Other studies investigating the temperature effects on microbial community structure in natural and engineered aquifers that are characterized by high temperature and salinity are extremely rare. However, the effects of temperature decreases in marine oil reservoirs caused by the introduction of sulfate-rich sea-water have been studied in detail. Several studies showed that the injection of sea-water in oil production systems altered the physical and chemical conditions in the reservoir, and favored the growth of mesophilic sea water bacteria, particularly SRB, near the injection well (Sunde and Torsvik 2005; Vance and Thrasher 2005). Subsequently, the activity of SRB caused severe economic problems due to the produced H_2S, leading to microbiologically influenced corrosion, reservoir souring, and decreasing efficiency of oil production due to plugging by SRB biomass and precipitated metal sulfides. Probably similar processes occurred at the cold well of the investigated geothermal plant due to the injection of cooled geothermal fluids at a temperature below the original aquifer fluid temperature; although the availability of organic matter is considerably lower than in oil reservoirs. Subsequently, the drop in aquifer fluid temperature from 54°C to 46°C and the availability of ferrous iron in the casing probably stimulated the SRB in the aquifer. In accordance to oil production systems, failures in plant operation caused by corrosion and mineral precipitation were observed and the detected SRB were probably causative for. Even if the availability of organic matter in the investigated geothermal aquifer is lower than in fluids of oil reservoirs, inferences between the investigated geothermal aquifer and oilfield reservoirs can be done since commonalities concerning the casing and physico-chemical properties of fluids like temperature, salinity, and sulfate content exist. In addition, sulfate reducers closely related to *Desulfotomaculum* and *Desulfohalobium* are also commonly found in oil reservoirs (Magot et al. 2000; Birkeland 2005; Grigoryan and Voordouw 2008; Gieg et al. 2011) as seen for the saline heat store. Thus, chemical constraints in deep reservoirs select for similar microbial communities, while abundant sulfate reducers lead to the widespread phenomenon of biological sulfide production that adversely affects operational materials in reservoirs or in topside processing facilities.

Conclusions

Fluid temperature, increased availability of organic carbon, and hydrogen gas seemed to be the most important environmental factors affecting the composition of the bacterial communities and their activity in the two wells of the heat store. Higher cell counts as well as higher 16S rRNA and *dsrA* gene copy numbers in fluids produced from the cold well of the geothermal plant that was seriously affected by corrosion and precipitation processes indicated that microorganisms were favored by lower fluid temperature and higher availability of hydrogen as an electron donor. Predominance and higher diversity of SRB in the cold well showed that microbial mediated corrosion processes seriously affected the operational reliability of the plant. Geochemical analyses were not sensitive enough to detect slight changes in organics in reservoir and topside conditions caused by plant operation with a high fluid flow. Thus, fermentative bacteria and SRB served as indicators for differences in the chemical conditions in the fluids of the two wells.

Acknowledgments

We thank the public utilities of the city Neubrandenburg providing access to the topside facility of the heat store. In addition, we thank AMODIA Bioservice GmbH (Braunschweig, Germany) for SSCP analyses and MicroPro GmbH (Gommern, Germany) for MPN counting. In addition, we thank Rickard Lindner for the analyses of the SRB diversity using *dsrB* gene fragments. We are thankful to Alexandra Vetter and Andrea Vieth-Hillebrand for the DOC measurements and the analyses of the dissolved organic acids in the fluid samples. This work was supported by Federal Ministry of the Environment, Natural Conservation and Nuclear Safety (BMU, grant No. 0327634).

References

Aerts JMFG, Lauwers AM, Heinen W (1985) Temperature-dependent lipid content and fatty acid composition of three thermophilic bacteria. Anton Leeuw 51:155-165

Akpabio EJ, Ekott EJ, Akpan ME (2011) Inhibition and control of microbiologically influenced corrosion in oilfield materials. Environ Res J 5(2):59-65

Alawi M, Lerm S, Vetter A, Wolfgramm M, Seibt A, Würdemann H (2011) Diversity of sulfate-reducing bacteria in a plant using deep geothermal energy. Grundwasser 16(2):105-112

Amy PS (1997) Microbial dormancy and survival in the subsurface. In: Amy P, Haldeman D (eds) The microbiology of the terrestrial subsurface. CRC Press, pp 185-203

Avidano L, Gamalero E, Cossa GP, Carraro E (2005) Characterization of soil health in an Italian polluted site by using microorganisms as bioindicators. Appl Soil Ecol 30:21-33

Balkwill D, Reeves R, Drake G, Reeves J, Crocker F, King M, Boone D (1997) Phylogenetic characterization of bacteria in the subsurface microbial culture collection. FEMS Microbiol Rev 20:201-216

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive staining of DNA in polyacrylamide gels. Anal Biochem 196:80-83

Bastin ES, Greer FE, Merritt CA, Moulton G (1926) The presence of sulphate reducing bacteria in oil field waters. Science 63:21-24

Beech IB, Gaylarde CC (1999) Recent advances in the study of Biocorrosion - An overview. Rev Microbiol 30:177-190

Bennett PC, Rogers JR, Choi WJ (2001) Silicates, Silicate Weathering, and Microbial Ecology. Geomicrobiol J 18:3-19

Birkeland NK (2005) Sulfate-reducing bacteria and archaea. In: Olivier B, Magot M (eds) Petroleum Microbiology. ASM Press, Washington, pp. 35-54

Bonch-Osmolovskaya EA, Miroshnichenko ML, Lebedinsky AV, Chernyh NA, Nazina TN, Ivoilov VS, et al. (2003) Radioisotopic, culture-based, and oligonucleotide microchip analyses of thermophilic microbial communities in a continental high-temperature petroleum reservoir. Appl Envion Microbiol 69(10):6143-6151

Bothe H, Jost G, Schloter M, Ward BB, Witzel K **(**2000) Molecular analysis of ammonia oxidation and denitrification in natural environments. FEMS Microbiol Rev 24:673-690

Brons HE, Griffioen J, Appelo CAJ, Zehnder AJB **(**1991) (Bio)geochemical reactions in aquifer material from a thermal energy storage site. Wat Res 25:729-736

Burdige DJ (2007) Preservation of organic matter in marine sediments: controls, mechanisms, and an imbalance in sediment organic carbon budgets? Chem Rev 107:467-485

Burke V, Wiley AJ (1937) Bacteria in coal. J Bacteriol 34:475-481

Campbell IL, Postgate JR (1965) Classification of the spore-forming sulfate-reducing bacteria. Bacteriol Rev 29:359-363

Cayol JL, Ollivier B, Patel BKC, Prensier G, Guezennec J, Garcia JL (1994) Isolation and characterization of *Halothermothrix orenii* gen. nov., sp. nov., a halophilic, thermophilic, fermentative, strictly anaerobic bacterium. Int J Syst Bacteriol 44(3):534-540

Cayol JL, Fardeau ML, Garcia JL, Ollivier B (2002) Evidence of interspecies hydrogen transfer from glycerol in saline environments. Extremophiles 6:131-134

Characklis WG (1990) Microbial fouling. In: Characklis WG, Marshall KC (eds) Biofilms. Wiley, New York, pp. 523-584

Chivian D, Brodie EL, Alm EJ, Culley DE, Dehal PS, DeSantis TZ, et al. (2008) Environmental genomics reveals a single-species ecosystem deep within earth. Science 322:275-278.

Coetser SE, Cloete TE (2005) Biofouling and biocorrosion in industrial water systems. Crit Rev

Microbial 31(4):213-232
Criaud A, Fouillac C, Marty B, Brach M, Wei HF (1987) Gas geochemistry of the dogger geothermal aquifer. Proceedings, Twelfth Workshop on Geothermal Reservoir Engineering Stanford University, Stanford, California, January 20-22, 1987 SGP-TR-109
Cullimore DR (1999) Microbiology of well biofouling. Lewis Publishers, Boca Raton, London, New York, Washington DC
Cullimore DR (2007) Practical manual of groundwater microbiology, 2nd edn. CRC Press/Taylor & Francis Group
Cuypers H, Zumft WG **(**1993) Anaerobic control of denitrification in *Pseudomonas stutzeri* escapes mutagenesis of an *fnr*-like gene. J Bacteriol 175:7236-7246
Dalas E, Koutsoukos PG (1989) Calcium carbonate scale formation on heated metal surfaces. Geothermics 18(1/2):83-88
Daumas S, Cord-Ruwisch R, Garcia JL (1988) *Desulfotomaculum geothermicum* sp. nov., a thermophilic, fatty acid-degrading, sulfate-reducing bacterium isolated with H_2 from geothermal ground water. Antonie Van Leeuwenhoek 54(2):165-78
Demadis KD (2003) Combating heat exchanger fouling and corrosion phenomena in process water. In: Shah RK (ed) Compact heat exchangers and enhancement technology for the process industries. Begell House Inc, New York, pp 483-490
Denger K, Warthmann R, Ludwig W, Schink B (2002) *Anaerophaga thermohalophila* gen. nov., sp nov., a moderately thermohalophilic, strictly anaerobic fermentative bacterium. Int J Syst Evol Microbiol 52:173-178
Dohrmann AB, Tebbe CC (2004) Microbial community analysis by PCR-single-strand conformation polymorphism (PCR-SSCP). In: Kowalchuk GA, Bruijn FJ de, Head IM, Akkermans AD, van Elsas JD (eds) Molecular Microbial Ecology Manual. 2nd edn. Dodrecht: Kluwer Academic Publisher 3.16, pp 809-838
D´Hondt S, Rutherford S, Spivack AJ (2002) Metabolic activity of subsurface life in deep-sea sediments. Science 295:2067-2070
Drysdale G, Kasan H, Bux F **(**1999) Denitrification by heterotrophic bacteria during activated sludge treatment. Water SA 25:357-362
Flemming HC (2002) Biofouling in water systems – cases, causes and countermeasures. Appl Microbiol Biotechnol 59:629-640
Fry JC, Parkes RJ, Cragg BA, Weightman AJ, Webster G (2008) Prokaryotic biodiversity and activity in the deep subseafloor biosphere. FEMS Microbiol Ecol 66:181-196
Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiol 156:609-643
Gallup DL (2002) Investigations of organic inhibitors for silica scale control in geothermal brines. Geothermics 31:415-430
Gallup DL (2009) Production engineering in geothermal technology: A review. Geothermics 38: 326-334
Geets J, Borremans B, Diels L, Springael D, Vangronsveld J, van der Lelie D, Vanbroekhoven K (2006) DsrB gene-based DGGE for community and diversity surveys of sulfate-reducing bacteria. J Microbiol Methods 66:194-205
Gieg LM, Jack TR, Foght JM (2011) Biological souring and mitigation in oil reservoirs. Appl Microbiol Biotechnol 92:263-282
Goldscheider N, Hunkeler D, Rossi P (2006) Review: Microbial biocenoses in pristine aquifers and an assessment of investigative methods. Hydrogeol J 0:1-16
Griebler C, Lueders T (2009) Microbial biodiversity in groundwater ecosystems. Freshwat Biol 54:649-677

Grigoryan A, Voordouw G (2008) Microbiology to help solve our energy needs: methanogenesis from oil and the impact of nitrate on the oil-field sulfur cycle. Ann NY Acad Sci 1125:345-352

Hamilton WA (1985) Sulphate-reducing bacteria and anaerobic corrosion. Ann Rev Microbiol 39:195-217

Hamilton WA (2003) Microbiologically influenced corrosion as a model system for the study of metal microbe interactions: A unifying electron transfer hypothesis. Biofouling 19:65-76

Harshey RM (2003) Bacterial motility on surface: many ways to a common goal. Annu Rev Microbiol 57:249-273

Howsam P (1988) Biofouling in wells and aquifers. J Inst Water Environ Manag 2:209-215

Hubert C, Arnosti C, Brüchert V, Loy A, Vandieken V, Jørgensen BB (2010) Thermophilic anaerobes in Arctic marine sediments induced to mineralize complex organic matter at high temperature. Environ Microbiol 12(4):1089-1104

Jakobsen TF, Kjeldsen KU, Ingvorsen K (2006) *Desulfohalobium utahense* sp. nov., a moderately halophilic, sulfate-reducing bacterium isolated from Great Salt Lake. Int J Syst Evol Microbiol 56:2063-2069

Javaherdashti R (2011) Impact of sulphate-reducing bacteria on the performance of engineering materials. Appl Microbiol Biotechnol 91:1507-1517

Jesußek A, Grandel S, Dahmke A (2012) Impacts of subsurface heat storage on aquifer hydrogeochemistry. Environ Earth Sci. DOI 10.1007/s12665-012-2037-9

Johnson SS, Hebsgaard MB, Christensen TR, Mastepanov M, Nielsen R, Munch K, Brand T, Gilbert P, Zuber MT, Bunce M, Rønn R, Gilichinsky D, Froese D, Willerslev E (2007) Ancient bacteria show evident of DNA repair. PNAS 104:14401-14405

Jørgensen BB (1982) Mineralization of organic matter in the sea bed - the role of sulphate reduction. Nature 296:643-645

Kabus F, Wolfgramm M (2009) Aquifer thermal energy storage in Neubrandenburg – Monitoring throughout three years of regular operation. In: Proceedings of the 11th International Conference on Energy Storage 2009, Stockholm, Sweden, 8 pp

Kalbitz K, Solinger S, Park JH, Michalzik B, Matzner E (2000) Controls on the dynamics of dissolved organic matter in soils: A review. Soil Sci 165:277-304

Kalmbach S, Manz W, Wecke J, Szewzyk U (1999) *Aquabacterium* gen. nov., with description of *Aquabacterium citratiphilum* sp. nov., *Aquabacterium parvum* sp. nov. and *Aquabacterium commune* sp. nov., three in situ dominant bacterial species from the Berlin drinking water system. Int J Syst Bacteriol 49:769-777

Kaye JZ, Baross JA (2004) Synchronous effects of temperature, hydrostatic pressure, and salinity on growth, phospholipid profiles, and protein patterns of four *Halomonas* species isolated from deep-sea hydrothermal-vent and sea surface. Appl Environ Microbiol 70(10):6220-6229

Kempf B, Bremer E (1998) Uptake and synthesis of compatible solutes as microbial stress compounds to high-osmolality environments. Arch Microbiol 170:319-330

Klotzbücher T, Kappler A, Straub KL, Haderlein SB (2007) Biodegradability and groundwater pollutant potential of organic anti-freeze liquids used in borehole heat exchangers. Geothermics 36:348-361

Kumar S, Nussinov R (2001) How do thermophilic proteins deal with heat. CMLS Cell Mol Life Sci 58:1216-1233

Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics. Wiley, Chichester, United Kingdom, pp 115-175

Lerm S, Alawi M, Miethling-Graff R, Wolfgramm M, Rauppach K, Seibt A, Würdemann H (2011a) Influence of microbial processes on the operation of a cold store in a shallow aquifer: impact on well injectivity and filter lifetime. Grundwasser 16(2):93-104

Lerm S, Alawi M, Miethling-Graff R, Wolfgramm M, Rauppach K, Seibt A, Würdemann H (2011b) Mikrobiologisches Monitoring in zwei geothermisch genutzten Aquiferen des Norddeutschen Beckens. Z geol Wiss 39(3-4):195-212

Lin LH, Wang PL, Rumble D, Lippmann-Pipke J, Boice E, Pratt LM, et al. (2006) Long-term sustainability of a high-energy, low-diversity crustal biome. Science 314:479-482

Little BL, Wagner PA, Hart KR, Ray RI (1996) Spatial relationships between bacteria and localized corrosion. Corrosion/96, paper no. 278 (Houston, TX: NACE International), pp 8

Little BJ, Lee JS (2007) Microbiologically Influenced Corrosion. Wiley Series in Corrosion. John Wiley & Sons, New Jersey

Lovley DR, Chapelle FH (1995) Deep subsurface microbial processes. Rev Geophys 33(3):365-381

Ludwig W, Strunk O, Westram R, Richter L, Meier H, Yadhukumar et al. (2004) ARB: a software environment for sequence data. Nucleic Acids Res 32:1363-1371

Magot M, Ollivier B, Patel BKC (2000) Microbiology of petroleum reservoirs. Antonie van Leeuwenhoek 77:103-116

Miranda-Herrera C, Sauceda I, González-Sánchez J, Acuña N (2010) Corrosion degradation of pipeline carbon steels subjected to geothermal plant conditions. Anti-Corrosion Methods and Materials 57(4):167-172

Möller P, Weise SM, Tesmer M, Dulski P, Pekdeger A, Bayer U, Magri F (2008) Salinization of groundwater in the North German Basin: results from conjoint investigation of major, trace element and multi-isotope distribution. Int J Earth Sci (Geol Rundsch) 97:1057-1073

Morse JW, Millero FJ, Cornwell JC, Rickard D (1987) The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. Earth-Sci Rev 24:1-42

Muyzer G, Smalla K (1998) Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. Antonie Leeuwenhoek Int J Gen Mol Microbiol 73:127-141

Muyzer G, Stams AJM (2008) The ecology and biotechnology of sulphate-reducing bacteria. Nat Rev Microbiol 6:441-454

Neria-Gonzalez I, Wang ET, Ramirez F, Romero JM, Hernandez-Rodrigues C (2006) Characterization of bacterial community associated to biofilms of corroded oil pipelines from the southeast of Mexico. Anaerobe 12:122-133

Obst K, Wolfgramm M (2010) Geothermische, balneologische und speichergeologische Potenziale und Nutzungen des tieferen Untergrundes der Region Neubrandenburg. Neubrandenburger Geol Beitr 10:145-174

Ohta H, Hattori R, Ushiba Y, Mitsui H, Ito M, Watanabe H, Tonosaki A, Hattori T (2004) *Sphingomonas oligophenolica* sp. nov., a halo- and organo-sensitive oligotrophic bacterium from paddy soil that degrades phenolic acids at low concentrations. Int J Syst Evol Microbiol 54:2185-2190

Parkes RJ, Cragg BA, Bale SJ, Getlifff JM, Goodman K, Rochelle PA, et al. (1994) Deep bacterial biosphere in Pacific ocean sediments. Nature 371:410-413

Parkes RJ, Cragg BA, Wellsbury (2000) Recent studies on bacterial populations and processes in subseafloor sediments: a review. Hydrogeol J 8:11-28

Parkes RJ, Sass (2007) The sub-seafloor biosphere and sulphate-reducing prokaryotes: their presence and significance. In: Barton L, Hamilton WA (eds) Sulphate-reducing bacteria: environmental and engineered systems. Cambridge University Press, Cambridge, United Kingdom, pp 329-358

Patureau D, Zumstein E, Delgenes JP, Moletta R (2000) Aerobic denitrifiers isolated from diverse natural and managed ecosystems. Microb Ecol 39:145-152

Philpotts AR (1990) Principles of Igneous and Metamorphic Petrology. Englewood Cliffs, New Jersey:

Prentice Hall. pp 498
Pryfogle PA (2005) Monitoring Biological Activity at Geothermal Power Plants. Idaho National Laboratory
Reardon EJ (1995) Anaerobic Corrosion of Granular Iron: Measurement and Interpretation of Hydrogen Evolution Rates. Environ Sci Technol 29:2936-2945
Roberts MF (2005) Organic compatible salutes of halotolerant and halophilic microorganisms. Sal Syst 1:5-30
Rogers JR, Bennett PC, Choi WJ (1998) Feldspars as a source of nutrients for microorganisms. J Am Miner 83:1532-1540
Sand W (2003) Microbial life in geothermal waters. Geothermics 32:655-667
Sass H, Cypionka H (2004) Isolation of sulfate-reducing bacteria from the terrestrial deep subsurface and description of *Desulfovibrio cavernae* sp. nov. System Appl Microbiol 27:541-548
Schmidt T, Mangold D, Müller-Steinhagen H (2004) Central solar heating plants with seasonal storage in Germany. Solar Energy 76:165-174
Schwieger F, Tebbe CC (1998) A new approach to utilize PCR–Single-Strand-Conformation Polymorphism for 16S rRNA gene-based microbial community analysis. Appl Environ Microbiol 64:4870-4876
Seibt P, Kabus F (2006) Aquifer thermal energy storage – Projects implemented in Germany. Proceedings of Ecostock, New Jersey, pp 8
Seibt A, Thorwart K (2011) Untersuchungen zur Gasphase geothermisch genutzter Tiefenwässer und deren Relevanz für den Anlagenbetrieb. Z geol Wiss 39 (3-4):261-274
Shock EL (2009) Minerals as Energy Sources for Microorganisms. Econ Geol 104:1235-1248
Skinner BJ, White DE, Rose HJ, Mays RE (1967) Sulfides associated with the Salton Sea geothermal brine. Econ Geol 62:316-330
Steube C, Richter S, Griebler C (2009) First attempts towards an integrative concept for the ecological assessment of groundwater ecosystems. Hydrogeol J 17:23-35
Straub KL, Schönhuber WA, Buchholz-Cleven BEE, Schink B (2004) Diversity of ferrous iron-oxidizing, nitrate reducing bacteria and their involvement in oxygen independent iron cycling. Geomicrobiol J 21:371-378
Struchtemeyer CG, Davis JP Elshahed MS (2011) Influence of the drilling formulation mud process on the microbial communities in thermogenic natural gas wells of the Barnett shale. Appl Environ Microbiol 77(14):4744-4753
Sunde E, T Torsvik (2005) Microbial control of hydrogen sulfide production in oil reservoirs. In: Ollivier B, Magot M (eds) Petroleum microbiology. ASM Press, Washington, D.C., pp 201-213
Suzina NE, Mulyukin AL, Kozlova AN, Shorokova AP, Dmitriev VV, Barinova ES, Mokhova ON, El'-Registan GI, Duda VI (2004) Ultrastructure of resting cells of some non-spore-forming bacteria. Microbiology 73:516-529
Taylor SW, Lange CR, Lesold EA (1997) Biofouling of contaminated ground-water recovery wells: Characterization of microorganisms. Groundwater 35(2):973-980
Teske A, Stahl DA (2002) Microbial mats and biofilms: Evolution, structure, and function of fixed microbial communities. In: Staley JT, Reysenbach A-L Biodiversity of microbial life. Wiley-Liss, New York, pp 49-100
Valdez B, Schorr M, Quintero M, Carrillo M, Zlatev R, Stoytcheva M, de Dios Ocampo J (2009) Corrosion and scaling at Cerro Prieto geothermal field. Anti-Corrosion Methods and Materials 56(1):28-34
Vance I, Thrasher DR (2005) Reservoir souring: mechanisms and prevention. In: Ollivier B, and Magot M (eds). Petroleum microbiology. ASM Press, Washington, DC, pp 123-142

Van Hamme JD, Singh A, Ward OP (2003) Recent advances in petroleum microbiology. Microbiol Mol Biol Rev 67(4):503-549

Vetter A, Mangelsdorf K, Schettler G, Seibt A, Wolfgramm M, Rauppach K, Vieth-Hillebrand A (2012) Fluid chemistry and impact of different operating modes on microbial community at Neubrandenburg heat storage (Northeast German Basin). Organ Geochem 53:8-15

Videla HA (2002) Prevention and control of biocorrosion. Intern Biodet Biodeg 49:259-270

Vieth A, Mangelsdorf K, Sykes R, Horsfield B (2008) Water extraction of coals –potential for estimating low molecular weight organic acids as carbon feedstock for the deep terrestrial biosphere. Organ Geochem 39(8):985-991

Wagner M, Roger AJ, Flax JL, Brusseau GA, Stahl DA (1998) Phylogeny of dissimilatory sulfite reductases supports an early origin of sulfate respiration. J Bacteriol 180:2975-2982

Wellsbury P, Mather I, Parkes RJ (2002) Geomicrobiology of deep, low organic carbon sediments in the woodlark basin, Pacific Ocean. FEMS Microbiol Ecol 42:59-70

Whitman WB, Coleman DC, Wiebe JW (1998) Prokaryotes: The Unseen Majority. Proc Natl Acad Sci USA 95:6578-6583

Wilms R, Sass H, Kopke B, Cypionka H, Engelen B (2007) Methane and sulfate profiles within the subsurface of a tidal flat are reflected by the distribution of sulfate reducing bacteria and methanogenic archaea. FEMS Microbiol Ecol 59(3):611-621

Wilson JT, McNabb JF, Balkwill DL, Ghiorse WC (2006) Enumeration and characterization of bacteria indigenous to a shallow water-table aquifer. Ground water 21(2):134-142

Wolfgramm M, Seibt A (2006) Geochemisches Monitoring des geothermalen Tiefenspeichers in Neubrandenburg, GTV-Tagung, Karlsruhe, Germany, pp 388-397

Wolfgramm M, Rauppach K, Thorwarth K (2011) Mineralneubildung und Partikeltransport im Thermalwasserkreislauf geothermischer Anlagen Deutschland. Z geol Wiss 39 3-4:213-239

Yu Y, Lee C, Kim J, Hwang S (2005) Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction. Biotechnol Bioeng 89:670-679

Zhu XY, Lubeck J, Kilbane II JJ (2003) Characterization of microbial communities in gas industry pipelines. Appl Environ Microbiol 69(9):5354-5363