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Diversity of sulfate-reducing bacteria in a plant using deep geothermal energy

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Abstract Abstract Enhanced process understanding of engineered geothermal systems is a prerequisite to optimize plant reliability and economy. We investigated microbial, geochemical and mineralogical aspects of a geothermal groundwater system located in the Molasse Basin by fluid analysis. Fluids are characterized by temperatures ranging from 61°C to 103°C, salinities from 600 to 900 mg/l and a dissolved organic carbon content (DOC) between 6.4 to 19.3 mg C/l. The microbial population of fluid samples was analyzed by genetic fingerprinting techniques based on PCR-amplified 16S rRNA- and dissimilatory sulfite reductase genes. Despite of the high temperatures, microbes were detected in all investigated fluids. Fingerprinting and DNA sequencing enabled a correlation to metabolic classes and biogeochemical processes. The analysis revealed a broad diversity of sulfate-reducing bacteria. Overall, the detection of microbes known to be involved in biocorrosion and mineral precipitation indicates that microorganisms could play an important role for the understanding of processes in engineered geothermal systems.

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Diversität sulfatreduzierender Bakterien im Prozesswasser einer geothermischen Anlage

Zusammenfassung Die Verbesserung des Prozessverständnisses ist eine grundlegende Voraussetzung für eine Optimierung der Betriebssicherheit und der Ökonomie geothermischer Anlagen in Bezug auf die Partikelbildung und Korrosion. Daher wurden Prozessfluide einer Anlage im Molassebecken unter mikrobiologischen, geochemischen und mineralogischen Gesichtspunkten untersucht. Die Fluidtemperatur der vor und nach dem Wärmetauscher entnommenen Fluide betrug zwischen 103 °C und 61 °C. Die Salinität variierte zwischen 600 und 900 mg/l und der gelöste organische Kohlenstoff (DOC) lag zwischen 6,4 und 19,3 mg C/l. Die mikrobielle Lebensgemeinschaft in der Anlage wurde mithilfe einer genetischen Fingerprinting-Methode charakterisiert. Hierzu wurde das 16S rRNA Gen sowie die für sulfatreduzierende Bakterien (SRB) spezifische dissimilatorische Sulfitreduktase untersucht. In allen Fluidproben konnten Mikroorganismen nachgewiesen werden. Die Zuordnung der Organismen zu stoffwechselphysiologischen Gruppen lieferte Hinweise auf verschiedene biogeochemische Prozesse. Die Untersuchungen zeigen eine beachtliche Diversität von SRB auf. Diese sind für ihre Rolle bei biologisch induzierten Korrosions- und Fällungsprozessen bekannt.

Introduction

The predominant usage of the geothermal plants in Germany is the allocation of district heating for directly connected

buildings or thermal baths. In 2008, about 13 geothermal plants served for district heating, three of them produced electricity. The geothermal energy sector is still expanding; from 2008 to 2009 around 35 new geothermal projects were realized. Further development of geothermal plants will also depend on enhanced long-term reliability and cost effectiveness.

The influences of microbial activities in geoengineered systems are rarely described and many bio-geological processes are still insufficiently understood (Sand 2003, Inagaki et al. 2003, Basso et al. 2005). In general, it is known that processes such as corrosion, scaling and precipitation of minerals could be induced or enhanced by microorganisms and seriously harm the economy and reliability of different technical plants (Honegger et al. 1989, Flemming 2002, Beech & Sunner 2004, Coetser & Cloete 2005, Little & Lee 2007, Valdez et al. 2009). It was also shown for cold storage that the microbial biomass, in addition to precipitated minerals, could cause clogging of installed filter units (Lerm et al. 2011).

Examples for the impact and characteristics of thermophilic (45–80 °C) and hyperthermophilic (80–120 °C) microbes in comparable hot ecological systems, with temperatures above 45 °C, are mostly given from studies of oil production (Nilsen et al. 1996, Dahle & Birkeland 2006). In comparison with oil production, the substrate availability for organic substances in the deep biosphere is low, but nevertheless there is adequate energy for metabolic processes (Kimura et al. 2010). Oxygen as an energetically favorable electron acceptor for heterotrophic growth is below the detection limit of monitoring tools. An anaerobic growth with alternative electron acceptors such as Mn^{4+}, Fe^{3+}, SO_4^{2-}, or CO_2 is possible, while only traces of NO_3^- were detected. Fluids from geothermal plants contain usually enough salts, minerals and sulfur compounds for microbial life (Sand 2003). Of particular importance for the reliability of geothermal plants are partially or fully oxidized sulfur compounds which can be microbially reduced. Many studies indicate that obligate anaerobic sulfate-reducing bacteria (SRB) play an important role in the process of microbial induced corrosion (MIC) and precipitation of minerals (Javerhadashti 2008). In hot anoxic geothermal fluids SRB are probably one of the most important group of microorganisms that could be linked to biocorrosion and precipitation of minerals. Furthermore, iron and heavy metal reducing microorganisms, methanogens, as well as iron-oxidizing bacteria could influence these processes. SRB can cause serious problems for industries, such as the offshore oil industry, due to the production of sulfide which is highly corrosive and precipitates as FeS in form of corrosive scales on metal surfaces (e.g. plant components). Some studies assume that produced FeS could lead to clogging in the closer well area and thereby reducing injectivity of wells (Schreurs 1970, van Beek & Kooper 1980, Würdemann et al. 2010, Zettlitzer et al. 2010).

Biofilms are accumulations of microorganisms and extracellular polymeric substances (EPS). They have been described for most aqueous systems supporting life (Wimpenny et al. 2000). The structure of biofilms ranges from thin monolayers of single cells to thick structures of macroscopic dimensions. Microorganisms within biofilms often act syntrophically to produce optimal growth conditions. Biochemical processes within the biofilm are known to accelerate the rates of electrochemical reactions leading to corrosion. The importance of biofilms in engineered systems are shown by Nivens et al. (1986), they observed that an increase in the corrosion rate was correlated to the formation of EPS.

The SRB are anaerobic microorganisms that use sulfur and its oxidized forms like sulfate or thiosulfate as terminal electron acceptors. SRB are also able to enzymatically reduce and precipitate heavy metals using organic compounds or hydrogen as electron donor (Sitte et al. 2010). The dissimilatory sulfate reduction is ubiquitous in anoxic environments, and is an important link in the sulfur and carbon cycle. Within the group of SRB, different species are adapted to extreme pH conditions. Therefore a pH range of 4–9.5 is covered by SRB (Barton & Tomei 1995). Three metabolic pathways of dissimilatory sulfate reduction have been described. The lithoautotrophic SRB use hydrogen as the only energy source. The heterotrophic group degrades organic matter to acetate and the third group oxidizes organics to carbon dioxide. Until now, none of the characterized species of SRB has shown a temperature optimum above 90 °C, however, sulfate reduction has been observed at temperatures up to 110 °C (Jørgensen et al. 1992). So far temperatures about 120 °C are the highest temperatures at which microbial growth has been observed in pure culture. The *Crenarchaeota* strain 121 grows at 121 °C under laboratory conditions. The strain was detected at a hydrothermal vent (up to 300 °C), in the Northeast Pacific Ocean and is capable of reducing Fe(III) (Kashefi & Lovley 2003). Takai et al. (2008) carefully described microbial cell proliferation from hyperthermophilic methanogens at 122 °C. Microorganisms adapted to high temperatures are protected against the irreversible denaturation of their proteins and nucleic acids. Membrane structure and composition lead to decreased membrane fluidity and thereby ensure a better thermostability (Rothschild & Mancinelli 2001, Pakchung et al. 2006).

The extent to which microorganisms can grow with increasing depth depends on the availability of energy sources and the ambient temperature. Remarkably, life in the deep biosphere is not limited by pressure. Studies have documented that microorganisms survive pressures equivalent to depth of 50 km below the Earth's crust (Sharma et al.

2002). Takai et al. (2001) described a hyperthermophilic archaeal community in a depth of 6 km, where pore-waters support enough energy resources. Bacteria were even found in the deepest sediment in the Mariana Trench at a depth of 10898 m (Kato et al. 1998).

In order to study the autochthonous microbial community and its activity in an engineered system of the Molasse Basin (South Germany), fluids and particles in the fluid of a geothermal plant were analyzed for microbial community composition, geochemistry and mineralogy. Since it is known that less than 0.3 % of the microbes in soils or sediments can be cultivated or isolated under laboratory conditions (Amann et al. 1995, Torsvik et al. 1990), modern molecular biological methods such as the analysis of universal 16S rRNA genes which are essential for the protein biosynthesis in every prokaryote, are used to identify the population. DGGE (Denaturing gradient gel electrophoresis) genetic fingerprinting was applied for separating the PCR amplified gene fragments from DNA extracted from the fluid samples. Subsequent DNA sequencing allows a phylogenetic affiliation by using public databases. In this study another functional gene, specific for SRB, the dissimilatory sulfite reductase (DSR) was additionally analyzed to perform a specific detection for this important group of bacteria.

Site description

A geothermal plant located in the Molasse Basin (South Germany) used for district heating was monitored for a period of 12 months. The plant uses two wells as a geothermal doublet (depth: 3 km to 3.5 km), they are situated 2 km away from each other. One well is used for production and the other one for re-injection of the thermal fluids. No additives such as corrosion inhibitors were added to the fluids. The malm aquifer structure consists of fractured limestone (Wolfgramm & Seibt 2008). Approximately 100 m^3/h of hot fluids are processed by the geothermal plant. The retention time of fluids in the surface facility is less than six minutes. Fluids are filtered by a coarse metal screen with a mesh size of approx. 5 mm before passing the heat exchanger. Produced fluids have a temperature of 103 °C, after the passage through the heat exchanger the re-injected fluids are cooled to 61 °C.

Material and Methods

Sampling

Fluid samples were taken from a permanently installed sampling device with a cooling system. In order to avoid contaminations by sampling ports, at least the first 30 liters of fluids were discarded prior sampling. Sampling was performed before and after the heat exchanger. All samples were cooled at 4 °C until vacuum filtered under aseptic conditions in the laboratory, using 0.22 µm cellulose acetate filters (Sartorius, Goettingen, Germany). Until further processing, filters were stored at −80 °C.

DNA extraction and PCR-DGGE analysis

For microbial analysis two liters (for the samples taken before the heat exchanger, four liters) of fluid were filtered (0.2 µm) using a vacuum manifold. Total DNA was chemically and mechanically extracted from these filters using a bead beater according to the manufacturer's instructions (MP Inc.). Partial 16S rDNA fragments were amplified by PCR using the eubacterial primer set 341F/907R (Muyzer et al. 1997), the archael primers UA571F/UA1204R (Baker & Cowan 2004) and primer set DSR2060F-GC/DSR4R (Geets et al. 2006), encoding the dissimilatory sulfite reductase β-subunit in the SRB communities. As usual for DGGE analysis, a GC-clamp was added to the forward primer. The following PCR conditions were used: initial denaturation at 96 °C, 3 min. 30 × 95 °C, 50 s; annealing 55 °C (archaea, eubacteria), 56 °C (DSR), 50 s; elongation 72 °C, 50 s; final elongation, 5 min. Denaturing gradient gel electrophoresis (DGGE) was performed as described by Muyzer et al. (1997) at 59 °C with a gradient from 50 % to 80 % denaturants. The polyacrylamide gel was than silver stained and digitized. Dominant bands were extracted from the gel, reamplified and the partial 16S rDNA sequences were compared with those on publicly accessible databases (NCBI GenBank) by using the program Basic Local Alignment Search Tool (BLAST, NCBI) (Altschul et al. 1990).

Mineralogical and geochemical analyses

The minerals, concentrated by filtration of the fluids with 0.4 µm filters, were analyzed using a scanning electron microscope (Cambridge S200) with energy dispersive X-ray spectroscopy (SEM-EDX). For the quantification of the DOC the fluid passed a 0.45 µm membrane syringe filter and was measured as non-purgeable organic carbon (NPOC) after acidification with hydrochloric acid. The acidified sample is purged with carbon dioxide free high purity air for 5 min. The residual dissolved organic carbon is transformed by catalytic oxidation into CO_2 which is measured by IR spectroscopy at 680 °C (TOC-2000A, Shimadzu). For the determination of the inorganic and organic anions all fluid samples were analyzed three to five times using ion chromatography (ICS 3000, Dionex Corp.). The equipment used for this purpose included a conductivity detector, a KOH eluent generator and an ASRS Ultra II 2 mm suppressor. For the separation of the anions an analytical column (AS11HC; 2 × 250 mm, Dionex Corp.)

at a constant temperature of 35 °C was used. The characterization and quantification of the DOC and its fractions were conducted using size-exclusion-chromatography with UV detection ($\lambda = 254$ nm) and organic carbon detection (IR) (Huber & Frimmel 1996). Phosphate buffer (pH 6.6; 9 mM Na_2HPO_4, 18 mM KH_2PO_4) was used as the mobile phase with a flow of 1 ml $\cdot$ min^{-1}. The sample passed a 0.45 µm membrane syringe filter before entering the chromatographic column (Novo-Grom GP2, 300 mm × 8 mm, Alltech Grom GmbH). After chromatographic separation into individual fractions (biopolymers, humic substances, building blocks, and different groups of LMW compounds), these fractions were characterized by UV detection. Quantification was possible after UV oxidation ($\lambda = 185$ nm) in a Gräntzel thin-film reactor by IR-detection. The ratio between the spectral absorption coefficient (SAC in m^{-1} at 254 nm) and the DOC (in mg/l) was then calculated as an indicator of the proportion of aromatic structure of the humic substances. For molecular weight calibration, humic and fulvic acid standards of the Suwannee River (IHSS 2010) were used. The redox potential, pH and fluid temperature were determined during the sampling procedures using a pH/mV/temperature meter (WTW).

Results

The predominant observed mineral precipitates are carbonates and iron sulfides. By SEM analyses pyrite framboids were observed (Fig. 1). Fluids from the investigated aquifer are characterized by a low redox potential of around −350 [mV] (Ag/AgCl). The pH value of the fluids was 6.6 to 7.3, the salinity varied between 600 to 900 mg/l and the dissolved organic carbon content (DOC) varied between 6.4 to 19.3 mg C/l. The chromatographic DOC was dominated (50 to 83 %) by low molecular organic acids (formate 0,8–1,2 mg/l, acetate 9,7–15,1 mg/l, propionate 2,0–3,1 mg/l and butyrate 0,4–0,7 mg/l). Between the fluids taken before and after the heat exchanger, no significant differences in the amounts of organic acids were observed. It is known that a low amount of natural crude oil exists in the aquifer, and is also found in the process fluids. The sulfate concentration varied between 30–39 mg/l. The Fe^{2+} concentration in fluids amounted 55 µg/l (Schröder & Hesshaus 2009). The gaseous phase of the thermal waters in the plant was analyzed and comprised around 20 vol % gases (at 70 °C) including CH_4 (∼50 vol %), CO_2 (∼40 vol %), N_2 and H_2S (∼10 vol %).

By analyzing the 16S rRNA genes of bacteria two sequences of SRB were detected (Table 1). *Desulfotomaculum reducens*-like bacteria were found in all samples (from 03-2008 until 09-2009), even in the 103 °C hot fluids before passing the heat exchanger. Candidatus *Desulforudis audaxviator*-like bacteria were solely found in the fluid samples taken after the heat exchanger at a temperature of 61 °C. By the use of PCR primers specific for archaea, *Methanothermobacter thermoautotrophicus* was detected, showing a 100 % DNA sequence similarity to the database entry (Table 1).

By the use of SRB specific primers targeting a subunit of the dissimilatory sulfite reductase gene seven different DNA sequences were obtained by DGGE analyses and sequencing (Table 1). The comparison with the public database revealed that five different sequences assigned to the genera *Desulfotomaculum* were present in the fluids taken after the heat exchanger at 61 °C. Sequence similarity on the partial DSR gene ranged between 85 to 89 %. Week DNA bands with a similar banding pattern to the above mentioned *Desulfotomaculum*-like sequences were also visible in samples taken at 103 °C, but could not be sequenced in a good quality because of a low DNA content. However, one sequence of *Desulfotomaculum*-like bacteria with a similarity of 87 % was exclusively detected in the 103 °C hot fluids. Additionally two sequences with a lower similarity to characterized species were found. One DSR sequence detected in the fluids obtained before the heat exchanger is corresponding to a *Desulfococcus oleovorans*-like bacterium (92 %) and the other sequence, detected in fluids taken after the heat exchanger, is affiliated to the genera *Desulfotomaculum* (85 %). The 10 sequences reported in this paper have been deposited in GenBank under the accession numbers HQ844560 to HQ844569.

Discussion

In the investigated geothermal plant with fluid temperatures from 61 °C after and 103 °C before the heat exchanger, microorganisms were detected by genetic fingerprinting in all fluid samples. Nevertheless it should be mentioned that the applied PCR analysis detects active as well as dead organisms. Therefore further analysis have to be performed to assess the activity of the detected microorganism, e.g. before the heat exchanger. The microbial monitoring performed by PCR-DGGE analysis indicates that SRB with a diversity of nine different DNA sequences assigned to three bacterial genera were detected in the fluids. A higher diversity of SRB was observed in the fluids that passed the heat exchanger (61 °C), indicating active organisms. The availability of different organic substrates could explain the presence of different genera of SRB in the same habitat as an example of ecological micro niches. The SRB detected are closely related to thermotolerant and thermophilic species of *Desulfotomaculum*. Additionally, archaea were detected as well as DNA sequences from yet unknown taxa.

The presence of iron sulfides and pyrite framboids correlates with the characterized SRB detected in these fluids.

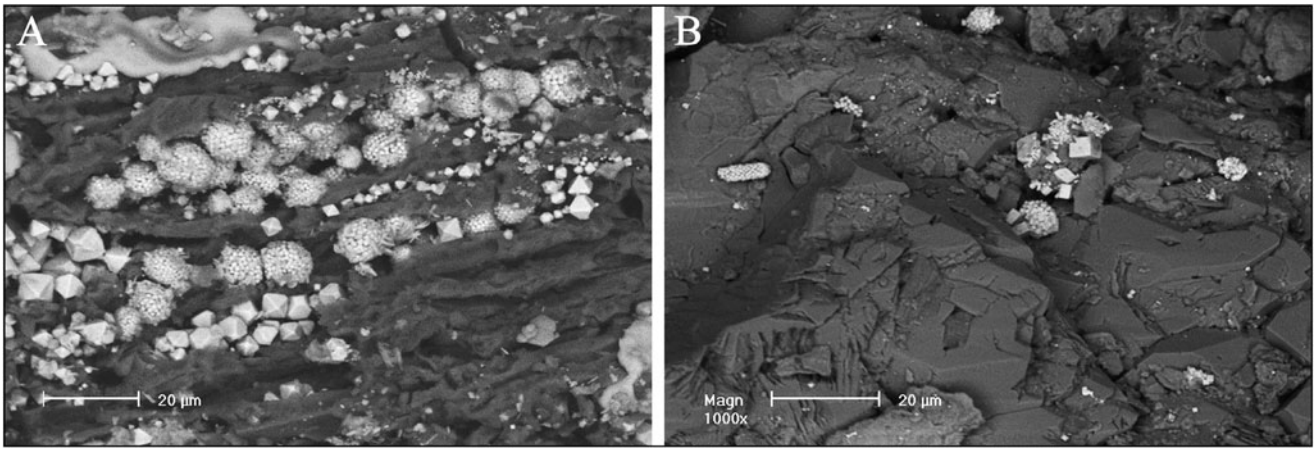

Fig. 1 SEM analysis, backscattered pictures: Cubic and framboidal pyrite on calcite. K. Rauppach, GTN

Interestingly and corresponding to the molecular biological results, it is described that pyrite framboids could be generally built up through microbial activities (Machel 2001, Schieber 2002).

During the time period of one year of microbial monitoring, the population structure remained quite constant. As to be expected due to very high temperature, low amounts of PCR products indicate lower cell numbers in fluid samples of about 103 °C compared to samples taken after passage through the heat exchangers with a temperature of about 61 °C. One sequence of a *Desulfotomaculum*-like bacterium was exclusively observed at a fluid temperature of 103 °C. Thus, this potentially hyper-thermophilic species seems to be outcompeted by the other detected *Desulfotomaculum*-like bacteria at temperatures around 61 °C. This finding also fits to the observation that many of the microbes in aquatic systems are aggregated in biofilms attached to surfaces (Wimpenny et al. 2000), otherwise different populations before and after the heat exchanger are not expected regarding the short retention times of the fluids. Although the PCR reaction is a sensitive method, DNA of underrepresented species can not be identified in the presence of relative high amounts of other species, like we observed after the passage through the heat exchanger. Thus it could not be ruled out that the strain that was found solely at 103 °C is not present after the heat exchanger at 61 °C in low cell numbers. Beside the competition in anoxic environments for hydrogen, formate and acetate (Robinson & Tiedje 1984), several studies described a syntrophic relation between sulfate-reducing bacteria and methanogens (Walker et al. 2009). Therefore the presence of the lithoautotrophic, hydrogenotrophic archaeon *Methanothermobacter thermoautotrophicus* identified in 61 °C hot fluids by DGGE analysis could be an indication for a syntrophic correlation with SRB. No changes in the archaeal community in fluids were observed during the 12 months of fluid monitoring. These syntrophic interactions are usually associated with interspecies hydrogen or even formate transfer (Schink & Stams 2002). Galouchko & Rozanova (1996) characterized a syntrophic relation between a non-sulfate-reducing bacterium, that grow by acetate oxidation coupled to reduction of Fe(III), elemental sulfur, or fumarate as electron acceptor. Alternatively, it can also grow on acetate, releasing electrons via interspecies electron transfer to a SRB. Additionally, ethanol-oxidizing sulfate reducers such as *Desulfovibrio vulgaris* have shown the ability to oxidize ethanol in the absence of sulfate by hydrogen transfer to a hydrogen-oxidizing methanogenic bacterium (Bryant et al. 1977). By whole-genome transcriptional analysis it was revealed that the expression of numerous genes involved in electron transfer and energy generation was up-regulated if growing syntrophically in co-culture. Beside potential activity-enhancing syntrophic effects, the relatively high abundance and diversity of SRB and the presence of methanogens at this site could be linked to the availability of fermentable organic substrates (DOC 6.4–19.3 mg C/l), presumably originated from organics in the natural crude oil of the malm aquifer. The characterization of the microbial community and geochemistry allows further insight into metabolic reactions which could take place after passing the heat exchanger: SRB and methanogens are able to sustain the extreme environmental conditions by syntrophic growth. Fluids from the observed plant are indeed characterized by a relative large methane fraction (50 vol %) in the gaseous phase. It is known for several species of *Desulfotomaculum* that sulfate reduction with propionate only takes place in the presence of archaeal methanogens such as *M. thermoautotrophicus* (Imachi et al. 2006). With these findings evidence is given for the interaction of these two microbial processes in engineered geothermal systems. Thereby studies, only focusing on SRB as main source for microbial mediated corrosion or precipitation processes, might overlook the importance of such syn-

Table 1 Detected microorganisms by DGGE fingerprinting

Gene Sequence Nr. Acc. Nr.	Sample origin	Temp [°C]	Sulfate [mg/l]	DOC [mg C/l]	Closest division	Similarity [%]
DSR a1_ dsr52-55 HQ844562	03-2008 until 09-2009 after heat exchanger	61	30–38	6.4–19.3	*Desulfotomaculum putei*	*100*
DSR a1_dsr59 HQ844565	03-2008 until 09-2009 before heat exchanger	103	30–39	7.3–12.3	*Desulfotomaculum* sp.	*87*
DSR a1_ dsr43 HQ844566	03-2008 until 09-2009 before heat exchanger	103	30–39	7.3–12.3	*Desulfococcus oleovorans* Hxd3	*92*
DSR a1_dsr58 a1_ dsr46 a1_ dsr47 HQ844564 HQ844560 HQ844561	03-2008 until 09-2009 after heat exchanger	61	30–38	6.4–19.3	*Desulfotomaculum* sp.	*85–89*
DSR a1_dsr57 HQ844563	03-2008 after heat exchanger	61	30–38	6.4–19.3	*Desulfotomaculum* sp.	*85*
16S rRNA bacteria a1_eub70 HQ844567	03-2008 until 09-2009 before and after heat exchanger	61–103	30–39	6.4–19.3	*Desulfotomaculum reducens*	*94*
16S rRNA bacteria a1_eubM1 HQ844568	09-2008 until 09-2009 after heat exchanger	61	30–38	6.4–19.3	Candidatus *Desulforudis audaxviator* MP104C	*94*
16S rRNA archaea a1_arch1-2 HQ844569	09-2008 until 09-2009 after heat exchanger	61	30–38	6.4–19.3	*Methanothermobacter thermoautotrophicus*	*100*

trophic growth. Under laboratory conditions the temperature optimum for growth of *M. thermoautotrophicus* is in the range of 65–70 °C, thereby the habitat temperature of 61 °C is serving good growth conditions. In the same fluids a Candidatus *Desulforudis audaxviator*-like bacterium was identified. Candidatus *D. audaxviator* is a SRB which was found in depths from 1.5 km to 3 km below the Earth's surface in the groundwater (Chivian et al. 2008). Genome analyses are indicating a motile, sporulating, chemoautotrophic and thermophilic organism able to fix nitrogen and carbon by syntrophic relations to archaea. Candidatus *D. audaxviator* is capable of an independent life-style well suited to long-term isolation within Earth's crust. The low DNA similarity of some detected sequences to members of the genera *Desulfococcus* and *Desulfotomaculum* makes assumptions concerning their ecological function more difficult. Further molecular biological analyses and the classical approach of cultivation could help to unravel the environmental importance of these microbes.

The detection of SRB and microbial species living syntrophically with sulfate reduction provides evidence that microbes may play an important role in biogenic processes that can impact the reliability of geothermal plants. No significant differences in the amounts of organic acids were observed although the detected low molecular weight organic acids are suitable substrates for SRB and methanogens. It is probable that due to the high flow rate serving a continuous supply with organic substrate did not allow monitoring of concentration changes due to consumption of microorganisms. However, the observation of an increased diversity of sulfate reducers after the heat exchanger indicates an active biocoenosis and that temperature is one of the cru-

cial environmental factors in this engineered ecosystem. It is remarkable that the detected microbes resisted *in-situ* temperatures up to 103 °C. For genera such as *Desulfotomaculum* and *Thermodesulfobacter* hyperthermophilic growth is described, and sulfate reduction was observed in hot thermal waters up to 110 °C (Jørgensen et al. 1992). The so called strain 121 (*Crenarchaeota*) that reduces Fe(III) under laboratory conditions up to 121 °C, was found in a hydrothermal vent with a temperature of up to 300 °C. Thus it is assumable that the estimated upper temperature limit for life is not finally established (Kashefi & Lovley 2003, Takai et al. 2008). Respecting that SRB, especially *Desulfotomaculum* are spore-forming microorganisms, it is assumable that they could resist higher *in-situ* temperatures than the observed temperatures in laboratory experiments. Rosnes et al. (1991) experimentally showed that spores of *Desulfotomaculum* sp. were extremely heat resistant and survived 131 °C for 20 min. Nevertheless the measured *in-situ* temperature of the fluids is an average value, therefore, on the borders to surrounding sediments temperatures could be slightly lower.

Conclusions

Overall, the detection of microbes known to be involved in biocorrosion and precipitation products such as iron sulfides indicated that microorganisms could have an influence on the reliability and operating life of the geothermal plant. SRB seem to play an important role in these ecosystems although the investigated geothermal system had a quite low sulfate content compared to the plants operating in saline aquifers such as in the Northern German Basin.

The increase of diversity of SRB after the heat exchange can be regarded as a first evidence for the relevance of microbial activity for the re-injection of fluids and the subsequent cooling of the near-well area over long-term periods. A further geochemical, mineralogical and microbiological monitoring of fluids from the geothermal plant and a physiological characterization of the occurring species will deliver comprehensive insight into the economical significance of microbial mediated processes. This enhances the overall process understanding of mineral creation and alteration as well as corrosion of plant components.

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