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Biogeochemical Characterisation of Geothermally Used Groundwater in Germany

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ABSTRACT

The multidisciplinary research project “AquiScreen” investigates the working reliability of geothermal utilization of aquifers, especially, considering microbial activity as well as particle transport and relocation. Major interest is the impact of microbial populations on aquifer systems. Therefore, the project integrates microbiological, biogeochemical, mineralogical, and petrologic investigations to qualify and quantify the variability of subsurface microbial communities in the fluid and solid phases of shallow and deep geothermal systems in Germany.

Organic compounds as well as sulfate (as an electron acceptor) are monitored to assess the operational state of geothermal plants and energy storages with respect to the working reliability. Our study shows, that the investigated parameters (such as low molecular weight organic acids – LMWOA - and sulfate) can be indicative markers for the evaluation of these plants. Sulfate concentration in shallow geothermally used aquifer systems (energy storages) seems to be suited to estimate microbial impact in plants. For deep energy storages, variations in LMWOA and DOC (dissolved organic carbon) concentration is suggested to be a marker for changes in fluid chemistry and can be an initial indication of fermentative microbial colonization. In the investigated geothermal plants and energy storages, sufficient amounts of electron acceptors and -donors are detected and are available as a potential feedstock for microbial life. Furthermore, we detect in all investigated geothermally used plant systems in the North German Basin microbial communities by 16S rDNA profiles and biomarker analyses (phospholipids). In some cases the bacterial metabolism may result in scale formation, filter clogging and corrosion processes.

1. INTRODUCTION

The scientific question about life in the deep biosphere and how the microorganisms can survive is of great interest from an industrial point of view. Since it is known that microorganisms can play a major role in damaging, e.g. industrial wells, cooling towers, and heat exchanger by biofouling, microbial influenced corrosion (MIC) as well as iron clogging (Pedersen, 1990; Sand, 2003; Ungemach, 2003; Coetser & Cloete, 2005), research groups work on the understanding of these phenomena and how the impact of microbes can be avoided. In the light of decreasing conventional energy resources, and increasing CO_2 emissions, nonpolluting geothermal energy and energy storages attract soaring notice in recent years (Bertani, 2005; Schellschmidt *et al.*, 2007).

For the working reliability of geothermal plants and energy storages, the monitoring program “AquiScreen”, started in 2007, investigates the role of microorganisms in geothermally used groundwater systems and the controlling factors of the microbial impact on the plants. For the assessment of the working reliability it is important to know, if there is any microbial alteration during extraction and reinjection of these volumes of ground water over time. Microorganisms seem to occur in nearly every environment of the earth subsurface where water, pore space, and possible carbon and energy sources for the microbial metabolism are available and adequate temperature conditions are prevailing. To date, only less is known about the biogeochemical interactions in the subsurface and within a geothermal plant.

Microorganisms in subsurface environments gain their energy from biogeochemical reactions for maintenance and growth and influence directly or indirectly the physical properties and chemical composition of sediments and waters (Fredrickson & Onstott, 2001). The metabolism of microbes can yield to the dissolution and precipitation of inorganic compounds as well as the degradation of organic matter (Lovley & Chapelle, 1995; Parkes *et al.*, 2000). In the absence of light and oxygen, microorganisms evolved a wide range of different strategies to survive in the subsurface depending on their environment. For instance, if biodegradable organic matter is available, fermentative microorganisms oxidize organic compounds into smaller organic species like e.g. acetate (Westermann, 1996). Low molecular weight organic acids (LMWOA) such as formate and acetate are essential substrates for microbial processes particularly in anaerobic ecosystems, where the substrate can be oxidized by fermentation or can be reduced by respiration. Fermentation takes place in the absence of exogenous electron acceptors while in respiration an electron acceptor is present as a terminal electron acceptor. In addition to organic species, a number of inorganic compounds (e.g. SO_4^{2-}, NO^{3-}, Fe^{3+}) can provide sufficient energy for microbial life by anaerobic respiration (Madigan & Martinko, 2006).

Dissolved organic carbon (DOC) is defined by 0.45 µm filtration and represents the most crucial energy and carbon source for microorganisms in groundwater systems (Clay *et al.*, 1996; Neff & Asner, 2001). This bulk parameter is composed of different amounts of compounds from several substance classes like humic substances (HS), biopolymers, neutral compounds, hydrophilic acids and hydrophobic substances. The DOC content provides a diversity of such compounds, but in general it is assumed that only 5 to 25% of the dissolved organic carbon is biodegradable. The major fraction is refractory (like humic substances) and not available for microbial metabolism (Griebler & Mösslacher, 2003). For natural groundwater systems an average value of almost 1 to 5 mg C/L is provided in literature (Leenheer *et al.*, 1974; Spalding *et al.*, 1978). The natural source of dissolved organic carbon in groundwater derives either from soils and sedimentary organic matter like peat, kerogen and coal or from water infiltrating through rivers, lakes and marine systems (Aiken, 2002). Detailed information about the origin and fate of DOC as electron donor for microbial metabolism can be provided by the isotopic composition of $\delta^{13}C_{DOC}$. Understanding the origin of dissolved organic carbon may provide additional information about the nutrient availability for microorganisms. Carbon isotope ratios of the bulk parameter DOC from aquifer systems are rarely presented in the literature. However, the $\delta^{13}C_{DOC}$ of aquifer systems ranges between -20‰ to -28‰ (Leenheer *et al.*, 1974; Spalding *et al.*, 1978; Aravena & Wassenaar, 1993) and is significantly lighter than the marine dissolved organic carbon which ranges between -18 ‰ to -20 ‰ (Avery *et al.*, 2006).

The current study focuses on the detection of small dissolved organic compounds (e.g. acetate), being a potential feedstock for microbial life, the analysis of microbial biomarkers and the determination of the carbon isotopic composition of these substances. Compound specific isotope analysis is used to specify the origin and fate of these compounds in the investigated geothermal systems. Intact phospholipids being microbial cell membrane constituents and indicating viable microorganisms (White *et al.*, 1979) are subject of our analysis of groundwater as well as filter material from the technical geothermal plants to document microbial variability. The analysis of the ester-linked lipid classes, like Phosphatidylglycerol (PG), Phosphatidylethanolamine (PE), Phosphatidylcholine (PC), and Phosphaytidylserine (PS) provides information on the domain of Bacteria. Furthermore, numerous investigations e.g. (Lechevalier & Moss, 1977; Green & Scow, 2000) demonstrates that the fatty acid side chains of the phospholipids (PLFA) gives information about microbial groups and their adaption to the specific environmental conditions. Furthermore, dissolved organic carbon (DOC) is characterized in more detail to estimate its origin and relevance as an energy and carbon source for microorganisms. Additionally, scales like carbonates and iron sulfides are investigated, and phylogenetic analysis is done based on 16S rRNA genes with respect to the properties of the aquifer and the operation mode of the geothermal plants. In this study we will present results of biogeochemical, microbiological, and mineralogical investigations from different geothermally used shallow and deep groundwater systems in Germany.

2. STUDY SITES

For the first biogeochemical screening of geothermally used groundwater systems, we investigated nine different aquifer systems. These systems are located in the two of the three major zones for geothermal energy production in Germany. The first zone is the North German Basin (NGB) with different energy storage systems and depth ranges from 30 to 1650 m below surface. The second zone is located in southern Germany in the Molasse Basin (MB) closed to the Alps with maximum depth of the investigated plants of > 3500 m below surface. The third zone, the upper Rhine Valley, was not investigated.

In the North German Basin two shallow geothermally used aquifers (<< 400 m) were examined. These two quaternary aquifer systems are used as heat and cold energy storages with temperature ranging between 5 to 50 °C, depending on energy storage type and operation mode (charge/discharge). Furthermore, one deep Mesozoic heat storage was screened showing temperatures of 45 to 78 °C and representing a high saline aquifer. For a better understanding of deep high saline aquifer systems, two balneological used thermal waters (initial temperature: 64 °C) were included in the monitoring project.

In the Molasse Basin four geothermal plants were investigated being all located in the upper Jurassic so called Malm aquifer with depth from 2200 to 3450 m below surface. These plants are used for district heating and power production. Temperatures of the deep aquifer systems can reach more than 120 °C.

3. MATERIALS AND METHODS

Since 2007 the aforementioned geothermal plants and energy storages were monitored using fluid and filter samples in the North German Basin and only fluid samples in the Molasse Basin. Process water has been taken from the production flow of the aquifer, and additionally, whenever possible, water was taken directly from the aquifer.

After sampling, the fluid samples are immediately analysed by ion chromatography for inorganic and organic anions like sulfate and LMWOA to detect and quantify the potential nutrients and metabolites of microbial processes. For anion detection all fluid samples were analyzed repeatedly (3-5 times) by ion chromatography (ICS 3000, Dionex Corp.) equipped with conductivity detection, 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.) was used at 35 °C.

The DOC was measured by IR-spectroscopy (TOC-2000A, Shimadzu) as non-purgeable organic carbon (NPOC) after acidification with hydrochloric acid. The characterization and quantification of the dissolved organic carbon (DOC) and its fractions were conducted by liquid chromatography-organic carbon detection (LC-OCD). The chromatographic system is composed of size exclusion chromatography combined with organic carbon detection and UV detection (λ=254 nm) (Huber & Frimmel, 1996).

Our method for the bulk carbon isotope analysis of the dissolved organic carbon (DOC) uses the LC IsoLink interface (Thermo Fisher Scientific), which is coupled to a continuous-flow Delta V Advantage (Thermo Fisher Scientific) (Krummen *et al.*, 2004). This bulk isotope analysis can be done by direct injection of the water sample into the LC IsoLink interface. Reference materials and unknown bulk samples can be analyzed with high sensitivity and speed.

To measure intact phospholipids (PL), filter samples from the plants were used. The accumulated sediments in filters are freeze-dried and are extracted using flow-blending (Radke *et al.*, 1978) and a modified Bligh and Dyer method (Bligh & Dyer, 1959). Subsequently, to separate major compound classes of the crude lipid extract, a column chromatography was performed and the PL fraction was measured by HPLC-ESI-MS. To obtain the phospholipid fatty acids (PLFA), a mild alkaline hydrolysis for the transesterification and derivatisation was used on the PL fraction according to the method by (Mueller *et al.*, 1990) and measured by GC-MS.

Microorganisms in process water and filters were studied using 16S rDNA analyses. The structural composition of the microbial community and the effects of energy recovery on community structure in geothermal aquifers were evaluated by a cultivation independent approach, based on PCR amplification of partial 16S rRNA genes from DNA. Single strand conformation polymorphism (SSCP) was chosen as a genetic profiling technique allowing direct comparison of the community composition from different geothermal plants and the identification of differences by DNA sequencing and phylogenetic analysis (Dohrmann & Tebbe, 2004).

Furthermore, standard characterization of the fluids were conducted, like measurements of pH, Eh, conductivity, oxygen content, temperature, titration of C-species (HCO_3^- and CO_3^{2-}), anions, cations and gas content are also measured. Additionally, filter bags of the geothermal plants are investigated by scanning electron microscopy (SEM).

4. RESULTS AND DISCUSSION

4.1 Composition and origin of fluids and solids of geothermally used groundwater systems

Generally, three types of water can be distinguished: (1) "normal" shallow freshwater aquifers, (2) low saline infiltration fluids and (3) high saline basinal fluids. Fluids from the investigated plants in the Molasse Basin are type two water with less than 1 g/L of salts and the fluids are dominated by HCO_3^-. In contrast, the deep aquifers in the North German Basin mostly consist of type three water with salinity of 100-300 g/L and are NaCl-dominated fluids. The shallow aquifers of the North German Basin contain type one water and the water chemistry represents a nutrient-poor environment.

The general composition of these shallow freshwater aquifers from the North German Basin suggest an oligotrophic environment (Total Dissolved Solid = 700 mg/L). Cations in these freshwater aquifers are mainly dominated by calcium (ca. 35 meq%) and minor sodium and magnesium (5-10 meq%). Iron in groundwater is observed with concentration of 2 mg/L, while sulfate and bicarbonate occurs in similar amounts with around 20 meq%. The concentration of chloride in shallow aquifers reaches to 10 meq%. CO_2 as dissolved gas in groundwater occurs with 20 mg/L. According to source rocks and deep fluids, "newly formed minerals" can be classified due to types of water. Shallow freshwater aquifers contain mostly iron hydroxide, iron sulfides and carbonates as new mineral formations.

The deep high saline aquifers in the North German Basin are characterized by newly formed minerals like lead, galena (lead sulfide), barite (barium sulfate), celestine (strontium sulfate), carbonates and miner iron sulfides. The amount of Total Dissolved Solids is 200 g/L and the main cation is sodium, while chloride is the dominant anion in these fluids and therefore the thermal waters are classified as Na-Cl-type. The pH value is slightly acidic with pH 6.0. Furthermore, the fluids contain about 15 mg/L of dissolved iron ions and ammonium is present with 25 mg/L. Sulfate concentration can have maximum values of 4 g/L and silica is with up to 20 mg/L comparatively low. The gas composition ranges between 5 to 10 Vol.% and is composed 55 Vol.% of CO_2 and 42 Vol.% of N_2. Trace gases are methane, helium, hydrogen, argon and ethane.

The predominant water type in the Molasse Basin is Na-HCO_3-Cl with Total Dissolved Solids of 1 g/L. The other parameters, like pH, are highly depended on the specific study site. Slightly acidic fluids (pH 6.5) are common in this area. High H_2S concentrations providing strong reducing environmental conditions are characteristic for low saline infiltrations fluids. Depending on the local conditions, gas content and composition differs in the Molasse Basin. The gas-water ratio is about 1:2, which means, in 1 liter thermal fluid 0.5 L of gas is dissolved. The gas content is composed of CO_2 and H_2S (50-70 Vol.%), methane, ethane as well as hydrocarbons (ca. 30-50 Vol.%) and minor nitrogen (around 10 Vol.%). Carbonates and iron sulfides are observed as mineral formations in the calcareous aquifer in the Molasse Basin.

Overall, in all investigated geothermally used aquifer systems newly formed minerals were detected. New carbonates are caused by degassing of CO_2. Sulfides and hydroxides are formed by changes in Eh- and pH-values and temperature. In addition to these abiotic reactions, at least in one case there are indications that microorganisms are involved in the formation of new minerals.

4.2 Nutrients for microbes in geothermally used groundwater systems and their origin

Two shallow aquifer systems located in the North German Basin which are used as energy storages (cold storage and heat storage with seasonal charge and discharge mode) provided sufficient amounts of DOC (2.9 to 4.9 mg/L) which – in both cases - is mainly composed of humic substances (HS). Humic substances can increase to 69 % of the DOC and are mainly composed of fulvic acids. We assume that HS are derived from the soil organic matter due to the shallow depth of the aquifers (max. 60 m below surface). The isotopic compositions of DOC confirm this suggestion. Process water, that have been taken from the plant during operation mode, and fluids directly taken from the aquifer show almost the same isotope values of about -27‰. For the shallow energy storages, there was no observed correlation between the isotope data and the operation mode (charge and discharge).

Within the plants, organic acids like formate and acetate are detected in the fluid in small amounts by ion chromatography. These LMWOA may be interpreted as an indication for fermentative microbes within these geothermal plants. For the energy yielding process of fermentation the organic molecules serve as both electron acceptors and donors and no inorganic terminal electron acceptors are required. Furthermore, sulfate in groundwater being a potential electron acceptor and can play a major role for anaerobic microbial respiration, especially for sulfate reducing microorganisms (SRM). For the shallow aquifers, sulfate is available in sufficient amounts, up to 270 mg/L. During an microbial induced disturbance in the cold storage, resulting in a decreasing injectivity, sulfate concentration significantly decreases by 30 mg/L. Due to the shallow depth of the aquifer, sulfate concentrations vary seasonally. Therefore, if the annual variation of anions is known, sulfate concentration can be used as a marker for the actual condition in the plant and, therefore, to assess its working reliability. Our monitoring is still ongoing.

The deep heat storage in the North German Basin located in the lower Jurassic and upper Triassic aquifer 1250 m below surface has a high mineralization potential. Chloride concentration, as an inert tracer, reaches up to 76 g/L. Sulfate concentration is about 1 g/L and no seasonal or operation mode depending variation has been observed. LMWOA (formate and acetate) are available and seem to build up around 30% of the DOC (average 4 mg C/L). In correlation to a reduced injectivity of the heat storage an increase in the content of LMWOA and a decrease in DOC content were observed. This could be interpreted as an indication for enhanced fermentation processes in the aquifer system.

Two balneological used thermal deep waters were also monitored, both revealing also high mineralization potentials with more than 100 g/L of chloride and about 4 g/L of sulfate. LMWOA were not observed in the fluids. The DOC content ranges between 4 and 8 mg/L and probably seems to consist of complex organic molecules with higher molecular weight like refractory organic matter.

Summarizing the monitoring results for the highly saline deep aquifers in the North German Basin, concentrations of LMWOA and content as well as composition of the DOC seem to be indicators for microbial processes within the geothermal system. The concentration of sulfate does not seem to be suitable to discover microbial activities because of the high background concentration (g/L!). In contrast, sulfate concentrations of shallow aquifer systems with their lower sulfate background concentrations (mg/L) appear as an adequate parameter to detect microbial activity of SRM. However, this parameter is only applicable as an indicator, if the annual variability is known. To date, no correlation between LMWOA, DOC and technical problems in the shallow aquifer systems are observed.

Four geothermal plants for district heating and power production in the Molasse Basin have been monitored. All geothermal plants are connected to the calcareous Malm aquifer with depth >2000 m below surface and temperatures ranging from 65°C to 120°C. Three geothermal plants show relatively low amounts of DOC concentration (average 0.9 mg C/L), but their composition of the DOC differs. With increasing depth and temperature, the dominant fraction of the DOC changes from neutral compounds (e.g. alcohols, ketones and aldehydes) to more LMWOA. The organic acids increase significantly up to 66% of DOC. One geothermal plant exhibits high DOC content of about 12 mg C/L, however, in this region the Malm aquifer has been described to transport minor amounts of natural oil. The composition of the LMWOA in water samples from the Molasse Basin shows a higher compositional variability than in the North German Basin. In addition to formate and acetate, propionate and butyrate have been detected. Moderate sulfate (~30 mg/L) and chloride concentrations (~270 mg/L) are occurring. The monitoring of these geothermal plants is still ongoing.

4.3 Microbes in geothermally used groundwater systems

First biogeochemical indication for the occurrence of living microbial populations in aquifers is provided by the detection of intact phospholipids (PLs) in the filter material. PLs are only stable in viable intact cells and can, therefore, be used as indicators for living microorganisms (Zink *et al.*, 2003). For all investigated samples, significant amounts of phosphatidylglycerols (PG) and phosphatidylethanolamines (PE) and minor amounts of phosphatidylcholines (PC) were detected. The occurrence of these phospholipids reflects the presence of living bacterial organisms in the aquifers.

The main fatty acids linked to the different PLs in shallow aquifer systems are the typical saturated and mono-unsaturated C_{16} and C_{18} fatty acids. On the other hand, filter samples from the deep energy storage contained saturated and mono-unsaturated fatty acids, having odd chain length with 15 and 17 carbon atoms as main constituent. The PLFA profiles of the samples from the North German Basin showed different amounts of terminally branched saturated (*iso* and *anteiso*) and mid-chain-branched fatty acids, with 15, 16, and 17 carbon atoms. A striking feature of the PLFA distributions from geothermal applications is the ubiquitous proportion of branched saturated fatty acids, which are indicative for bacterial contribution, especially, for the contribution of sulfate-reducing bacteria.

A detailed screening of the PL composition and fatty acid side chain inventory for a possible adaption of microorganisms to the specific environment conditions (e.g. salt content, pressure, temperature) is currently in progress.

Bacteria were also detected in process water and filter samples of the investigated geothermal plants by 16S rDNA profiles. The analysis of the bacterial composition in samples originating from the operational circulation of the geothermal plants showed distinctive 16S rDNA profiles, indicating different microbial communities for the investigated plants. These differences are presumably caused by the environmental conditions in the aquifers, defined by depth, temperature, salinity and presence or absence of inorganic and organic nutrients. Only well adapted organisms are able to survive and proliferate in these environments. They are found to be located in sedimentary pore space and attached to the surfaces of technical plant materials.

The analysis of bacterial profiles revealed associations to different physiological groups, catalyzing turn over processes of certain nutrients being present in the aquifer or derived from plant materials. They can be classified in organoheterotrophic and lithoautotrophic organisms. Organoheterotrophic primary production bases on degradation of organic carbon (carbohydrates, lipids, organic acids), whereas a lithoautotrophic metabolism bases on inorganic carbon, in form of CO_2 and electron donors like H_2, CO, S^0, Fe^{2+} and NH_4.

Genetic profiles of microorganisms detected in the investigated geothermal plants are involved in cycles of basic elements of life: carbon, sulfur, iron, and nitrogen. Microbial metabolisms and nutrient cycles were partly connected by syntrophic relations between different bacteria types due to arrangement in complex biofilms. Biofilms are composed of bacterial cells and excreted extrapolymeric substances (EPS) and are attached to different solid surface materials and in porous media. The attachment of bacteria into filter fibres resulted in accumulation of bacteria, hold together by EPS. This biofilm probably retained fluid containing nutrients for bacterial metabolism and growth. Additional trapping of suspended particles that have been derived from the aquifer resulted in filter clogging.

One physiological group, relevant for the investigated energy storage systems in the North German Basin is, in addition to fermentative bacteria, sulfate reducing microorganisms (SRM). This study shows that SRM ubiquitously occur in the investigated North German geothermally used aquifers. SRM obtain energy by dissimilatory sulfate reduction resulting in sulfide production. Sulfide is of special interest because it will favor precipitation of iron sulfide in the presence of Fe^{2+}. Additionally, SRM are involved in corrosive processes, attacking the surface of metallic plant materials (Beech & Gaylarde, 1999).

Sulfur oxidizing bacteria are also negatively affecting plant relevant processes. Due to the partly filamentous cell structures these organisms easily accumulate and form biofilms, influencing the filter permeability.

5. SUMMARY AND CONCLUSION

Sufficient amounts of nutrients for microbial metabolism are detected in all investigated studied sites. Moreover, the monitored parameters (such as LMWOA and sulfate) can be indicative markers for the operational state of the geothermal plants. For shallow aquifer systems which serve as energy storages, sulfate can be used as marker for microbial impact, if "background" values of the annual variation of sulfate concentration are known. Variation in LMWOA and DOC concentration for deep energy storages, especially for high saline aquifers with high sulfate concentration (g/L), seems to be a marker for changes in the fluid chemistry and can be an initial indication of fermentative bacteria colonization.

The occurrence of microorganisms in most of the geothermally used plant systems have been shown by 16S rDNA profiles and biomarker analyses (phospholipids). The microbial communities in process waters and filters were phylogenetic and metabolic diverse. The different nutrients monitored in the aquifer waters form a potential feedstock for these microorganisms. On different sites in the North German Basin, bacterial metabolism resulted in scale formation, filter clogging and corrosion.

Our investigations showed that shallow geothermal systems and also deep geothermal systems with temperatures below 100°C reveal indications of microbial colonization. This statement has to be confirmed for the high temperature systems of the Molasse Basin. Our monitoring activities provided a deeper insight into the natural variability of microbial communities in geothermally used aquifer systems possibly leading to severe technical problems in the individual geothermal plants.

Since the monitoring is still ongoing, data compilation and final conclusions are not yet drawn.

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