

The Influence of Geothermal Plants on the Biogeochemistry of the Microbial Ecosystems in Aquifers

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Alexandra Vetter

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Vorsitzender: 1. Prof. Dr. G. Franz

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Alexandra Vetter

List of Publications

Articles:

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Abstract

With increasing importance of geothermal energy as renewable energy providing huge amounts of sustainable, CO₂-effective and commercial base load power, also possible disturbances within the utilisation of geothermal techniques have to be addressed. The efficiency of geothermal plants can be deteriorated by the presence and activity of microbial organisms as well as by their metabolic products. Known consequences of microbial activity in geothermal systems are corrosion, scaling, and clogging. For an improved understanding of these phenomena, microbial communities in plant-integrated filter systems as well as fluid chemistry have been investigated in a long-term study on geothermal plants in the North German Basin and the Molasse Basin.

The aim of this study was the characterisation of the induced changes of the indigenous microbial communities to the individual operation modes of various geothermal plants with different operating conditions (e.g. temperature, depth, fluid chemistry). The microbial communities in the geothermal plants were characterized using molecular marker compounds (biomarkers). The biomarkers applied were intact phospholipids being characteristic for living bacteria (“life marker”) and its linked fatty acids side chain inventory (phospholipid fatty acid, PLFA). Another focus was placed on the characterisation of fluid chemistry at different geothermal sites with special emphasis on the quality and quantity of dissolved organic carbon (DOC) and the changes in fluid composition related to operation of the geothermal plants as well as to microbial activity.

It could be concluded that the microbial communities are remarkably resistant to the different operation modes of the geothermal plants and developed various adaptations to survive the prevailing temperature regimes as well as the variability in nutritional state in such environments.

In a shallow cold storage (German Parliament, Berlin) strong indications of a microbial contribution to clogging processes have been observed by changes in PLFA composition between times of normal operation and plant deterioration. A newly developed PLFA ratio can be applied to assess the microbial contribution to clogging and provides a helpful tool in the selection of a proper cleaning technique. In addition, biomarker analysis revealed the adaptation of bacterial membrane lipids to unfavourable living conditions by transformation of *cis* to *trans* monounsaturated fatty acids.

Furthermore, in a shallow heat storage (Rostock) the PLFA composition showed an adaptation to decreasing temperature conditions during discharge operating mode.

In the deep heat storage Neubrandenburg microbial communities were observed that are adapted to the specific temperature range as well as operating modes (charge/discharge, cold/warm side). According to the PLFA results, the dominating species in the microbial community on the cold site are sulfate-reducing bacteria. It is likely that the sulfate-reducing bacteria were involved in corrosion occurring on the pump at the cold side.

Water samples from all geothermal sites were analysed for inorganic and organic anions (e.g. sulfate and acetate), DOC and its isotopic composition ($\delta^{13}\text{C}_{\text{DOC}}$). At all investigated sites the most important electron acceptor for microbial respiration was sulfate, although in the Molasse Basin high concentrations of dissolved gases (e.g. CO_2) may also play a crucial role in redox reactions. Observed changes in fluid chemistry have not been indicative for geothermal plant deterioration. This limited applicability of fluid chemistry parameters is possibly related to the high volumes of process water that flow through the geothermal systems and provide continuous and sufficient nutrient supply. For the Molasse Basin a depth-related correlation of the concentration and composition of DOC has been shown. In addition, concentration and composition of DOC seem to be valuable parameters to characterize the decrease in drill mud contamination over operation time.

Kurzfassung

Mit der zunehmenden Bedeutung der geothermischen Energie als erneuerbarer Energieträger, die große Mengen nachhaltiger, CO₂-effizienter und ökonomischer Energie für die Grundlastversorgung zur Verfügung stellen kann, müssen auch mögliche Störungen in der Nutzung dieser Technik untersucht werden. Die Effektivität geothermischer Anlagen kann durch die Anwesenheit und Aktivität von Mikroorganismen sowie durch deren Stoffwechselprodukte beeinträchtigt werden. Bekannte Folgen mikrobieller Aktivität in geothermischen Systemen sind Korrosion, Ablagerungen und Verstopfungen. Um ein besseres Verständnis dieser Prozesse in geothermischen Anlagen zu bekommen, wurden sowohl die mikrobielle Gemeinschaft in den integrierten Filtersystemen als auch der Fluidchemismus in einer Langzeitstudie an verschiedenen geothermischen Anlagen und Energiespeichern im Molassebecken und im Norddeutschen Becken untersucht.

Das Ziel der vorliegenden Arbeit war die Erforschung der indigenen mikrobiellen Gemeinschaft der geothermischen Anlagen und deren Anpassung an die verschiedenen Betriebsbedingungen (z.B. Temperatur, Tiefe, Fluidzusammensetzung). Die mikrobielle Biozönose wurde anhand von molekularen Markern (Biomarker) charakterisiert. Bei den untersuchten Biomarkern handelt es sich um intakte Phospholipide, die ausschließlich in lebenden Bakterien vorkommen („life marker“) und um deren Inventar an Fettsäuren (Phospholipidfettsäuren, PLFA). Ein weiterer Fokus richtete sich auf die Charakterisierung des Fluidchemismus der verschiedenen Geothermie-Standorte mit besonderem Augenmerk auf die Qualität und Quantität der im Thermalwasser gelösten organischen Kohlenstoffverbindungen (dissolved organic carbon, DOC) und die Änderungen in der Fluidzusammensetzung in Abhängigkeit von der Betriebsweise der geothermischen Anlage bzw. der mikrobiellen Aktivität.

Anhand der vorliegenden Ergebnisse konnte geschlussfolgert werden, dass die mikrobiellen Gemeinschaften ausgezeichnet angepasst sind an die jeweiligen vorherrschenden Betriebsbedingungen und Strategien entwickelt haben, um den Veränderungen in Temperatur und Nährstoffgehalt zu begegnen.

Für den flachen Kältespeicher (Reichstag, Berlin) konnte nachgewiesen werden, dass der Rückgang der Injektivität mikrobiell induziert ist, da sich die Zusammensetzung der PLFA von Betriebszeiten mit normaler Injektivität zu Zeiten mit reduzierter Injektivität reproduzierbar verändert hatte. Aus diesen Ergebnissen konnte ein PLFA-Parameter

abgeleitet werden, mit dem der mikrobielle Beitrag zu den Injektivitätsproblemen abgeschätzt und das optimale Reinigungsverfahren ausgewählt werden kann. Zusätzlich zeigte die detaillierte Analyse der bakteriellen Zellmembranbestandteile die Anpassung an weniger vorteilhafte Umweltbedingungen durch die Transformation der einfach ungesättigten Fettsäuren vom *cis*- zum *trans*-Isomer.

Weiterhin konnte in einem flachen Wärmespeicher (Rostock) mit der PLFA-Analyse die Anpassung der indigenen mikrobiellen Biozönose an die sinkenden Fluidtemperaturen während der Ausspeicherung nachgewiesen werden.

Für den tiefen Wärmespeicher Neubrandenburg konnte gezeigt werden, dass die mikrobiellen Gemeinschaften sowohl an die Temperaturschwankungen als auch die Betriebsweisen (Einspeicherung/Ausspeicherung, kalte Seite/warme Seite) sehr gut angepasst sind. Aus den Ergebnissen der Filteruntersuchungen konnte geschlossen werden, dass sulfatreduzierende Mikroorganismen auf der kalten Seite dominieren. Daher kann angenommen werden, dass die Sulfatreduzierer an der Pumpenkorrosion auf der kalten Seite des Wärmespeichers beteiligt waren.

Von den Thermalwässern aller Untersuchungsstandorte wurden die Konzentrationen der gelösten anorganischen und organischen Anionen (z.B. Sulfat und Acetat), der DOC und seine Isotopenzusammensetzung ($\delta^{13}\text{C}_{\text{DOC}}$) bestimmt. An allen Standorten war Sulfat der wichtigste Elektronenakzeptor für die mikrobielle Stoffwechselprozesse, auch wenn an den Standorten im Molassebecken hohe Gehalte an gelösten Gasen (CO_2) gemessen wurden, die ebenfalls für die mikrobiellen Redoxprozesse von Bedeutung sein können. Die Veränderungen im Fluidchemismus konnten nicht mit dem jeweiligen Zustand oder der Betriebsweise der geothermischen Anlagen korreliert werden. Diese eingeschränkte Anwendbarkeit der Fluidparameter kann auf die hohen Volumenströme in den geothermischen Anlagen zurückgeführt werden, die für ein kontinuierliches und ausreichendes Angebot an Nährstoff sorgen. Für die Standorte im Molassebecken konnte gezeigt werden, dass sich Konzentration und Zusammensetzung des DOC in Abhängigkeit von der Tiefe des Malm-Aquifers verändern. Weiterhin konnte anhand der Konzentrationen sowie der jeweiligen Zusammensetzung des DOC die Anwesenheit von Resten der Bohrspülung im Thermalwasser nachgewiesen werden.

Abbreviations

ATES	Aquifer thermal energy storage
BB	Building blocks
Bio	Biopolymers
C	Charge of heat or cold
DC	Discharge of heat or cold
DOC	Dissolved organic carbon in mg C/L
$\delta^{13}\text{C}_{\text{DOC}}$	Ratio of ^{13}C and ^{12}C isotopes in ‰ relative to PDB on DOC
EDM	Early discharge mode
EPS	Exopolymeric substances
FA	Fatty acid
HE	Heat exchanger
HOC	Hydrophobic organic carbon
HS	Humic substances
LC-OCD	Liquid chromatography-organic carbon detection
LDM	Late discharge mode
LMWNS	Low molecular weight neutral substances
LMWOA	Low molecular weight organic acids
MB	Molasse Basin
M1 to M5	Study sites in the Molasse Basin
NC	Neutral Compounds
NGB	North German Basin
NOOP	Normal operating mode
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PG	Phosphatidylglycerol
PLFA	Phospholipid fatty acid
RI	Reduced injection
SRB	Sulfate-reducing bacteria

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1 Introduction

Today, renewable energy technologies and energy saving strategies are becoming increasingly important within the light of the current public debate on energy shortage and environmental protection. The use of conventional energy resources such as fossil fuels (e.g. gas, oil and coal) is thought to influence Earth's climate resulting in a rapid climate change in recent years towards warmer conditions caused by emission of the anthropogenically produced greenhouse gas CO₂ (IPCC, 2007). Furthermore, nuclear power will obviously remain a high risk technology for mankind and natural ecosystems as implied by nuclear disasters such as in Chernobyl, Ukraine (1986) or in Fukushima, Japan (2011). Geothermal energy is a possibility for a clean and sustainable energy resource in addition to photovoltaic, wind power, biogas plants, hydropower stations, and tidal power. Geothermal energy appears to be one of the most promising alternative energy sources because of its constant availability, presence in many areas, independence of weather and climate, safety and environmental friendliness. The generated and stored thermal energy of the Earth in combination with hydrothermal systems (aquifers) provides many applications for energy supply (power production, direct utilisation of heat), for balneology (thermal spa), as well as for energy storage (cold and heat storage) and represents a technology supporting careful and sustainable handling of our resources.

The installation of a geothermal used plant is a disturbance of the natural systems, not only having an effect on the hydrology, chemistry and rock properties but also on the indigenous microbial communities in the deep subsurface. The impact of geothermal plants on the microbial ecosystem in aquifers and in return the impact of microorganisms on the performance of geothermal plants is up to now very poorly investigated. Thus, the topic of this work is to improve the understanding on the interaction of geothermal plants and the indigenous microbial communities.

1.1 Geothermal Energy

The main idea is to utilize the generated and stored thermal energy of the Earth as a clean and sustainable energy resource. This is most efficient in regions where the geothermal heat flow is high, and as a consequence, economically usable amounts of geothermal heat reaches accessible shallow lithospheric successions or even the surface.

1.1.1 Heat Inventory of the Earth

Sources of Heat

The heat of the Earth's interior derives on the one hand from primordial heat related to the formation of the Earth 4.5 billion years ago (gravitation energy) and on the other hand from the decay of natural radioisotopes such as uranium (^{238}U , ^{235}U), thorium (^{232}Th), and potassium (^{40}K). In a recent publication by Gando *et al* (2011) it is indicated that almost half of the Earth's total heat loss derives from the Earth's primordial heat supply.

Transport Processes of Heat

Transport processes of Earth's heat to the surface are mainly controlled by conduction, convection, and radiation (Allen & Allen, 1990). The heat flow in the shallow lithosphere, which can be reached by geothermal applications occurs mainly in the form of conduction and convection and is defined as the product of the geothermal gradient and thermal conductivity (Stüwe, 2000).

Heat conduction as the primary process in the shallow lithosphere is controlled by thermal conductivity which describes the ability of a material (e.g. rocks) to transfer thermal energy via conduction (Hantschel & Kauerauf, 2009). The thermal conductivity depends on mineral content, porosity, pore fluid and the anisotropy of a specific rock types (Clauser & Huenges, 1995). If rocks are isotropic such as volcanic and plutonic rocks, the heat flow will be predominantly vertical. Lateral heat flow prevails in the case of anisotropic rocks such as sedimentary and metamorphic rocks (Clauser & Huenges, 1995).

Heat convection is the dominant transport mechanism in the Earth's mantle, the ductile asthenosphere (Allen & Allen, 1990), but can be also relevant within sedimentary basins by processes such as fluid flow of pore water, liquid petroleum and gas, or by movements of solids such as salt.

Advection is a combination of conduction and convection and proceeds e.g. via heating of grains by groundwater flow (Hantschel & Kauerauf, 2009), intrusion of magma (contact metamorphism), or erosion (exhumation, sedimentation) (Stüwe, 2000). For instance, high erosion rates may increase the surface heat flow by exposure of hot rocks (England & Richardson, 1980). In contrast, high sedimentation rates result in a decrease of surface heat flow by deposition of "fresh/cold" sediments that have not yet reached thermal equilibrium (Hutchison, 1985). Groundwater flow can have profound effects on the heat and mass transport in sedimentary basins (Smith & Chapman, 1983; Deming *et al.*, 1992). Groundwater

flow systems can produce thermal anomalies on a kilometre scale and are capable of producing hundreds of megawatts of thermal energy (Chapman & Rybach, 1985). However, groundwater flow can also decrease the heat flow by recharge of cold, meteoric water into the sediments (Majorowicz *et al.*, 1984).

Radiation of heat via electromagnetic waves is another heat transport mechanism (Hantschel & Kauerauf, 2009). Radiation contributes only sizeably and efficiently to heat transfer at temperatures higher than 1200°C (Clauser & Huenges, 1995).

Geological Controls on Heat Flow

The total heat loss of the Earth is 44.2×10^{12} W (Pollack *et al.*, 1993) of which 70 % is transferred through deep oceans and marginal basins and 30 % through continents and continental shelves (Sclater *et al.*, 1980).

On continents, the heat flow depends on a variety of geological factors, including the amount of radiogenic elements that are enriched in the continental crust, the orogenic history, magmatic sources and erosion (Sclater *et al.*, 1980; Chapman & Rybach, 1985). The mean continental heat flow is 65 mW/m^2 (Pollack *et al.*, 1993). In active tectonic areas, e.g. volcanic and/or extensional tectonic areas, heat flow is high. In contrast, in tectonically inactive areas the heat flow is inversely correlated with the age of the rocks (Vitarello & Pollack, 1980). The heat flow decreases with increasing age of the radioactive elements bearing rocks (Allen & Allen, 1990). Therefore, for old cratonic shields (e.g. Scandinavian Shield) a low heat flow of 40 mW/m^2 can be expected, while for rift provinces (e.g. Upper Rhine Graben) much higher values around 90 mW/m^2 are characteristic (Chapman, 1986).

Heat flow variations observed in oceans are mainly related to the cooling of the lithosphere plate or to heat supply from the mantle, whereas the influence of radioactivity plays only a minor role. The mean heat flow in oceans is 101 mW/m^2 (Pollack *et al.*, 1993). Highest heat flows of up to 1000 mW/m^2 occur on ocean ridge spreading centres during the creation of lithospheric plate material by crystallization of basaltic magma (Chapman & Rybach, 1985). With increasing distance from ridges and rising age of the oceanic lithosphere, heat is lost and the plates cool down. Therefore, a strong positive correlation exists between the age of oceanic crust and decrease in heat flow.

However, even though oceans are providing more overall energy, the usage of Earth's heat portfolio by geothermal applications is restricted mainly to the subsurface of continental landmasses up to a depth of 15 km (England & Thompson, 1984).

1.1.2 Tapping Earths Heat Energy

Principles of Geothermal Systems and Utilisation

For the usage of ground heat by geothermal plants a carrier medium is necessary to transport heat to the surface. This carrier medium is mainly provided by water, mostly from aquifers but also from crystalline bedrocks, which can be produced, if the stimulated rocks provide enough fault structures (e.g. Hot Dry Rock (HDR) technique in Soultz-sous-Forêts). Aquifers can be used for geothermal applications, if the formation possesses sufficient porosity (>20 %), permeability (>500 mD) and thickness (>20 m) (Feldrappe *et al.*, 2008).

In general, geothermal systems are distinguished into deep and shallow systems with different geothermal applications associated. Deep geothermal systems are located deeper than 400 m, whereas shallow systems extend from the surface down to 400 m (Haehnlein *et al.*, 2010). In our latitudes, the subsurface is influenced by seasonal insolation down to 20 m depth, leading to a mean annual temperature of about 10°C in this depth range. Down to about 40 m is a neutral zone where seasonal temperature variations from the surface have no impact. Below this zone, the subsurface is influenced by the geothermal heat flow (Kaltschmitt *et al.*, 2006).

Shallow Geothermal Energy Applications:

Aquifer thermal energy storage (ATES) is one application for the use of shallow geothermal energy. This technology is used for space and water heating as well as for cooling systems. A minimum of two wells (warm side/cold side) is necessary for the seasonal operating mode. For space and water heating the heat can be sourced from cogeneration plants or solar units, generating surplus heat in summer when heat demand is low. The heat is stored in the aquifer during the summer season and extracted in winter when heat demand increases. The transformation of the energy is conducted by a heat exchanger. When used for cooling units, e.g. for air conditioning systems in buildings, the system works vice versa.

Shallow geothermal energy is also tapped by geothermal (ground-sourced) heat pumps (GHP). This is the worldwide fastest-growing application of renewable energy (Lund *et al.*, 2004). The system uses direct heat or cold from ground-coupled (closed loop) or groundwater

(open loop) systems providing heating, cooling and domestic hot water for buildings. The required subsurface temperatures are relatively low ranging approximately between 5 and 30°C, making this technology applicable in all countries of the world (Lund *et al.*, 2004).

Deep Geothermal Energy Applications:

In the case of deep (>400 m) geothermal energy systems (hydrothermal energy generation) the heat from the deep aquifer can be used directly via a heat exchanger. The technique offers great potential for saving fossil energy (Lottner & Mangold, 2000). Most of the geothermal plants that use deep geothermal energy producing both, direct heat for district heating and electrical power. The main principle is a doublet of wells, a production well and an injection well. The thermal water from the production well is re-injected in the injection well after energy extraction to keep the pressure and the mass balance of the aquifer constant.

Water with a temperature below 100°C is defined as warm water, above this temperature it is called hot water, and above 150°C steam occurs (Kaltschmitt *et al.*, 2006). For direct power generation temperatures above 150°C are necessary. However, there are technologies (binary systems) such as the ‘Organic Rankine cycle’ (ORC) and ‘Kalina cycle’ that generate power at temperatures below 150°C (Lund, 2004). For ORC an organic fluid with a low boiling point (e.g. *n*-pentane, *i*-butane) is heated by the thermal water. The organic fluid is evaporated, passes a turbine which produces power and is subsequently re-condensed. The Kalina cycle works with a mixture of two fluids (ammonia and water) with different boiling points. The advantage is that evaporation and condensation take place at a greater temperature range than for the pure liquids (Kaltschmitt *et al.*, 2006).

Brief History of Geothermal Usage

In 2010, 78 countries used geothermal energy for direct utilization such as ground-source heat pumps (47.2 %), bathing and swimming (25.8 %), space heating (14.9 % of which 85 % is for district heating), greenhouses and open ground heating (5.5 %), industrial process heating (2.8 %), aquaculture pond and raceway heating (2.7 %), snow melting and cooling (0.5 %), and agricultural drying (0.4 %) (Lund *et al.*, 2011). The worldwide installed power for direct utilization of geothermal energy accounted for 48,493 MWt at the end of 2009 and reflects an increase of almost 72 % compared to the installed power in 2005 (Lund *et al.*, 2011). The countries with the largest installed capacity are USA, China, Sweden, Germany, and Japan,

accounting for 63 % of the worlds total capacity (Lund *et al.*, 2011). Beside the direct use of geothermal energy, geothermal power generation is reported from 24 countries. Here, the top five producing countries are USA, Philippines, Indonesia, Mexico and Italy (Bertani, 2012).

The country with the most extensive use of geothermal energy is Iceland due to its favourable geologic conditions and an efficient hot water distribution network (Fridleifsson, 2001; IEA, 2007). Nevertheless, Italy (in Larderello) was the first country in history that used geothermal energy for power production in 1904, followed by the USA (The Geysers) in the 1930s, New Zealand (Wairakei) in 1958, Mexico (Pathé) in 1959, Japan (Honshu) in 1966, Russia (Paratunka) in 1967, Iceland (Námafjall) in 1969, and China (Fengshun) in 1970 (Lund, 2004). After the oil crisis in the 1970s, more and more new geothermal plants were installed around the world. Due to today's policy and progress in technology the expansion of geothermal energy utilisation, as a renewable and clean energy is still ongoing. In Germany, the first plant using direct heat from the deep (1.500 m, 60°C, Rätzh formation) for district heating was installed in Waren (Müritze) in 1984. Later, in 2003, the first binary geothermal plant was put into operation in Neustadt-Glewe (2250 m, 99°C, Rätzh formation) producing power by ORC and using the cooled thermal water for district heating. In Unterhaching, the Kalina cycle technology for power generation was realized for the first time in Germany (3446 m, 123°C, Malm formation).

1.1.3 Geothermal Energy in Germany

The Energy Mix in Germany

Germany's energy supply is a mixture of different energy resources. Since the German Renewable Energy Act (Erneuerbare-Energien-Gesetz, EEG) came into force in 2000, the amount of geothermal direct use increased from 27 major centralized installations in 1999 (Schellschmidt *et al.*, 2000) up to 162 geothermal installations and 3 geothermal plants for power generation at the end of 2009 (Schellschmidt *et al.*, 2010). Up to now the amount of geothermal energy (power generation, heat supply) accounts for only 1.6 % within the renewable energy resources (Fig. 1.1), which is mainly due to high drilling costs for tapping the hot water from the deep subsurface.

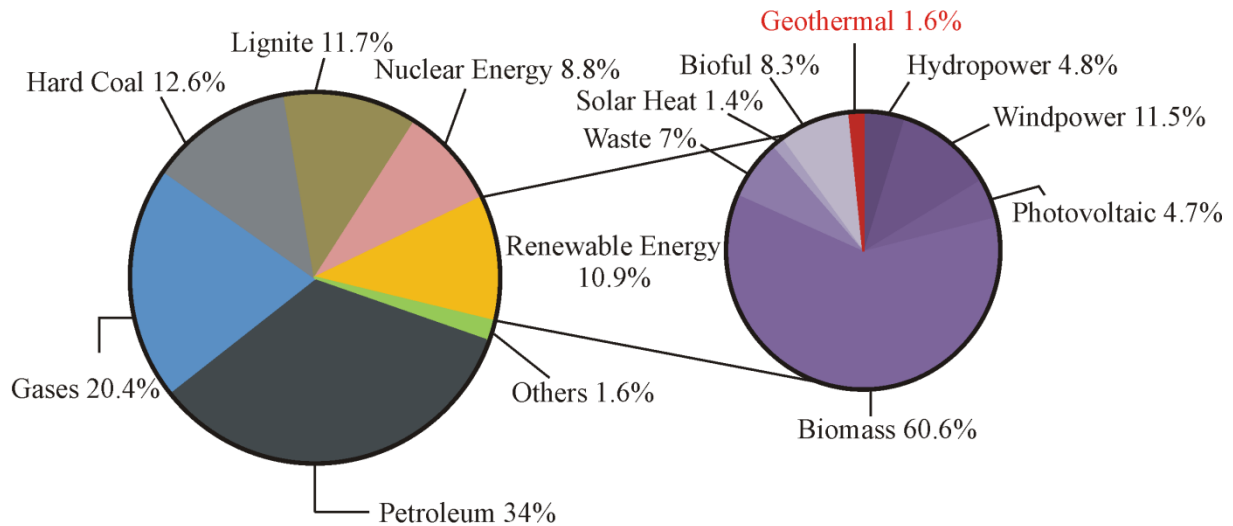


Fig. 1.1: Germany's Energy mix in the year 2011 adapted from Arbeitsgemeinschaft Energiebilanzen, Berlin (2012).

Nevertheless, small decentralized units for space heating by ground source heat pumps represent the fastest growing market in Germany with around 34.500 newly installed units in 2008 (Schellschmidt *et al.*, 2010). The potential for geothermal energy is high because of its constant availability and its cost-efficient provision. Especially in the light of recent economical and political developments, like the moratorium on nuclear power, rising oil prices, and public acceptance, geothermal energy appears to be an attractive alternative.

Important Geothermal Regions in Germany

In Germany three geographical regions are known for the usage of geothermal energy: the North German Basin, the Upper Rhine Graben, and the Molasse Basin (Fig. 1.2). These areas provide enough exploitable waters with adequate temperatures between 60 and 120°C at depth ranges not exceeding 3000 m (Kaltschmitt *et al.*, 2006).



Fig. 1.2: Map of Germany indicating the potential areas for geothermal utilisation (dark grey): North German Basin, Upper Rhine Graben, and Molasse Basin (modified after Seibt *et al.*, (2010)).

While the Upper Rhine Graben, located in a tectonically active and extensional zone, offers high heat flows with geothermal gradients of up to 100°C/km (Buntebarth, 1978; Pauwels *et al.*, 1993), the North German Basin and the Molasse Basin in southern Germany exhibit ideal conditions for geothermal applications due to thick, laterally extended accumulations of aquifer-bearing sediments.

The potential layers for geothermal applications in the **Upper Rhine Graben** are sandstones from Early Permian (Rotliegend) and Early Triassic (Buntsandstein) as well as granites from the basement, limestones from Middle Triassic (Muschelkalk) and Middle Jurassic (Hauptrogenstein), and Tertiary sandstones (Jodocy & Stober, 2008; Stober & Jodocy, 2009; Wolfgramm *et al.*, 2009). The most interesting sandstone layers for geothermal energy use in the **North German Basin** are from Early Permian (Rotliegend), Early Triassic (Buntsandstein), Upper Triassic/Lower Jurassic (Rhät/Lias), Middle Jurassic (Dogger) and Lower Cretaceous (Rockel *et al.*, 1997). In the **Molasse Basin** the limestones and marls of the Upper Jurassic (Malm) are of geothermal interest (Feldrappe *et al.*, 2007; Wolfgramm *et al.*, 2009). A distinct characteristic of the Malm aquifer is the relatively constant occurrence of

freshwater even in great depths (Andrews *et al.*, 1987), which is of advantage for the plant equipment. In contrast, fluids from the North German Basin show higher corrosional effects due to increasing salinity with depth and often occurring as brines (Wolfgramm *et al.*, 2011).

1.2 The Deep Biosphere – Occurrence and Exploration of Microbial Life in the Depth

Search for Deep Microbial Life

The deep biosphere in the continental subsurface comprises habitats beneath the rhizosphere or bioturbated zones (Whitman *et al.*, 1998) down to depth where water, nutrients, space and temperature are still sufficient to support life (Gold, 1992; Pedersen, 1993; Fredrickson & Onstot, 1996; Heim, 2011). For long time it was generally believed that microorganism only inhabit the uppermost layers of soils or sediments (Morita & ZoBell, 1955), although Edson S. Bastin found the first hints for microorganisms in the deep subsurface already in the 1920s (Fredrickson & Onstot, 1996). The existence and extension of life in the deep subsurface was, therefore, unclear until the nineties of the last century. Due to the improvement of the sensitivity in microbiological analysis techniques more and more evidences for the existence of a deep biosphere was obtained (e.g. Parkes *et al.* (1994)). Meanwhile, the deepest finding of microbial life below the seabed was detected in 1626 m depth (Roussel *et al.*, 2008) and in the terrestrial subsurface in 2 to 3 km deep gold mines in South Africa (Onstott *et al.*, 2006 ; Chivian *et al.*, 2008).

The presence of subsurface microbial communities was established by developing new drilling and sample techniques that minimized contamination (Ghiorse & Balkwill, 1983; White *et al.*, 1983; Chapelle *et al.*, 1987). Tracer techniques (Phelps *et al.*, 1989; Colwell *et al.*, 1992; Pedersen *et al.*, 1997), the use of fluorescent microspheres (Kieft *et al.*, 1997a; Onstott *et al.*, 2003; Kallmeyer *et al.*, 2006), and comparative community profiling (Lehman *et al.*, 1995; Moser *et al.*, 2003) allow to estimate the degree of surface contamination introduced by drilling fluids into the core material. Furthermore, by the use of molecular approaches (e.g. analysis of 16S rRNA sequences), phylogenetic information of microbes are obtained to functional groups and taxa without culturing the microbes (Nealson, 1997). Geochemical methods provide information on microbial activity by kinetic isotope fractionation (Pedersen, 1993).

Origin and Survival of Microbial Life in the Deep Subsurface

There are different theories on the origin of microorganisms in the deep subsurface. One possibility is that microorganisms are progenies of microbial communities that were buried at times of deposition and were isolated within connate water or trapped in fluid inclusions of minerals (e.g. Vreeland *et al.* (2000)). This suggests that the microbial communities forming the deep biosphere are millions of years old. Another possibility is microbial migration by groundwater recharge (Murphy *et al.*, 1992), percolating through the sediment or fracture systems. Although the groundwater circulation in the intermediate and regional scale of the subsurface (Lovley & Chapelle, 1995) is relatively slow with time intervals of hundreds to thousands of years (Seiler & Lindner, 1995), over geological times microbial migration is a conceivable scenario.

Regardless of their origin in the deep subsurface, microorganisms must survive for long-time periods (Chapelle & Lovley, 1990; Fredrickson *et al.*, 1995; Colwell *et al.*, 1997). Thus, microbial communities in the deep subsurface must be well adapted to specific environmental conditions in the absence of light and oxygen (photosynthesis). Microorganisms have some strategies for long-term survival, forming spores or use their internal energy reserves resulting in a shrink of the cell size (Kieft *et al.*, 1994; Kieft *et al.*, 1997b).

Microbial Diversity in the Deep Biosphere

In deep sedimentary rocks microbes utilizing metabolic processes such as Fe(III) and Mn (IV) reduction and fermentation (Boone *et al.*, 1995; Colwell *et al.*, 1997), SO_4^{2-} reduction and acetogenesis (Krumholz *et al.*, 1997). Microbial communities found in oilfield waters can comprise saline-tolerant, thermophilic fermenting, Fe(III)-reducing bacteria, and sulfate-reducing bacteria (SRB) (Onstott *et al.*, 1998), as well as thermophilic bacteria and archaea (L'Haridon *et al.*, 1995). These well adapted oil-associated microorganisms are capable of anaerobic hydrocarbon degradation (Townsend *et al.*, 2003; Aitken *et al.*, 2004) and have profound effects on the quality of oils. In deep coastal plains, methanogens, SRB (Chapelle *et al.*, 1987) and acetogens (Chapelle & Bradley, 1996) are recovered. These ecosystems are based on the primary production from the surface or the upper few layers of the water column, producing organic matter that is buried into sediments, feeding the heterotrophic communities. With the discovery of subsurface lithoautotrophic microbial ecosystems (SLIMES) (Stevens & McKinley, 1995; Kotelnikova & Pedersen, 1997; Pedersen, 1997) and

the radiolytic H₂ production in the continental crust (Lin *et al.*, 2005) in deep igneous rocks an H₂-driven biosphere was assumed independent of the products of photosynthesis such as organic carbon and molecular oxygen.

Application of Biological Processes

The knowledge of all these active and diverse microbial communities adapted to the deep subsurface is not only of scientific interest (origin of life) but is also used in diverse technological applications. For instance, *bioremediation* is a technology that uses the metabolic potential of microbes to clean up a contaminated environment (Watanabe *et al.*, 2002). The natural attenuation of contaminants such as the fuel additive methyl-*tert*-butyl ether (MTBE) (Davis & Erickson, 2004), petroleum components such as benzene, toluene, ethylbenzene, and xylene (BTEX) (Lin *et al.*, 2002), as well as polycyclic aromatic hydrocarbons (PAHs) (Meckenstock *et al.*, 2004) can occur naturally or by the addition of electron acceptors or donors to stimulate microbial communities or introducing specific microorganisms to enhance *in situ* biodegradation of the target compound (bioaugmentation). In addition, the monitoring of contaminant concentration, metabolic products and/or isotope fractionation can provide evidence for a successful *in situ* biodegradation (Morrill *et al.*, 2005). Other useful tools for which microbial activity plays a crucial role include e.g. *bioleaching*, conversion of insoluble metal sulfide into soluble metal sulfates (Rohwerder *et al.*, 2003), *biomining*, as a microbial pre-treatment process to open up structures of minerals for a better treatment of the target elements (e.g. gold) (Krebs *et al.*, 1997; Rawlings, 2005).

To summarize: viable microbial communities occur widespread in the deep subsurface. They utilize different metabolic processes than those living in the photic zone. The growing knowledge of these microbial processes is now being used for applications in biotechnology. Investigation of microbial communities found in geothermal plants and their specific metabolism might teach us more about life in the deep subsurface and concomitantly this knowledge might be very useful to reduce the negative impact of microorganisms on the working reliability of geothermal applications.

1.3 Current State of Biogeochemical Research in Geothermal Plants

The first studies in the 1980s focused on the general existence and distribution of microbes in different environments and, therefore, addressed geothermal plants as a potential habitat for microbial ecosystems. Using isolation and cultivation techniques, hyperthermophilic archaea (*Pyrobaculum* gen. nov.) were discovered in the geothermal power plant of Krafla, Iceland (Huber *et al.*, 1987). Daumas *et al.* (1988) found a new species of sulfate-reducing bacteria (SRB, *Desulfotomaculum geothermicum* sp. nov.) in produced fluids for district heating in Creil, Paris Basin. Early surveys focused on sulfide scaling and corrosion of pipe material, but the contribution of microorganisms was only poorly understood during that time. Criaud & Fouillac (1989) as well as Honegger *et al.* (1989) mentioned that a contribution of bacterially mediated sulfide scales by SRB cannot be excluded. In the 1990s, fluids from deep geothermal production wells were analysed with the aim to investigate the presence of viable microorganisms (Takai & Horikoshi, 1999). Later, in the 1990s, first attempts were made to describe the impact of temperature variations on the general mass reproduction of microbes in an artificial ATES pilot plant in Stuttgart, Germany (Adinolfi *et al.*, 1994). Similar investigations were carried out on core segments that are related to geothermal wells by Schippers & Reichling (2006). Both studies concluded that the operation of a geothermal plant (temperature increase/decrease) had no significant influence on the general abundance of microorganisms. Recently, a more comprehensive study evaluated the influence of an aquifer thermal energy discharge in Bavaria, Germany, and its temperature changes on the diversity of microbial and faunal populations in the aquifer (Brielmann *et al.*, 2009). Fluid samples were taken at observation wells during one cooling period. The authors found no significant changes on the bacterial or faunal abundance. However, the diversity of bacterial fingerprints and faunal populations was found to be clearly temperature dependent (Brielmann *et al.*, 2009).

Enrichment cultures from geothermal plants in Germany were investigated by Köhler *et al.* (1997). In their approach, cultivable microorganisms were recovered such as endospore forming gram-positive bacteria, sulfate-reducing bacteria, halophilic gram-negative bacteria as well as methanogenic archaea. Schulze-Makuch & Kennedy (2000) used a combined evaluation of fluid chemistry and microbiology in fluids from the Tortugas Mountain Geothermal Area in New Mexico, USA. They assumed that the microbial composition in hydrothermal fluids could be related to the fluid chemistry, formation and origin. In their

study they were able to reconstruct the regional groundwater flow using DNA analysis and phospholipid fatty acid analysis.

First culture-independent investigations on microbial contribution in scale formations are conducted on silica deposits at the Otake geothermal power plant in Kyushu, Japan (Inagaki *et al.*, 1997; Inagaki *et al.*, 2003). At the Cerro Prieto geothermal field in Mexico microbially-influenced corrosion (MIC) was determined by sulfate-reducing bacteria and thermophilic archaea (Valdez *et al.*, 2009). In the course of time, knowledge on microorganisms, their metabolic activity as well as the consequences for the technical equipment gained attention in studies concerning geothermal plants (cf. Sand, 2003).

Monitoring of dissolved organic carbon (DOC) in geothermal aquifer systems received up to now only little attention. Organic compounds can serve as essential carbon and energy source for microorganisms and can become highly reactive with increasing temperature (Horsfield *et al.*, 2006). Batch experiments focussing on DOC from heated ATES sediment samples were conducted by Brons *et al.* (1991). The results showed an enhanced release of organic compounds (fulvic acids) at 45°C, and an abiotically release of CO₂ at 55°C. Furthermore, the mobilization of fulvic acids is suggested to control the precipitation of Ca and Mg minerals. Kárpáti *et al.* (1999) studied dissolved organic compounds in thermal water samples in Hungary and found increasing DOC with increasing temperature and decreasing humic acids. Additionally, they found a high variety of aromatic hydrocarbons in thermal waters that is not connected to oil sources, with temperatures above 65°C, while waters below 50°C were almost free of this compound class.

As presented above, some studies have been conducted with a focus on the presence of microorganisms in geothermal plant systems and temperature effects of geothermal applications on the prevailing ecosystem in the aquifer. Most of these studies have been performed using different molecular biological or microbiological methods. Biogeochemical approaches were in most studies not applied. Analysis of molecular biomarkers such as intact phospholipids being main membrane constituents of bacterial cell membranes is an appropriate tool to monitor changes in the bacterial populations over time. This technique is culture-independent and provides information about the entire viable bacterial communities (Green & Scow, 2000). Most of the studies used fluid samples to isolate and cultivate microbes, which might only represent a part of the original composition of the indigenous microbial communities (Hirsch & Rades-Rohkohl, 1988). This is supported by observations

that 90 % of the microorganisms live attached on particle surfaces and only 10 % are of planktonic living (Hazen *et al.*, 1991; Alfreider *et al.*, 1997; Griebler *et al.*, 2002).

Up to now, information on changes and adaptations of microbial community composition with the operation of geothermal plants (seasonal energy storages or geothermal doublet) is not available. Additionally, most studies did not evaluate the availability of electron donors and acceptors in the process fluids for microbial metabolism; especially data on DOC is missing.

1.4 The ‘AquiScreen’ Project

This thesis is part of the AquiScreen project (Operational reliability of geothermal used **aquifers** with special consideration to microbial activity and particle relocation – **screening** at representative sites; “*Betriebssicherheit der geothermischen Nutzung von **Aquiferen** unter besonderer Berücksichtigung mikrobiologischer Aktivität und Partikelumlagerung – **Screening** an repräsentativen Standorten*”) commenced in February 2007 as a joint interdisciplinary collaboration of biogeochemists and microbiologists from *GFZ Potsdam*¹, geologists from *GTN Neubrandenburg*² and geochemists from *BWG*³. The AquiScreen project was funded by the Federal Ministry for the Environment, Nature Conservation and Nuclear Safety (BMU) to evaluate the nature and compositional variability of the indigenous microbial ecosystems with regard to changing plant conditions caused by different seasonal operating modes. This focus addresses to the working reliability of geothermal plants. To guarantee a reliable operation of geothermal plants working with natural waters, indigenous processes leading to system impairments have to be avoided. In general, there are three main processes which can be observed: scaling, clogging, and corrosion. These phenomena can reduce the permeability of the aquifer and/or deteriorate the technical equipment such as the tubing, pumps and heat exchanger resulting in immense repair costs. Scaling, clogging and corrosion result from abiotic as well as biotic reactions. Microbial processes and their by-products can alter the hydraulic properties of the aquifer (Chapelle, 2000). Examples on microbial-mediated precipitations are presented by studies from Vasconcelos *et al.* (1995) and Warthmann *et al.* (2000), showing dolomite precipitations by sulfate-reducing bacteria (SRB)

¹ Helmholtz Centre Potsdam, German Research Centre for Geosciences (GFZ), Telegrafenberg, 14473 Potsdam, Germany

² Geothermie Neubrandenburg GmbH (GTN), Seestr. 7a, 17033 Neubrandenburg, Germany

³ BWG Geochemische Beratung GbR, Seestr. 7a, 17033 Neubrandenburg, Germany

under anoxic hypersaline conditions. SRB are also indirectly responsible for the formation of pyrite and other metal sulfides by producing hydrogen sulfide (Berner, 1984), which reacts with metals to iron sulfides (FeS), galena (PbS) and sphalerite (ZnS) (Labrenz *et al.*, 2000; Machel, 2001). Furthermore, SRB are also indirectly involved in corrosion processes on steel by producing H₂S through bacterial sulfate reduction. From dissolution of the clay mineral smectite by Fe(III)-reducing bacteria, it is known that these bacteria release Fe²⁺, Ca²⁺, silica, and bicarbonate to the surrounding pore waters and the mineral transformation mechanism results in the formation of new minerals such as quartz and amorphous silica, pyrite or calcium carbonate (Dong *et al.*, 2003; Kim *et al.*, 2004; Vorhies & Gaines, 2009). Thus, microorganisms can play a crucial role as a source of disturbances in geothermal plants.

1.5 Goals and Objectives of this Thesis

In this thesis, a biogeochemical approach was performed to characterize the indigenous microbial communities using specific biomolecules (biomarkers). Especially, bacterial cell membrane phospholipids were investigated. Intact phospholipid esters are indicators for living bacterial communities, because they become usually rapidly degraded after cell death and are only stable in viable organisms over longer periods of time (White *et al.*, 1979; Zink *et al.*, 2003; Logemann *et al.*, 2011). The membrane phospholipid fatty acid (PLFA) pattern obtained from a natural sample represents the whole bacterial community (known and unknown species) and shows a high adaptation capability concerning different environmental conditions (e.g. temperature, pressure, nutritional status) to sustain the membrane fluidity (Guckert *et al.*, 1986; Suutari & Laakso, 1994). Therefore, phospholipids provide an extremely useful tool for long-term screening studies.



Fig. 1.3: Filter samples from a geothermal plant in the North German Basin. On the left side two used filters loaded with sediments and for comparison on the right side a new filter.

PLFA analyses were conducted using filter samples. These filters (Fig. 1.3) are originally installed to retain aquifer particles from the process water and also accumulate microorganisms passing the filters together with the process water or being attached to mineral particles present in the fluid. Therefore, such filters are well suited for the analysis and assessment of microbial communities in the geothermal system.

In this study, geothermal fluids were screened and monitored for the composition and quantity of DOC compounds (bulk DOC, DOC fractions and low molecular weight organic acids) and inorganic anions, being potential electron donors (DOC) and acceptors (e.g. SO_4^{2-}) for microbial metabolisms. The detection of dissolved substances can be carried out by ion chromatography of anions (e.g. acetate, sulfate) as well as quantification and characterisation of dissolved organic carbon (DOC) by liquid-chromatography organic carbon detection (LC-OCD). Additionally, carbon isotope composition of DOC and its variability can provide information on the origin of the organic carbon as well as the activity of microorganisms. Detailed description on the methods used in this study can be found in Chapters 4, 5, and 6.

In this study, 7 geothermal plants with different applications (heat/cold storage, district heating, power production, and balneology) have been monitored over three years. If possible, the time series of sampling were conducted monthly. The depth of the tapped aquifers ranged between 20 and 3590 m with temperatures between 6°C and 123°C. In total 65 fluid and 26 filter samples from the North German Basin and 33 fluid samples from the Molasse Basin have been analysed.

The key questions addressed in this study are:

- How does the indigenous microbial community react in terms of composition and/or adaptation to varying ambient conditions during plant operation such as different operation modes, temperature conditions and nutrient levels?
- Are microbial communities involved in processes causing operation failures such as reduced injection due to clogging or corrosion of plant installations?
- What is the general fluid chemistry at the investigated sites and does the fluid chemistry show any changes over time, with depth or due to different operating modes?
- Does the applied microbiological (e.g. variability in the phospholipid fatty acid composition) and biogeochemical (fluid geochemistry) approaches provide parameters which can be used as indicators for the assessment of the working reliability of the geothermal plants?

The thesis shows the following structure:

The following Chapter 2 characterizes the fluid organic geochemistry of the Malm aquifer in the Molasse Basin related to depth and over time as well as its possible changes with operation of the binary geothermal plants. Chapter 3 is an introduction to the geology and fluid evolution of the Northeast German Basin that serves as background information to the subsequent chapters. Chapter 4 describes the prevailing conditions in a central solar heating plant with seasonal heat storage in a shallow aquifer of 20 m depth. The monitoring of this solar ATES system focused on the possible temperature adaptation of microorganisms by using phospholipid analysis. In addition, Chapter 5 characterizes a shallow cold storage in the North German Basin where two events of reduced injection occurred during the long-term monitoring of ca. 3 years. Here, changes in the PLFA pattern have been found to be valuable indicators of the state of the ATES. The following Chapter 6 depicts biogeochemical processes in a deep heat storage in the North German Basin and the variability of microbial communities during different operation modes (charge and discharge) and with time. Finally, Chapter 7 provides the overall conclusions.

2 Dissolved Organic Carbon in the Malm Aquifer of the Molasse Basin and its Variability with Depth and Implications for Geothermal Plants

Parts of this chapter have been published in:

Mashal Alawi, Stephanie Lerm, **Alexandra Vetter**, Markus Wolfgramm, Andrea Seibt, Hilke Würdemann (2011): Diversity of sulfate-reducing bacteria in a plant using deep geothermal energy; Grundwasser 16 (2), 105-112

2.1 General Introduction

With the current debate on the impact of fossil energy resources on the Earth's climate alternative energy resources such as geothermal energy moved into the focus of public interest. Geothermal energy is a clean and renewable energy resource and uses the heat stored in the upper, accessible part of the Earth crust. In Germany, the Molasse Basin (MB) with its Malm aquifer is one of the geothermal regions, providing ideal conditions for geothermal power production, district heating and balneological applications. From 2007 to 2009 four geothermal plants were investigated in the German MB and one in the Austrian MB. The focus was placed on the occurrence and concentration of dissolved organic compounds (DOC) in the produced fluids to get insight into the natural variability of the organic fluid chemistry within the MB as well as their potential to act as substrates to sustain a microbial ecosystem within the geothermal plants. Additionally, changes in the organic compound inventory over time and during plant operation were investigated, especially with decrease in temperature after the heat exchanger.

2.2 Introduction to the Molasse Basin

2.2.1 Geological Evolution of the Molasse Basin

The MB is located in the northern foreland basin of the Central and Eastern Alps, and extends over 700 km from the French Savoie in the west to Lower Austria in the east (Sissingh, 1997). The basin history of the MB is characterized by three stages, each separated by unconformities: Permo-Carboniferous graben sedimentation, Mesozoic mixed carbonate-siliciclastic shelf sedimentation, and Cenozoic Molasse sedimentation (Bachmann & Müller, 1992). The basin is arcuate, reaches its maximum width (ca. 150 km) in Germany, and is

filled by predominantly siliciclastic sediments of Cenozoic age with a thickness of up to 5000 m in front of the Alps (Bachmann *et al.*, 1987). The MB exhibits a high mean heat flow of 87 mW/m^2 (Vedova *et al.*, 1995). The so-called ‘Molasse’ are the accumulated deposits of the rising Alps mainly during the Oligocene and the Miocene (Sissingh, 1997). The MB is surrounded by the Alps in the south, the Black Forest in the west, the Swabian and Franconian Alb in the north and northwest, and the Bohemian Massif in the east (Fig. 2.1). The Tertiary folded and thrust sediments of the MB together with its Mesozoic and partly younger Cenozoic sediments extend at least 50 km under the Alpine nappe system (Bachmann *et al.*, 1987). The geology of the molasse infill of the MB is well known since it is a mature oil and gas province with reservoirs mainly in Eocene to Miocene sediments. Additionally, oil and gas-bearing horizons are known from the Muschelkalk (“Trigonodus-Dolomite”) and Keuper (Schilfsandstein, Kieselsandstein, Stubensandstein, and R  th-Sandstein; (Boigk, 1981)).



Fig. 2.1: Topographic map of Germany. The MB is surrounded by the Alps, the Black Forest, the Swabian and Franconian Alb, and the Bohemian Massif.

The crystalline basement of the MB consists of gneisses and granites which outcrop in the Black Forest and the Bohemian Massif (Bachmann *et al.*, 1987). As a result of the consolidation of the Variscides, many grabens and troughs subsided in the area of the MB during Permo-Carboniferous times and are partly filled with 1000 m of sediments (Wrobel *et al.*, 2002). During the following Triassic, the area of the MB was part of the German ‘Vindelician High’ and formed the northern margin of the Tethys. The Vindelician High

sourced siliciclastic erosional material into the northern and southern basin parts until the mid-Jurassic (Bachmann *et al.*, 1987). In the eastern MB the crystalline barrier of the Landshut-Neuötting High started to uplift during the Keuper period (Lemcke, 1973) and separated two basins during the Jurassic: the Braunauer Trough in the east and the Wasserburger Trough in the west (Boigk, 1981). During the Liassic, the TOC-rich Toarcian Posidonia Shale was deposited and represents a prominent source rock for oil (Bachmann *et al.*, 1987). Lias α sandstones can serve as hydrocarbon reservoir (Boigk, 1981). During the Middle Jurassic a prominent bituminous claystone (Opalinus Clay) was deposited and potential reservoirs for generated oil and gas are Dogger β sandstones (Boigk, 1981). The Vindelician High was leveled during the Upper Jurassic and became fully inundated by the Tethys. Up to 600 m of limestones and marls were deposited as part of the Tethys shelf (Bachmann *et al.*, 1987). Malm outcrops today occur in the Swabian and Franconian Albs and dip below the MB. The Upper Jurassic shelf sediments are characterized by the occurrence of sponge and algal reefs. These carbonates are frequently dolomitized and change to the south into a thick bedded, black, micritic limestone (“Quintner Kalk”) which is part of the Helvetic facies. The TOC content of the Quintner Kalk can be as high as 10 wt. % (Boigk, 1981). Black Malm limestones and claystones with TOC contents up to 3 % can be also found in the Franconian Alb (Meyer & Schmidt-Kaler, 1993). Today the Upper Jurassic sediments are located in the MB centre and are as deep as 3000 to 4000 m. In contrast, the crystalline barrier of the Landshut-Neuötting High is covered by 400 m thick Malm sediments in a depth of around 790 m below surface (Wrobel *et al.*, 2002). Karstification was the result of eustatically induced regression of the Tethys during the transition from the latest Jurassic to the earliest Cretaceous. In general, the Cretaceous is characterized by eustatic sea level changes which resulted in alternating deposition of marl, lime and sand. Some of these Cretaceous sediments are fractured and enable production of oil and gas (Bachmann *et al.*, 1987). From the Late Cretaceous to Early Tertiary inversion tectonics affected the foreland area presumably as a result of the collision of the Adriatic-African plate with the shelf margin of Europe (Ziegler, 1987). In addition, the inversion was accompanied by upthrusting of major basement blocks such as the Bohemian Massif and Landshut-Neuötting High (Ziegler *et al.*, 1995). With the initial development of the elevated Alps during the Late Eocene and Oligocene and the final collision of the orogenic wedge with the Helvetic Shelf, the pre-Molasse stage is reflected by the deposition of fluvial and shallow-marine sandstones, shales and carbonates.

Contemporaneously, the narrowing of seaways led to the occurrence of the Paratethys north of the Alps and the Mediterranean Sea in the south. During the Early Oligocene (Rupelian) the basin deepened rapidly (Bachmann *et al.*, 1987), widened abruptly, and the Western Alps were uplifted (Sissingh, 1997). Thus, sedimentation in the eastern MB part changed from calcareous deposits into organic-rich rocks (Schöneck Formation) that are considered to be source rocks for hydrocarbons (Wehner & Kuckelkorn, 1995; Schulz *et al.*, 2002). Basically, Eocene (Priabon), Oligocene (Sannois, Ruppel, Chatt) and Miocene (Aquitain, Burdigal) deposits are the most productive hydrocarbon reservoirs (Boigk, 1981). During the Early Oligocene to Early Miocene (Rupelian – Aquitanian) the first megacycle set with upward-coarsening deposition occurred, whereas marine sedimentation was cyclically replaced by fully continental sedimentation, leading to the Lower Marine Molasse (1500 m) and the Lower Freshwater Molasse (4000 m). Subsequently, during the Miocene (Burdigalian – Tortonian) the Central and Eastern Alps were uplifted and the Upper Marine Molasse (1300 m) and Upper Freshwater Molasse (1500 m) were deposited (Sissingh, 1997). Both are part of the second megacycle which correlates with global sea-level changes. The post-Molasse stage (Late Miocene to Pliocene) was characterized by continuing uplift and erosion of the Alps (Lemcke, 1984; Sissingh, 1997). During the Pleistocene several glacial advances from the Alps into the Alpine foreland led to glacial and glaciﬂuvial deposits. The typical Alpine relief, with U-shaped valleys, hanging valleys, and steep mountain peaks is consequence of the Pleistocene glaciations. With the retreatment of the glaciers during the Holocene, the current uplift rates in the Alps are in the order of 1–2 mm/y and denudation is the main process in the Alps today (Fitzsimons & Veit, 2001).

2.2.2 Characteristics of the Malm Aquifer

Malm sediments represent the most important aquifer in the MB with ideal conditions (e.g. temperature, permeability, etc.) for geothermal energy applications. The excellent aquifer properties are provided by fractures linked to fault zones (Fig. 2.2). Therefore, only the Malm aquifer is considered in this work. An overview about the hydrochemical evolution of the Malm waters is provided by Birner *et al* (2011).

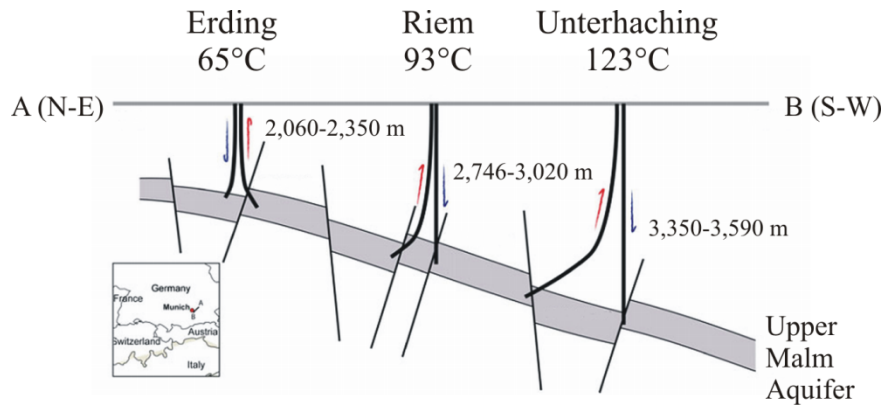


Fig. 2.2: Schematic cross section of the dipping Malm limestone in the MB from northern foreland of the Alps (NE) towards the Alps (SW). Temperature increases with increasing depth. Geothermal plants are located on fault structures for hydraulic properties reasons (modified after Goldscheider *et al.*, (2010)).

The karstified Malm aquifer was confined until Pliocene times, then large discharge systems (Danube and Rhine) occurred and cutted deeply into Tertiary sediments down to Malm sediments (Lemcke, 1977). Therefore, deep groundwater discharges into the Danube, Inn, and Isar rivers today (Andres & Egger, 1985). Furthermore, there is a continuous recharge of groundwater from percolation of meteoric water from the surficial basin borders Swabian and Franconian Albs; (Stichler *et al.*, 1987; Goldbrunner, 2000), and the Bohemian Massif (Andrews *et al.*, 1987) into deeper parts of the MB. Thus, the age of the fluids in the Malm aquifer is relatively young (10,000 to 50,000 yrs) and of meteoric origin (Andrews *et al.*, 1987; Goldbrunner, 1997). The Malm aquifer is mainly a freshwater unit (infiltration water) with total dissolved solids (TDS) of less than 1 g/L (see Table 2.1 and Fig.2.3).

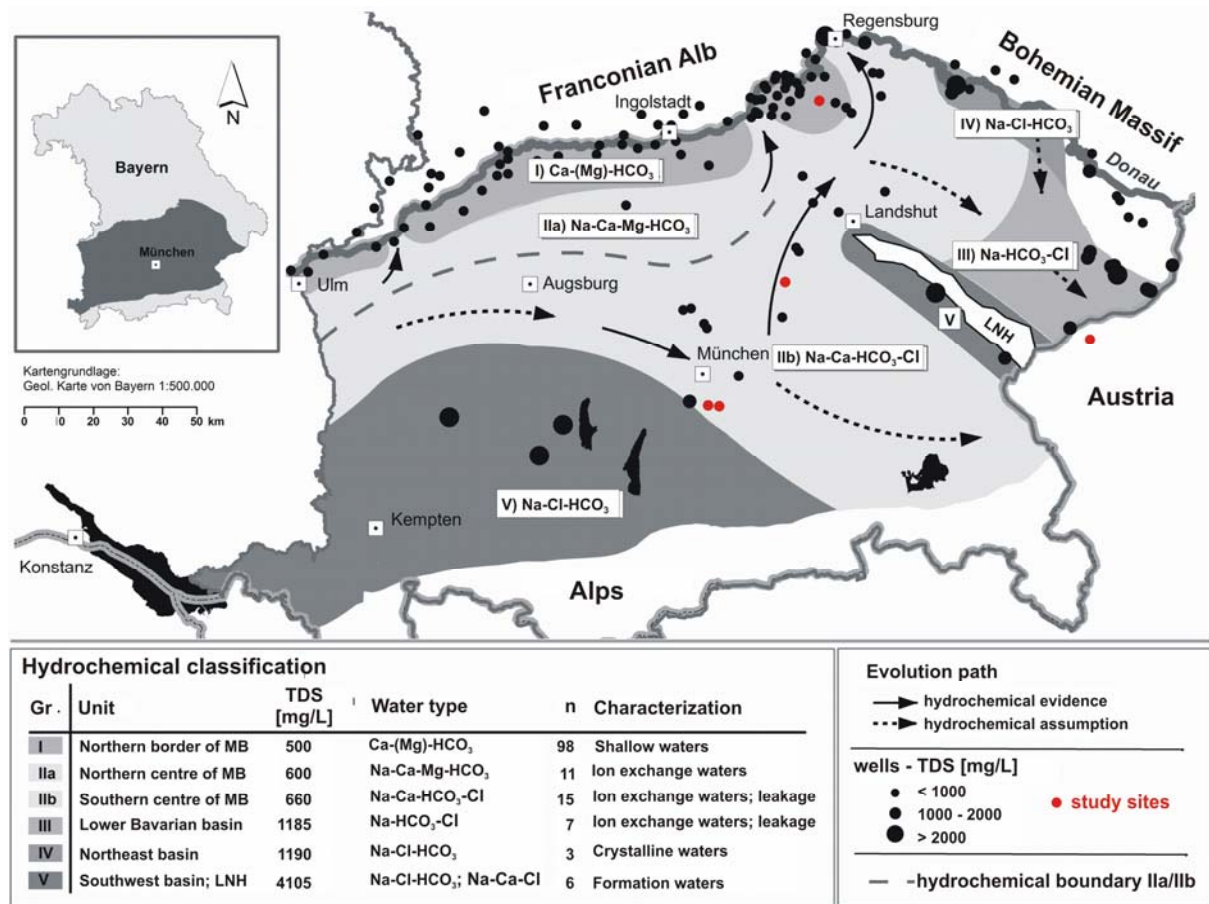


Fig. 2.3: Map of hydrochemical provinces of the Malm aquifer in the MB (modified after Birner *et al.*, (2011)). The red points represent the investigated study sites.

Sometimes mixing phenomena occur and are the result of discharge from Tertiary aquifers. These processes lead to locally restricted increases in salinity up to 5 g/L (Wolfgramm & Seibt, 2008). Additionally, east of the Landshut-Neuötting High the Malm waters of the deep basin show a higher mineralization and higher contents of trace elements (e.g. fluoride and iodide). This change in fluid chemistry could be due to admixing of saline pore waters or inflow of highly saline water from the recharge area of the crystalline Bohemian Massif (Bay.StWiVT, 2010). The principal flow direction of the Malm water is from west to east with less intense mineralization (Bay.StWiVT, 2010). The fluids are of Ca-(Na)-(Mg)-HCO₃-(Cl)-type. With increasing depth of the Malm aquifer, Ca²⁺, Mg²⁺, and HCO₃⁻ concentrations decrease whereas Na⁺ and Cl⁻ concentrations show the opposite tendency (Wolfgramm & Seibt, 2008). A characteristic feature of the Malm waters with depth is Na⁺ excess compared to the Cl⁻ content, which can be explained by admixing of waters. Contrary to sodium, sulfate concentrations decrease from the margins to the basin centre due to sulfate reduction. H₂S

contents are, thus, detectable in all deep waters of the Malm aquifer (Bay.StWiVT, 2010). Gases dissolved in the fluid are mainly CO₂ and CH₄ varying between 50 to 500 mL_{gas}/L_{Fluid} as well as H₂S (up to 25 mg/L) (Wolfgramm & Seibt, 2008).

Tab.2.1 Fluid chemistry of the lithological units in the MB (Bay.StWiVT, 2010). The blue box marks the fluid composition of the Malm aquifer.

S-Bavaria (MB)	Series	Stage	Lithology	Water Type	TDS	Remarks
Tertiary	Miocene	Burdigal	sand-marl-succesion	Na-Cl	1-11.5g/L	
		Aquitain	sand-marl-succesion	Na-Cl	1-16g/L	
		Chatt (east MB)	sandstone with embedded clay and marls	Na-Cl	1-60g/L	Increasing with depth.
	Oligocene	Baustein layers (west MB)	sandstone	Na-HCO ₃	low	With hydraulic connection to Malm.
		Sannois (Lattdorf)	sandstone	Na-Cl	<5g/L	
		Eocene	Priabon	sandstone	Na-Cl	<10g/L
Mesozoic	Cretaceous	Cenoman	sandstone	Na-HCO ₃ -Cl	<1g/L	
		Jurassic	Malm (border of basin)	carbonates (limestone, dolomite, marl)	Ca-Mg-HCO ₃	<1g/L
	Malm (basin centre)		Na-(Ca)-HCO ₃ -Cl	<1g/L		
N-Bavaria (Franconian Basin)						
Mesozoic	Jurassic	Dogger	Fe dominated sandstone	Ca-(Na)-HCO ₃	<1g/L	
			sandstone with embedded clay	Ca-Mg-HCO ₃	<1g/L	
		Keuper	sandstone with embedded gypsum	Ca-Mg-HCO ₃ -SO ₄ ²⁻	>1g/L	
				Na-HCO ₃ -(Cl)	<1g/L	Along Danube river.
			carbonates	Ca-Mg-HCO ₃	<1g/L	Shallow aquifers.
		Triassic	Muschelkalk	carbonates and dolomites with embedded clay, gypsum, and salt	Ca-Mg-SO ₄ ²⁻ /Na-Cl	>10g/L / 250g/L
			sandstone	Na-(Ca)-HCO ₃ -(SO ₄ ²⁻)	<1g/L	Border of facies
	Buntsandstein		sandstone with embedded clay and silt, sometimes embedded anhydrite	Ca-Mg-HCO ₃ -(SO ₄ ²⁻)	<1g/L	Autochtone
	Perm		Inflow of Zechstein waters	Na-Cl	>100g/L	Allochtone
		Zechstein	carbonates	Na-Cl	>100g/L	Central facies
		carbonates	Ca-SO ₄ ²⁻	<5g/L	Border of facies, increase of Ca, Mg, SO ₄ ²⁻ , and HCO ₃	
Paleozoic	Rotliegend		sandstone, conglomerates, volcanics	Ca-Mg-HCO ₃ -SO ₄ ²⁻	low	Shallow aquifers
				Na-HCO ₃ -Cl	0.4 - <100g/L	Autochtone
	crystalline bedrock	Prä-Perm	granite, gneiss, shists, phyllite, quartzite, diabase, diorite, shales, carbonate, greywacke	Na-SO ₄ ²⁻ /Na-HCO ₃	low	Close to surface (rich in earth-alkaline salts).
			Na-Cl	high	Deeper parts (rich in gases: N ₂ , CO ₂ , CH ₄ , and H ₂).	

2.2.3 Dissolved Organic Carbon in Aquifers

Dissolved organic carbon is defined as the organic carbon passing through a 0.45 µm filter and comprises a multitude of organic compounds such as fatty acids, carbohydrates, amino acids, hydrocarbons, hydrophilic acids, fulvic acids, humic acids, viruses, and clay-humic-metal complexes (Fig. 2.4). Pyrolysis experiments on humic substances by Schulten & Gleixner (1999) showed also a wide range of building units (carbohydrates, phenols and lignin monomers, lignin dimers, lipids, alkylaromatics, aromatic nitrogen compounds, sterols, peptides, suberins, and loosely bound fatty acids). In aquifers, DOC derives either from surface organic matter (e.g. soil) or from deposited organic matter in sediments and rocks of

the aquifer (Thurman, 1985b). In general, the amount of DOC in groundwater systems ranges usually from 0.2 to 15 mg C/L with an average concentration of 0.7 mg C/L (Leenheer *et al.*, 1974; Thurman, 1985b). Nevertheless, the amount of DOC in aquifers can vary significantly with the associated environment. In groundwater associated with coals DOC concentrations can be 5 to 10 mg C/L (Thurman, 1985b), in oil-field waters high concentrations up to 4900 mg C/L have been detected (Carothers & Kharaka, 1978), and in oil shale waters DOC may range from 2 to 5 mg C/L (Thurman, 1985b).

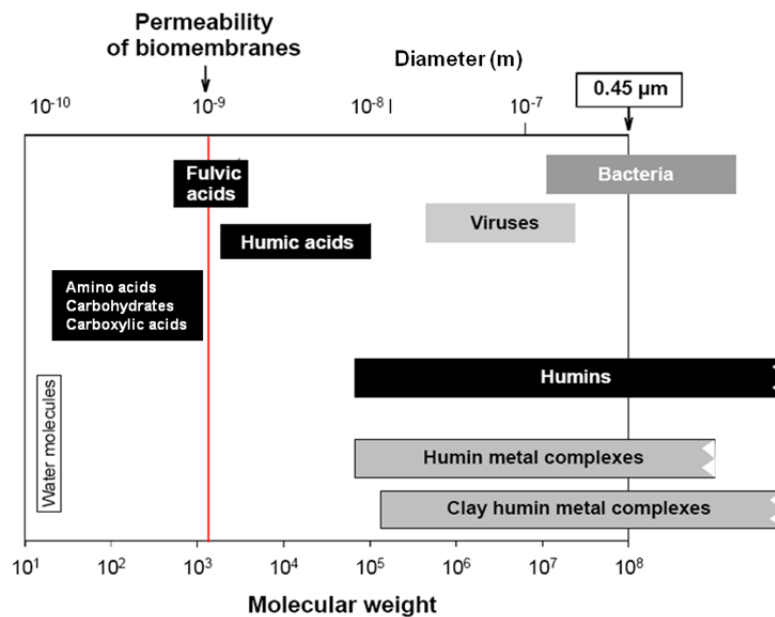


Fig. 2.4 Possible fractions of dissolved organic carbon defined by 0.45 μm filtration (modified after Thurman (1985b)).

In shallow aquifers that are highly influenced by surface processes, the main contribution of DOC derives from soils and plants. The dissolved organic compounds that are directly diluted by plants comprise low molecular weight carbohydrates, phenols, aldehydes and carboxylic acids (Hoffman *et al.*, 1980). These compounds are transported by leachate and rapidly metabolized by microorganisms. Thus, DOC concentration decreases with depth and only less amounts of DOC reach the shallow aquifers. Another contribution to DOC concentration in the aquifer is the buried sedimentary organic matter. The deposited organic matter can be leached and microbial processes can attack the organic material, resulting in release of DOC (Filip & Smed-Hildmann, 1992). With increasing depth and temperature, when microbial degradation of organic matter is strongly reduced, thermal decomposition of sedimentary macromolecular organic matter becomes of greater importance and leads to release of organic

compounds into the aqueous phase and contributes to an enhanced DOC concentration (You & Gieskes, 2001). It was shown, that the macromolecular organic matter in organic matter rich deposits (e.g. lignites and coal seams) release LMWOA into the pore water during ongoing maturation (Glombitza *et al.*, 2009) and thus, contributing to DOC. In this context, also abiotic release of organic compounds by hydrolysis of the sedimentary organic matter is suggested. However, the input from sedimentary macromolecular organic matter to DOC by abiotic, thermal decomposition seemed to be obviously more relevant in deep formation waters at higher temperatures.

The aim of this study is the characterization of DOC in the Malm aquifer by its molecular and isotopic composition with increasing depth. Therefore, water samples from the Malm aquifer, representing a depth range from 240 to 3446 m below surface have been taken in geothermal plants. Water samples were analysed for DOC concentration and isotopic composition and the content of low molecular weight organic acids (LMWOA). Additionally for two geothermal plants, the variability of organic substrates with plant operation as well as their potential to act as substrates for microorganisms will be discussed.

2.3 Study Sites and Fluid Sampling

Fluid samples have been taken and investigated from 4 different geothermal plants within the German and Austrian MB and 1 study site is used for other purposes. Concerning the project agreements the individual names of the investigated geothermal plants have to be kept confidential. Therefore, the different sites examined were labeled Molasse 1 to 5. The study site Molasse 1 (M1) is located in the northern part of the MB close to the Franconian Albs. It is used for industrial purposes and does not serve for geothermal energy production. Only one sample from the production well of site M1, pumped up from the Malm aquifer at a depth of ca. 240 m below surface, was investigated. The study sites M2 (3 samples), M4 (8 samples), and M5 (17 samples) are located in the centre of the MB west of the Landshut-Neuötting High around Munich. M3 (4 samples) is situated in the eastern part of the MB in Austria east of the Landshut-Neuötting High. All geothermal plants are equipped with a production well, heat exchanger and an injection well. All plants are kept under pressure to avoid degassing and mineral precipitation. A specific feature of site M3 is a significant higher temperature compared to M2 (almost same depth). This is due to the artesian aquifer at study site M3. Study site M4 stands out due to incidental production of oil with the hot water. The

geothermal plants M2, M3, M4, and M5 are all used for district heating (Table 2.2). Additional applications are thermal spa (M2, M3) and power production (M3 and M5). In total, 33 fluid samples were analysed. If possible, samples were taken from both sides of the geothermal plant, before and after the heat exchanger. At site M5, an additional fluid tapping point was installed on the injection well side (after heat exchanger). All analytical data can be found in the Appendix ‘geothermal plants’.

Tab. 2.2: Overview of the study sites in the MB and its installation characteristics. DH = district heating, Spa = thermal spa, ORC = Organic Rankine Cycle, P = power generation. Data are compiled from literature (Wolfgramm *et al.*, 2007; Goldscheider *et al.*, 2010; Kaelcke, 2010; Alawi *et al.*, 2011; Butuzov, 2011).

Study sites	Geothermal application	Well depth (m) Production/Injection	Temperature (°C) Production/ Injection	Flow rate (l/s)	Thermal Power (MW)
M1	-	240/-	-	-	-
M2	DH, Spa, potable water	2250/2060	65/17	25-57	8
M3	DH, P by ORC, Spa	2300/2165	106/60	46	11.5
M4	DH	3443/3370	107/61	50	6
M5	DH, P by Kalina cycle	3446/3590	123/62	150	40

2.3.1 Analysis of the Fluids

The Malm aquifer fluids were sampled and analysed using methods described in chapter 4 and 5. Concentrations of inorganic and organic anions in the fluids were determined by ion chromatography. DOC was characterized and quantified using the LC-OCD system (for details see chapter 4 and 5). Different organic fractions can be classified by their molecular sizes and UV activity such as biopolymers (bio), humic substances (HS), building blocks (BB), hydrophobic organic carbon (HOC), and different groups of low molecular weight (LMW) compounds (for details see chapter 4 and 5). Additionally, the bulk carbon isotope ratios of the DOC have been determined by LC-Isolink coupled to Delta V Advantage (Thermo Fisher Scientific).

2.4. Results and Discussion

2.4.1 Fluid Composition of the Malm Aquifer

Most of the fluids show a freshwater composition with total dissolved solids (TDS) below 1 g/L (Table 2.3). An exception is formed by study site M3 where fluids have slightly higher TDS (1.3 g/L).

Tab. 2.3 Basic information on the fluid chemistry of the study sites. Data are compiled from literature: (Pernecker, 1999; Wolfgramm *et al.*, 2007; Kaelcke, 2010; Seibt *et al.*, 2010; Alawi *et al.*, 2011). No data available for study site M1.

Study sites	Depth (m)	T (°C)	Water type	TDS (g/L)	pH	Gases(%)
M2	2200	65	Na-Ca-HCO ₃ -Cl	0.6	7.3	CH ₄ , H ₂ S, CO ₂ , N ₂
M3	2300	106	Na-HCO ₃ -Cl	1.3	7.0	CH ₄ (61), N ₂ (28), CO ₂ (7), H ₂ S (4)
M4	3443	107	Na-HCO ₃ -Cl	0.9	6.6-7.3	CH ₄ (50), CO ₂ (40), N ₂ +H ₂ S (10)
M5	3446	123	Na-HCO ₃ -Cl	<1	6.1-6.3	CO ₂ (45), CH ₄ (30) H ₂ S+N ₂ (25)

Although the water type did not change significantly with depth as mentioned above, an increase in chloride concentration is observed with depth as shown in Fig 2.5. An exception represents M5 that showed a similar chloride content as M2. For the locations west of the Landshut-Neuötting High (M1, M2, M4 and M5) the sulfate concentration appears to increase with depth from basin border to basin centre. East of the Landshut-Neuötting High sulfate concentration showed the lowest values.

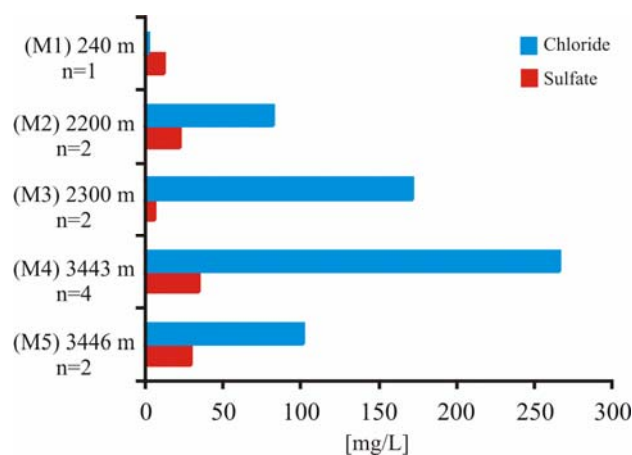


Fig. 2.5: Chloride and sulfate concentrations in natural fluids from different depth of the Malm aquifer. Samples have been taken from the production wells before heat exchanger in geothermal plants. The presented concentrations are average values (M2 to M5).

2.4.2 Dissolved Organic Carbon and its Characterization in the Malm Aquifer

Average values for DOC concentration of each study site (Table 2.4) ranged from 0.4 M2 to 10.5 mg C/L M4 with an increase in DOC with increasing depth of the Malm aquifer. The high DOC content at study site M4 is possibly correlated to the natural occurrence of heavy oil at this site (Kaelcke, 2010). Please note that the DOC content is given in mg carbon per litre fluid, whereas the concentrations of the individual organic acids are presented in mg per litre. For direct comparison of DOC and organic acids, the acid concentrations have to be recalculated to concentrations of acid-carbon in mg per litre. In addition to increasing DOC concentration, an increase in LMWOA with depth is also observed for the respective sites (Table 2.4). The concentration ranges from below detection limit M1 up to 17 mg/L in plant M4, with acetate being the dominant organic acid up to 12.6 mg/L. The predominance of acetate in formation waters associated with oils is a common observation (Carothers & Kharaka, 1978; Fisher, 1987; Means & Hubbard, 1987). LMWOAs such as acetate are known to be components of the polar oil fraction with good solubility in the water phase and thus, oil-water contact leads to solution of hydrophilic acids in water and is suggested to increase the LMWOA content in the DOC (Reinsel *et al.*, 1994). With increasing depth a lower turnover rate for the microbial utilisation of the LMWOA is expected due to an increase in the recalcitrant proportion of the organic matter and the decreasing microbial activity limiting the consumption of LMWOAs. However, there are observations where high LMWOA concentrations can occur at comparably low temperatures due to mixing of water (Fisher, 1987) and that organic matter can be thermally activated. Such a process increases again the bioavailability of the buried sedimentary organic matter stimulating microbial activity in the deep subsurface (Wellsbury *et al.*, 1997; Horsfield *et al.*, 2006).

Tab. 2.4: Concentration of DOC and individual low molecular weight organic acids (LMWOA) with depth. All concentrations are presented as average of all fluids sampled from the production wells of the different geothermal plants. n.a. = data not available; n = number of samples.

study sites	Depth (m)	Temp (°C)	DOC (mg C/L)	Formate (mg/L)	Acetate (mg/L)	Propionate (mg/L)	Butyrate (mg/L)	Valerate (mg/L)	Σ LMWOA (mg/L)
M1 (n=1)	240	n.a.	0.7	-	-	-	-	-	-
M2 (n=2)	2200	65	0.4	<0.1	-	-	-	-	<0.1
M3 (n=2)	2300	106	0.9	0.1	0.6	-	-	-	0.7
M4 (n=4)	3443	107	10.5	1.2	12.6	2.4	0.5	0.2	16.9
M5 (n=2)	3446	123	2.1	0.3	2.5	0.4	0.1	-	3.3

The results of the DOC-characterization using size exclusion chromatography are presented in Table 2.5. Here, it is quite obvious that large and complex molecular structures like humic substances (HS) are only present in fluid from the shallowest side M1 with a proportion of 10 % of total DOC. For this sample the ratio between the spectral absorption coefficient (SAC in m^{-1} at 254 nm) and the DOC content in (mg C/L) of the humic substances was calculated as an indicator for the proportion of aromatic structures in this fraction. The HS fraction showed very low aromaticity of 0.86 L/(mg*m). With this analysis also the mean molecularity of the HS fraction has been determined to be 535 g/mol. The very low aromaticity and the low mean molecular weight of the HS fraction indicate the origin from marine fulvic acids according to the modified humification pathway (Huber *et al.*, 2011). This suggests that the HS fraction derives from deposited material within the aquifer or its proximate surrounding and has not been supplied by surface material.

The fraction of neutral substances (LMWNS) represents the main fraction of DOC in samples from M1 and M2 with 76 % and 36.6 %, respectively. LMWNS comprises compound classes of mono- and oligosaccharides, alcohols, aldehydes, ketones, and amino sugars (Huber *et al.*, 2011). Due to the lack of further molecular information on this fraction, the origin of LMWNS remains unclear. Nevertheless, this fraction is found to be the major fraction until depth of 2200 m and temperatures up to 65°C.

In M2, the biopolymer fraction (polysaccharides; polypeptides) is present only with a low percentage of 2 %, whereas no biopolymers occur in M1. The presence of biopolymers at site M2 could be due to extracellular polymeric substances (EPS) from microorganisms as polysaccharides being considered to be the dominating material of EPS (Flemming *et al.*, 2007). Nevertheless, the percentage proportion and amount of biopolymers is low and should not be over-interpreted.

Tab.2.5: Average concentration of DOC and percentage proportions of DOC fractions in fluids sampled from production wells of sites M1 to M5. HOC = hydrophobic organic carbon, Bio = biopolymers, HS = humic substances, BB = building blocks, LMWOA = low molecular weight organic acids, LMWNS = low molecular weight neutral substances. $\delta^{13}\text{C}_{\text{DOC}}$ are given as average values. n = number of samples

					>20,000 (g/mol)	1000 (g/mol)	300-500 (g/mol)	<350 (g/mol)	<350 (g/mol)
study sites	Depth (m)	$\delta^{13}\text{C}_{\text{DOC}}$ (‰)	DOC (mg C/L)	HOC (%)	Bio (%)	HS (%)	BB (%)	LMWOA (%)	LMWNS (%)
M1 (n=1)	240	-	0.7	9	-	10	6	-	76
M2 (n=2)	2200	-31.5	0.4	20	2	-	32	11	36
M3 (n=2)	2300	-31.2	0.9	30	0.2	-	41	23	18
M4 (n=2)	3443	-25.3	9.6	13	0.4	-	4	64	20
M5 (n=1)	3446	-29.6	1.9	17	-	-	8	67	8

A change in the overall DOC composition along the depth gradient of the Malm aquifer is observable for the study sites M2 and M3, where DOC is dominated by building blocks, LMWOA and LMWNS. Building blocks can be generated by the alteration/degradation of humic substances and can be considered as an intermediate stage on oxidation pathway to LMWOA (pers. comm. DOC-Labor Dr. Huber). It can be assumed that at this depth of the Malm aquifer the humic substances, that are initially present (see site M1) are already degraded to building blocks and with further increase in depth (M4 and M5), LMWOA fraction will become dominant, representing the final degradation products of the initially present humic substances. Interestingly, the $\delta^{13}\text{C}_{\text{DOC}}$ in samples from M2 and M3 (depth: >2200 m) are relatively similar.

In depths > 3400 m and temperatures >106°C, LMWOA dominate the DOC and acetate has been detected as the prevailing organic acid. This is in accordance to observations from other formation waters. The maximum concentrations of LMWOAs (up to 5000 mg/L) have been observed in formation waters sampled from oil fields between temperatures of 80 to 140°C (Carothers & Kharaka, 1978; Fisher, 1987; Lundegard & Kharaka, 1994). In shallow formation waters with temperatures well below 80°C and the occurrence of sufficient electron acceptors (e.g. sulfate), LMWOA can be easily consumed by microorganisms and with this the amount of organic acids is lower. At higher temperatures abiotic thermal decarboxylation of LMWOA takes place, leading to production of CO_2 and CH_4 and a decrease in the

concentration of LMWOA (Carothers & Kharaka, 1980; Kharaka *et al.*, 1983; Palmer & Drummond, 1986).

LMWOA can be generated by thermal degradation of kerogen in source rocks (Kawamura *et al.*, 1986; Barth *et al.*, 1988; Lundegard & Kharaka, 1990), by in situ thermal alteration of oil (Boles, 1992; Kharaka *et al.*, 1993; Borgund & Barth, 1994; Seewald, 2001) or by hydrolysis from macromolecular organic matter e.g. in kerogen (Glombitza *et al.*, 2009). The process of the LMWOA formation is suggested to derive either from thermal cracking reactions (Barth *et al.*, 1988) or from oxidation of *n*-alkanes (Seewald, 2001) or from hydrolysis of esters which are cross-linked within the macromolecular network of kerogen (Siskin & Katritzky, 1991). Oxidation processes of hydrocarbons or kerogen is assumed to derive from hydrolytic disproportionation (Helgeson *et al.*, 1993) or by mineral oxidants (Shebl & Surdam, 1996). Furthermore, the presence of organic acids dissolved in formation fluids may contribute to the dissolution of minerals, leading to weathering processes and secondary porosity in the respective environments (Thurman, 1985b; McMahon *et al.*, 1995; Mazzullo, 2004).

Carbon isotope composition of DOC for M4 and M5 showed distinct difference in $\delta^{13}\text{C}$ compared to M2 and M3. Fluid samples from production wells of sites M4 and M5 have DOC with relatively heavy isotopic composition of -22.3‰ (n=4) and -26.4‰ (n=2) respectively, compared to DOC from M2 (-31.5‰) and M3 (-31.2‰). These variations in isotope composition can be derived either from different level of maturation of the organic material or from different sources.

The results show that the deep Malm aquifer possesses a large pool of LMWOAs forming a potential feedstock for microbial life in the subsurface or in the above ground installations of the geothermal energy plant. At appropriate temperature conditions this substrate pool might be able to stimulate microbial activity causing problems (clogging and corrosion) in the geothermal plant. Thus, energy extraction from the aquifer water has to be adjusted carefully not to fall below a specific temperature threshold below which microbial activity is significantly stimulated.

2.4.3 Monitoring of the Fluid Chemistry at Study Site M4

A more detailed monitoring of the DOC content and its bulk isotopic composition as well as the composition of the LMWOAs was conducted for the geothermal plant M4 (used for

district heating) from March 2008 to October 2009 (Fig. 2.6). The highest DOC concentration during operation of the plant was observed at the beginning of the monitoring in March 2008 (19.3 mg C/L). Afterwards the DOC concentration was rather constant varying around an average of 10 mg C/L (range 6.4-12.3 mg C/L). In October 2009 the DOC values decrease to about 6.4 mg C/L. When samples have been taken before (107°C) and after (61°C) the heat exchanger, only minor variations in DOC concentration could be observed and the LMWOA fraction (sum of organic acid carbon detected by IC) seemed to be almost unaffected. In contrast $\delta^{13}\text{C}_{\text{DOC}}$ showed a large variability (from -16.0 to -25.7‰) over time and also in samples taken before or after the heat exchanger within a narrow time window.

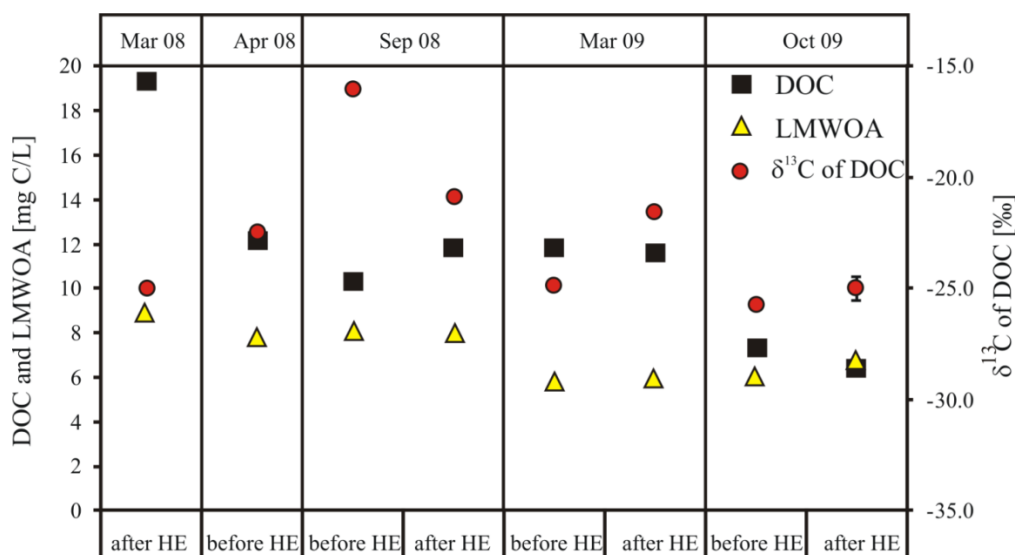


Fig. 2.6: Concentration of dissolved organic carbon (DOC; black squares) and low molecular weight organic acids (LMWOA; yellow triangles) in mg carbon per litre fluid at the study site M4. The red circles represent $\delta^{13}\text{C}_{\text{DOC}}$. HE = heat exchanger.

This is an unexpected observation, because DOC is mainly composed of LMWOA and the concentrations of individual acids did not change. One reason for the changing $\delta^{13}\text{C}$ of DOC might be microbial activity. Alawi *et al* (2011) were able to show that some sulfate-reducing bacteria (SRB) are present in the natural fluids before and after the heat exchanger (106°C/61°C), however, with a higher diversity in fluids sampled after the HE. Thus, microbial communities might influence the carbon isotope composition of DOC before and after the HE, whereas the higher diversity might suggest that sulfate reduction rates are higher in fluids after the HE. As a consequence sulfate concentration should be lower after the

HE while the carbon isotope composition of the DOC might be enriched in ^{13}C due to the preferential utilization of the lighter carbon species by microorganisms. Such a scenario for the carbon isotopes might be observed in March 2009 and to a lower extent in October 2009 (Fig. 2.6, see Appendix ‘geothermal plants’), however, in September 2008 the opposite trend is observable. Furthermore, a significant decrease in sulfate concentration while passing the HE is not seen (see Appendix ‘geothermal plants’). Due to the high flow rate within the geothermal plant of up to $100 \text{ m}^3/\text{h}$ providing a continuous supply of organic substrates and electron acceptors such as sulfate, it is conceivable that the monitoring of concentration changes in sulfate caused by microbial turnover processes is simply not possible.

Another explanation is simply natural variability in the DOC. Thus, although the DOC concentration is not much changing before and after the heat exchanger, natural inhomogeneities might have caused the variability in the carbon isotope data, especially since M4 occasionally contains oil hydrocarbons. This might be supported by the fact that samples before and after the heat exchanger were taken indeed in a narrow time window but never at the same time.

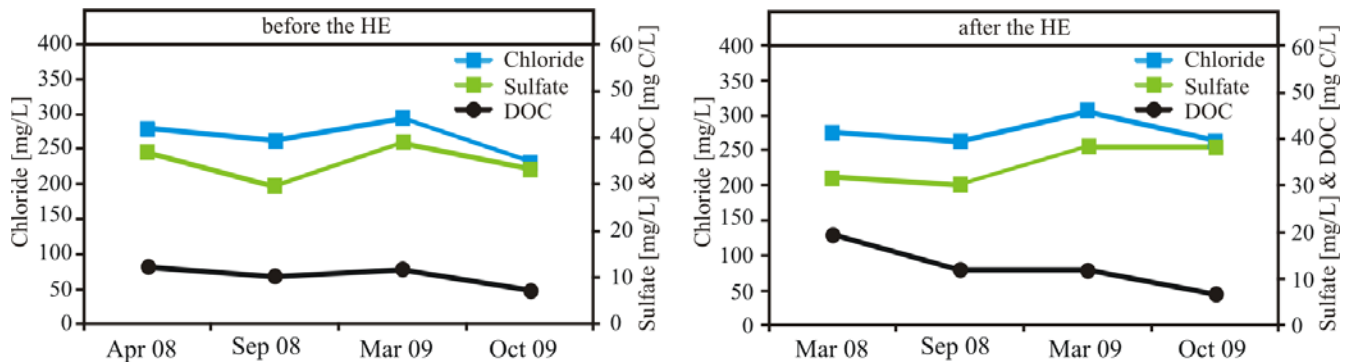


Fig. 2.7: Sulfate, chloride and DOC concentration in fluids from site M4 sampled before and after the heat exchanger (HE).

As shown in Fig. 2.7, chloride and sulfate concentrations vary in a similar way. Since chloride is used as an inert tracer, this similarity might indicate that the changes in the sulfate concentration reflect the natural variability of sulfate in the produced fluids. In comparison to chloride and sulfate profiles the DOC concentrations in samples especially taken after the HE varied in a different manner. This may indicate that concentrations of DOC are influenced by additional processes within the HE. It is known that carbonate precipitation occurs in the HE (pers. comm. of the plant operator). This is presumably caused by a small pressure release due to technical design (creation of swirl) in the HE, leading to CO_2 degassing and enhanced

carbonate precipitation. These carbonate scales may act as an adsorption surface for organic compounds (Thomas *et al.*, 1993) and could have changed the concentration of DOC as well as to a smaller extent also the composition of DOC and probably its carbon isotope composition. Changes in the DOC concentration and composition in the natural fluids before and after passing the HE are shown in Fig. 2.8. The LC-OCD results show a small decrease in the total DOC concentration and differences in the distribution of the different DOC-fractions.

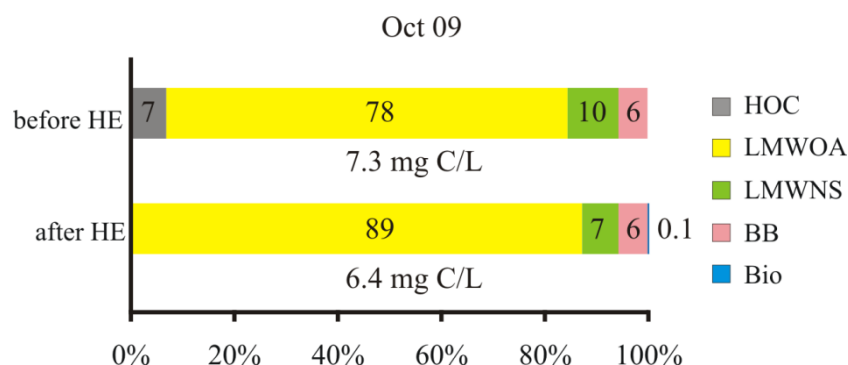


Fig. 2.8: Composition of DOC (%) and the concentration of DOC (mg C/L) in fluids taken before and after the heat exchanger (HE) of study site M4 on the same day. For abbreviations see Table 2.5.

2.4.4 Monitoring of the Fluid Chemistry at Study Site M5

The geothermal plant M5 used for the production of power due to the high indigenous fluid temperature of 123°C has been monitored from January 2007 until October 2009. The Monitoring started in January and May 2007 during a period where hydraulic tests of the production and injection well were conducted (Fig. 2.9). The relatively high DOC concentrations of 10 mg C/L and 3.5 mg C/L, observed during these start-up phases are presumably artefacts from the previous drilling process and the applied drill mud. On the injection side the concentration of LMWOA (10 mg C/L) also seemed to be influenced by the previous drilling activities. Similar to study site M4, acetate is the dominating LMWOA in the produced fluid during the start-up phase.

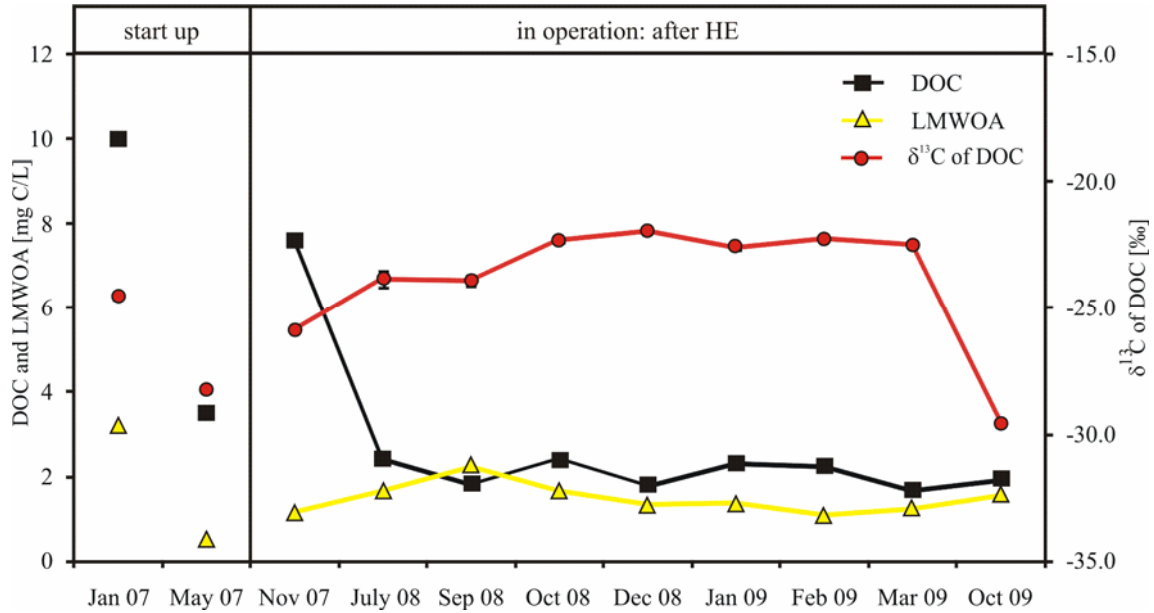


Fig. 2.9: Concentration of dissolved organic carbon (DOC; black squares) and low molecular weight organic acids (LMWOA; yellow triangles) in mg carbon per litre. The red circles give $\delta^{13}\text{C}_{\text{DOC}}$. Samples from January (injection well) and May 2007 (production well) represent the start-up phase of this plant. HE = heat exchanger.

With the beginning of the regular operation of this geothermal plant most samples have been collected from tapping point after the heat exchanger. The fluid sampled in November 2007 had a strong odor of hydrocarbons (7.8 mg C/L of DOC). The reason is not clear yet but might still reflect an influence from the start-up phase or might be explained by a malfunction of technical equipment (e.g. loss of pumping oil). However, during the rest of the monitoring period from July 2008 to October 2009 DOC concentrations remained almost stable around 2 mg C/L. In contrast to site M4 the carbon isotopic composition of DOC in fluids from study site M5 is after a slight enrichment in ^{13}C from July to October 2008 (from -23.8 to -22.3‰) relatively constant between October 2008 and March 2009 (around -22‰). In October 2009 a significant depletion in ^{13}C has been detected in samples (-29.6‰). The reason for this is still puzzling since there are no large variations in the DOC concentration (see Fig. 2.10B).

The relative proportions of different DOC fractions have been analysed for fluids from site M5. Fluid from November 2007, that tends to show an influence of drilling activities or malfunction of the technical equipment, has a high DOC (7.6 mg C/L) which is dominated by LMWOA (62 %) and LWMNS (34 %) (Fig. 2.10A). This pattern is significantly different from the pattern observed for the other DOC samples supporting the suggestion that this sample was not yet in a steady state with the surrounding. Since July 2008 the DOC

composition became more similar. DOC is still dominated by LMWOA, but the fraction of HOC increases (16 to 46 %) and the percentage of LMWNS is reduced to 9 to 14 %. It is quite reasonable that these deviations in DOC composition may be reflected in the carbon isotope composition of the DOC and might explain the changes in the $\delta^{13}\text{C}$ signal between November 2007 and October 2008.

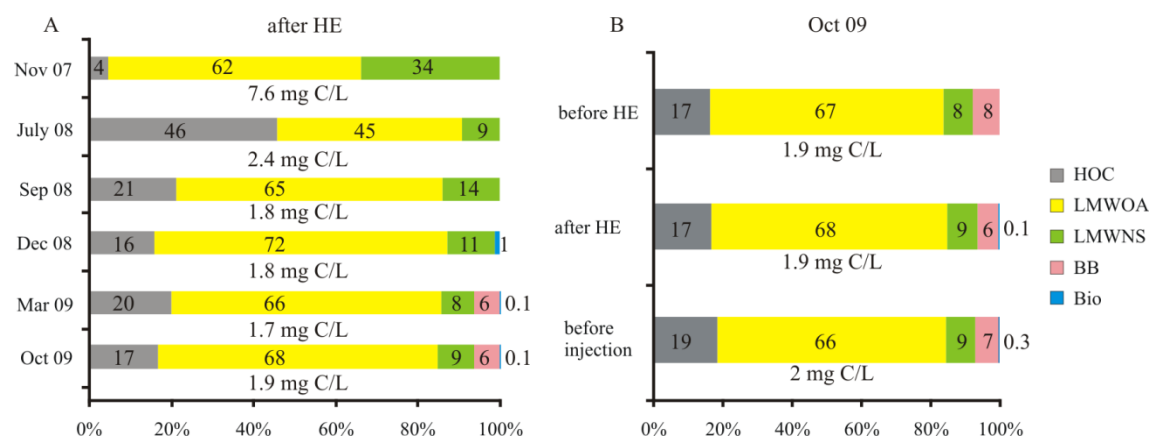


Fig. 2.10: Characterization (%) and concentration of DOC (mg C/L). A) Fluids sampled after the heat exchanger (HE) over time and B) DOC concentration and composition in fluids taken before and after the HE and before injection well in October 2009. For abbreviations see Table 2.5.

A significant change in DOC composition could be observed in fluids sampled since March 2009 (Fig. 2.10A). Here, organic compounds, belonging to the fraction of building blocks (BB), occurred for the first time, whereas the relative amount of LMWNS decreased. This compositional change seems not to influence the carbon isotope composition of DOC which remained relatively constant in March 2009 (-22.3 to -22.6‰). The general DOC composition between March and October 2009 is quite similar and provides no clue for the significantly lighter $\delta^{13}\text{C}$ -signal of DOC in October 2009 (-29.6‰). To improve the knowledge about what determines the $\delta^{13}\text{C}$ -signal of DOC, it might be helpful to analyse the carbon isotope composition of individual DOC fractions.

In October 2009, three fluid samples have been taken, before and after the heat exchanger and prior to re-injection. These samples were taken to monitor possible changes that occur in fluid chemistry when passing the technical equipment of the geothermal plant. In contrast to the results at site M4 (see above) DOC concentration (1.9 mg C/L) is nearly similar for these three samples (Fig. 2.10B). The same comes true for the DOC composition, indicating that no

significant changes tend to occur during residence time of the geothermal fluid in the plant at site M5.

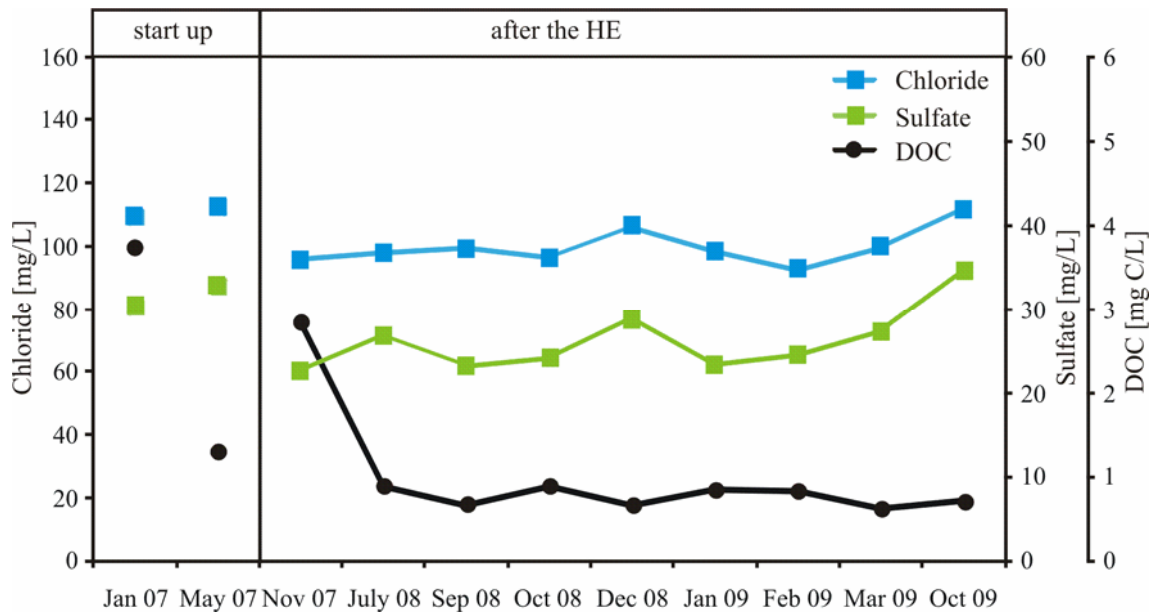


Fig. 2.11: Sulfate, chloride and DOC concentration during the monitoring at site M5. Samples are taken after heat exchanger.

At site M5 the sulfate concentration being an indicator for microbial activity, vary in a similar way than the chloride (inert tracer) concentration (Fig 2.11). This suggests the sulfate concentration is not much influenced by microbial processes and that the variations mostly rather resemble overall changes in the natural fluid flow (pumping of different volumes of water). However, as discussed above it also has to be taken into account that it might be quite difficult to monitor concentration changes related to microbial processes due to the high flow rate within the geothermal plant ($100 \text{ m}^3/\text{h}$).

2.5 Summary and Conclusions

Dissolved Organic Carbon in Natural Fluids in the Molasse Basin

DOC concentration and composition in deep natural fluids in the MB was investigated using samples from four different geothermal plants and on one production well for industrial purposes. The plants differ in depth (240-3446 m) and temperature ($65\text{-}123^\circ\text{C}$). With increasing depth DOC concentration increases. The maximum concentration of up to 9.6 mg C/L on average was measured in a geothermal plant that produces occasionally crude oil. Additionally, the DOC composition changed with increasing depth. LMWNS (76-36 %)

are the dominated fraction of DOC in depths between 240 to 2200 m with temperatures up to 65°C. Subsequently, in depth of 2300 m and 106°C the fraction of building blocks (41 %) dominated the DOC and finally at depth of around 3440 m the LMWOAs are the main fraction of DOC. Acetate is the dominating LMWOA in the studied deep Malm aquifer in addition to propionate, formate, butyrate and valerate. Thus, especially the deep Malm aquifer contains a large pool of potential substrates for microbial life. It is known that microorganisms can deteriorate the reliability of geothermal plants by causing scaling, clogging, and corrosion processes. The deep Malm fluids provide sufficient amounts of potential electron acceptors (e.g. sulfate, CO₂) and donors (e.g. DOC, H₂S, CH₄) for microbial metabolism. Therefore, this substrate pool bears the potential to cause problems in the geothermal plant by stimulating microbial activity at certain (lower) temperature conditions of the aquifer water. Therefore, the amount of energy extraction from the aquifer water has to be set with care.

Fluid Monitoring of the Geothermal Plants (M4/M5) in the Molasse Basin

Long-term monitoring was conducted on two geothermal plants in the MB sites M4 from March 2008 to October 2009 and site M5 from January 2007 to October 2009. The Malm aquifer is located in depth down to 3446 m with maximum temperature of 107°C (M4) and 123°C (M5). Sulfate concentrations varied between 23 and 39 mg/L. DOC concentrations ranged between 6.4 to 19.3 and 1.7 to 10.0 mg C/L for site M4 and M5, respectively. The high DOC concentrations are related to the sporadic production of crude oil. The DOC mainly consists of LMWOA (up to 89 %), mainly acetate. During operation, the fluid composition at side M4 varied slightly before and after the heat exchanger. This indicates that either (adsorption-) processes within the heat exchanger or microbial activity lead to changes in DOC concentration and composition at tapping points before and after the HE. The detection of SRB was already shown by Alawi *et al* (2011). Certain variability in organic fluid chemistry may occur at site M4 due to the sporadic production of hydrocarbons.

At study site M5 variations in fluid chemistry also occurred over time, less change have been observed with passage through the technical plant. The results showed clear differences in DOC concentration and composition between start-up phases (after drilling) of the production/injection wells, the initial and the regular plant operation.

3 Geology and Fluid Evolution of the North German Basin

Subdivisions of the Basin

The investigated aquifer thermal energy storage (ATES) sites Rostock, German Parliament (Reichstag), and Neubrandenburg are all located in the North German Basin (NGB), which comprises the Northeast German Basin (NEGB) and the Northwest German Basin (NWGB). Their modern fluid composition, and in a broad sense their nutrient and organic carbon contents forming a potential feedstock for prokaryotic life, are strongly correlated to the geological history and basin evolution. The NGB is part of the Southern Permian Basin (SPB) in Central Europe (Ziegler, 1990). The SPB extends for 1700 km from England across the Southern North Sea through Northern Germany into Poland and the Baltic countries (van Wees *et al.*, 2000). The SPB can be subdivided into four basins (Gast *et al.*, 1998). The Polish Trough in the east, separated by a barrier from the NEGB, the NWGB which is separated by the Altmark-barrier from the NEGB, and finally in the west the Dutch-England Basin (Gast *et al.*, 1998). The NGB is situated between the Precambrian Baltic Shield to the north and Variscan-influenced areas to the South (Franke, 1993; Benek *et al.*, 1996). The NGB comprises strata from Carboniferous to Quaternary (Bachmann & Grosse, 1989). The above mentioned study sites all belong to the NEGB.

Basin Development



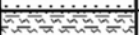




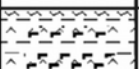




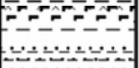
The evolution of the NGB was defined for the first time by Nöldeke & Schwab (1977), dividing the basin evolution into four main stages and was supplemented by a fifth stage by Scheck & Bayer (1999) (Table 3.1).

Table 3.1: Overview of the basin development of the NGB after Nöldecke & Schwab (1977) and Scheck & Bayer (1999)

Time	Main Stages
Cenozoic	Cenozoic subsidence
Late Cretaceous	basin inversion
Keuper - Early Cretaceous	basin differentiation
Early Permian - Middle Triassic	main subsidence
Late Carboniferous - Early Permian	initial rifting

The NEGB formed after the Variscan Orogeny, where crustal thinning occurred (initial rifting) as a consequence of abnormal heat flow, resulting in volcanic rocks during late Carboniferous/Lower Permian (Glennie, 1990; Benek *et al.*, 1996; Bachmann & Hoffmann, 1997; Scheck & Bayer, 1999). During the Upper Rotliegend thick red colored sandstones were deposited under arid to semiarid climate conditions (Gaupp *et al.*, 1993; Platt, 1994; Gast *et al.*, 1998; Rieke *et al.*, 2001). During this period, up to 1800 m of sediments were deposited in the NGB (Scheck & Bayer, 1999). The Rotliegend hosts considerable gas reservoirs due to the migration of gases from underlying Carboniferous coals (Gaupp *et al.*, 1993; Krooss *et al.*, 1995; Lüders *et al.*, 2005). From Early Permian (Rotliegend) to Middle Triassic (Muschelkalk) the maximum subsidence occurred in the basin (Nöldeke & Schwab, 1977; Scheck & Bayer, 1999). The transition from Rotliegend to Zechstein is represented by a change from continental desert to marine environment (Legler *et al.*, 2005). The Zechstein period is characterized by repeating marine transgressions being associated to the Milankovic cycles (Strohmenger *et al.*, 1996), resulting in the deposition of a giant carbonate-evaporite succession. Up to seven evaporitic cycles can be distinguished in the NGB (Richter-Bernburg, 1955; Tucker, 1991) with an ‘ideal’ cycle starting with either clay or carbonate overlain by sulfate and salt (halite, K- and Mg salts) deposits. These thick evaporitic Zechstein units, with average thickness of 2 km (Bayer *et al.*, 1997), played a crucial role in the fluid evolution of the NGB (Schmidt Mumm & Wolfgramm, 2002), because almost the whole Zechstein salt has moved into diapirs or pillows (Reinhardt, 1993) and serves at the same time as a seal for the underlying fluids (Ziegler, 1990; Gaupp *et al.*, 1993) (Fig. 3.1).

The Zechstein salt formations play an important role in the thermal regime of the basin owing much higher mean value of thermal conductivity (Bayer *et al.*, 1997). Therefore, thermal anomalies occur in the vicinity of salt diapirs (Giesel & Holz, 1970). At the top of salt diapirs temperature is higher and at the base of the salt structure temperature is lower than in comparable depth without salt structures. These positive and negative temperature anomalies are due to the different thermal conductivities of the salt and the surrounded sediment, resulting in a ‘chimney effect’ (Giesel & Holz, 1970). In general, heat flow values of the Zechstein unit and Permian-Carboniferous volcanic rocks of the NEGB is in maximum of about 60-65 mW/m² (Vosteen *et al.*, 2004).

Age		Stratigraphy and hydrogeological complexes		Geothermal resources
Cenozoic	Quaternary		Glacial deposits	Freshwater complex
	Neogene		Fluvial to lacustrine clay and silt sequences; lignite bearing sands	
	Paleogene		Clays and calcareous marls (Rupelian)	
Mesozoic	Cretaceous		Clastic material and sands	Salt water complex
			Chalky limestones	
	Lower		Marine marls; coarse-grained deltaic sequences	
	Jurassic		Marine limestones and marls; clays and coarse-grained deltaic sequences	
	Triassic		Playa lake deposits; limnic to fluvial deposits	Regional aquitard
			Carbonate-evaporite platform deposits	
			Fluvial to lacustrine sediments; playa lake deposits	
Paleozoic	Permian		Cyclic evaporites (salt & anhydrite); carbonates	Regional aquitard
			Fluvial fans at the southern basin margin passing laterally into salt lake deposits towards N.	Salt water complex
	Lower (Rotliegend)		Volcanics	









	Gravels		Sand/-stones		Marls/-stones		Clay, shales and mudstones
	Volcanics		Carbonates		Anhydrite		Rock salt

Fig. 3.1: Generalized stratigraphy and hydrogeological complexes with its potential for geothermal resources of the North German Basin, (modified after Möller *et al* (2008)).

The following Mesozoic is characterized by sedimentation during transgressive and regressive phases of the Tethys. At the base of the Mesozoic (Buntsandstein) successions terrestrial red bed sequences occur (Bachmann, 1998) with a thickness of 1400 m in the depocentre (Scheck & Bayer, 1999) consisting of alluvial fans, lacustrine, and playa deposits. These deposits are also of interest for geothermal utilisation. Subsequently, in Mid Triassic (Muschelkalk) time shallow marine carbonates with thickness of 250 to 350 m were deposited (Scheck & Bayer, 1999) followed in the Upper Triassic (Keuper) by mainly terrestrial facies of sandstones such as the deltaic deposits present at the geothermal energy storage of Neubrandenburg (Obst & Wolfgramm, 2010). The Keuper in the NEGB is, for instance, also used for the CO₂ storage pilot project at Ketzin (Förster *et al.*, 2006). During the

Buntsandstein and Muschelkalk, subsidence of the basin was still ongoing (Meinhold & Reinhard, 1967). According to Scheck & Bayer (1999) the basin configuration changed in the Keuper and basin subsidence decreased, while basin differentiation started which is documented in local thickness variations and development of smaller sub-basins continuing into Cretaceous times. Sediments of Jurassic age were preserved in the southern and eastern part of the NEGB due to ongoing subsidence, while the NWGB was uplifted (Scheck & Bayer, 1999). Salt diapirism of underlying Zechstein salt started during the Upper Triassic (Trusheim, 1957; Wienholz, 1965; Meinhold & Reinhard, 1967; Scheck *et al.*, 2003a). Intensive halokinetic movement also took place during Late Jurassic to Early Cretaceous (Kockel, 1998; Kossov *et al.*, 2000), suggesting a close correlation to tectonic pulses (Scheck *et al.*, 2003a). Basin inversion with compressional deformation occurred in the NGB during Late Cretaceous (Nöldeke & Schwab, 1977; Kossov & Krawczyk, 2002), leading to the present-day vertical basement offset of 5 km (Scheck & Bayer, 1999; Scheck *et al.*, 2003b), and most of the salt domes are re-activated (Meinhold & Reinhard, 1967; Scheck *et al.*, 2003b). The Late Cretaceous is characterized by uplift of basement blocks such as the Flechtinger High, the Harz Mountains, and Thüringer Wald (Reinhardt, 1993). The Mesozoic period ends with mainly marine deposits of Jurassic and Cretaceous age.

Heat flow modeling of the NEGB for sediments younger than Permian age yielded in a high average value 77 mW/m^2 (Norden *et al.*, 2008) that is assumed to correlate with higher quartz-cemented Mesozoic sandstones (Fuchs & Förster).

Cenozoic deposits consist of unconsolidated sediments. In the Paleogene and Neogene (formerly Tertiary) the lithology varies from brackish-marine to clastic fans, and especially in the NEGB, lignite seams, Rupelian clay and Miocene sands are deposited. The Rupelian clay serves as an important aquitard with a thickness between 25 to 260 m separating brines from freshwaters. The Quaternary is characterized by glacial sediments such as till, loam, and sands. They are widely distributed in the NGB and form today important aquifers. Southward-moving glaciers in Pleistocene shaped today's morphology and formed the North German Plain. The shallow freshwater aquifers consists of inhomogeneous Pleistocene sequences of gravel, sand, silt and clays, which are highly variable in thickness and lateral extension (Möller *et al.*, 2008). According to the movement of the ice shields during the last periods of glaciation, locally deep Pleistocene channels occurred that are cutting deep into the

underlying strata and displaced or eroded the important Rupelian clay aquitard (Tesmer *et al.*, 2007). This locally leads to a mixing of deep saline fluids with meteoric water. However, subglacial valleys are an important groundwater reservoir and are extensively used by waterworks in Northern Germany (Gabriel *et al.*, 2003).

Fluid Composition in the NEGB

Classification of water is still arbitrary and no satisfactory classification system exists. One system developed by Davis (1964) and Carpenter (1978) is based on salinity or total dissolved solids (TDS), respectively (Table 3.2). Another classification is based on the origin of the fluids. Meteoric water or infiltration water is surface water (e.g. rain, freshwater, sea water) percolating into the subsurface. It is known that these waters can descend into depth of 15 km (Nesbitt & Muehlenbachs, 1989). Basinal brines or connate water is water trapped in the pores of the sediment during formation of the rock and is also described as fossil water. In contrast, juvenile water or primary water resulted from the inner Earth and was never in contact within the hydrological cycle. However, the term formation water is simply referred to the water within a rock unit independent of the origin of the fluid.

Table 3.2: Classification of water based on total dissolved solids (TDS)

Classification	TDS g/L
Freshwater	<1
Brackish Water	1-10
Saline Water	10-100
Brine	>100

Subsurface fluids in the NEGB can be generally divided into five genetic groups (anthropogenic, contaminated water is not concerned): 1) Freshwater near to surface in Quaternary and Tertiary units, that is primary used as drinking water, 2) shallow groundwater salinization (coastal areas, vicinity of salt diapirs) (Grube, 2000), 3) deep basinal saline water (brine) (Wolfgramm *et al.*, 2011), 4) more or less mixed water of deep fluids along faults in Carboniferous-Permian to Zechstein units (Gaupp *et al.*, 1993; Schmidt Mumm & Wolfgramm, 2002; Wolfgramm & Seibt, 2003; Wolfgramm *et al.*, 2003); 5) mixed water of deep fluids in faults (mainly on the margin of NGB) and rims of salt domes (Magri *et al.*,

2008). In general, the salinity in the NEGB increases by 10 g/L per 100 m depth (Wolfgramm *et al.*, 2011).

Characteristics of Paleozoic Fluids

The fluid composition of the deep aquifers (Carboniferous to Zechstein) in the NEGB are strongly influenced by the development of the Zechstein evaporates and its evolution to salt diapirs, pillows and walls. Schmidt Mumm and Wolfgramm (2002) assume migration of brines and local input of external fluids, possibly derived from deep-seated sources, during early stages of thermal basin subsidence in the Upper Rotliegend. Main subsidence of the basin (Rotliegend to Muschelkalk) leads to the development of faults and fractures and offers pathways for fluid migration. Thus, saturated fluids from the Zechstein salt layer, with salinities in average of 330 g/L (Lehmann, 1974), descending deeply into the underlying sediments mixing with ascending less saline fluids derived from devolatilisation of the submerging Carboniferous sediments and Rotliegend volcanic rocks (Schmidt Mumm & Wolfgramm, 2002). Despite this, significant proportions of original Paleozoic formation waters are preserved and seawater evaporation was the principle source of salinity of these formation waters (Lüders *et al.*, 2010). The fluids of the Rotliegend unit are often Na-Ca-Cl solutions with salinities of 280 g/L on average (Lehmann, 1974; Wolfgramm *et al.*, 2011). A further distinct feature of the Rotliegend units in the NEGB is the high dissolved gas content. Nitrogen is the predominant gas in natural reservoir in the NEGB. The gaseous phase of Groß Schönebeck, for example, includes nitrogen (83.4 Vol. %), methane (14.4 Vol. %), CO₂ (1.7 Vol. %) helium, and hydrogen (0.4 Vol. %) (Wolfgramm *et al.*, 2003). The major source rocks for these gases are Carboniferous coals and marine black shales (Krooss *et al.*, 1995; Bandlowa, 1998; Mingham *et al.*, 2003; Mingham *et al.*, 2005).

Characteristics of Mesozoic Fluids

Fluids of different lithologic Mesozoic units are mostly linked to the formation (Wolfgramm *et al.*, 2011). However, fault structures favored the migration and mixing of fluids from regions and in different reservoirs. Basinal deep water prevails in the central basin (Wolfgramm, 2005). Formation waters stored in Buntsandstein (Lower Triassic), Râth (Upper Triassic) and Liassic reservoirs (Lower Jurassic) are less influenced by mixing with other fluids and are typically Na-Cl waters possibly reflecting their parent composition. However,

the connate water with high salinity is assumed to be a result of clay membrane effects (Wolfgramm *et al.*, 2011). The salinity for these fluids increases by 10-13 g/L per 100 m depth. Exceptions are the fluids in Buntsandstein reservoirs, which show higher salinities between 15 to 25 g/L per 100 m depth (Wolfgramm *et al.*, 2011). During Triassic and early Jurassic, movements of evaporates was initiated and became more strong in Late and post-Cretaceous time (Scheck & Bayer, 1999). Thus, the halokinetic movement resulted in hydraulic connection of the Zechstein fluids with the accumulated Mesozoic sandstones (Lower Cretaceous, Upper and Middle Jurassic) on the rim of the diapirs (Schmidt Mumm & Wolfgramm, 2002) by thermohaline convection (Magri *et al.*, 2005a; Magri *et al.*, 2005b). Therefore, aquifers that belong to the rim of the salt diapirs show Na-Cl fluids with concentrations of 20-25 g/L per 100 m depth (Wolfgramm *et al.*, 2011).

Characteristics of Cenozoic Fluids

In Germany shallow waters account for more than 85 % of groundwater recharge and range in depth between 10 and 100 m (Seiler & Lindner, 1995). For the NEGB, shallow freshwater of Ca-HCO₃-type are stored in Quaternary and Upper Tertiary unconsolidated sediments with TDS below 1 g/L (Grube, 2000). The fluid composition for these aquifers is characterized by recent and Pleistocene recharge conditions (Tesmer *et al.*, 2007; Möller *et al.*, 2008). An important seal is the Tertiary Rupelian Clay which separates the freshwater from saline fluids below the Upper Tertiary sandstones. Beside coastal areas, where seawater infiltration took place, locally, Pleistocene channels cut deep into the underlying strata and eroded the aquitard. In these areas groundwater salinization occurs according to ascending deep saline waters and dissolution of salt from diapirs (Grube, 2000).

In summary, the North German Basin and especially the Northeast German Basin exhibit ideal conditions for geothermal applications due to thick, laterally extended accumulations of aquifer-bearing sediments.

4 Biogeochemical Monitoring of a Shallow Geothermally Used Aquifer in the North German Basin

Biogeochemisches Monitoring eines flachen, geothermisch genutzten Aquifers im Norddeutschen Becken

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4.1 Zusammenfassung

Mit der bevorstehenden Verknappung konventioneller Energieträger wie Erdöl und Kohle erfährt die Geothermie eine wachsende Bedeutung in der aktuellen Energiediskussion. Bei der Nutzung der Geothermie kann es in den technischen Systemen zu bekannten Problemen wie Korrosion, der Bildung von Scales (Festphasen) und zu Verockerungen kommen, welche die Nutzbarkeit des geothermischen Aquifers und der technischen Anlage gefährden und den zuverlässigen Betrieb der gesamten geothermischen Anlage herabsetzen. Im Rahmen dieser Studie wurde ein biogeochemisches Monitoring eines oberflächennahen Aquifers durchgeführt, der als Energiespeicher (aquifer thermal energy storage, ATES) genutzt wird. Ziel dieses Monitorings war es, die Wechselwirkungen zwischen der technischen Anlage und dem natürlichen Aquifer zu untersuchen. Die Arbeiten umfassten die Charakterisierung der Fluidgeochemie sowie den Nachweis einer lebenden und aktiven mikrobiellen Gemeinschaft in der geothermischen Anlage und deren Variabilität in Abhängigkeit von der Betriebsweise. Im Zeitraum zwischen Juli 2007 und Dezember 2008 wurden die Zusammensetzung und Quantität der gelösten organischen Verbindungen (DOC) und der anorganischer Anionen untersucht, die von den in der Anlage lebenden Mikroorganismen als Kohlenstoff- und Energiequelle genutzt werden können. Weiterhin wurden die betriebsbedingten Unterschiede in der mikrobiellen Gemeinschaft detailliert mit Hilfe von charakteristischen Lipidbiomarkern

(Phospho- und Etherlipide) analysiert. In den Fluiden des Grundwasserleiters konnten ausreichende Mengen von potentiellen Elektronenakzeptoren (z.B. Sulfat 240 mg/L) und –donatoren (DOC 2,3 mg C/L) für die mikrobiellen Stoffwechselprozesse nachgewiesen werden, wobei die Variabilität in der Fluidgeochemie eher von saisonalen Schwankungen der obertägigen Umweltbedingungen als von der Betriebsweise der geothermischen Anlage abhängig zu sein scheint. Der Nachweis signifikanter Mengen an intakten Phospholipiden (PL) wies deutlich auf die Anwesenheit einer lebenden bakteriellen Gemeinschaft innerhalb der Anlage hin. Die weitere Analyse der Membranphospholipidfettsäuren (PLFA) zeigte, dass sich die Bakterien durch verschiedene Modifikationen der Zellmembranen an die betriebsbedingten Temperaturschwankungen innerhalb der geothermischen Anlage anpassen können. Beispiele für die Anpassung sind der relativ höhere Anteil an ungesättigten Fettsäuren (13,4 %) und der höhere Anteil an Fettsäuren mit kürzeren Kettenlängen (10,8 %) in den Filterproben, die bei geringeren Temperaturen aus der geothermischen Anlage entnommen wurden. Die Anteile von *iso*- und *anteiso*-Fettsäuren sind insgesamt kleiner bei den Proben, die geringeren Temperaturen in der geothermischen Anlage ausgesetzt waren. Dies deutet darauf hin, dass Mikroorganismen, die über diesen Anpassungsmechanismus verfügen, weniger häufig in den Proben bei niedrigeren Temperaturen vorkommen. Nichtsdestotrotz, zeigt ein höheres *anteiso/iso*-Fettsäurenverhältnis in diesen Proben eine Anpassung dieser Mikroorganismen bei niedrigeren Temperaturen an. Die Phospholipidanalyse weist also auf eine lebende und aktive mikrobielle Gemeinschaft in der geothermischen Anlage hin, die durch die Bildung von Biofilmen unter Umständen zu Störungen in der Anlage beitragen kann.

4.2 Abstract

Since conventional energy resources such as oil and coal will run short in the future, geothermal energy has become increasingly important in present energy discussions. With the use of geothermal energy, well-known problems such as corrosion, scaling and iron clogging often occur within the geothermal systems, leading to a reduced utilizability of the geothermally used aquifer as well as reduced working reliability of the geothermal plant. Within this study biogeochemical monitoring of an aquifer thermal energy storage (ATES) has been conducted to investigate interactions of the technical plant with the natural environment that may cause the above-listed disturbances. The main focus was laid on the

characterization of possible changes in the fluid geochemistry and on the proof of a microbial community in the geothermal plant and its variability due to different operating modes. In this context, from July 2007 to December 2008, we monitored the composition and quantity of dissolved organic compounds (DOC) and inorganic anions that may serve as potential carbon and energy sources for microbial processes and the variations in the microbial population using biomarkers such as phospholipids and ether lipids. Analysis of the fluid geochemistry showed that sufficient amounts of electron acceptors (e.g. sulfate 240 mg/L) and donors (DOC 2.3 mg C/L) for potential microbial respiration and energy consumption are present. However, fluid chemistry seemed to be more influenced by seasonal changes in the environmental conditions than by the operating mode of the geothermal plant. The occurrence of significant amounts of intact phospholipids (PLs) clearly indicated the presence of a substantial viable bacterial community within the geothermal plant. Moreover, the analysis of the phospholipid fatty acids (PLFA) inventory revealed an active microbial population that is capable of adapting its cell membranes to seasonal temperature changes caused by the different operating modes of the ATES. These variations are reflected by a relative shift in the PLFA composition. In comparison to the sample taken at warmer temperatures, the sample obtained during cooler temperature conditions in the ATES exhibited a shift of 13.4 % towards more unsaturated fatty acids (FA) and of 10.8 % towards more fatty acids with shorter chain length. The proportions of *iso*- and *anteiso*-fatty acids were generally lower in the sample at cooler temperatures, which might indicate that those microorganisms adapting their cell membrane fluidity via the *iso/anteiso* system were less abundant in the ATES at cooler temperatures. However, the *anteiso/iso* ratio was higher in the sample taken at cooler temperatures indicating a membrane temperature adaptation of these microorganisms. Overall, the phospholipid analysis indicates a viable and active microbial community in the ATES, and with its potential to form biofilms might be able to cause disturbances in the geothermal plant.

4.3 Introduction

The technical utilization of aquifer systems for geothermal plants, energy storages, and balneological applications has become increasingly importance with the general increase in energy/heat demand and the knowledge on limited fossil resources, such as coal, oil and gas. In May 2000 the first central solar heating plant with aquifer thermal energy store (ATES) was realized in Rostock in the North German Basin (NGB) (Bauer *et al.*, 2010). The utilization of the natural aquifer system for such technical applications provides a new challenge for the investigation and understanding of the biogeochemical reactions in the natural subsurface as well as the biogeochemical processes in the technical installations. Changes in groundwater chemistry that are related to changes in redox potential, temperature, pressure and/or microbial activity can have profound effects on the working reliability of these geothermal applications. Iron clogging of wells (Van Bleek & Van der Kooij, 1982; Smith & Tuovinen, 1985; Ralph & Stevenson, 1995), building of scales (Thomas & Gudmundsson, 1989; Møller *et al.*, 1998; Houben, 2003), and corrosion (Kritzer *et al.*, 1999; Potekhina *et al.*, 1999; Coetser & Cloete, 2005) are well-known phenomena which can all lead to reduced injectivity and failure of the plant. The mechanisms behind these phenomena are driven by abiotic but also by biotic processes. Therefore, the main focus of this study is a biogeochemical monitoring to prove the occurrence of a viable microbial community in the ATES and its variability to the operating mode. To investigate the relevance of the biologically driven processes, a general knowledge about microorganisms and their life strategies in geothermally used aquifer systems is necessary.

In natural groundwater environments living conditions such as temperature, pressure, salt content and availability of carbon and energy sources can vary significantly and microorganisms show a wide range of adaptation mechanisms to exist under these conditions. For instance, they are able to change the lipid composition of their cell membrane to sustain membrane fluidity under different environmental conditions (Hazel & Williams, 1990; Männistö & Puhakka, 2001; Konings *et al.*, 2002; Russell, 2002; Denich *et al.*, 2003). Main cell constituents in bacteria are different groups of polar lipids such as phospholipids (PL) that form bilayers in the cell membranes and are composed of a phosphatidyl head group and generally two fatty acids (FAs), that are both linked to a glycerol backbone (for structure see Appendix A). In case of the geothermal use of an aquifer system it is expected that temperature variations have a strong effect on the PL composition. Thermal adaptation of the

membrane PLs can be regulated by changes of 1) the chain length of the PLFA, 2) the degree of unsaturation of PLFA, and 3) the ratio of branched-chain PLFA (Boggs, 1986; Russell & Fukunaga, 1990; Kaneda, 1991; Mangelsdorf *et al.*, 2009). All of these biochemical adaptations have been described to maintain the fluidity of the cell membrane (e.g. Russell *et al.*, 1995; Beney & Gervais, 2001) and along with this its permeability for nutrient uptake (Russell, 1989). Furthermore, PLs rapidly degrade after cell death (White *et al.*, 1979; Harvey & Macko, 1997). Thus, the detection of intact PLs provides evidence that living bacteria are present and can be used as *life marker* for viable bacteria (White *et al.*, 1979; Zink *et al.*, 2003) in the ATES.

In addition to bacteria, another domain of prokaryotic life are archaea which are known to inhabit extreme (Blöchl *et al.*, 1997; Stetter, 1999) as well as moderate environments (Hershberger *et al.*, 1996; DeLong & Pace, 2001; Chaban *et al.*, 2006). Detmers *et al.* (2004) showed that the total cell counts of archaea in a pristine aquifer can make up up to 25 % of the microbial community. Their membrane lipids consist predominantly of diether bilayers or tetraether monolayers (De Rosa & Gambacorta, 1988). Diethers bear C₂₀ phytanyl chains with ether-linkage to a glycerol backbone connected to a polar head group. Tetraethers are characterized by two isoprenoidal C₄₀ alkyl chains with both ends linked via an ether bond to a glycerol backbone that is additionally connected to a polar head group. Another group of tetraethers containing branched instead of isoprenoidal side chains is interpreted to derive from bacteria due to its stereochemical configuration characteristic for bacteria (Weijers *et al.*, 2006a). Thus, there are specific bacteria that are able to synthesize ether lipids in their cell membranes as well (Langworthy *et al.*, 1983; Huber *et al.*, 1992; Huber *et al.*, 1996; Rütters *et al.*, 2001). After cell death the tetraethers lose their head groups. The glycerol ether cores (glycerol-dialkyl-glycerol tetraethers (GDGT), for structure see Appendix B), however, are relatively stable against degradation processes and can therefore often be found in natural systems, indicating fossil archaeal (isoprenoidal side chain) (Chappe *et al.*, 1982; De Rosa *et al.*, 1986) or bacterial (branched side chain) biomass (Weijers *et al.*, 2006a).

In the subsurface, the microbial biota is adapted to life without light and oxygen using, for instance, buried organic carbon (e.g. formate and acetate) as electron donor and inorganic compounds (e.g. CO₂, H₂, NO₃⁻, SO₄²⁻) as electron acceptor for their metabolism. The low molecular weight organic acids (LMWOA) such as acetate serve as important substrates and are metabolites of anaerobic processes. In pore waters of near-surface sediments acetate

concentrations are at low levels due to rapid turnover (Wellsbury & Parkes, 1995; Wu *et al.*, 1997). Changes in LMWOAs may reflect disturbances of the natural system such as decreasing/increasing rates of LMWOA consumption and production and therefore seem to be suitable indicators for the microbial communities. LMWOAs represent one fraction of the bulk dissolved organic carbon (DOC). DOC in pristine aquifer systems is reported to have low concentrations ranging from 0.2 to 5 mg C/L, with the majority below 2 mg C/L (Leenheer *et al.*, 1974; Spalding *et al.*, 1978; Thurman, 1985a). In groundwater systems, DOC results from the decomposition of plant and animal residues in the soil (MacCarthy, 2001), sedimentary organic matter like peat, kerogen and coal or from water infiltrating from rivers, lakes and marine systems (Aiken, 2002). Thus, the bioavailability and biodegradability of dissolved organic carbon may depend on the diagenetic alteration of the organic matter (Mindl *et al.*, 2000).

DOC comprises various compound classes and can be separated into different fractions based on molecular weight using size exclusion chromatography (SEC): 1) low molecular weight (LMW) compounds comprising e.g. LMW organic acids and LMW neutrals such as alcohols, aldehydes, ketons and amino sugars; 2) building blocks interpreted as breakdown products of humic substances; 3) humic substances such as fulvic and humic acids; and 4) biopolymers such as polysaccharides, polypeptides, and proteins. The residual fraction, which is not amenable to chromatographic separation, is assumed to consist of hydrocarbons and/or humins (Huber & Frimmel, 1996). Detailed information about the origin and fate of DOC as an electron donor for microbial metabolism can be derived from the isotopic composition of the DOC. Carbon isotope ratios of DOC in natural groundwaters are rarely presented in literature. However, $\delta^{13}\text{C}_{\text{DOC}}$ in aquifer systems range between -20‰ to -28‰ (Leenheer *et al.*, 1974; Spalding *et al.*, 1978; Aravena & Wassenaar, 1993) and are lighter than marine DOC which range between -18‰ to -20‰ (Avery *et al.*, 2006).

In the present study, biogeochemical monitoring was performed for an ATES system in the NGB to evaluate the changes in the microbial community over time and during operation of the system. Groundwater of the geothermal plant was investigated for its fluid chemistry (inorganic and organic anions, DOC). Filter samples from the technical plant were analysed for their biomarker composition to prove the existence of a viable microbial community in the ATES and to evaluate possible adaptation of microbial cell membranes to the specific conditions of the heat storage (charge/discharge) during operation. These filters are excellent

samples for studying the microbial community because they represent an in situ enrichment of particles and associated microorganisms. In the literature, some studies are available on e.g. screening and monitoring of the indigenous microbial community in the vicinity of ground source heat pumps in the Molasse Basin, Germany, (Brielmann *et al.*, 2009), on incubation experiments related to temperature changes in borehole heat exchangers (Schippers & Reichling, 2006), or on the biogeochemical evaluation of an ATEs in the Netherlands using batch experiments (Brons *et al.*, 1991). However, to the author's knowledge, this investigation is the first study on biogeochemical monitoring (July 2007 to Dec 2008) of an operating ATEs in the NGB using biogeochemical methods such as the phospholipid and ether lipid analysis in combination with the geochemical fluid analysis.

4.4 Material and Methods

4.4.1 Shallow Energy Storage Rostock

The solar assisted heat storage in Rostock, Germany, was established in April 2000 and since then has been working efficiently in the fields of solar energy production and the storage of surplus heat for space heating and domestic hot water preparation in multi-family houses. On the roof of these buildings, solar collectors have been installed and the surplus heat is stored in an aquifer that is located between 15 and 30 m below the surface in quaternary sand layer. The ATEs consists of one doublet of wells, each well being equipped with pumps. Thus, it can be used either to produce or to inject the heated or cooled water (Fig. 4.1). During summertime, water is pumped up from the cold side with a temperature of 20 to 8°C, heated with the surplus solar heat and reinjected into the warm side with a temperature of 50°C (charge mode). In wintertime, water is taken from the warm side, is pumped through a heat exchanger and the cooled water (around 20°C) is injected back into the cold side (discharge mode).

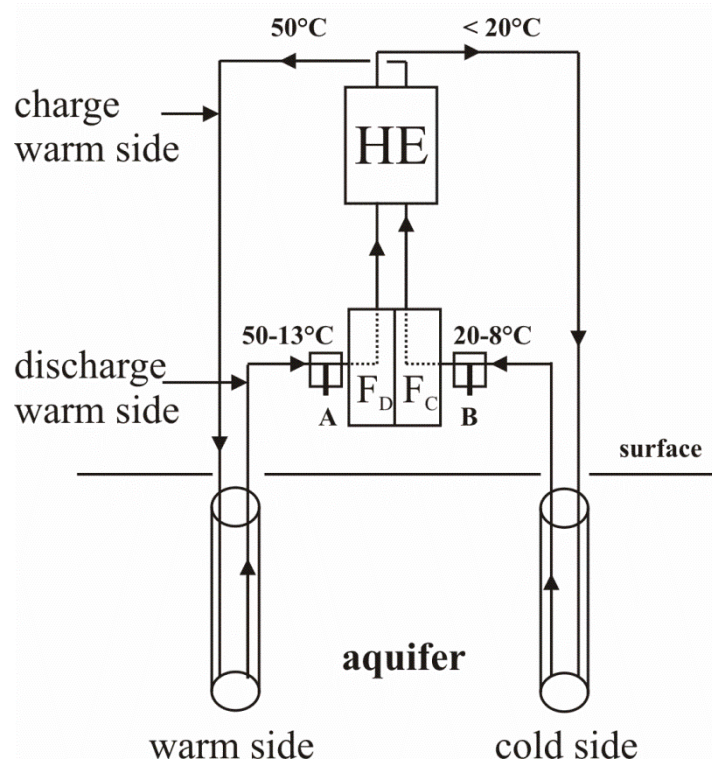


Fig. 4.1: Schematic view of the operating mode from the Ates. During charge mode (summer) water is pumped from the cold side, loaded with surplus heat of the solar system in the heat exchanger and reinjected at the warm side of the aquifer. During discharge mode (winter) water is pumped from the warm side via the heat exchanger to the cold side. A = tapping point at the warm side (used during discharge operating mode from warm to cold side); B = tapping point at the cold side (used during charge operating mode from cold to warm side). HE = heat exchanger; FD = particle filter in discharge mode, FC = particle filter in charge mode.

During one season of charge or discharge mode, the water temperature shifts towards warmer or colder temperature respectively in the aquifer depending on the energy demand. Filters (10 μm pore size) are integrated into the plant to refine particles before the fluid is transported through the heat exchanger. The Ates is designed for a maximum flow rate of 15 m^3/h . To avoid introduction of oxygen into the plant system and to keep hydrostatic pressure, all wells are filled with nitrogen gas above groundwater level (Schmidt *et al.*, 2004).

4.4.2 Fluid and Filter Sampling

Since 2007, we investigated the Ates using fluid and filter samples taken during the charge and discharge operating mode. We collected six fluid samples (Tab. 4.1) from the production flow of the aquifer (Fig. 4.1), one fluid downhole sample from the warm side, and three filter samples (Tab. 4.2). The six water samples were taken using appropriate sampling devices.

The downhole sample was collected directly from the aquifer using a steel bailer that had been cleaned and sterilized with ethanol, dried, and subsequently washed with purified water (Millipore). Water was filled into sterilized glass bottles without entrapped air, stored on freezer packs and analysed within 24 hours. Three filter samples were taken during different operating modes. The first filter sample taken in March 2008 represents the late discharge mode (LDM) with temperatures decreasing from 20 to 13.7°C during the time the filter was used. The second filter was taken in May 2008 during charge mode (CM) at operating temperature from 8 to 10.2°C and the third filter that has been sampled (Nov 2008) was installed during the early discharge mode (EDM) with temperatures between 50 and 20.9°C. The filters were taken directly from the plant, wrapped in pre-heated and clean aluminium foil and analysed immediately or stored in the freezer for later analysis at -80°C.

4.4.3 Ion Chromatography

For the determination of the inorganic and organic anions all fluid samples were analysed 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 x 250 mm, Dionex Corp.) at a constant temperature of 35°C was used. Further details and standard deviations of this method have already been described by Vieth *et al* (2008).

4.4.4 Quantification of DOC

The DOC was measured as a non-purgeable organic carbon after acidification with hydrochloric acid. The acidified sample was purged with CO₂-free high purity air for 5 min. The residual dissolved organic carbon was transformed into CO₂ by catalytic oxidation at 680°C, and measured using IR-Spectroscopy (TOC-2000A, Shimadzu). The quantification of the non-purgeable organic carbon was performed by external calibration using potassium hydrogen phthalate.

4.4.5 Liquid Chromatography-Organic Carbon Detection (LC-OCD)

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 Na₂HPO₄, 18 mM KH₂PO₄)

was used as the mobile phase with a flow of 1 mL min⁻¹. The sample passed a 0.45 µm membrane syringe filter before entering the chromatographic column (Novo-Grom GP2, 300 mm x 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 C/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.

4.4.6 Stable Carbon Isotope Analysis

For the bulk carbon isotope analysis of the DOC, the LC IsoLink (Thermo Fisher Scientific) was used, which is coupled to a continuous-flow Delta V Advantage (Thermo Fisher Scientific). Further details of this instrument and the bulk isotope analysis have been described by Krummen *et al* (2004). To get rid of particulate and inorganic carbon, the samples were filtered (0.45 µm), amended with concentrated phosphoric acid, shaken and placed in the fumehood over night. For the preparation of the reagents and the mobile phase, we used deionized water treated with UV photooxidation (Simplicity 185, Millipore) to remove organic compounds. To obtain CO₂-free mobile phase and reagents, the solutions were degassed under vacuum in an ultrasonic bath (30 min at 40°C) and purged with a constant flow of He during operation. The carbon isotope composition of the DOC is reported in δ -notation (per mill) relative to Vienna Pee Dee Belemnite Standard (V-PDB).

4.4.7 Extraction of Filters for Phospholipid Analysis

The freeze-dried and ground sediments from the filters of the geothermal plant were extracted using flow-blending (Radke *et al.*, 1978) applying a modified version of the Bligh and Dyer (1959) method. The method used for our sample extraction is also described in Zink & Mangelsdorf (2004). A solvent mixture of methanol-dichloromethane (DCM)-ammonium acetate buffer with a ratio of 2:1:0.8 (v/v) was applied. To separate the extract from the sediment residue, a centrifuge was used (10 min, 2500 rpm). The residue of the sediment was re-extracted twice with the same solvent mixture by ultrasonication (5 min) and the

supernatants were combined. The combined extracts were changed to a ratio of 2:2:0.9 by adding DCM and ammonium acetate buffer resulting in a lipid and an aqueous phase. Prior to phase separation, a deuterium-labelled lyso-PC (1-palmitoyl-(D_{31})-2-hydroxy-3-phosphocholine) was added as internal standard. After removal of the organic phase the residual aqueous phase was re-extracted twice using DCM. The combined organic phases were concentrated by TurboVap, dried under a stream of nitrogen and stored at -28°C .

The filter sample of November 08 (EDM) did not contain enough sample material to directly remove it from the filter. In this case, the material was finely dispersed on the filter matrix. Therefore, a visual inspection of the filter was done and the part of the filter that contained an adequate amount of sample material was selected and extracted twice for 20 min by ultrasonication using the same solvent mixture as described above. The combined organic phases were processed as described above. A clean filter was extracted as a blank, no phospholipids could be detected.

4.4.8 Chromatographic Separation of Lipids

To separate major compound classes of crude lipid extract a chromatographic separation using two columns in sequence filled with dried pure silica (1 g silica gel 60, 63-200 μm , upper column) and Florisil (1 g magnesium silica gel, 150-200 μm) was carried out. The extract was dissolved in 1 mL DCM – methanol, 9:1, (v/v). Four fractions were obtained by elution with the following solvents: neutral lipids (20 mL chloroform), free fatty acids (50 mL methyl formate with 0.025 % glacial acetic acid), glycolipid fraction (20 mL acetone), and phospholipid fraction (25 mL methanol). Subsequently, all fractions were reduced to dryness and stored at -28°C .

To obtain the phospholipid fatty acids (PLFA), a mild alkaline hydrolysis was applied to half of the PL fraction for transesterification according to the method described in White *et al.* (1979). The fraction aliquot was dissolved in 1 mL toluene-methanol, 1:1 (v/v), using ultrasonication for 5 min. 1 ml 0.2 N methanolic KOH was added and the mixture was placed in a cabinet drier at 37°C for 15 min. Afterwards the sample was transferred into a glass tube and acidified to pH 6 with acetic acid, diluted with 2 mL chloroform and 2 mL H_2O , and shaken vigorously for 5 min. Finally, the solution was centrifuged (6 min, 3000 rpm) to separate the organic phase from the aqueous phase. After removal of the organic lipid phase, the PLFA were dried under a nitrogen stream. To ensure that all fatty acids are derivatized,

additionally diazomethane was used to convert fatty acids into their corresponding methyl esters.

4.4.9 Analysis of Intact Phospholipids (PLs)

Analysis of intact PLs was performed using a Shimadzu SCL-10a VP HPLC system connected to a Finnigan MAT TSQ 7000 triple-quadrupole mass spectrometer (MS) coupled via an electrospray interface (ESI). Here, the PL classes were separated by their head groups with a normal-phase HPLC on a pure silica gel column (125x2 mm; Hypersil Si-3 μ , 120 Å). The LC was equipped with a 5 μ L sample loop. The mobile phase was a gradient prepared from chloroform (A), methanol (B) and ammonium acetate buffer (C). The initial mobile phase composition was 30 % A, 65 % B, and 5 % C; B was decreased to 55 % and C increased to 15 % after 3 min. After 8 min the mobile phase was reset to the initial conditions and held isocratically for 7 min, resulting in a total analysis time per sample of 15 min. The flow rate was set to 150 μ L min⁻¹. After 9 min the PLs were completely separated and eluted. Full-scan mass spectra were recorded in the negative ion mode over an m/z range of 400-1800 amu for 12 min. For structure elucidation of individual PLs collisionally activated dissociation (CAD) MS-MS experiments were performed over an m/z range of 100-800 amu.

4.4.10 Analysis of Phospholipid Fatty Acids (PLFA)

The PLFA fractions were analysed by gas chromatography (Agilent 6890 Series GC) coupled to an MS (Finnigan MAT 95 XL). The GC was equipped with a programmable temperature vaporization inlet (PTV, Agilent Technology, USA) working in splitless mode and a BPX 5 fused-silica capillary column (SGE, 50 m x 0.22 mm ID x 0.25 μ m FT). The injector temperature was 230°C. The temperature program of the GC started at 50°C and increased at a rate of 3°C min⁻¹ to 310°C and then kept constant at that temperature for 15 minutes. Helium, set to a flow rate of 1.0 ml min⁻¹, was used as carrier gas. For compound identification the MS was operating in electron impact mode at 70 eV. Full-scan mass spectra were recorded from m/z 50-650 amu at a scan rate of 1 s per decade and an interscan delay of 0.2 s, resulting in a scan cycle time of 1.3 s.

4.4.11 Analysis of Ether Lipids

The analysis of the ether bound glycerols was performed on a Shimadzu SCL-10a VP HPLC instrument coupled to a Finnigan MAT TSQ 7000 MS with an atmospheric pressure chemical ionization (APCI) interface. Samples were separated at 30°C with a Prevail Cyano column (2.1 x 150 mm, 3 µm; Alltech) equipped with a precolumn filter. The mobile phase consisted of *n*-hexane (A) and *iso*-propanol (B) held at 99 % A, 1 % B for 5 min, followed by a linear gradient to 1.8 % B within 40 min. Afterwards, the mobile phase changed to 10 % B within 1 min and was held at that level for 5 min to clean the column and back to the initial conditions within 1 min, held for 16 min for equilibration. The flow rate was set to 200 µl/min. The APCI conditions were a corona current of 5 µA giving a voltage of around 5 kV, a vaporizer temperature of 350°C, a capillary temperature of 200°C and nitrogen sheath gas at 60 psi without auxiliary gas. Mass spectra were generated by selected ion monitoring in the positive ion mode at a scan rate of 0.33 s using the masses 1295, 1302.1, 1049.5, 1035.5, 1021.5, and 654.2 at a width of 7 amu.

4.5 Results

4.5.1 Fluid Samples from the ATES System in Rostock

The fluid of the freshwater aquifer was characterized by pH values around 7.2, redox potential between 90 and 143 mV and conductivity of 920 µS/cm. Dissolved oxygen concentration accounts for 0.02 mg/L and the content of total dissolved solids (TDS) is about 500 mg/L. The major cations are sodium (16 mg/L), potassium (1.5 mg/L), magnesium (26 mg/L), calcium (144 mg/L), manganese (0.3 mg/L), total iron (1.2 mg/L), and ammonium (0.1 mg/L); the major anions are chloride (65 mg/L), sulfate (240 mg/L), hydrogen carbonate (232 mg/L), and nitrate (1.3 mg/L). These values are given as average concentrations of the long term hydrochemical monitoring done at the ATES. The measured temperature of the fluid at the time of sampling (Tab. 4.1) was between 10.2 to 11.7°C during the charge mode of the warm side (Fig. 4.1, tapping point B) and between 13.7 and 20.9°C during the discharge mode of the warm side (Fig. 4.1, tapping point A).

In our fluid samples, inorganic anions were detected in moderate concentrations (Tab. 4.1). Since the beginning of the monitoring, the sulfate concentration varied between 271 mg/L and 221 mg/L. Only three water samples (July, Sept and Nov 2007) had detectable concentrations

of nitrate (0.1 to 2.9 mg/L), whereas the downhole sample (Nov 07) taken directly from the aquifer showed a nitrate concentration of 0.1 mg/L.

Table 4.1: Overview of fluid samples representing different operating modes (charge of and discharge from the warm side) and concentrations of organic and inorganic anions as well as DOC and its carbon isotopic composition; ws = warm side; A = tapping point at warm side; B = tapping point at cold side; n.m. = not measured; n.d. not detected.

Sampling time	Operating mode	T (°C)	tapping point	Formate (mg/L)	Acetate (mg/L)	Nitrate (mg/L)	Chloride (mg/L)	Sulfate (mg/L)	DOC (mg C/L)	$\delta^{13}\text{C}_{\text{DOC}}$ (‰)
July 2007	charge	11.7	B	<0.5	<0.5	0.8	70	271	1.2	-27.9
Sep 2007	charge	10.9	B	n.d.	n.d.	2.9	53	233	2.6	-25.4
Nov 2007	not in use	n.m.	downhole sample:ws	0.2	0.2	0.1	60	225	5.0	-27.3
Mar 2008	discharge	13.7	A	n.d.	n.d.	n.d.	71	253	3.3	-27.9
May 2008	charge	10.2	B	n.d.	n.d.	n.d.	65	226	2.4	-27.4
Nov 2008	discharge	20.9	A	n.d.	n.d.	n.d.	65	250	2.9	-27.2
Dec 2008	discharge	15.9	A	n.d.	n.d.	n.d.	68	221	1.2	-27.5

The freshwater samples exhibited DOC content between 1.2 and 5 mg C/L. The highest DOC (5 mg C/L) was detected in the downhole sample (Nov 07), whereas water samples taken from the geothermal plant were characterized by an average DOC of 2.3 mg C/L. Fluid samples were analysed by LC-OCD to characterize the composition of DOC. Around 86 % of total DOC in the fluid sample from July 2007 taken during the charge mode of the warm side were amenable to size exclusion chromatography. The chromatographic DOC was dominated by humic substances (44.5 %) that were characterized by molecularity of 665 g/mol and aromaticity of 2.2 L/(mg*m) and could therefore be classified as fulvic acids (Huber & Frimmel, 1996). Besides humic substances, building blocks (32.7 %), LMW compounds (22.6 %) subdivided into e.g. neutral compounds (11.2 %) and acids (3.0 %), as well as biopolymers (0.2 %) were detected. This DOC composition was confirmed for other fluid samples from the ATES (data not shown) implicating that, despite the varying temperature, the composition of DOC seems to be similar.

The carbon isotopic composition of the DOC (Tab. 4.1) ranged between -27.9 and -25.4‰ for the process water. Only one sample from Sept 2007 showed a deviating isotopic composition (-25.4‰) being slightly enriched in ^{13}C compared to the average isotope value of the other fluid samples (-27.6‰). The DOC of the downhole sample had a similar isotopic composition (-27.3‰) to the fluids sampled within the plant. LMWOAs like formate and acetate (Tab. 4.1) were only detected in the fluid sample from July 2007 (1 mg/L) and in the downhole sample (0.4 mg/L).

4.5.2 Filter Samples from the ATES System in Rostock

An overview of the available filter samples and the corresponding operating mode of the ATES during the installation of these filters is provided in Table 4.2. Additionally, the temperature range during the installation of the filters is indicated, where the first number represents the temperature on the day of installation and the second one the temperature on the day of removal of the filters.

Table 4.2: Membrane phospholipid composition of the bacterial community in three filter samples taken during charge and discharge mode of the warm side. ¹temperature measured at day of filter installation; ²temperature measured at day of filter sampling; PG = phosphatidylglycerol, PE = phosphatidylethanolamine; PC = phosphatidylcholine; LDM = late discharge mode; CM = charge mode and EDM = early discharge mode of the warm side.

Sampling time	Mar 08	May 08	Nov 08
operating mode	LDM	CM	EDM
T (°C) ¹	20.0	8.0	50.0
T (°C) ²	13.7	10.2	20.9
PG (%)	6.2	2.9	8.9
PE (%)	88.3	89.1	66.4
PC (%)	5.4	8.0	24.7

Three filters were collected, one in the charge CM, (taken from filter F_C), and two in the discharge mode EDM and LDM, (taken from filter F_D) of the warm side (Fig. 4.1) and analysed for phospholipids and ether lipids (Tab. 4.2 and 4.5). In all filters, intact phospholipids have been detected indicating the existence of viable microorganisms in the ATES system (Tab. 4.2 and Fig. 4.2). The dominating PLs were phosphatidylethanolamines (PE) with 66 – 89 % followed by phosphatidylcholines (PC) with 5 – 25 % and phosphatidylglycerols (PG) with 6 – 9 % (Tab. 4.2). The amount of PEs increased with decreasing temperature rising from 66.4 % (20.9°C, EDM) to 88.3 % (13.7°C, LDM) and 89.1 % (10.2°C, CM), while the percentage of PGs decreased with decreasing temperature dropping from 8.9 % (20.9°C) to 6.2 % (13.7°C) and 2.9 % (10.2°C). The percentage of PCs showed certain variability and no clear temperature trend. At the highest temperature (20.9°C) around 25 % of PL consisted of PC, whereas at the lower temperatures (10.2 and 13.7°C) the percentage of PC was low at 8.0 and 5.4 % respectively. Figure 4.2 shows the mass spectra of PGs, PEs, and PCs and the dominating combinations of fatty acid side chains are indicated.

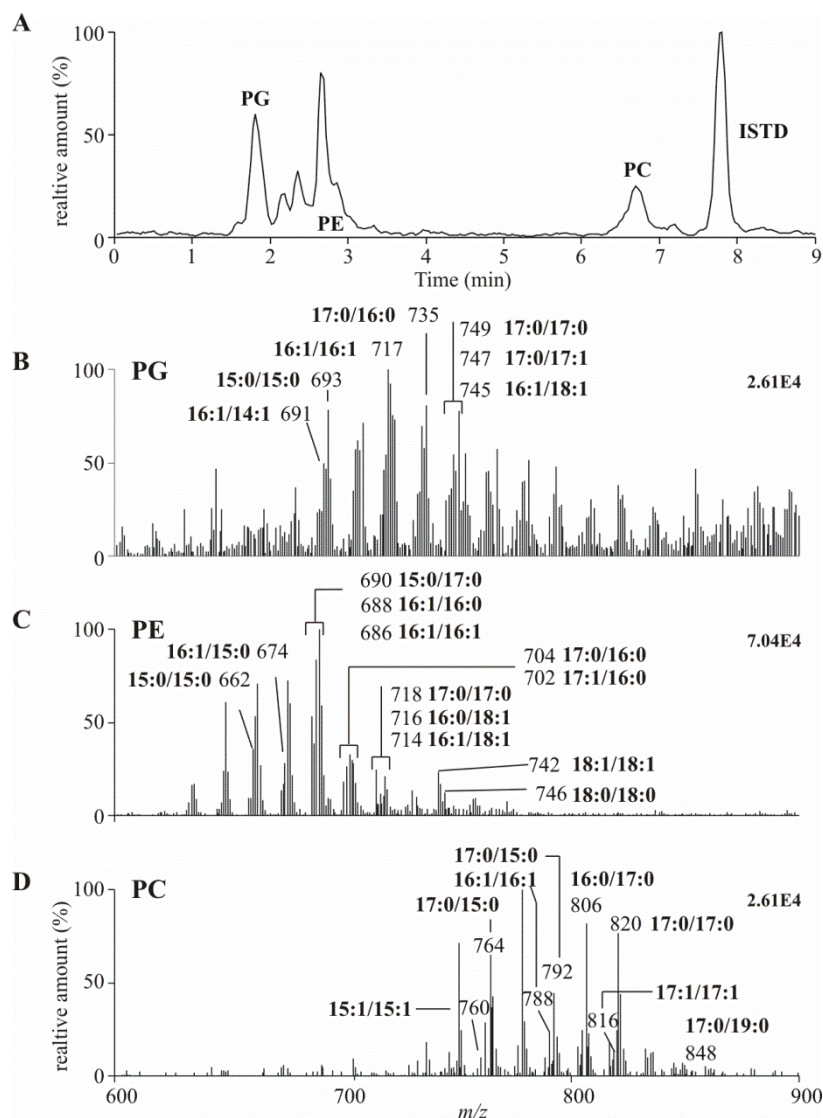


Fig. 4.2: A) HPLC-ESI-MS chromatogram of the phospholipid distribution from the filter sample taken in Nov 2008 and mass spectra of B) phosphatidylglycerols (PG), C) phosphatidylethanolamines (PE), and D) phosphatidylcholines (PC) showing the main fatty acid combinations of intact phospholipids. ISTD = internal standard: deuterium-labelled Lyso-PC (1-palmitoyl-(D_{31})-2-hydroxy-glycero-3-phosphocholine). X:Y = carbon number of PLFA : number of double bonds.

The two filters that were sampled during the discharge mode of the warm side represent early (EDM) and late (LDM) discharge modes. Please note that these samples were not sampled in a chronological sequence as indicated by the sampling dates. In contrast to the filter sampled during charge mode, the water for both filter samples taken during discharge mode had the same origin (warm side of the ATES). For that reason, the PLFA profiles obtained from these two filter samples were compared (Tab. 4.3). In our samples the fatty acids linked to the different head groups were typically saturated, monounsaturated and branched chain mid-

chain branched (mcb), and terminal branched *iso* (*i*), and *anteiso* (*ai*) fatty acids with 12 to 20 carbon atoms with the main compound being *n*-C_{16:0} FA.

Table 4.3: Comparison of phospholipid fatty acid (PLFA) composition obtained after alkaline hydrolysis of intact phospholipids from LDM and EDM. Branched fatty acids are indicated in italics; *i* = *iso*; *ai* = *anteiso*; mcb = mid-chain branched; br = not identified branching position; C_x:_y = carbon number of PLFA : number of double bonds.

PLFA (%)	LDM Mar 08	EDM Nov 08
C _{12:0}	-	0.5
<i>br</i> -C _{13:0}	-	1.1
C _{13:0}	1.8	0.5
<i>i</i> -C _{14:0}	-	2.0
C _{14:0}	2.0	6.3
<i>i</i> -C _{15:0}	5.2	12.0
<i>ai</i> -C _{15:0}	6.1	13.6
C _{15:1}	-	1.1
C _{15:0}	2.5	2.5
<i>i</i> -C _{16:0}	-	2.1
C _{16:1}	20.5	10.2
C _{16:0}	21.8	16.6
mcb-C _{17:0}	18.4	6.6
<i>i</i> -C _{17:0}	0.7	1.5
<i>ai</i> -C _{17:0}	1.8	2.0
C _{17:0}	2.4	1.9
C _{18:1}	7.3	3.1
C _{18:0}	6.7	14.1
C _{19:0}	2.9	-
C _{20:0}	-	2.3
T (°C) at sampling day	13.7	20.9

The broadest distribution of PLFAs occurred in the EDM sample (Nov 08). Besides the main compound, the *n*-C_{16:0} FA (16.6 %), intermediate to low amounts of saturated PLFAs were detected for *n*-C_{18:0} (14.1 %), *n*-C_{14:0} (6.3 %), *n*-C_{15:0} (2.5 %), *n*-C_{17:0} (1.9 %), and *n*-C_{20:0} (2.3 %) FA. With exception of the *n*-C_{16:1} isomers (C_{16:1}ω7 and C_{16:1}ω5; 10.2 %) monounsaturated PLFAs could be detected only in low amounts of 3.1 % for *n*-C_{18:1}, and 1.1 % for *n*-C_{15:1} FA. Terminally and mid-chain branched PLFAs such as *i*-C_{15:0} (12.0 %), *ai*-C_{15:0} (13.6 %), mcb-C_{17:0} (6.6 %), *i*-C_{17:0} (1.5 %), *ai*-C_{17:0} (2.0 %), as well as *i*-C_{14:0} (2.0 %) and *i*-C_{16:0} (2.1 %) FAs occurred in intermediate to low proportions. The sample from the LDM (Mar 08) showed a lower range of carbon atoms in PLFAs compared to the sample

from the EDM (Nov 08). The n -C_{16:0} FA was present with 21.8 %, followed by a high proportion of the monounsaturated PLFAs with 16 carbon atoms (C_{16:1}ω7; 20.5 %) and the saturated mcb-C₁₇ FA with 18.4 %. Monounsaturated and saturated C₁₈ FA were detected in relative amounts of 7.3 % and 6.7 %, respectively. Furthermore, terminally branched fatty acids with 15 carbon atoms were present in relative amounts of 5.2 % (*iso*) and 6.1 % (*anteiso*). Saturated PLFAs with 13, 14, 15, 17, and 19 carbon atoms only accounted for a small proportion of the fatty acid distribution ranging between 1.8 and 2.9 %.

For a better visualization of the differences in the saturated and unsaturated PLFA patterns between the sample from the EDM (Nov 08, 20.9°C) and the LDM (Mar 08, 13.7°C) a PLFA difference diagram was created by subtracting the percentage proportion of the PLFA obtained from the EDM sample (20.9°C) from those obtained from the LDM sample (13.7°C) (Fig. 4.3).

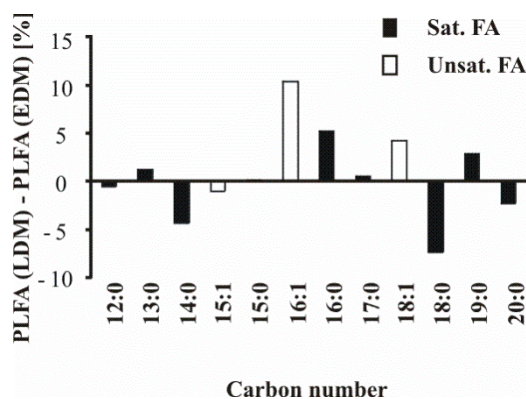


Fig 4.3: Difference diagram of saturated and unsaturated phospholipid fatty acids (PLFA) from the EDM (warmer temperature) subtracted from those from the LDM (cooler temperature). X:Y = carbon number of PLFA : number of double bonds.

The LDM sample showed a higher proportion of short chain FA (n -C₁₂ to n -C₁₆ FA) as indicated mainly by a shift from 18 to 16 carbon atoms but also by a shift from 20 to 19 and 14 to 13 carbon atoms (Fig. 4.3). In total, the proportion of short chain FA increased by 10.8 % from 37.8 % in the EDM sample to 48.6 % in the LDM sample (Tab. 4.4). Furthermore, there was a higher proportion of unsaturated FA in the LDM sample which was indicated by the increase of the n -C_{16:1} and n -C_{18:1} FAs (Fig. 4.3). Their relative amount increased by 13.4 % from 14.4 % in the EDM sample to 27.8 % in the LDM sample (Tab. 4.4).

Table 4.4: Relative proportion of different groups of PLFAs in filter extracts from the EDM (higher temperature) and the LDM (lower temperature) of the warm side.

PLFA (%)	LDM - Mar 08	EDM - Nov 08	LDM-EDM
T (°C) at sampling day	13.7	20.9	
saturated <i>n</i> -FA	40.0	44.8	-4.8
unsaturated <i>n</i> - FA	27.8	14.4	13.4
ratio unsat/sat	0.7	0.3	-
<i>n</i> -C ₁₂ to <i>n</i> -C ₁₆	48.6	37.8	10.8
<i>n</i> -C ₁₇ to <i>n</i> -C ₂₀	19.3	21.4	-2.1
ratio short/long	2.5	1.8	-
<i>iso</i> -FA	5.9	17.6	-11.7
<i>anteiso</i> -FA	7.8	15.5	-7.7
ratio <i>anteiso/iso</i>	1.3	0.9	-
mcb-C ₁₇	18.4	6.6	11.8

The proportions of the *iso*- and *anteiso*-branched FA were generally lower in the LDM sample. The sum of these two FA groups decreased by almost 20 % (Tab. 4.4). On the other hand, the mid-chain branched C₁₇ FA increased significantly in the LDM sample by 11.8 %. Thus, the comparison of the PLFA distributions revealed four distinct trends from the EDM to the LDM sample: 1) a shift to shorter FA chain length, 2) a shift to more unsaturated FAs, 3) lower proportions of *iso*- and *anteiso*-branched FAs, and 4) a higher proportion of the mid-chain branched C₁₇ FA.

Six different isoprenoidal glycerol dialkyl glycerol tetraethers (GDGT I-IV, caldarchaeol, and crenarchaeol; for structures see Appendix B), being molecular markers for archaeal biomass (Woese *et al.*, 1978; De Rosa *et al.*, 1986; Kates, 1997; Hopmans *et al.*, 2000), were identified in the 3 filter extracts. These GDGTs varied in their internal cyclization containing 0 (caldarchaeol) to 4 cyclopentyl moieties. Crenarchaeol contains one additional cyclohexyl ring to the 4 cyclopentyl moieties. The most abundant compounds were caldarchaeol (31 – 37 %) and crenarchaeol (22 – 25 %) (Tab. 4.5). In addition, compounds I, II and IV with average amounts of around 12 % were identified, while GDGT III was present only with 6 %. In all 3 filter extracts diether glycerol core lipids could not be detected.

Table 4.5: Archaeal ether lipids and ether lipids of bacterial origin from filters sampled during EDM, LDM, and CM of the warm side. Temperatures indicate temperature at day of sampling.

	M+	Compound	Cyclization	Methylation	20.9°C (%) EDM	13.7°C (%) LDM	10.2°C (%) CM
archaeal etherlipids	1302	Caldarchaeol	0	isoprenoidal	37.2	30.8	36.6
	1300	I	1	isoprenoidal	13.2	12.1	10.9
	1298	II	2	isoprenoidal	9.9	12.9	11.2
	1296	III	3	isoprenoidal	5.7	5.8	6.2
	1294	IV	4	isoprenoidal	12.1	13.0	12.2
	1292	Crenarchaeol	5	isoprenoidal	21.9	25.4	23.0
bacterial etherlipids	1050	V	0	6	17.9	25.1	14.9
	1036	VI	0	5	15.1	19.1	18.8
	1022	VII	0	4	38.7	40.4	51.3
	1020	VIII	1	3	24.4	11.6	8.1
	1018	IX	2	2	2.1	2.1	2.1
	1046	X	2	4	0.2	0.6	1.4
	1034	XI	1	4	1.5	1.1	3.4
	1032	XII	2	3	0.1	0.0	0.0

Additionally, eight branched membrane-spanning GDGT lipids, being biomarkers for bacterial biomass (Weijers *et al.*, 2006a; Weijers *et al.*, 2007), were identified in the filter samples of the ATES (Tab. 4.5). The branched GDGTs vary in the number of methyl groups and cyclopentane rings (see Appendix B for structure V to XII). The most abundant ether lipids of bacterial origin were the branched GDGTs without any pentacyclic ring system (V, VI, VII) with relative amounts of up to 85 % in the CM sample taken at the lowest temperature of 10.2°C. The ring-containing GDGTs (VIII to XII) accounted for 15 to 28 % the highest percentage was detected in the filter extract from the EDM with the highest temperature of 20.9°C.

4.6 Discussion

The present results on fluid geochemistry were obtained during the monitoring of the ATES from July 2007 to Dec 2008. The range of the DOC concentration from 1.2 to 3.3 mg C/L was in accordance with the reported values in literature (0.2 to 5 mg C/L) for pristine groundwater (Leenheer *et al.*, 1974; Spalding *et al.*, 1978). The samples taken during normal operation of the plant (Mar 08 to Dec 08) reflected a certain variability of the DOC concentration. The

slightly higher values from Mar 08 (3.3 mg C/L) and Nov 08 (2.9 mg C/L) can be seen as an indication for increased spring and fall run-off when the soil is saturated (Reardon *et al.*, 1980; Antweiler & Drever, 1983; Wassenaar *et al.*, 1989). Exemplary, a detailed DOC characterization was performed by LC-OCD for the sample from July 07. The result revealed a dominance of humic substances. Based on molecularity and aromaticity, these substances could be classified as pedogenic fulvic acids (Huber & Frimmel, 1996). This means that the humic substances seemed to derive from soil organic matter. This is quite plausible due to the shallow depth of the aquifer (15-30 m). In addition, the molecular biological analysis of the fluid and filter samples from this ATES system resulted in the conclusion that the shallow aquifer is influenced by surface processes (Lerm *et al.*, this volume). A comparison of the DOC concentration and the operating mode of the ATES showed no correlation. Within the first months of our monitoring (July 07 – Nov 07), the increase in DOC concentration over time had to be considered with caution due to the malfunction of the plant. This problem may also have caused some variations occurring in the sample of Sept 07 enrichment in ^{13}C and increasing nitrate concentration, (Tab. 4.1).

During the normal operation of the ATES there was no indication for a correlation of charge or discharge phases with DOC concentration or the concentration of selected anions in the fluid. The high proportion of humic substances in the DOC could be discussed as a bioavailable pool of organic carbon for microorganisms. For instance, microorganisms such as *Geobacteraceae* are capable of reducing humic substances for respiration in anaerobic habitats (Lovley *et al.*, 1996; Coates *et al.*, 2002). A study by Amon & Benner (1996) showed that the high molecular weight substances (HMW, >1 kDa) of DOC in river systems are a more reactive pool for growth and respiration of microbes than low molecular weight substances (LMW, <1 kDa) of DOC. These authors state that the HMW fraction is more recent and the LMW classes are more diagenetically/biogenically altered and therefore less available for microorganisms. This might imply a highly available carbon pool for microbes within the freshwater aquifer. However, other studies show a greater biodegradability of LMW compounds (Meyer *et al.*, 1987; Covert & Moran, 2001) and they are usually considered to be easily biodegradable for the respiration and energy consumption of microorganisms (del Giorgio & Cole, 1998; van Hees *et al.*, 2005; Berggren *et al.*, 2007).

The isotopic compositions of DOC in the fluids showed a mean value of -27.6‰ and this was consistent with the $\delta^{13}\text{C}_{\text{DOC}}$ from the downhole sample (-27.3‰). As it has been assumed that

DOC predominantly consists of fulvic acids, these isotope results were in accordance with the mean isotopic composition of fulvic acids that is assumed to be around -27‰. Reported isotope data of fulvic acids in groundwater systems ranges between -23.7 and -30.5‰ (Wassenaar *et al.*, 1990a; Wassenaar *et al.*, 1991; Grøn *et al.*, 1996; Einsiedl *et al.*, 2007) and the commercially available Suwannee River fulvic acid has a reported value of -27.9‰ (IHSS 2010). The $\delta^{13}\text{C}_{\text{DOC}}$ in the water sample from Sept 2007 has been excluded here and has been considered as an outlier that probably could be related to the malfunction of the plant.

The high amount of sulfate (average 240 mg/L) in the aquifer, as well as the absence or presence of only minor amounts of other possible electron acceptors, suggested that the sulfate reduction could be the dominant electron accepting process for anaerobic microbial processes in this aquifer (Routh *et al.*, 2001). Considering the concentration of sulfate and DOC from Mar 2008 to Dec 2008, there seemed to be a correlation between the concentrations of both compounds. Higher concentrations of sulfate in Mar 08 and Nov 08 were related to higher DOC, and lower concentrations of sulfate in May 08 and Dec 08 were also mirrored by lower DOC at both sampling dates. In addition, a small variability in the isotopic composition of DOC occurred. It could be assumed that these changes and correlations of different compounds were the result of seasonal influxes into the aquifer.

The dominant ether lipids are caldarchaeol (30.8 – 37.2 %) and crenarchaeol (21.9 – 25.4 %). Both were detected in the filter samples. These compounds are core membrane lipids of archaea, mainly of the kingdom Crenarchaeota (Gambacorta *et al.*, 1995; Schouten *et al.*, 2000; Sinninghe Damsté *et al.*, 2002), which is one of the two big phyla next to the Euryarchaeota. The Crenarchaeota are a diverse group of archaea which are found in terrestrial and marine habitats (Teske & Sørensen, 2008), and in extreme to non-extreme or moderate environments (DeLong & Pace, 2001). Branched GDGTs are first described in peat (Sinninghe Damsté *et al.*, 2000) and are now found to be ubiquitous and dominant in soils (Weijers *et al.*, 2006b) and are related to bacterial origin (Weijers *et al.*, 2007).

Studies on archaea in pristine groundwaters are rare in literature (Kotelnikova & Pedersen, 1997; Chapelle *et al.*, 2002; López-Archilla *et al.*, 2007) and do not deal with tetraether membrane lipids. These lipids will not serve as biomarkers for living archaea due to the fact that they are already partly degraded (loss of head groups). However, it can be assumed that living archaea are present and have been accumulated in the filter samples, since other

microbiological investigations show that archaea exist in groundwater environments and can even make up up to 90 % of the groundwater community in subsurface water as described in Chapelle *et al* (2002). Moreover, because it is known that archaea are capable of forming biofilms (Rinker & Kelly, 1996) the domain of archaea should receive more attention in aquifer systems with industrial applications.

The detection of intact phospholipids (PG, PE, and PC) in the filter samples indicated the occurrence of a viable bacterial community in the ATEs Rostock. The analytical results of the filter samples showed significant variations in the PL inventory regarding the fatty acid side chain composition as well as the PL head groups. Ambient temperature is an important environmental factor that influences the PL inventory of bacterial cell membranes (Russell & Fukunaga, 1990; Russell *et al.*, 1990; Beney & Gervais, 2001). Microorganisms adapt their membrane composition to the ambient temperature conditions to maintain cell membrane fluidity and functionality (Cullis *et al.*, 1996; Nedwell, 1999). Former studies showed that at lower temperatures the proportion of unsaturated and short chain FAs increases, preventing the solidification of the cell membranes (e.g. Hazel & Williams, 1990). Unsaturated and shorter chain FAs have lower melting temperatures leading to a lower solid-liquid phase transition temperature of the cell membrane (Sinensky, 1974; Kaneda, 1991; Suutari & Laakso, 1994) which is required to keep the membrane in the fluid stage when the ambient temperature decreases. The incorporation of a higher proportion of bulky and repulsive head groups can also lower the cell membrane solid-liquid phase transition temperature (Boggs, 1986).

In the current study the PLFA composition seemed to reflect temperature changes in the ATEs during the operating mode. During the EDM, the temperature dropped from 50°C to 20.9°C (sample Nov 08) and from 20°C to 13.7°C (sample Mar 08) during the LDM. Thus, the microbial biomass in the two filter samples represented different temperature conditions during operation. The PLFA composition in the filter sample from the LDM (lower ambient temperatures) showed a 13.4 % higher proportion of unsaturated fatty acids compared to the sample from the EDM (higher ambient temperature), indicating an adaptation of the microbial community to the lower temperature conditions during the LDM (Fig. 4.3, Tab. 4.4). This alteration was associated with the monounsaturated fatty acids with 16 and 18 carbon atoms. Moreover, there was a shift of 10.8 % towards fatty acids with shorter chain length in the

LDM sample which is an additional indication for membrane adaptation of the microbial community to cooler ambient temperature conditions.

In addition to the regulation of the cell membrane fluidity by the ratio of saturated and unsaturated FA and the chain length of the membrane PLFAs, other microorganisms are able to adjust their membrane composition to the ambient temperature conditions by incorporating different levels of *iso*- and *anteiso*-FA. As for the unsaturated FA, the solid liquid phase transition temperature (T_m) of *iso*- and *anteiso*-FA is lower than T_m of the corresponding saturated straight chain FA, where this effect is larger for the *anteiso*-congeners (e.g. PC(15:0/15:0): $T_m = 34.2^\circ\text{C}$; PC(*i*-15:0/*i*-15:0): $T_m = 6.5^\circ\text{C}$; and PC(*ai*-15:0/*ai*-15:0): $T_m = -16.5^\circ\text{C}$, Kaneda (1991)). In general, the proportions of *iso*- and *anteiso*-branched fatty acids were significantly lower in the LDM sample by 11.7 % and 7.7 %, respectively (Tab. 4.4). This might indicate that microorganisms regulating their cell membrane fluidity by *iso*- and *anteiso*-FA were less abundant in the LDM sample and this is in accordance with studies indicating that thermophilic bacteria are capable of membrane regulation using *iso*- and *anteiso*-FA (Kaneda, 1991).

Rilfors *et al.* (1978) reported that the relative proportion of *iso*-FA increases with an increasing growth temperature in *Bacillus megaterium* while the proportion of *anteiso*-FA decreases. Although the general amount of the *iso*- and *anteiso*-FA was different between the EDM and LDM sample, the ratio of these branched fatty acids indicates membrane adaptation during both operating intervals. In the LDM sample with lower average temperatures *anteiso*-FAs were more abundant relative to the *iso*-FAs, while in the EDM sample with higher average temperatures the *iso*-FAs showed a higher proportion. These trends are resembled in the *anteiso*- and *iso*-FA ratio in Table 4.4. Thus, for microorganisms regulating their cell membrane fluidity via these branched FAs, the ratio of *anteiso*- to *iso*-FA also indicated an adaptation to the ambient temperature conditions.

Additionally, the relative proportion of the mcb-C_{17:0} FA was significantly higher (by 11.8 %) in the LDM sample. To our knowledge there is no information on the T_m of this compound. However, due to the branching position it can be speculated that this compound might have an effect on the cell membrane fluidity that is similar to or even higher than the *anteiso*-FA. Therefore, its higher abundance in the LDM sample might also represent an adaptation to the lower ambient temperature in the ATES system during that time and in that operation mode.

The relative proportions of the PL head group inventory of the LDM (Mar 08) sample were quite similar to the composition in the CM sample (May 08), both samples representing cooler temperature conditions (Tab. 4.2). In contrast, the EDM sample (Nov 08), representing warmer ambient temperatures, showed a significantly different PL distribution. For temperature adaptation, the incorporation of more repulsive and bulky head groups (PG and PC) would be expected to occur at cooler ambient temperature to lower the cell membrane compaction (Boggs, 1986). However, the samples representing cooler conditions (CM and LDM) in the ATES were not characterized by higher proportions of these compounds (Tab. 4.2). In contrast, the EDM sample contained higher abundances of PGs and PCs. Thus, in the current study, temperature adaptation did not appear to be regulated via the head group inventory. However, the similar head group composition in the samples representing cooler conditions during CM and LDM might point to similarities in microbial communities.

The PL data showed that an active bacterial community dwelled in the Rostock ATES. The detailed investigation of the fatty acid inventory revealed an adaptation to changing temperature conditions in the system during different operating modes. This adaptation might include both an internal shift of the microbial species within the microbial community towards better-adapted microorganisms and a direct membrane adaptation of specific microorganisms.

This study provides the first biogeochemical insight into microbial processes in an ATES and improves the understanding of how microorganisms adapt to the specific conditions in geothermal applications. The presented results clearly indicate the existence of a viable and active bacterial community in the ATES Rostock. The microbial community shows a remarkable resistance. They are able to adapt to a temperature range of 10°C to 50°C as being defined by the operating mode of the ATES. With their capability to form distinct biofilms microorganisms might be able to cause disturbances in a geothermal plant with time or under specific circumstances favoring an intensified growth of the microbial communities.

4.7 Conclusion

A biogeochemical monitoring of a geothermally used groundwater system was conducted on fluid and filter samples from the aquifer thermal energy storage (ATES) in Rostock by evaluating the potential carbon and energy sources for microorganisms as well as the biomarker analysis (phospholipids and ether lipids) of the indigenous microbial community.

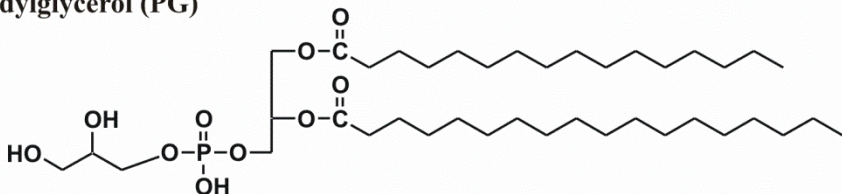
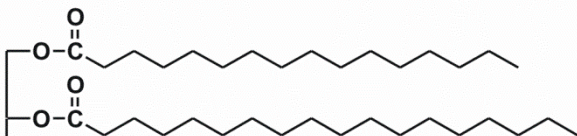
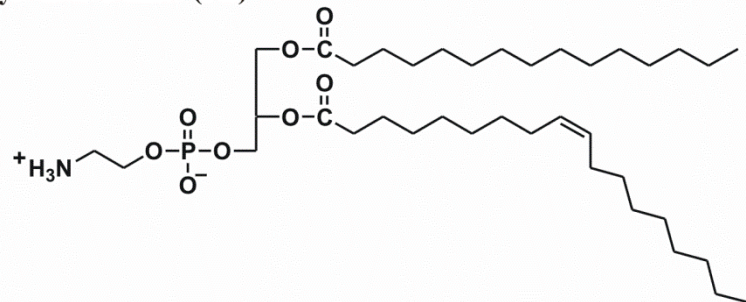
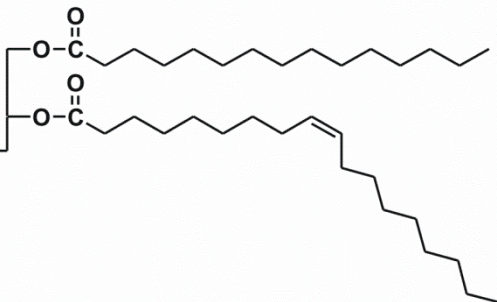
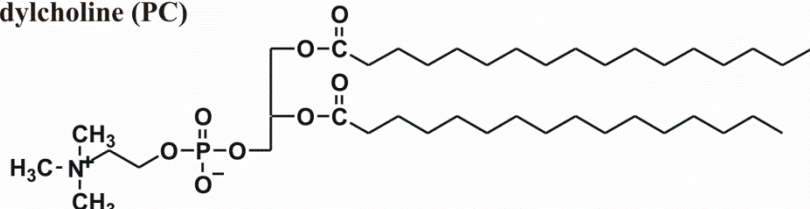
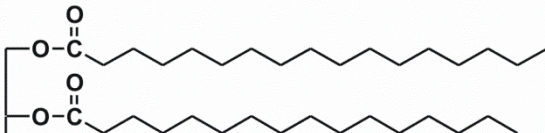
To sustain microbial life in this geothermally used aquifer, there were sufficient amounts of potential energy and carbon sources available in the ATEs. The variability in fluid geochemistry seemed to be more influenced by environmental conditions at the surface than by the operation mode (charge/discharge) of the geothermal plant. The influence of surface processes was indicated i.e. by the dominance of fulvic acids in DOC from groundwater. The variability in sulfate and DOC concentrations appeared to be independent of the operation mode.

The presence of living microorganisms in the ATEs in Rostock was indicated by the detection of intact phospholipids (PG, PE, PC). Moreover, differences in the composition of phospholipid fatty acids (PLFA) concerning chain length, degree of unsaturation, as well as *iso*- and *anteiso*-FA demonstrated the capability of the active bacterial community to adapt to temperature changes in the natural environment caused by the geothermal plant.

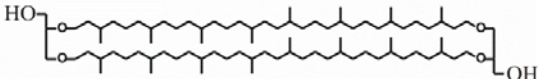
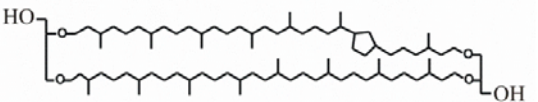
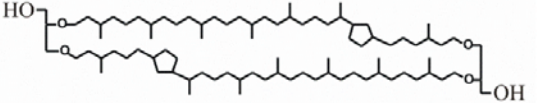
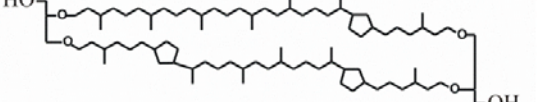
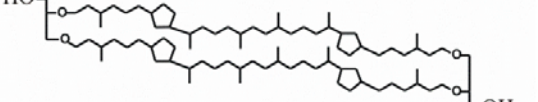
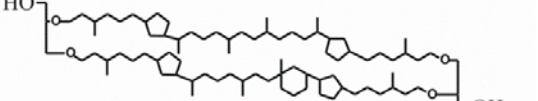
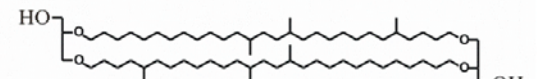
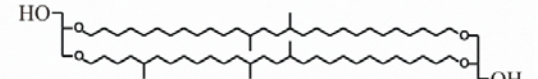
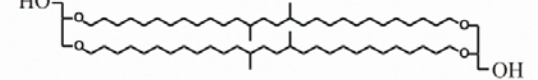
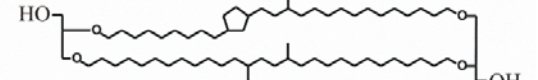
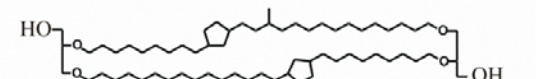
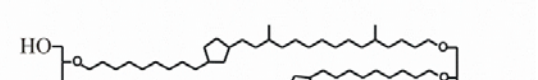
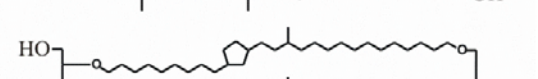
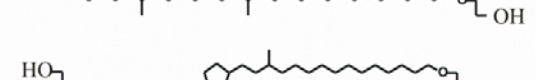
4.8 Acknowledgement

We are grateful to Kristin Günther, Bastian Kasek, Cornelia Karger, Anke Kaminsky and Ursula Kegel for their technical and analytical support. We would like to thank the persons in charge of the ATEs from WIRO (Wohnen in Rostock) for granting us permission to monitor the ATEs and to publish our results. This work was conducted as part of the research project “AquiScreen” which has been funded by the German Federal Ministry for Environment, Nature Conservation and Nuclear Safety (BMU, grant no. 0327634). The responsibility for the content of this work is carried by the authors of this publication. Furthermore, special thanks are given to Prof. Dr. André Lipsky and Frank Remmler for their highly constructive reviews of this article.

4.9 Appendix A

hydrophilic head		hydrophobic tail		e.g.	m/z
polar head group	glycerol	fatty acid side chain			
Phosphatidylglycerol (PG)					
				<i>n</i> -C _{16:0} <i>n</i> -C _{18:0}	749
Phosphatidylethanolamine (PE)					
				<i>n</i> -C _{15:0} <i>n</i> -C _{18:1}	702
Phosphatidylcholine (PC)					
				<i>n</i> -C _{17:0} <i>n</i> -C _{16:0}	806

4.10 Appendix B

Compound	m/z	Structure
Caldarchaeol	1302	
I	1300	
II	1298	
III	1296	
IV	1294	
Crenarchaeol	1292	
V	1050	
VI	1036	
VII	1022	
VIII	1020	
IX	1018	
X	1046	
XI	1034	
XII	1032	

5 Variations in Fluid Chemistry and Membrane Phospholipid Fatty Acid Composition of the Bacterial Community in a Cold Storage Groundwater System during Clogging Events

This chapter was published in:

Alexandra Vetter, Kai Mangelsdorf, Georg Schettler, Markus Wolfgramm, Kerstin Rauppach, Andrea Vieth-Hillebrand (2012); Variations in fluid chemistry and membrane phospholipid fatty acid composition of the bacterial community in a cold storage groundwater system during clogging events; *Applied Geochemistry* 27 (6), 1278-1290

5.1 Abstract

In order to monitor the operating mode of the cold storage of the German Parliament (North German Basin) the fluid chemistry and the phospholipid fatty acid (PLFA) composition of the indigenous microbial community have been monitored from August 2006 to August 2009. During this time two periods of reduced injection (clogging events) characterized by Fe precipitates and microbial biofilms in filters occurred in the injection wells impairing the operating state of the investigated cold storage. The fluid monitoring revealed the presence of sufficient amounts of potential C and energy sources (e.g. DOC and SO_4^{2-}) in the process water to sustain microbial life in the cold storage. In times of reduced injection the PLFA inventory of the microbial community differs significantly from times of normal operating phases indicating compositional changes in the indigenous microbial ecosystem. The most affected fatty acids (FAs) are 16:1 ω 7c (increase), 16:1 ω 7t (decrease) and 18:1 ω 7c (increase), interpreted to originate mainly from Fe and S oxidizers, as well as branched FA with 15, 16 and 17 C atoms (decrease) most likely representing sulfate-reducing bacteria (SRB). Based on this variability, PLFA ratios have been created to reflect the increasing dominance of biofilm forming S and Fe oxidizers during the disturbance periods. These ratios are potential diagnostic tools to assess the microbiological contribution to the clogging events and to find appropriate counteractive measures (e.g. mechanical cleaning vs disinfection). The correlation between changes in the PLFA composition and the operational state suggests that microbially

mediated processes play a significant role in the observed clogging events in the investigated cold storage.

5.2 Introduction

In recent years, Aquifer Thermal Energy Storage (ATES) systems have become increasingly important for cooling and heating of buildings by storing natural cold or surplus heat in subsurface formations. For a reliable operation of an ATES, it is important to understand the impact of the geothermal plant on the groundwater system. Problems in the technical plant can occur in terms of corrosion, scaling, and clogging all reducing the geothermal utilizability of the aquifer and the operational reliability of the geothermal plant.

Clogging processes can be classified into three categories: physical, chemical and biological (Baveye *et al.*, 1998). The physical clogging progression is mainly caused by suspended solids leading to the accumulation of the suspended solids in the downhole plant tubes and the formation of a filter cake as well as the blocking of pore spaces in the aquifer (Baveye *et al.*, 1998).

The chemical clogging process is linked to chemical parameters in the respective aquifers such as electrolyte concentration, fraction of organic compounds in the aqueous phase, pH, Eh as well as the mineralogical composition of the solid phase, its surface characteristics, and the chemical reactions (precipitation/dissolution) which can lead to clay-sized particles lodging in the pores (Baveye *et al.*, 1998). Chemical plugging is related to scaling formed by precipitation of e.g. gypsum, carbonates or hydroxides (van Beek, 1989). Worldwide, the most often occurring chemical clogging events in water supply systems are induced by the precipitation and deposition of Fe-oxyhydroxides (van Beek, 1989). Abiotic Fe precipitation in wells can be caused by the contact of anoxic groundwater with atmospheric O₂ as e.g. realized by changing water tables (Van Beek *et al.*, 2009) or by mixing with O₂-containing groundwater.

The biologically mediated clogging is caused by the activity of microorganisms in the aquifer or plant (Ralph & Stevenson, 1995; Potekhina *et al.*, 1999; Rinck-Pfeiffer *et al.*, 2000; Inagaki *et al.*, 2003; Coetser & Cloete, 2005). The clogging material is often found to be slimy layers (Smith & Tuovinen, 1985). These biofilms consisting of bacterial populations surrounded by a thick film of microbially derived extracellular polymeric substances EPS are located at or outside the cell surface (Costerton *et al.*, 1995; Laspidou & Rittmann, 2002). They are highly hydrated and form a matrix keeping the cells together and retaining water (Flemming *et al.*,

2007). The biofilm EPS are composed of polysaccharides, proteins, and nucleic acids as well as microbially produced organic substances or the residue of dead cells (Stoodley *et al.*, 2002). The EPS can trap, bind and accumulate organic material as well as capture suspended solids and inorganic precipitates (Laspidou & Rittmann, 2002; Stoodley *et al.*, 2002). Sogaard *et al.* (2001) showed that rates of biotic Fe oxidation can be 1000 times faster than for abiotic Fe oxidation and the involved biofilm and its EPS serve as a catalyst for the oxidation/precipitation process as well as preventing re-dissolution of the Fe(III) precipitates (Sogaard *et al.*, 2000).

Furthermore biofouling and biocorrosion of construction materials within the plant caused by microbial biofilms can lead to problems in industrial process water and potable water (Sand, 2003; Ungemach, 2003; Coetser & Cloete, 2005). These phenomena can also cause severe disturbances in the technical equipment of geothermal plants such as pipes, pumps, screens, and heat exchangers leading to substantial operating expense. Additionally, the lifetime of geothermal systems is often limited not only by the wearout of the technical equipment but also by the formation and deposition of scale in the well and in the vicinity of the wells (clogging) (van Beek & van der Kooij, 1982; van Beek, 1989; Ralph & Stevenson, 1995).

Sometimes chemical, physical or biological clogging processes can also occur simultaneously. In these cases their interaction makes it difficult to determine which process has been the initial mechanism and which is the most prevalent.

To date, little is known about the biogeochemical interactions of microorganisms within a geothermal plant and the impact of seasonal changes during different operating modes of the plant on the indigenous microbial community. In the current study, biogeochemical monitoring of the fluid chemistry and bacterial community was conducted for the cold storage of the German Parliament buildings (Reichstag ATES) in Berlin from August 2006 to August 2009. During this time, two periods of reduced injection were observed in the plant. The aim of the study was to investigate the changes in the fluid chemistry and bacterial community with time, especially, related to these events. While microbiological approaches were covered by partners in the project (Lerm *et al.*, 2011b), the current biogeochemical approach aims to monitor changes in the bacterial communities using characteristic microbial lipid markers such as phospholipids. These biomarkers, forming a major part of the bacterial cell membranes, are regarded to be indicators for living bacterial communities due to their rapid degradation after cell death (White *et al.*, 1979; Harvey *et al.*, 1986). An advantage of this

method is that the phospholipid signal represents the whole indigenous bacterial community and, therefore, covers the compositional changes of the whole bacterial population during the monitoring. For the fluid analysis the focus was placed on selected fluid components being potential electron acceptors (SO_4^{2-}) and donors (dissolved organic C, DOC) for the indigenous microbial community in the ATES.

5.3 Study Site and Operating Modes of the Reichstag ATES

The energy supply system of the German Parliament buildings in Berlin, one of the first ATES projects in Germany (Poppei *et al.*, 1998), exhibits two independent energy storage systems supplying cold and heat to the Reichstag building and surrounding offices (Kabus & Seibt, 2000; Sanner, 2001). Both storage systems were established in 1999. In the current study only the seasonal cold storage was investigated.

The freshwater aquifer that is used for the cold storage of the German Parliament buildings is located in the inner city of Berlin, close to the Spree River at 30 to 60 m depth (Sanner, 2001) (Fig. 5.1). The study area is part of the ‘Warschau-Berliner’ glacial valley and its morphology was shaped during the last glaciation (Weichselian, Quaternary). The aquifer consists of melt water sands, gravel, and boulder clay intercalated as lenses (Knoblich *et al.*, 1994; Sanner, 1994; Kabus & Seibt, 2000). In the northern part of the study area (warm side), the groundwater is covered by a layer of till, while this layer is absent in the southern part (cold side). The Oligocene Rupelian clay is in 120 m depth (Knoblich *et al.*, 1994) and protects these groundwater reservoirs against blending with highly saline fluids occurring in deeper stratigraphic units of the North German Basin. The flow gradient of the groundwater is extremely low and the flow path is directed northwards to the river Spree (Wolfgramm *et al.*, 2010).

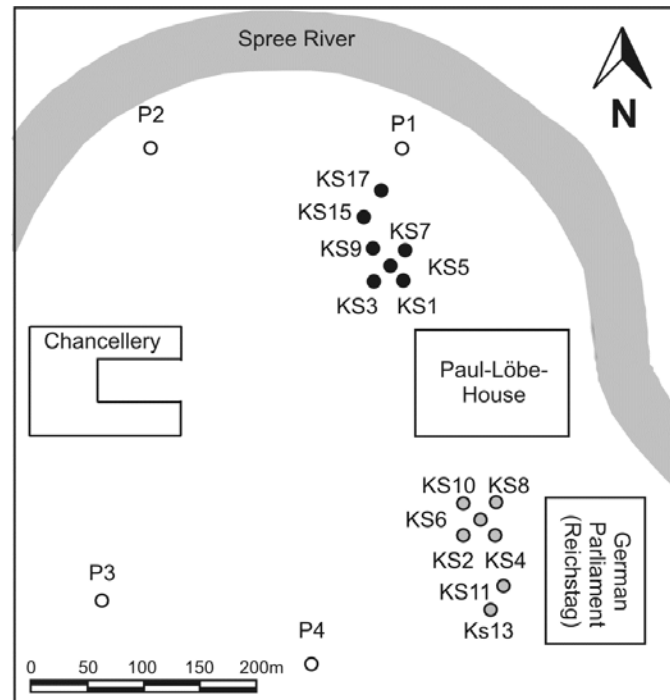


Fig 5.1: Locations of wells used for the cold storage in the German Parliament area. Gray circles represent the cold and black circles the warm wells of the system. The open circles indicate the positions of the observation wells of the shallow aquifer.

The groundwater system investigated has a natural temperature of 12°C. During winter time the groundwater from the warm side with temperatures between 22 and 14°C is pumped up, cooled down by an air cooler and subsequently re-injected with a temperature of around 5°C to the cold side, hereafter called charge mode (charge of cold; Fig. 5.2a). In summer, the groundwater from the cold side with temperatures between 6 to 10°C is discharged via a heat exchanger, hereafter called discharge mode (discharge of cold; Fig. 5.2b). The cold water is used to cool down the air conditioning system of the parliament buildings. Subsequently, the water is re-injected to the warm side with temperatures between 15 and 30°C depending on the cooling demand of the parliament buildings. Before passing the heat exchanger, the fluid flows through polypropylene filters where particles exceeding 25 µm are retained. Based on the storage design data, 60 % of the cold demand of the Parliament buildings in summer is covered by the ATES (Kabus & Seibt, 2000; Sanner *et al.*, 2005).

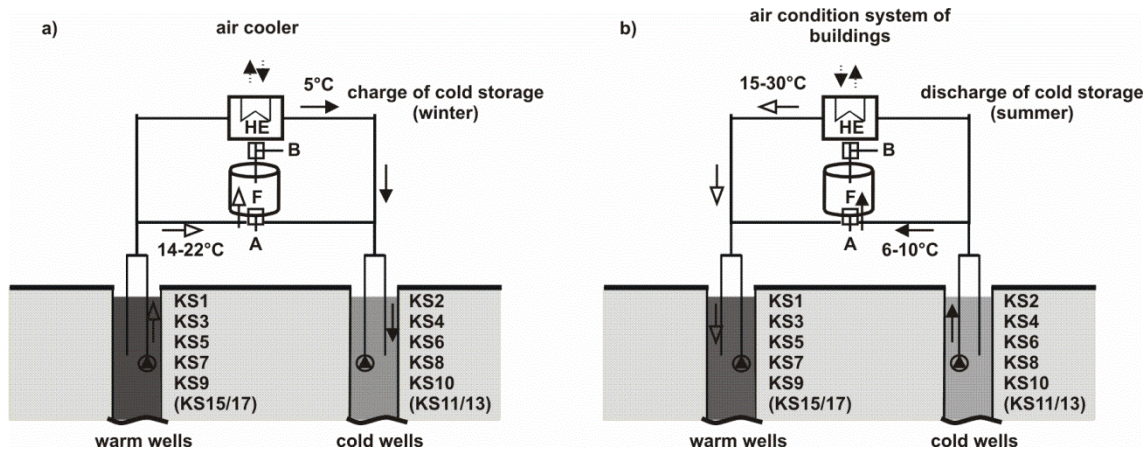


Fig. 5.2: Simplified scheme of the operating modes of the Reichstag cold storage. (a) In winter water is pumped up from the warm side, cooled in a heat exchanger (HE) with water coming from air coolers, and re-injected into the cold side of the aquifer (charge mode). During the charge mode, the average water temperature in the cold storage decreases to 6–10°C. (b) In summer water is pumped from the cold side via an HE to the warm side (discharge mode). The cold water is used to cool down the air of the parliament buildings. The water is re-injected with temperatures between 15 and 30°C. F = particle filter; A and B = tapping points before and after the particle filters.

The cold storage system comprises five groundwater wells for the cold side (KS2, KS4, KS6, KS8, KS10) and five wells for the warm side (KS1, KS3, KS5, KS7, KS9) where the water is pumped up and re-injected in alternate flow direction depending on the season (Sanner, 2001). The production rate for each well is about 60 m³/h (Kabus & Seibt, 2000). Thus, a maximum production rate of 300 m³/h is given for the entire system. Each storage side has two additional wells (cold side: KS11, KS13; warm side: KS15, KS17) serving as backup wells. The pipes of the cold storage are manufactured from synthetic material (PE100) and are under pressure (1.4 – 1.9 bar) at all times. To keep the plant free of O₂ and to avoid abiotic Fe clogging, the system is charged with N₂ (Sanner *et al.*, 2005). Four observation wells (P1, P2, P3, and P4) are installed around the cold storage (Fig. 5.1) to monitor the temperature regime and the groundwater table during the ATES operation.

5.4 Clogging Events in the Cold Storage

During discharge mode in summer 2006 (August to October) and 2008 (July to October) an increased injection pressure occurred on the warm side caused by clogging events. The first clogging event (summer 2006) is not well documented. The well slides were covered with Fe hydroxide crusts. Regeneration of the wells on the warm side was conducted in May and June 2007. A further decrease in the injection rate occurred in July 2008. Until October the

decrease in the injection rate developed to be a serious issue. The inner surface of the injection well was covered with Fe hydroxide precipitates whereas the riser pipe was free of any precipitation (Wolfgramm *et al.*, 2010).

In summer 2008, 85 % of solid particles in the filters were fine grained Fe hydroxides with a particle size of 5 µm (in normal operating phases this is <40 %), larger Fe hydroxide crusts were rarely detected (Wolfgramm *et al.*, 2010). During this time the capacity of filters to retain these particles before being clogged was reduced by a factor of 10, having major effects on the lifetime of the filters. Scanning electron microscope (SEM) analysis showed frequent round Fe hydroxide particles covered by thin filamentous structures (Wolfgramm *et al.*, 2010). These structures are interpreted as deriving from S-oxidizing bacteria of the genus *Thiothrix*. *Thiothrix* is suggested to be mainly responsible for the reduced filter lifetimes during the 2008 disturbance period (Lerm *et al.*, 2011b).

Single strand conformation polymorphism (SSCP) fingerprinting analysis based on 16S rRNA of filter material sampled in March 2006 revealed in addition to *Thiothrix* the occurrence of Fe-oxidizing bacteria of the genus *Gallionella* in the ATES system (Lerm *et al.*, 2011b). Lerm *et al.* (2011b) suggested that *Gallionella* might be involved in the formation of Fe hydroxides in the plant. SEM analyses also revealed the sporadic occurrence of *Gallionella* on the filter material during the disturbance phase in 2008. Thus, *Thiothrix* and/or *Gallionella* are both capable of forming biofilms (Hallbeck & Pedersen, 1995; Brigmon *et al.*, 1997) might play an essential role in the observed clogging events in 2006 and 2008. In November 2008, disinfection with H₂O₂ was conducted in the cold storage and since that time the plant has worked efficiently and reliably.

5.5 Material and Methods

5.5.1 Fluid and Filter Sampling

Between May 2007 and April 2009 the fluid chemistry in the storage system of the ATES system was monitored. All samples were taken from the Paul-Löbe-House (Fig. 5.1). Initially, fluid samples were only occasionally taken during charge and discharge operating modes between May 2007 and March 2008. Since May 2008 fluid samples have been sampled monthly during the different operating modes. In total 15 fluid samples were taken all from the tapping point prior to the filter unit tapping point A in (Fig. 5.2). The process water of the cold storage is taken at the tapping point A (Fig. 5.2) and represents a mixture of water of

between two to five wells depending on the cooling demand of the connected buildings with a maximum flow rate of 300 m³/h from either the warm side (charge mode) or the cold side (discharge mode).

During the monitoring period it was also possible to take fluid samples from selected wells of the warm and cold side as well as from the observation wells (Fig. 5.1). Most of the well samples were taken once during the discharging periods in May and June 2007. *In-situ* samples from wells of the cold side (KS2, KS4, KS6, KS8, and KS10) were taken from the bottom of the wells. Fluid samples of wells from the warm side (KS3, KS5, and KS7) were taken after passing the plant before re-injection into the cold side. *In-situ* fluids from the two warm wells KS1 and KS9 were sampled from the bottom of these wells in November 2008 during charge mode. Fluid samples from the observation wells P1 - P4 were recovered during the discharge mode in June 2008. These four groundwater samples were obtained from a depth range between 30 and 60 m below surface by pumping. A minimum of two well volumes of water was pumped before sampling. The fluids were collected in sterile glass bottles, stored on freezer packs and analyzed within 24 h.

Additionally, 13 filter samples were taken during different operating modes between August 2006 and August 2009 (Fig. 5.6). The filters were taken directly from the filter system prior to the heat exchanger (Fig. 5.2). Filters were wrapped in pre-heated and -cleaned Al foil and analyzed immediately in the home laboratory or stored at -80°C in the freezer until analysis. Since obtaining filters depending on filter lifetimes and filter material and because the filter samples were distributed between the different project partners, it was not possible to have replicate filter samples for a specific sampling time. Furthermore, to avoid inhomogeneities and due to the limited amount of sediment in the filters filter pieces were extracted, preventing analytical replicates from the allocated filter material. On the other hand, several different samples were taken during periods of normal and disturbed operation which can be regarded as replicates over time.

5.5.2 Analysis of Anions in Fluid Samples

For the determination of the concentration of inorganic and organic anions all fluid samples were analyzed using ion chromatography (IC, ICS 3000, Dionex Corp.) equipped with a conductivity detector, KOH eluent generator and an ASRS Ultra II 2 mm suppressor. For separation of the anions an analytical column (AS11HC; 2x 250 mm, Dionex Corp.) was used

at a constant temperature of 35°C. Further details of the method applied have been described in Vieth et al. (2008). Please note that the detection limit was improved from 0.5 mg/L in 2006/2007 to 0.1 mg/L in 2008 (e.g. see Table 5.1). The standard deviation was below 5 %. The determined data are an average of at least three replicate measurements. The standard deviation for Cl^- and SO_4^{2-} was below 4 %.

5.5.3 Characterization and Quantification of Dissolved Organic Carbon (DOC)

The characterization and quantification of the DOC and its fractions were conducted by size-exclusion-chromatography with subsequent ultra violet (UV) ($\lambda = 254 \text{ nm}$) and infra-red (IR) detection by a liquid chromatography-organic carbon detection (LC-OCD) device (Huber & Frimmel, 1996). Phosphate buffer (pH 6.85; 2.5 g KH_2PO_4 , 1.5 g Na_2HPO_4) was used as mobile phase with a flow of 1.1 mL/min (Huber *et al.*, 2011). The sample passed a 0.45 μm membrane syringe filter before entering the chromatographic column (250 mm x 20 mm, TSK HW 50S, 3000 theoretical plates, Toso, Japan). After chromatographic separation into individual fractions (biopolymers, humic substances, building blocks, hydrophobic organic C, and different groups of low molecular weight compounds), these fractions were characterized by UV detection. Quantification of DOC fractions by IR-detection of released CO_2 is possible after UV photooxidation ($\lambda = 185 \text{ nm}$) in a *Gräntzel* thin-film reactor. The ratio between the spectral absorption coefficient (SAC in m^{-1} at 254 nm) and the DOC (in mg C/L) were calculated as an indicator for the proportion of aromatic structures in the humic substances. For molecular weight calibration humic and fulvic acid standards of the Suwannee River (IHSS, 2011) have been used.

For the quantification of the DOC the fluid passes a 0.45 μm membrane syringe filter and was measured as non-purgeable organic C (NPOC) after acidification with HCl. The acidified sample was purged with CO_2 -free high purity air for 5 min. The residual DOC was transformed by catalytic oxidation into CO_2 which was measured by IR spectroscopy at 680°C (TOC-2000A, Shimadzu). Quantification of DOC was done by external calibration using potassium hydrogen phthalate. The determined data are an average of at least three replicate measurements. The standard deviation for DOC was below 1 %.

5.5.4 Stable Carbon Isotope Analysis of Fluid Samples

Bulk C isotope analysis of the DOC was conducted by continuous-flow analysis coupled to an isotope ratio mass spectrometer (Isolink with Delta V Advantage, Thermo Fisher Scientific). Details of this instrument and bulk isotope analysis have been described by Krummen et al. (2004). To remove particulate and inorganic C in the samples, they were filtered (0.45 μm), amended with concentrated H_3PO_4 , shaken and placed in the fumehood over night. For the preparation of the reagents and the mobile phase, deionized water (Simplicity 185, Millipore) was used that had been treated by UV photooxidation to remove organic compounds. To obtain CO_2 -free mobile phase and reagents, the solutions were degassed under vacuum in an ultrasonic bath (30 min at 40°C) and purged with a constant flow of He during operation. The C isotope composition of the DOC is reported in δ -notation (‰) relative to the Vienna Pee Dee Belemnite Standard (V-PDB). The standard deviation for this method is $\leq 0.5\text{‰}$.

5.5.5 Phospholipid Analysis of Filter Samples

The freeze-dried sediment from the filters was extracted with a modified Bligh and Dyer (1959) method. However, 10 of 13 filter samples contained only low amount of sediment being finely dispersed on the filter matrix. Therefore, pieces of the respective filter visually containing adequate amount of sample material were taken and extracted twice for 20 min by ultrasonication using a solvent mixture of methanol-dichloromethane (DCM)-ammonium acetate buffer with a ratio of 2:1:0.8 (v/v). Additionally, clean filters were extracted as blanks. Phospholipids were not detected in the blanks.

In a second step the solvent ratio of the extract was changed to 1:1:0.9 by adding DCM and ammonium acetate buffer resulting in a phase separation of an organic and an aqueous phase. Subsequently, the aqueous phase was re-extracted three times with 20 mL DCM. The combined organic phases were concentrated using a Turbo Vap system (Zymark). Afterwards, the extract was chromatographically separated into fractions of different polarity resulting in a phospholipid (PL) fraction as already described in Zink and Mangelsdorf (2004). Half of each PL fraction was analyzed for intact phospholipids (PLs) using a liquid chromatography mass spectrometry system (LC-MS). The determined PL data are an average of at least three replicate measurements. The second aliquot of each PL fraction was used for saponification to liberate the phospholipid fatty acids (PLFA) which were subsequently measured by gas

chromatography-mass spectrometry (GC-MS). Details of the methods and instrument parameters are described in Vetter et al (2011).

Due to the fact that the filters did not have the same lifetimes, that there were alternatively used parallel filter systems and that the volume flow was variable, each filter sample had been passed by different and unknown volumes of water. This prevented an absolute quantification of PL data. Therefore, only the relative proportions of PL and PLFA data within a sample were quantitatively assessed by normalizing the total PLs or PLFAs to 100 %. In doing so, changes in the relative percentage proportions of PLs or PLFAs can be compared between the samples from different times and operating periods. Although filter life times were different, in all cases the filter life times were long enough to obtain a representative sample of an *in-situ* enrichment of particles and associated microorganisms during a respective operation phase.

5.6 Results and Discussion

5.6.1 Potential Carbon and Energy Sources for the Microbial Community in the Fluids from the ATEs System

Groundwater samples from the individual wells of the cold and warm side (Table 5.1) showed variations in anion concentrations. Sulfate content from the warm wells (on average 205 mg/L) was lower compared to the SO_4^{2-} content of the cold side (on average 249 mg/L) and low molecular weight organic acids such as formate and acetate only showed a peak in the IC chromatograms on the cold side. However, the signal was too small for quantification (<0.5 mg/L). These results could be an indication of a higher microbial turnover rate on the warm side, with temperatures ranging from 15 to 30°C, compared to the cold side with temperatures of 6 to 10°C. Nevertheless, DOC concentrations are quite similar for both sides although $\delta^{13}\text{C}$ of DOC showed a broader range in the isotope signal for the wells of the cold side (-26.8 to -28.3‰).

Table 5.1: Selected hydrochemical data for cold, warm and observation wells of the ATES.

	Chloride (mg/L)	Sulfate (mg/L)	Nitrate (mg/L)	DOC (mg/L)	$\delta^{13}\text{C}$ (‰)	Formate (mg/L)	Acetate (mg/L)	Operating mode
cold side								
KS 2	58	274	-	4.2	-27.2	<0.5	<0.5	discharge
KS 4	56	248	-	3.9	-26.8	<0.5	<0.5	discharge
KS 6	57	243	1.6	3.8	-26.9	<0.5	<0.5	discharge
KS 8	62	214	-	3.7	-28.3	-	-	discharge
KS 10	58	265	1.0	3.5	-28.3	<0.5	<0.5	discharge
on average	58	249	1.3	3.8	-27.5	<0.5	<0.5	
warm side								
KS 1	64	197	0.2	4.3	-27.8	-	-	charge
KS 3	62	215	-	3.9	-27.3	-	-	discharge
KS 5	61	206	1.6	4.0	-27.3	-	-	discharge
KS 7	62	210	<0.5	3.6	-27.2	-	-	discharge
KS 9	60	197	0.2	4.3	-27.0	-	-	charge
on average	62	205	0.7	4.0	-27.3	-	-	
observation wells								
P 1	57	194	<0.1	4.3	-27.3	<0.1	<0.1	discharge
P 2	48	138	<0.1	4.9	-27.4	<0.1	<0.1	discharge
P 3	57	164	<0.1	4.2	-27.4	<0.1	<0.1	discharge
P 4	54	138	1.3	5.4	-27.0	<0.1	<0.1	discharge
on average	54	159	0.4	4.7	-27.3	<0.1	<0.1	

Fluids from the observation wells (Table 5.1) showed concentrations of inorganic anions ranging between 48 and 57 mg/L Cl^- and 138-194 mg/L SO_4^{2-} . These concentrations from P1 to P4 are lower compared to the contents of the temperature-influenced wells (cold/warm side) of the cold storage. Nitrate was below detection limit except in water from well P4. Formate and acetate were below detection limit (<0.1 mg/L) and DOC varied between 4.2 and 5.4 mg C/L. The C isotope composition of DOC ranged between -27.0‰ and -27.4‰.

Fluid sampling monitoring for the cold storage was conducted from May 2007 to April 2009. Average characteristics of the fluids are listed in Table 5.2.

Table 5.2: Average characteristic parameters of the fluid within the ATES; n.m. = not measured, b.d. = below detection limit.

Parameter	Average value	Reference
pH	7.4	Pers. comm. A.Seibt
Eh (mV) SHE	40-80	Pers. comm. A.Seibt
conductivity ($\mu\text{S}/\text{cm}$)	930	(Opel <i>et al.</i> , 2008)
Ca^{2+} (mg/L)	137	(Opel <i>et al.</i> , 2008)
Na^+ (mg/L)	38	(Opel <i>et al.</i> , 2008)
Mg^{2+} (mg/L)	12	(Opel <i>et al.</i> , 2008)
K^+ (mg/L)	11	(Opel <i>et al.</i> , 2008)
NH_4^+ (mg/L)	1	(Opel <i>et al.</i> , 2008)
Fe total (mg/L)	2	(Opel <i>et al.</i> , 2008)
HCO_3^- (mg/L)	247	Pers. comm. A.Seibt
SO_4^{2-} (mg/L)	196	this study
H_2S (mg/L)	n.m.	Frequently detected by odor
Cl^- (mg/L)	58	this study
PO_4^{3-} ($\mu\text{g}/\text{L}$)	52	(Opel <i>et al.</i> , 2008)
NO_3^- (mg/L)	b.d.	(Opel <i>et al.</i> , 2008)
O_2 (mg/L)	<0.1	Pers. comm. A.Seibt
CO_2 (mg/L)	24	Pers. comm. A.Seibt
DOC (mg/L)	4	this study
$\delta^{13}\text{C}$ (‰)	-27.1	this study

Since the beginning of the monitoring, SO_4^{2-} concentration (Fig. 5.3) have varied around an average value of 194 mg/L (discharge mode) or 200 mg/L (charge mode) with a minimum of 164 (November 2008) and a maximum of 221 mg/L (May 2007). The SO_4^{2-} concentration was relatively constant from May 2008 to August 2008 and from December 2008 to April 2009. However, two significant deviations have been detected from the general trend of the SO_4^{2-} concentration profile. From August to September 2008 SO_4^{2-} decreased by 32 mg/L and after a small increase in October 2008 a second decrease of 21 mg/L was observed in November 2008. These deviations occurred at the end phase of the period of reduced injection from summer to fall 2008.

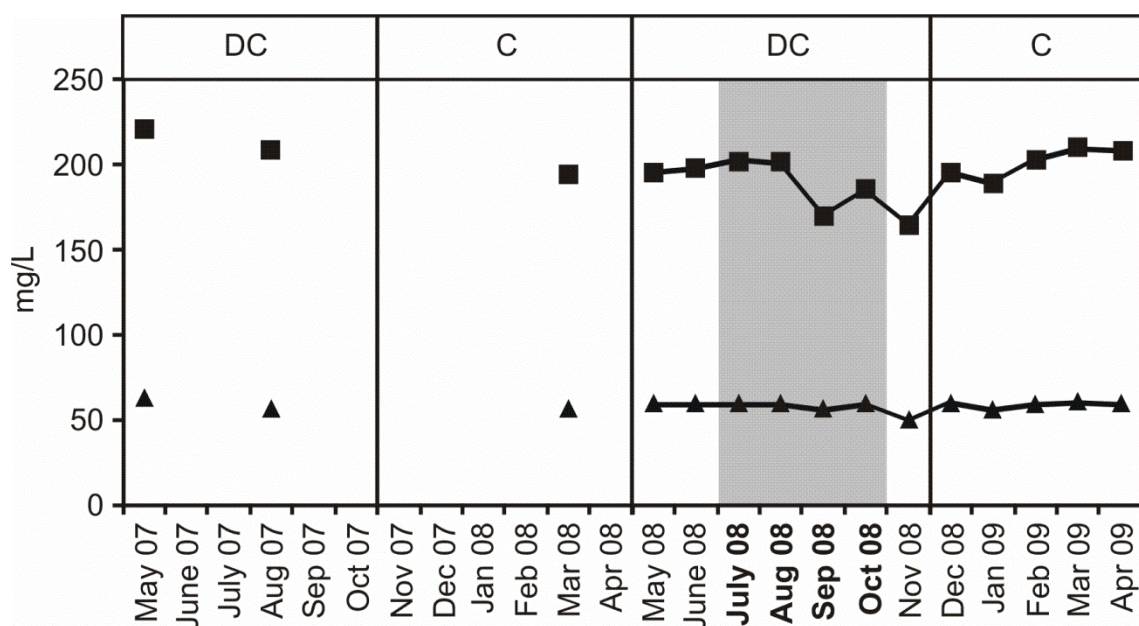


Fig. 5.3: Sulfate (squares) and chloride (triangles) concentrations measured during the fluid monitoring of the Reichstag cold storage (standard deviation below 4 %). The gray area marks a period of reduced injection. DC = discharge mode, C = charge mode (see Fig. 5.2).

The lower SO_4^{2-} concentrations might be related to a higher activity of SRB during this time. The concentration of Cl^- , regarded as an inert constituent, was quite constant during the monitoring (average 58 mg/L). However, the Cl^- concentration also showed a small decrease of 9 mg/L (Fig. 5.3) in November 2008 indicating that the SO_4^{2-} decrease during this time might be a dilution artefact, for instance by intensive pumping and disinfection with H_2O_2 . Overall, potential electron acceptors (such as SO_4^{2-} and in smaller amounts NO_3^-) for microbial processes are present in the process water of the ATES.

The DOC content of the fluid (Fig. 5.4) ranged between 3.6 (January 2009) and 6.2 mg C/L (August 2008). The average concentration was 4.7 mg C/L during the discharge phase and 3.8 mg C/L during the charge phase. From May 2007 to April 2008 in $\frac{2}{3}$ of the samples DOC concentrations show values near 4 mg C/L.

At the beginning of the monthly monitoring interval (May 2008 to April 2009) the DOC concentration was slightly higher (4.4 mg C/L) and increased to 6.2 mg C/L in August 2008. Subsequently, the DOC values showed a significant decrease back to about 4 mg C/L and remained more or less constant for the rest of the monitoring. The significant decrease of DOC from August to September 2008 might be interpreted as an enhanced consumption of DOC by microorganisms during the time of plant disturbance. A change in the $\delta^{13}\text{C}$ value due

to enhanced microbial consumption would suggest that the remaining DOC becomes isotopically heavier, because microorganisms would initially prefer to metabolize substrates with the lighter ^{12}C .

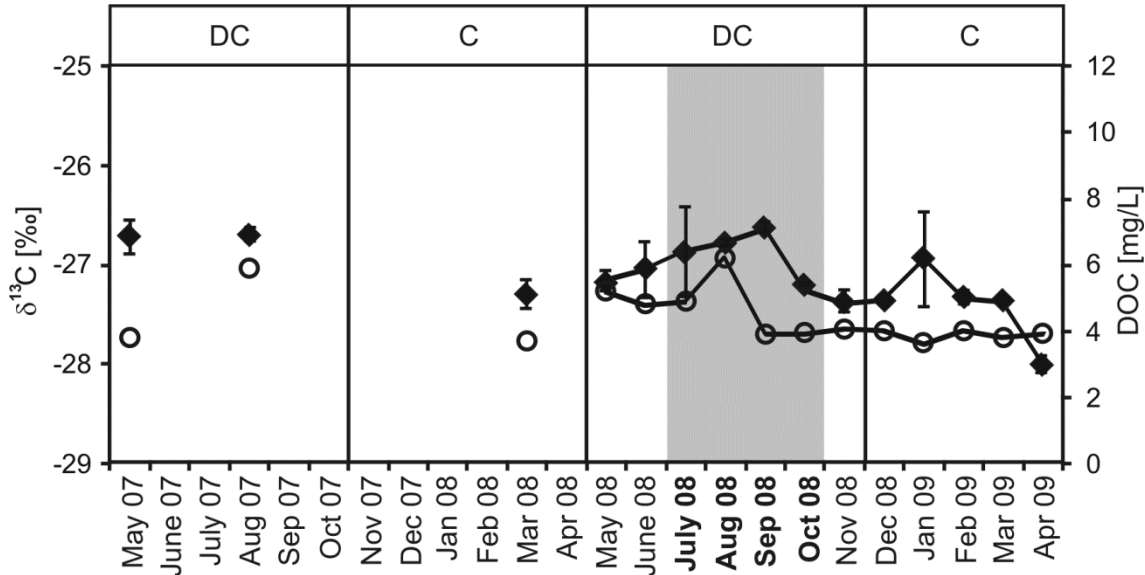


Fig. 5.4: Concentration (open circles; standard deviation below 1 %) and carbon isotope composition of DOC (diamonds) during the monitoring period. The gray area indicates period of reduced injection. DC = discharge mode, C = charge mode see (Fig. 5.2).

This seems to be the case initially (August 2008), but one month later (September to October 2008) a shift in the C isotope composition of DOC to lighter values was observed shift of (0.6‰). This decrease is puzzling, but might reflect an increase in the production of microbial biofilm (EPS) contributing to the biopolymer fraction of the DOC. From October 2008 the $\delta^{13}\text{C}$ value of DOC remains constant even when the system shifted back to the normal operating mode (two exceptions in January and April 2009). Therefore, it is difficult to answer, whether the DOC profile in any way resembles the variability of the microbial community in the ATES system. The variations in DOC during the time of reduced injection might also derive from changes in the DOC source or influx into the aquifer as a result of different pumping processes during this period.

Four fluid samples were investigated for their DOC composition using LC-OCD analysis. Three of these samples were taken during the monitoring period and one prior to the monitoring in January 2006 (Table 5.3). The determined DOC composition showed a clear dominance of humic substances (HS) accounting for 55 to 69 % of total DOC. Based on HS aromaticity and HS molecular weight (on average 3.5 L/mg m and 649 g/mol respectively) the

origin of HS was pedogenic and derived from fulvic acids (Chin *et al.*, 1994; Huber & Frimmel, 1996; Huber *et al.*, 2011). HS directly influence microbial processes by acting as a C and energy source (Jones, 1992; Lovley *et al.*, 1996; Steinberg & Bach, 1996) and by their metal and nutrient binding abilities (De Haan, 1992).

Table 5.3: Concentration, carbon isotope composition ($\delta^{13}\text{C}$), and size fractions of DOC from selected fluid samples taken from the plant during charge and discharge modes; HS = humic substances; BB = building blocks; NC = neutral compounds; HOC = hydrophobic organic carbon; LMWOA = low molecular weight organic acids; Bio = biopolymers.

	DOC (mg/L)	$\delta^{13}\text{C}$ of DOC (‰)	HS (%)	BB (%)	NC (%)	HOC (%)	LMWOA (%)	Bio (%)	Operating mode
Jan 06	4.0	-	55	21	11	6	1.4	0.1	charge
Sep 08	3.9	-26.6	66	17	13	2	0.6	0.6	discharge
Jan 09	3.6	-27.0	69	16	14	0.5	-	-	charge
Apr 09	3.9	-28.0	64	20	13	2	0.4	0.3	discharge

Building Blocks are the second major compound group and are assumed to reflect breakdown products of HS (Huber & Gluschke, 1998; Huber *et al.*, 2011). The fraction of neutral compounds such as alcohols, aldehydes, ketones, sugars and also amino acids is characterized by low molecular weight and low ion density (Sachse *et al.*, 2001; Sachse *et al.*, 2005; Huber *et al.*, 2011). Sugars and amino acids can be bound chemically in significant amounts to HS and thereby can enhance the bioavailability of HS as substrates for microbial metabolism (Volk *et al.*, 1997). The fraction of low molecular weight organic acids (LMWOAs) includes in addition several metabolites from biological and chemical processes, also accounting for the labile portion of the C pool. This fraction contributed to only a minor extent (up to 1.4 %) to the total DOC concentration. Reasons for the low detection of LMWOAs in process waters could be the high volume of water passing through the plant (up to 300 m³/h), but samples from the observation wells (Table 5.1) also had low concentrations of LMWOAs and these wells are not influenced by the operating modes of the cold storage. Therefore, another explanation is needed for this system. Wellsbury and Parkes (1995) and Wu *et al.* (1997) showed that in waters of near-surface sediments acetate concentrations are low due to rapid microbial turnover. Therefore, it is assumed that the presence of high concentrations of inorganic electron acceptors (e.g. SO_4^{2-}) provides ideal conditions for a rapid turnover of the easily utilizable LMWOAs needed as electron donors by microorganisms.

The biopolymer fraction is characterized by polysaccharides (Huber *et al.*, 2011) which is the dominating material of EPS (Flemming *et al.*, 2007). The process water sample for September 2008 showed only a slight enrichment (0.6 %) in the biopolymers fraction (Table 5.3). This enrichment of biopolymers during the time of reduced injection (July to October 2008) might support the assumption made above that a higher proportion of microbial EPS becomes part of the DOC at the end of the disturbance phase.

Overall, the measured DOC in the cycling fluids during the monitoring period is a likely C and energy source for heterotrophic microorganisms. Furthermore, fermentative degradation of DOC is known to lead to the release of CO₂ forming the substrate source for autotrophic microorganisms. The presence of heterotrophic and autotrophic bacteria (e.g. sulfate reducers, *Gallionella* and *Thiothrix*) in the process water was demonstrated by Lerm *et al.* (2011b). In general, the chemical composition of the fluid from the cold storage at the German Parliament (Tables 5.1 and 5.2) provides ideal conditions for the existence of these oxidizers as well as for SRB. Thus, both bacteria of the genus *Thiothrix* and *Gallionella* are possible candidates to cause or at least support the clogging events in the ATEs system.

5.6.2 Bacterial Variability during Normal and Disturbed Operating Modes in the ATEs System

5.6.2.1 Intact Phospholipids

In all filters intact phospholipids (PLs) have been detected indicating the presence of a viable bacterial community (Zink *et al.*, 2003) in the cold storage (Fig. 5.5; sample from August 2007). The dominating PLs were phosphatidylethanolamines (PEs), 72 – 89 %, followed by phosphatidylglycerols (PGs), 9 – 23 % and phosphatidylcholines (PCs), 2 – 9 % (Fig. 5.6). PE is known to be a major membrane constituent of for instance many gram-negative bacteria (Wilkinson, 1988; Dowhan, 1997) especially of many SRB (Makula & Finnerty, 1974; Rütters *et al.*, 2001; Rütters *et al.*, 2002; Seidel, 2009). In contrast, PGs are often dominant in the cells of gram-positive bacteria, whereas PEs form only a minor proportion in these microorganisms (O'Leary & Wilkinson, 1988; Dowhan, 1997). However, Heim *et al.* (2009) also reported the predominance of PGs in a microbial mat dominated by the gram-negative proteobacterium *Gallionella*. PCs are usually associated with eukaryotes, but they also

account for up to 10 % of bacterial membrane lipids (Sohlenkamp *et al.*, 2003), which coincides with the low abundance of PCs detected in the cold storage.

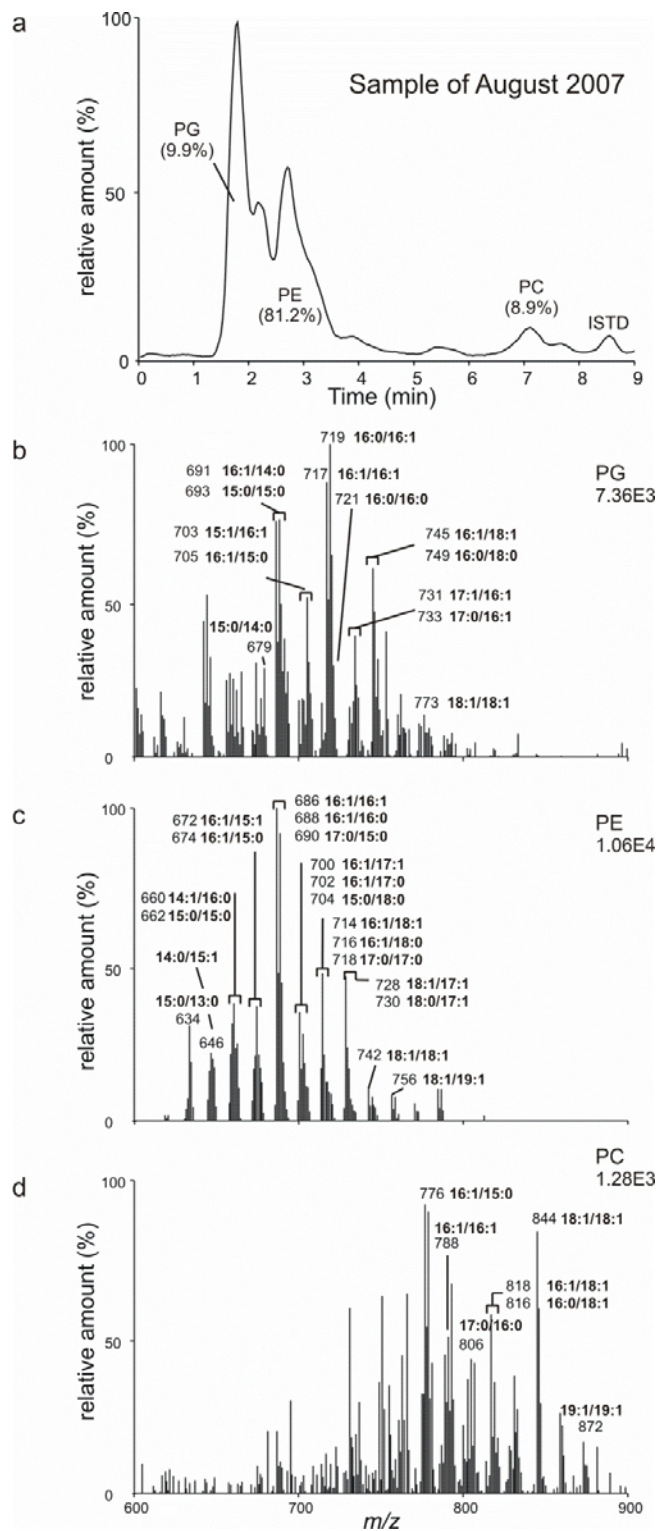


Fig. 5.5: a) HPLC-ESI-MS chromatogram of the phospholipid signal of the bacterial community from the filter sample taken in August 2007 and mass spectra of b)

phosphatidylglycerols (PG), c) phosphatidylethanolamines (PE), and d) phosphatidylcholines (PC) showing the main fatty acid combinations of intact phospholipids. ISTD = internal standard: deuterium-labeled Lyso-PC (1-palmitoyl-(D_{31})-2-hydroxy-glycerol-3-phosphocholine). X:Y = carbon number of PLFA : number of double bonds.

During times of reduced injection, two trends were observed within the PL composition. In summer 2008 (July to October) the PE content decreased from 87 to 77 %, while the PG distribution increased from 11 to 20 %.

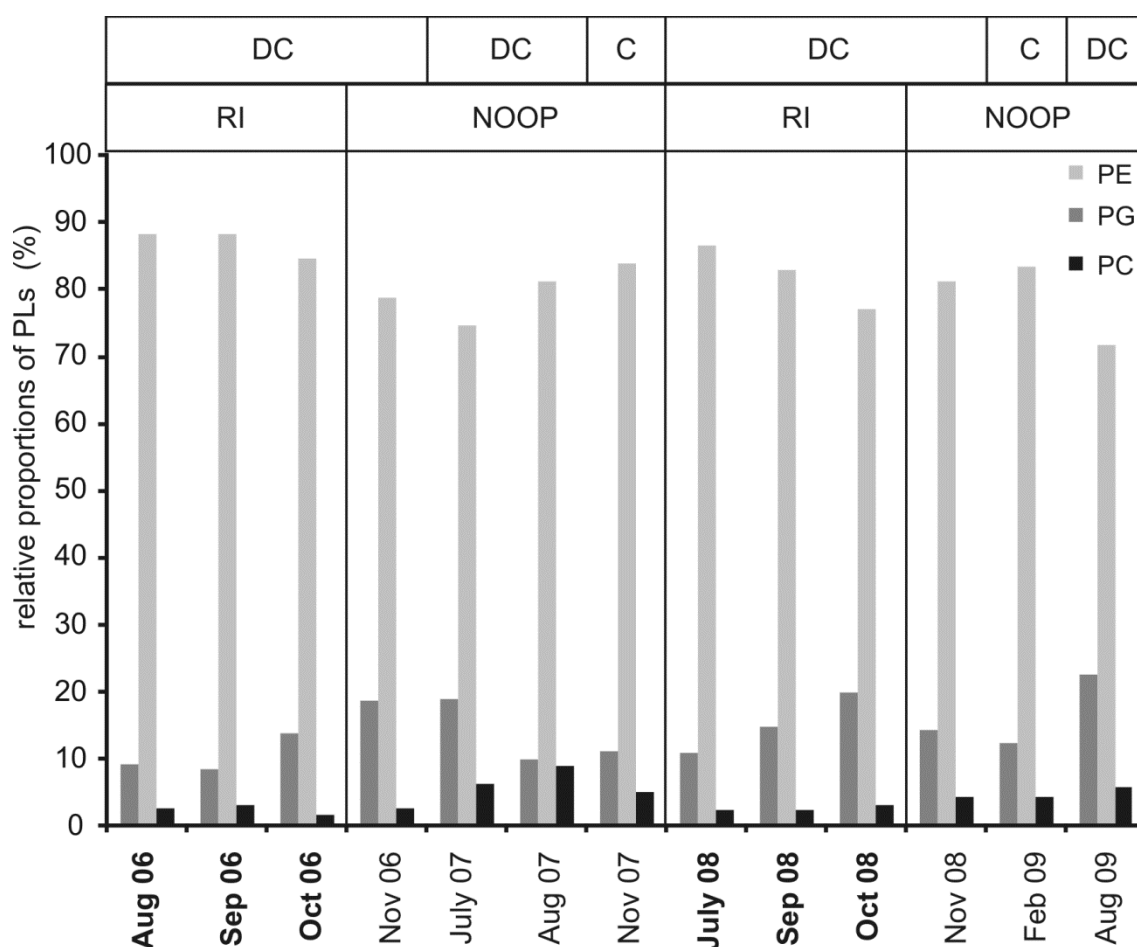


Fig. 5.6: Percentage proportions of the intact phospholipid (PL) pattern of filter samples from the Reichstag cold storage during the monitoring period. The months in bold indicate the two time intervals of reduced injection. PG = phosphatidylglycerol, PE = phosphatidylethanolamine, PC = phosphatidylcholine; DC = discharge mode, C = charge mode (see Fig. 5.2); RI = time of reduced injection, NOOP = normal operating mode.

In summer 2006 these trends were much less pronounced, but still visible. The PC contents were generally lower than during the times of normal operating mode. These variations are thought to represent changes in the microbial community, for instance a decrease in the

relative proportion of SRB and an increase in the Fe-oxidizing bacteria *Gallionella* during the disturbance phase.

However, due to the fact that only the most common and, therefore, of minor significance, PL classes were detected in the cold storage, the significance of the PL head groups on the taxonomy of bacteria is restricted in the current study and interpretations are not unambiguous. In general, no distinct correlation with charge/discharge operating modes can be observed.

5.6.2.2 Phospholipid Fatty Acid (PLFA) Side Chains

The PLFA inventory obtained after saponification of the filter extracts from the cold storage contains typical saturated, monounsaturated (*cis*, *trans*) and branched (*iso*, *anteiso* and mid-chain branched) fatty acids (Table 5.4 and Fig. 5.7) with 13 - 20 C atoms and showed clear changes during the monitoring (Table 5.4).

Branched Phospholipid Fatty Acids

Terminally branched FA can be derived from different sources. For instance, branched PLFAs such as *iso*- and *anteiso*-FA with 15 and 17 C atoms as well as the mid-chain branched 10-Me-16:0 FA are characteristic for the presence of SRB (Boon *et al.*, 1977; Taylor & Parkes, 1983; Dowling *et al.*, 1988; Kohring *et al.*, 1994; Rütters *et al.*, 2001; Rütters *et al.*, 2002). SRB are often found in anoxic aquifers (Jakobsen & Postma, 1994; Routh *et al.*, 2001; Detmers *et al.*, 2004; Fang *et al.*, 2006; Musslewhite *et al.*, 2007) using SO_4^{2-} as a terminal electron acceptor. Sulfate was measured in sufficient concentrations in the process water (mean 196 mg/L) and in the aquifer of the warm (205 mg/L) and cold (249 mg/L) side (Fig. 5.3, Table 5.1) and the occurrence of SRB in the cold storage has been demonstrated by Lerm *et al.* (2011b). Thus, SRB are a likely source of branched FA in the cold storage.

Compared to the normal operating mode phase (NOOP) the relative abundance of branched FA decreases distinctly (below 4 %) during the two periods of reduced injection indicating a change in the relative composition of the microbial community (Fig. 5.7). Assuming that branched fatty acids are indicators for SRB, it can be suggested that the relative percentage of SRB in the microbial community is diminished during times of reduced injection. This does not necessarily mean that the total number of SRB decreases. It is also possible that the abundance of other microorganisms increases significantly during the disturbance time

providing mostly unsaturated FA to the PLFA inventory (Fig. 5.7). An indication that enhanced SO_4^{2-} reduction also occurred during times of reduced injection might be given by the significant decrease in dissolved SO_4^{2-} concentration of 32 mg/L from August to September 2008 (Fig. 5.3).

Table 5.4: Phospholipid fatty acid (PLFA) compositions obtained after alkaline hydrolysis of intact phospholipids from times of reduced injection (RI) and times of normal operating mode (NOOP). *i* = *iso*, *ai* = *anteiso*, *meb* = mid-chain branched, *Me* = methyl branch, *br* = unidentified branching position; *c* and *t* = *cis* and *trans* configuration; Cx:y = carbon number of PLFA and number of double bonds. DC = discharge mode, C = charge mode (see Fig. 5.2); RI = time of reduced injection, NOOP = normal operating mode.

sampling time operating mode	Aug 06		Sep 06		Oct 06		Nov 06		Jul 07		Aug 07		Nov 07		Jul 08		Sep 08		Oct 08		Nov 08		Feb 09		Aug 09	
	DC	RI	DC	RI	DC	RI	DC	NOOP	DC	NOOP	DC	NOOP	C	NOOP	DC	RI	DC	RI	DC	NOOP	C	NOOP	DC	NOOP		
PLFA (%)																										
br-13:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.3	-	-	-	
13:0	-	-	-	0.1	-	-	-	-	-	-	0.6	-	-	-	-	-	0.1	0.1	0.1	0.2	0.1	-	-	-	-	
br-14:0	-	-	-	0.1	-	-	-	-	-	-	1.2	-	0.8	-	0.1	-	0.1	0.4	-	0.7	0.5	-	-	-	-	
14:1	-	-	-	0.2	-	-	-	-	-	-	-	-	-	-	0.3	-	0.2	-	-	-	0.8	-	-	-	-	
14:0	0.7	0.6	-	0.6	-	-	2.4	0.7	1.6	1.0	1.6	1.0	1.0	0.6	0.6	0.6	0.7	1.2	1.2	1.8	1.8	3.6	-	-		
meb-15:0	-	-	-	-	-	-	-	-	-	-	0.3	-	-	-	0.1	-	0.1	-	-	-	0.2	-	-	-	-	
i-15:0	0.6	0.6	0.4	-	3.0	2.7	3.4	2.7	3.4	2.7	6.2	2.7	2.7	0.9	0.5	0.3	1.1	2.2	2.2	2.3	3.6	5.6	-	-	-	
ai-15:0	1.1	0.8	0.6	-	7.1	2.7	6.2	2.7	6.2	2.7	-	-	-	-	0.9	0.6	0.9	3.3	3.3	3.6	5.6	-	-	-	-	
15:1	0.2	-	-	-	0.9	0.7	-	-	0.7	-	-	-	-	-	-	0.1	-	-	-	0.8	-	-	-	-	-	
15:0	0.2	0.3	-	-	2.0	0.5	0.2	0.8	0.1	0.1	0.2	0.8	0.8	0.2	0.3	0.4	0.3	0.4	0.8	0.8	2.6	-	-	-	-	
i-16:0	-	0.2	-	-	2.2	0.6	0.9	0.7	0.1	0.1	0.1	0.1	0.7	0.1	0.1	0.3	0.3	0.9	0.9	0.6	-	-	-	-	-	
16:1ω7c	64.3	68.0	64.7	-	19.2	42.1	33.9	29.0	67.6	66.2	55.7	44.1	31.3	38.1	-	-	66.2	55.7	44.1	31.3	38.1	-	-	-	-	
16:1ω7t	2.9	3.5	8.0	-	2.7	4.8	4.1	5.5	2.3	2.3	4.1	5.5	5.5	3.6	-	-	1.7	3.4	5.5	3.6	-	-	-	-	-	
16:1ω5c	10.5	5.5	3.5	-	10.0	15.2	12.6	10.4	2.6	2.0	12.6	10.4	5.2	14.7	-	-	2.0	3.9	5.2	14.7	16.7	-	-	-	-	
16:0	15.9	11.3	12.2	-	33.0	17.1	17.2	21.4	13.9	11.9	17.2	21.4	13.8	16.3	28.3	-	11.9	14.0	13.8	16.3	16.3	28.3	-	-	-	
10-Me-16:0	-	0.5	-	-	2.7	1.0	1.8	1.6	0.5	0.7	1.8	1.6	0.9	0.5	0.7	0.7	0.5	0.7	2.0	1.3	-	-	-	-	-	
i-17:0	-	0.1	-	-	0.6	0.6	0.9	0.8	0.1	0.1	0.1	0.8	0.8	0.1	0.2	0.4	0.2	0.4	0.5	-	-	-	-	-	-	
ai-17:0	-	0.1	-	-	1.7	0.6	0.8	0.9	0.1	0.1	0.8	0.9	0.9	0.7	0.1	0.1	0.2	0.6	0.7	-	-	-	-	-	-	
17:1	-	0.5	-	-	1.9	1.6	1.8	2.0	0.8	0.5	1.8	2.0	2.0	3.6	-	-	0.5	1.3	2.3	3.6	-	-	-	-	-	
17:0	0.6	0.1	-	-	1.1	0.8	0.5	0.7	0.1	0.1	0.5	0.7	0.7	0.6	-	-	0.1	0.1	0.4	0.6	-	-	-	-	-	
br-18:0	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-	
18:1ω9c	-	-	-	-	-	-	-	-	-	-	-	-	1.6	0.1	0.1	-	0.1	-	-	0.8	-	-	-	-	-	
18:1ω7c	2.9	7.0	9.0	-	4.3	7.4	8.6	6.7	9.0	13.7	8.6	6.7	9.5	12.4	-	-	13.7	14.1	12.1	12.4	-	-	-	-	-	
18:0	0.1	0.3	1.6	-	2.3	1.0	3.2	9.5	0.1	0.1	3.2	9.5	1.4	1.4	-	-	0.4	2.6	3.5	1.4	-	-	-	-	-	
19:1	-	-	-	-	1.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8	0.2	-	-	-	-	
19:0	-	-	-	-	1.0	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	
20:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Σ i-15:0 + ai-15:0 + 10-Me-16:0 + i-17:0 + ai-17:0	1.8	2.1	1.0	-	15.1	7.7	13.2	8.7	2.0	2.0	13.2	8.7	8.4	10.8	-	-	1.8	3.1	8.6	8.4	-	-	-	-	-	
Σ 16:1ω7c + 16:1ω7t + 16:1ω5c + 18:1ω9c + 18:1ω7c	80.5	84.0	85.3	-	36.2	69.4	59.2	53.2	81.6	83.6	59.2	53.2	62.9	54.8	-	-	83.6	77.1	66.9	62.9	-	-	-	-	-	
Σ n-saturated	17.5	12.7	13.8	-	41.9	20.0	22.8	33.5	14.9	13.3	22.8	33.5	21.1	34.4	-	-	13.3	17.8	19.5	21.1	-	-	-	-	-	
ratio 16:1ω7t / 16:1ω7c	0.05	0.05	0.12	-	0.14	0.11	0.12	0.19	0.03	0.03	0.12	0.19	0.11	0.11	-	-	0.03	0.06	0.12	0.11	-	-	-	-	-	
ratio (16:1ω7c + 16:1ω7t + 16:1ω5c + 18:1ω9c + 18:1ω7c) / (i-15:0 + ai-15:0 + 10-Me-16:0 + i-17:0 + ai-17:0)	45.8	39.4	88.9	-	2.4	9.1	4.5	6.1	40.5	47.5	4.5	6.1	7.8	7.5	-	-	47.5	25.0	7.8	7.5	-	-	-	-	-	

Monounsaturated and Saturated Fatty Acids

The most affected monounsaturated and saturated PLFA during the monitoring period were 16:1 ω 7, 16:1 ω 5, 18:1 ω 7, and 16:0 and 18:0 FA, respectively. Within the clogging events the amounts of 16:1 ω 7 and 18:1 ω 7 increased significantly, whereas 16:1 ω 5, and 16:0 and 18:0 decreased (Table 5.4, Figs. 5.7 and 5.8).

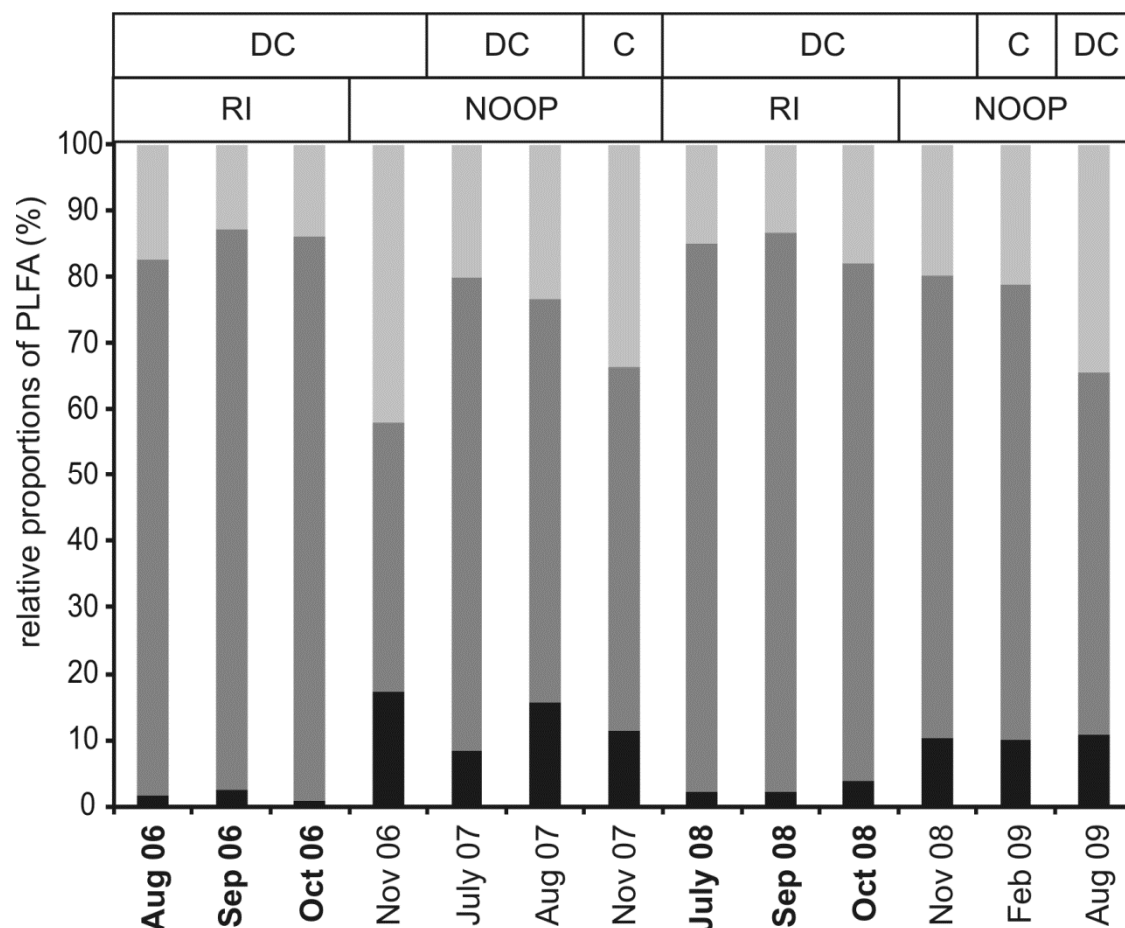


Fig. 5.7: Phospholipid fatty acid (PLFA) composition of filter extracts from the Reichstag cold storage during the monitoring period. Saturated PLFA are marked in gray, monounsaturated in dark gray, and branched PLFA in black. DC = discharge mode, C = charge mode (see Fig. 5.2); RI = time of reduced injection, NOOP = normal operating mode.

SEM analysis data has shown the occurrence of S-oxidizing bacteria of the genus *Thiothrix* and Fe-oxidizing bacteria of the genus *Gallionella* in the Reichstag ATES (Lerm et al., 2011b). *Gallionella* are suspected to be involved in the Fe hydroxide formation during the times of reduced injection in 2006 and 2008. However, the biofilm forming *Thiothrix* is interpreted to be mainly responsible for the filter clogging and reduced filter lifetimes during the clogging event in 2008 (Lerm et al., 2011b). PLFA studies on microbial mats of S-oxidizing bacteria, dominated by *Thiothrix* (Katayama-Fujimura et al., 1982; Jacq et al.,

1989; Zhang *et al.*, 2005) have shown that the main PLFAs were 16:1 ω 7, 16:0, and 18:1 ω 7 FAs. For Fe-oxidizing bacteria from borehole water, where *Gallionella* was the dominant species, Sahl *et al.* (2008) reported that 16:1 ω 7 and 16:0 FAs were the major cell membrane fatty acids. Thus, the 16:1 ω 7 FA, which is mainly responsible for the observed relative increase of monounsaturated FA during times of reduced injection (Fig. 5.7 and Table 5.4), most likely shows an increase of *Gallionella* and/or *Thiothrix* in 2006 and 2008, respectively. Although 16:0 FA seems to be present in the membrane composition of the oxidizers, this FA is not very specific and occurs in almost every membrane composition of pro- and eukaryotes.

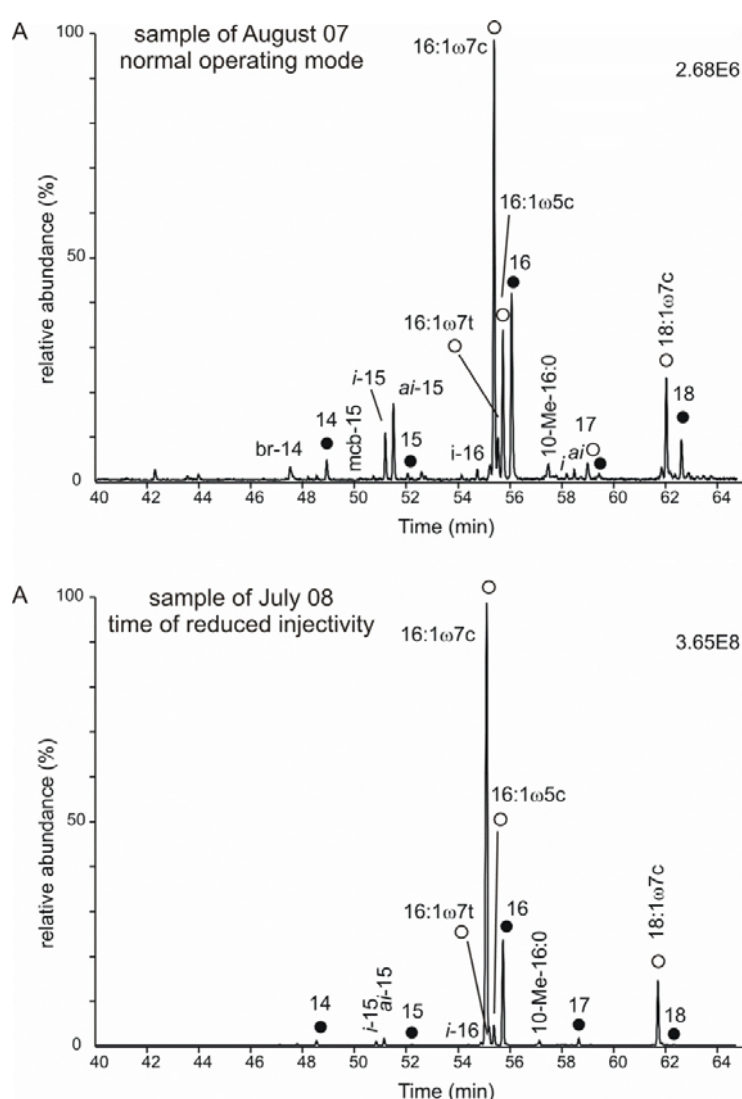


Fig. 5.8: Comparison of GC-MS chromatograms of PLFA distributions representing filter extracts obtained during a) time of normal operating mode and b) time of reduced injection. Numbers indicate carbon number of fatty acids; *i* and *ai* = *iso* and *anteiso* branching positions, *c* and *t* = *cis* and *trans* configuration of double bond, Me = methyl branch.

5.6.2.3 Phospholipid Fatty Acid Ratios as a Potential Operational State Parameter

For the assessment of the operational state of the cold storage a ratio was developed based on the most significant variations in the PLFA inventory (Fig. 5.9). This ratio relates the monounsaturated C₁₆ and C₁₈ FAs, most likely representing the S- and Fe-oxidizing bacteria, to the branched FA (*i*-15:0, *ai*-15:0, 10-Me-16:0, *i*-17:0, *ai*-17:0), presumably representing SRB (Fig. 5.9 and Table 5.4). During times of reduced injection this ratio clearly shows higher values, on average 47.8 (range from 25 to 89). In times of normal operating mode the ratio does not exceed a value of 10 (average 6.1). The ratio clearly seems to show the variations in the microbial population in the ATES during normal and disturbed operating periods, whereby Fe- and S-oxidizers became dominant during times of reduced injection rates. Obviously, these three groups occur with other bacteria in the energy storage but they appeared to be the most significant groups reflecting compositional changes in the ATES during normal and disturbed operating modes.

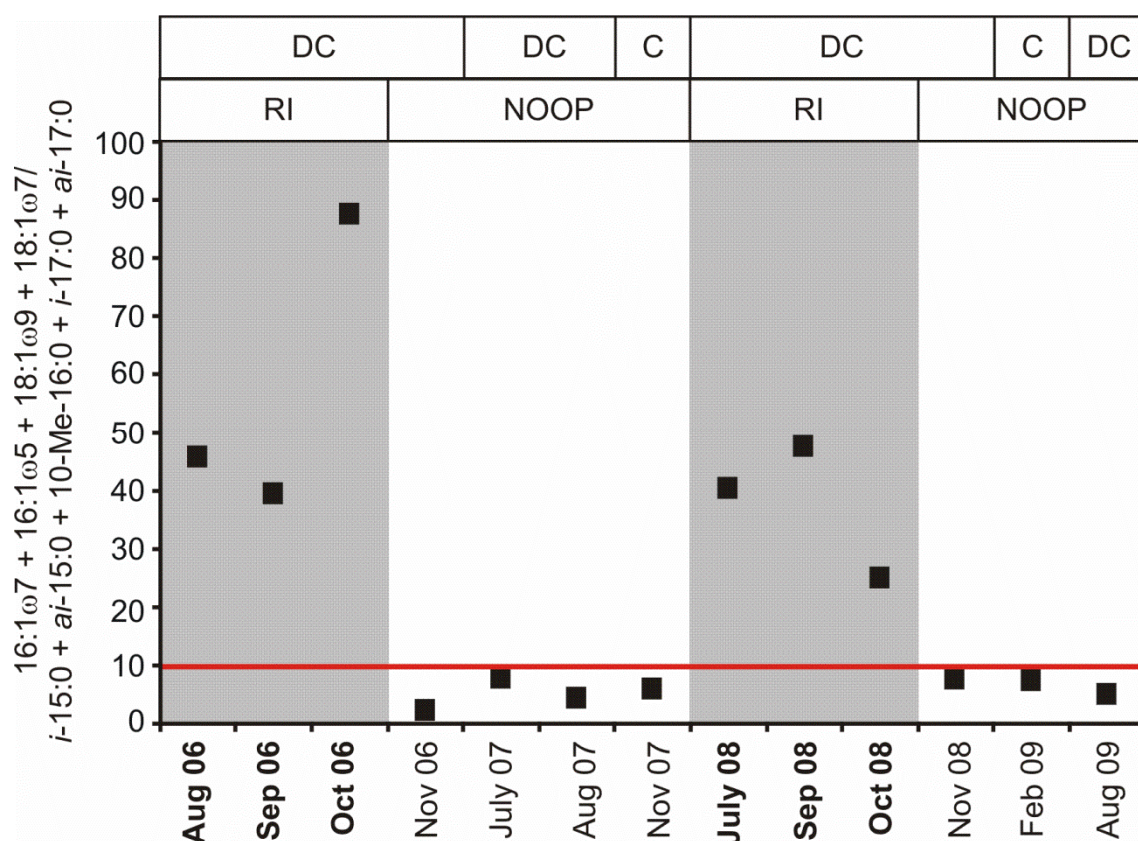


Fig. 5.9: Ratio of dominating monounsaturated PLFAs (16:1, 18:1) vs main branched PLFA (*i*-15:0, *ai*-15:0, 10-Me-16:0, *i*-17:0, *ai*-17:0) during the monitoring period. Average ratio value during normal operating mode (NOOP) is 6.1 and during time of reduced injection (RI) 47.8. DC = discharge mode, C = charge mode (see Fig. 5.2).

A second ratio of interest is the *cis/trans* isomers of the 16:1 ω 7 FA detected in the filter samples of the cold storage. The main isomer is the *cis* 16:1 ω 7 FA, whereas the *trans* 16:1 ω 7 FA only occurs in lower abundance (Fig. 5.8). The *trans/cis* ratio shows strong variability, which coincides with one exception (October 2006) to the periods of reduced injection and normal operating mode (Fig. 5.10). Variations in the *cis* and *trans* configuration of monounsaturated compounds may reflect a shift in the composition of the microbial community (Gillan *et al.*, 1981) as well as the response of the microbial community to environmental stress (Guckert *et al.*, 1985).

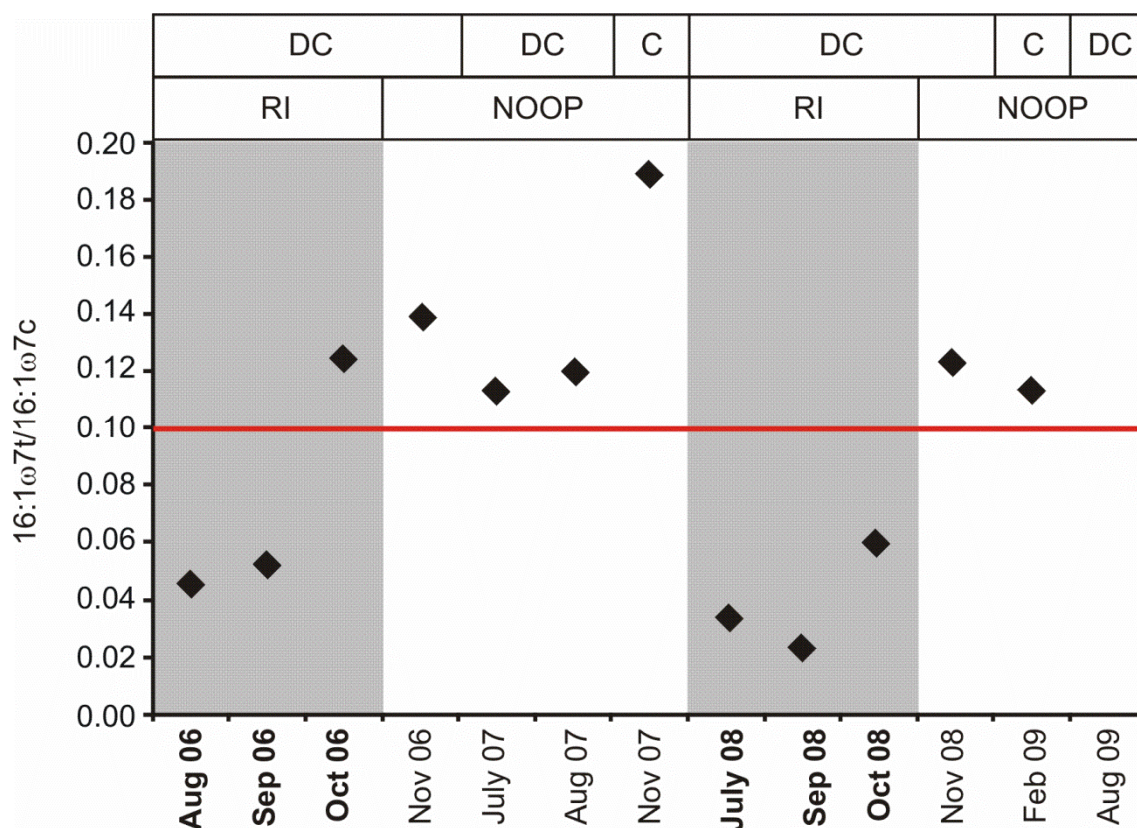


Fig. 5.10: Ratio of *trans* and *cis* configuration of C₁₆:1 ω 7 fatty acids during the monitoring period. Average ratio value during normal operating mode (NOOP) is 0.13 and during time of reduced injection (RI) 0.04 (whereas the October 2006 sample was not considered). DC = discharge mode, C = charge mode (Fig. 5.2)

The conversion of *cis* to *trans* unsaturated FAs can be caused by external stress factors such as starvation (Guckert *et al.*, 1986; Hedrick *et al.*, 1991; Borga *et al.*, 1994; Kieft *et al.*, 1994), toxic organic solvents (Heipieper *et al.*, 1992; Heipieper *et al.*, 1995; Ludvigsen & Albrechtsen, 1999), potentially harmful metals, and temperature changes (Heipieper *et al.*, 1996). The reason for this conversion is not yet completely clear, but as a consequence the

membrane fluidity is affected due to the different physical properties of monounsaturated FAs being in the *cis* or *trans* configuration (Diefenbach *et al.*, 1992). In response to environmental stress the membrane fluidity can be regulated by variations of saturated, unsaturated and/or branched FA e.g. (Green & Scow, 2000). However, these changes can only occur during *de novo* synthesis (Denich *et al.*, 2003) if cells grow and divide. Therefore, it can be an advantage for microorganisms to use the *cis/trans* isomerization as an alternative way of regulating membrane fluidity when growth is inhibited (Diefenbach *et al.*, 1992; Heipieper *et al.*, 2003).

Guckert *et al.* (1986) suggested a *trans/cis* ratio as a starvation index or stress index for microbial communities. It was suggested that a microbial population can be regarded as healthy, if the *trans/cis* ratio is below 0.1. In contrast, ratios higher than 0.1 indicate that the microorganisms providing these FA biomarkers are exposed to environmental conditions which are stressful (Guckert *et al.*, 1986; Ludvigsen & Albrechtsen, 1999).

As discussed earlier the 16:1 ω 7 FA increases during the phase of reduced injection and could reflect the increased relative abundance of *Thiothrix* and/or *Gallionella*. With the exception of October 2006 the *trans/cis* ratio is below 0.1 in the filter extracts indicating favorable living conditions for *Thiothrix* and *Gallionella* during times of reduced injection (Fig. 5.10). During the normal operating modes the ratio exceeds the value of 0.1, indicating unfavorable conditions for the oxidizers which might be the contributors of at least a significant proportion of the 16:1 ω 7 FA.

Thus, both ratios have the potential to indicate the involvement of microbial activity in processes causing a disturbance in the operating state of the Reichstag ATES and for decisions of counter measures, being appropriate to eliminate the clogging source (e.g. mechanical cleaning vs disinfection). In addition, the developed ratio might be a useful control parameter to assess the operating state of the Reichstag ATES. However, to use this parameter it would be necessary to improve the sampling frequency to enable the monitoring of the transition period from normal to disturbed operating state e.g. in a bypass system not disturbing the overall operation.

5.7 Conclusions

Biogeochemical monitoring was conducted for the cold storage of the German Parliament buildings in Berlin over three years. This monitoring comprised the characterization of the fluid chemistry in the process water and the indigenous microbial community by phospholipid fatty acid analysis of filter material. During the monitoring, two periods of reduced injection occurred in 2006 and 2008 due to clogging events. These clogging periods were characterized by increased Fe hydroxide formation and, at least documented in 2008, the formation of extensive biofilms from bacteria of the genus *Thiothrix*, significantly reducing the filter lifetimes in the top site facilities (Wolfgramm *et al.*, 2010; Lerm *et al.*, 2011b).

The monitoring of the fluid chemistry revealed the presence of sufficient amounts of DOC and inorganic anions (SO_4^{2-}) being potential C and energy sources for the indigenous microbial ecosystem in the ATES. Variations in the fluid chemistry reveal almost no correlation with the operational state.

The PLFA data show that the composition of the microbial community changed significantly from normal operating modes to times characterized by clogging events. During the clogging events, the relative proportion of branched FAs, presumably representing sulfate-reducing bacteria, decreased while the relative abundance of 16:1 ω 7 and 18:1 ω 7 FAs significantly increased. These monounsaturated FAs are suggested to be the main FAs in the Fe-oxidizing bacteria of the genus *Gallionella* and S-oxidizing bacteria of the genus *Thiothrix*, respectively. Both bacteria were detected in the cold storage (Lerm *et al.*, 2011b).

Based on the variations in the PLFA inventory two ratios were developed to assess the contribution of microbial activity to the disturbance of the operational state in the ATES system of the German Parliament. The ratio between monounsaturated FA (C_{16} and C_{18}) and branched FAs (C_{15} , C_{16} and C_{17}) clearly allows differentiation between normal and disturbed operating conditions reflecting the relative dominance of the S- and/or Fe-oxidizers during times of reduced injection. A second parameter is given by the *trans/cis* ratio of 16:1 ω 7 FA. This ratio seems to indicate favorable conditions for the oxidizers during the clogging events. Both ratios are suitable parameters to indicate the microbial impact on the operational state in the cold storage of the ATES system. Furthermore, the parameters can be of help in deciding appropriate counter measures.

5.8 Acknowledgements

We would like to thank the persons in charge of the ATES of the German Parliament buildings for granting us permission to monitor the ATES and to publish our results. In addition, we gratefully acknowledge Oliver Opel (Leuphana University of Lüneburg), Andrea Seibt (BWG, Neubrandenburg), and Stefan Kranz (GFZ Potsdam) for providing unpublished data from the Reichstag ATES. We are grateful to Kristin Günther, Bastian Kasek, Cornelia Karger, Anke Kaminsky and Ursula Kegel for their technical and analytical support. This work was conducted as part of the research project “AquiScreen” which has been funded by the German Federal Ministry for Environment, Nature Conservation and Nuclear Safety (BMU, grant no. 0327634).

6 Fluid Chemistry and Impact of Different Operating Modes on Microbial Community at Neubrandenburg Heat Storage (Northeast German Basin)

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6.1 Abstract

The efficient use of energy is an important issue of public interest. In the Neubrandenburg heat storage surplus heat from a gas and steam cogeneration plant is stored in an aquifer system for use of the stored energy during times of high heat demand. The reliability of such a plant can strongly be influenced by microbial communities. Therefore, biogeochemical monitoring of the heat storage of Neubrandenburg was conducted from March 2006 to January 2010 to characterize the natural variability of prokaryotic life by way of phospholipid fatty acid (PLFA) analysis and the availability of electron acceptors (e.g. sulfate) and donors [e.g. dissolved organic carbon (DOC)] under different operating modes of the plant. Analysis of the fluid chemistry showed that a sufficient amount of electron acceptors (sulfate ca. 1 g/L) and donors (DOC up to 19 mg C/L) for potential microbial respiration and energy consumption is present. Phospholipid analysis of filter samples from the heat storage revealed a viable microbial community in the plant with adaptation to changes in the operating mode (charge/discharge) and associated variation in temperature (45°-73°C). The PLFAs mainly influenced were saturated and branched FAs, most likely reflecting temperature adaptation by a variable microbial community in different parts of the heat storage. Furthermore, branched monoenoic FAs indicate the presence of sulfate-reducing bacteria within the plant.

6.2 Introduction

With the decrease of fossil energy resources in recent years, new concepts have been developed to improve energy efficiency. Storage systems for surplus energy have become increasingly important. One possibility is the storage of surplus energy in aquifers during periods of less energy demand (e.g. summer) and re-extraction of the energy from the water

during times of high energy demand (e.g. winter). Such a strategy of conscientious handling of energy is conducted in the Neubrandenburg heat storage system in the Northeast German Basin. Here, the surplus heat from a gas and steam cogeneration plant is stored in form of warm water in an aquifer during spring/summer, with the water being used for district heating during winter.

The operation of such plants can be influenced by abiotic and biotic processes, leading to massive plant disturbance by way of clogging events caused by e.g. iron hydroxide precipitation and biofilms. Microbiological and biogeochemical studies have shown that geothermally used aquifers form an appropriate life habitat for microbial communities (Brielmann *et al.*, 2009; Lerm *et al.*, 2011a; Vetter *et al.*, 2011; Vetter *et al.*, 2012). Due to their ability to form biofilms, microbial populations can have a strong impact on the working reliability of the plants (Lerm *et al.*, 2011b; Vetter *et al.*, 2012). From a surface perspective, heat storage systems form an extreme environment in which the microorganisms must show significant adaptability to the variable temperature conditions in the different parts of the geothermal plant. The temperature of the brine produced from the Neubrandenburg heat storage from an aquifer in 1250 m depth varies between 45 and 85°C, depending on the operating mode. Temperature is one of the limiting factors for deep microbial life (Parkes *et al.*, 2000). However, the current maximum temperature for bacterial [*Aquifex pyrophilus*, 95°C (Huber *et al.*, 1992)] and archaeal [*Methanopyros kandleri*, 122°C (Takai *et al.*, 2008)] life are far above the temperature range of the Neubrandenburg heat storage. The temperature conditions in the geothermal energy system in Neubrandenburg should be favorable for thermophilic (45-80°C) and hyperthermophilic (>80°C) microorganisms (Parkes *et al.*, 2000).

Temperature has a profound effect on the integrity of microbial cell membranes, causing variation in the membrane lipid inventory to adapt to the different temperature conditions. The fluidity of bacterial cell membranes is influenced by the lipid composition of the main membrane constituents, phospholipids (PLs). PL variation can occur in terms of different head groups and variable proportion of saturated, unsaturated, branched as well as cyclic fatty acid (FA) side chains (Russell & Fukunaga, 1990; Šajbidor, 1997). Adaptation to an increase in temperatures involves an increase in the chain length and a decrease in degree of unsaturation in the FA side chains to counteract an increase in membrane fluidity. In microorganisms, regulating their FA fluidity via the branched system an increase in the *iso*

FAs is observed while the proportion of *anteiso* FAs decreases with rising temperature (Rilfors *et al.*, 1978; Reizer *et al.*, 1985; Kaneda, 1991). In the presence of high salt content, it has been shown that the upper growth temperature limit is increased for specific bacteria (Hurst *et al.*, 1984) that is also reflected in alteration of the lipid composition (Hurst & Ofori, 1984; Miller, 1985). In this case, mesophilic bacteria (10-45°C) could be also of relevance in the hot and saline environment of the Neubrandenburg heat storage.

We have monitored changes of the cell membrane PL of the indigenous microbial communities during different operating modes within the Neubrandenburg heat storage at different sampling locations affected by different temperature conditions. Furthermore, we investigated variation in the fluid constituents that form potential carbon and energy sources for the indigenous microbial communities.

6.3 Study Site and Operating Modes of the Heat Storage, Neubrandenburg

The geothermal heating plant at Neubrandenburg was commissioned in 1989. During operation, corrosion damage occurred due to the presence of unprotected metal pipes in contact with the brine. In 1998, the operation of the plant had ceased due to severe damage by corrosion after oxygen entered the water cycle (Seibt & Kellner, 2003). However, in 2003 the plant was renewed for combined use as a geothermal plant and surplus heat aquifer storage (Kabus *et al.*, 2004). Since then, the heat storage has been in use to store surplus heat from a gas and steam cogeneration plant in summer and to provide this energy for district heating in winter.

The Neubrandenburg heat storage system is the deepest one in the world. It is located in northeast Germany in Mecklenburg-Western Pomerania close to the Baltic Sea and has been in operation since March 2004. The energy storage comprises two wells drilled down to the Upper Triassic (Keuper) sandstone aquifer (Postera formation) at 1250 m. The original aquifer temperature is 54°C. The aquifer consists of terrestrial clastic sediments, was deposited in channel-like deltaic environments and possesses a thickness of 15-25 m with a porosity of 20-25 % (Obst & Wolfgramm, 2010). Sandstones are of white or grey color and carbonaceous fragments are imbedded. The Postera formation consists predominantly of quartz and secondary of feldspar (plagioclase and microcline). Minor components are phyllosilicates of kaolinite, illite, glauconite, chlorite, smectite, and muscovite. The cementation consists of siderite, calcite, dolomite and anhydrite and, in the area with

carbonaceous components, pyrite occurs (Wolfgramm & Seibt, 2006). New mineral formations in filter samples consist mainly of Fe, Cu, and Ni sulfides (Obst & Wolfgramm, 2010). The Postera sandstone hosts an anoxic and hypersaline aquifer.

In summer, during charge mode (Fig. 6.1) water is pumped up from the cold well (Gt N4) with a temperature of 54-45°C, filtered (F2) before passing the heat exchanger (HE) and loaded with surplus heat to 80-90°C before the water is refiltered (F1) and injected into the warm well (Gt N1). Until September 2009 the warm well was charged at 80°C. Subsequently, the temperature was raised to 85°C. Nowadays, water with a temperature of 90°C is stored.

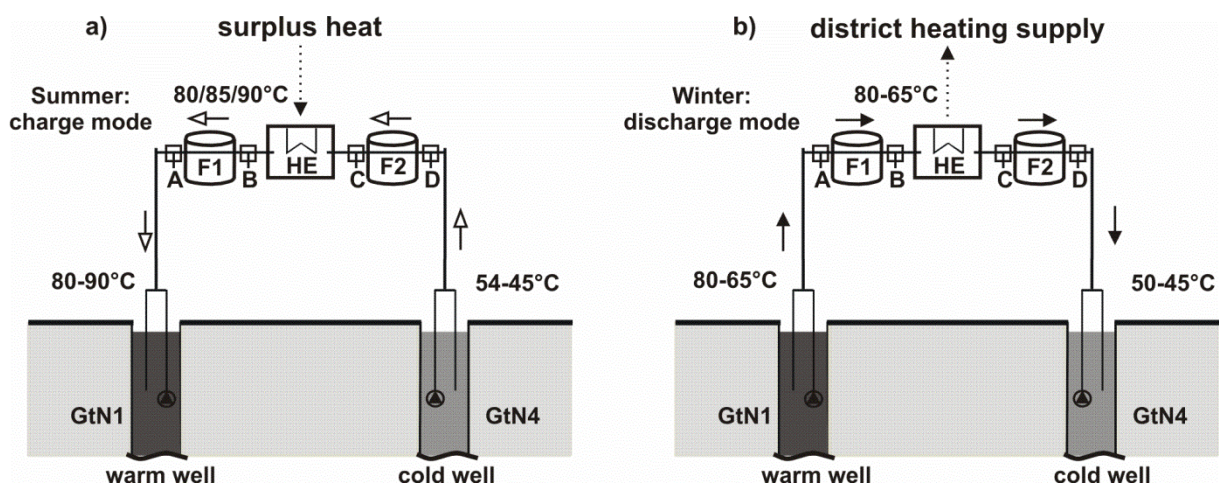


Fig. 6.1: Simplified schematic view of deep heat storage at Neubrandenburg during different operating modes. a) In summer (charge mode) the brine is pumped up from the cold well, heated with surplus heat from a gas and steam cogeneration plant in a heat exchanger (HE) to a temperature between 80 and 90°C, and re-injected into the warm well. During the charge period, the average water temperature in the cold well decreases from 54 to 45°C. b) In winter (discharge mode) the brine is pumped from the warm well via the HE to the cold side. The warm water is used for the district heating supply. During the discharge period the temperature of the brine decreases from 80 to 65°C in the warm well. (F1/F2, filter systems; A, B, C, and D, tapping points before and after the filter systems).

In winter, when heat is demanded, the stored heat is discharged, with initial temperature of ca. 80°C from the warm well (Gt N1). During discharge, the brine passes the filter system (F1) before entering HE, is filtered again (F2) and re-injected to the cold well at a temperature between 50 and 45°C. During discharge, the temperature of the warm well can decrease to 65°C. When the temperature of the charged water was increased from 80 to 85°C in September 2009, the temperature in the warm well did not fall below 70°C during the first discharge phase. The filter units (1 µm pore size, polypropylene) are integrated into the plant to retain particles before the fluid passes HE. The maximum production rate of the heat

storage amounts to 100 m³/h and provides energy for district heating of 2.5-3 MW (Kabus *et al.*, 2004). While the reservoir pressure accounts for 130 bar, the pressure in the plant is kept at 2-6 bar to avoid oxygen supply. During the monitoring period in 2008, the heat storage was temporarily (May to December 2008) not operating due to corrosion of the pump in the cold well.

6.4 Material and Methods

6.4.1 Fluid and Filter Sampling

Since March 2006 the heat storage has been investigated by monitoring the fluid chemistry in the storage system. Fluid samples were taken during charge and discharge operating modes, either from the production flow (tapping points A or D in Fig.6.1) or prior to injection to the recharge well (tapping points B or C in Fig. 6.1). In total, 17 fluid samples were taken from the warm side (12 from production flow and 5 prior to injection to the warm side) and 13 fluid samples from the cold side (8 from the production flow and 5 prior to injection to the cold side). In each case the fluid was transferred to sterile glass bottles without an air head space, stored on freezer packs, and analyzed within 24 h.

Additionally, 10 filter samples were taken between February 2008 and July 2009, representing distinct charge or discharge operating modes. In April 2008 and January 2009 (discharge mode), four filters were taken directly from filter systems before (F1) and after (F2) HE. Two additional filters were sampled from the filter system after HE (F2) in February 2008 and March 2009 (Table 6.1). Furthermore, two filters were collected during charge mode from the filter system before HE (F2) in June and July 2009. Due to technical reasons, it was not possible to get a sample from the filter system after HE (F1) during charge mode. Finally, during the disturbance phase from May to December 2008, filters were sampled from both filter systems (F1 and F2) with a filter operating time of 8 months. During this downtime phase, the filters were continuously in contact with the fluid and the system was kept under pressure to avoid oxygen penetration. Only these filters were used for analyzing PLFAs, which represent only one flow direction (operating mode). Filter operating times are listed in Table 6.1. With exception of the filter from June 2009, all filters were in use at least for 1 month before the PLFA inventory was investigated. Such accumulation times are desirable for obtaining a representative average of the PLFA signal from the respective side of the

geothermal plant. All filters were wrapped in pre-heated and -cleaned Al foil and analyzed immediately in the laboratory or stored at -80°C until analysis.

6.4.2 Detection of Anions in Fluid Samples

All fluid samples were analysed (3x to 5x) using ion chromatography (ICS 3000, Dionex Corp.) equipped with a conductivity detector, KOH eluent generator and an ASRS Ultra II 2 mm suppressor for determination of the concentration of inorganic and organic anions. For separation of the anions, an analytical column (AS11HC; 2x 250 mm, Dionex Corp.) was used at a constant 35°C. Details of the method have been described by Vieth *et al.* (2008). Due to the high salt content of the fluid samples the standard deviation was <10 %.

6.4.3 Quantification of Dissolved Organic Carbon (DOC) in the Fluid

For the quantification of the DOC, the fluid passed a 0.45 µm membrane syringe filter and was purged with N₂ for 5 min after acidification with HCl to remove the dissolved inorganic carbon. Afterwards, aliquots of the sample were automatically injected onto a heated catalyst (680°C), where DOC was transformed to CO₂ for measurement using infrared (IR) spectroscopy (TOC-2000A, Shimadzu). Quantification of DOC was carried out by way of external calibration using potassium hydrogen phthalate.

6.4.4 Extraction and Chromatographic Separation of Filter Samples for Phospholipid Analysis

The freeze-dried filters only contained a low amount of finely dispersed sediment. Therefore, pieces of the respective filter that visually contained an adequate amount of sample material were taken and extracted (2x 20 min, ultrasonication) using MeOH-dichloromethane (DCM)-ammonium acetate buffer with a ratio of 2:1:0.8 (v/v; see Bligh and Dyer (1959)). A clean filter was extracted as a blank and, no phospholipids (or PLFAs) could be detected. In a second step the solvent ratio of the extract was changed to 2:2:0.9 by adding DCM and ammonium acetate buffer, resulting in phase separation. The aqueous phase was re-extracted (3x) with 20 ml DCM. The combined organic phases were concentrated using a Turbo Vap system (Zymark). The extract was then chromatographically separated into polarity fractions described by Zink and Mangelsdorf (2004). To liberate the PLFAs the PL fractions was saponified (White *et al.*, 1979). The resulting PLFAs were measured using gas

chromatography-mass spectrometry (GC-MS). The performance of the GC-MS system was regularly controlled using a standard FA mixture containing most of the FAs which usually can be expected in PLFA analysis. Details of the methods and instrument parameters are described by Vetter *et al.* (2011). Occasional analysis of intact polar lipids revealed that the FAs obtained after saponification were derived mainly from intact phospholipids.

6.5 Results and Discussion

6.5.1 PLFA Composition

For the analysis of the PLFA signals in the heat storage, filter material from the warm well ($>70^{\circ}\text{C}$) and cold well ($<48^{\circ}\text{C}$) were investigated during discharge mode, during downtime of the plant from May to December 2008 (temperature decrease to 20°C) and during charge mode. When the heat storage was operated in charge mode, for technical reasons, only filters from the cold well (F2) could be sampled. The finding of PLs in all filter samples from the cold well and warm well indicate the presence of viable microorganisms on both sides. PLs are marker for living microorganisms, because they are only stable in intact cells over longer periods of time (White *et al.*, 1979; Harvey *et al.*, 1986; Logemann *et al.*, 2011). Representative GC-MS chromatograms of the PLFA signals (Fig. 6.2) during different operating modes and from different wells of the heat storage indicated significant differences in the relative proportions of saturated and branched FAs. Saturated PLFAs ranged from 14 to 18 carbons, the maximum varying between C_{15} and C_{16} . Branched FAs were dominated by *iso*- and *anteiso*-FAs with 14-18 carbons (C_{15} max.). Unsaturated FAs represented only a minor proportion (mainly with 17 and 18 carbons).

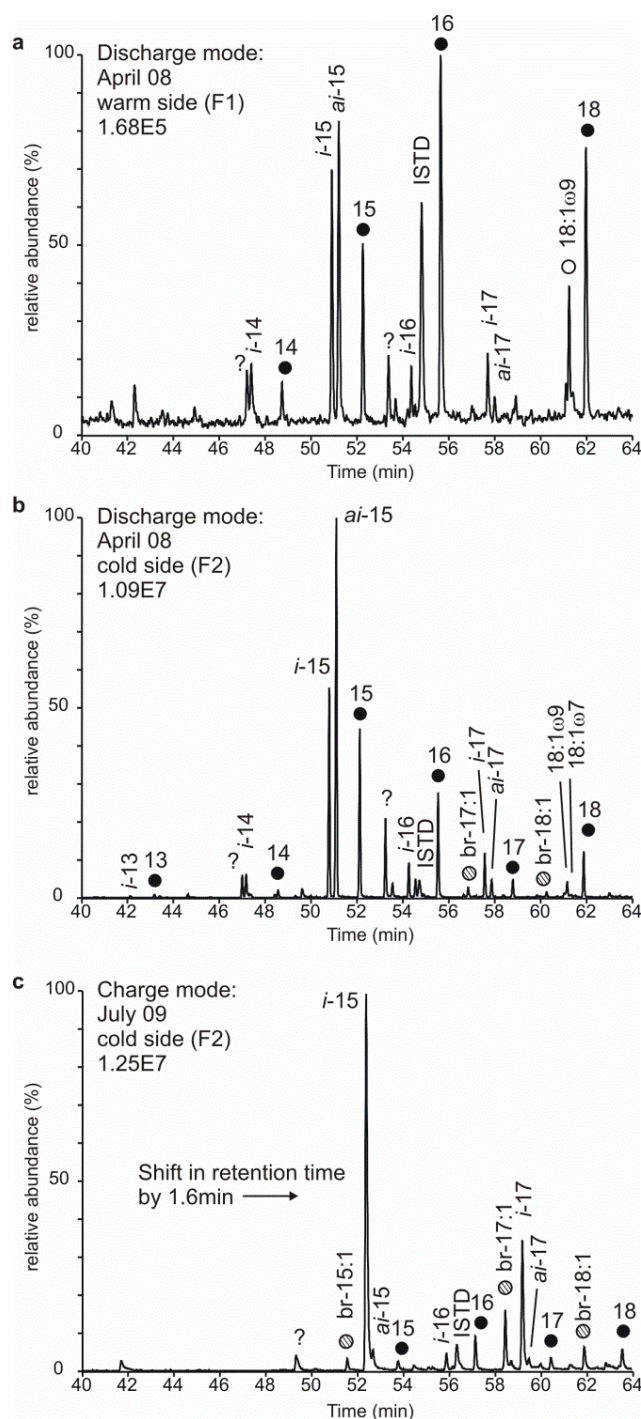


Fig. 6.2: Partial total ion current (TIC) chromatogram of PLFA distribution from filter samples taken in April 2008 (discharge mode) from a) F1 (warm well) and b) F2 (cold well) and from filters taken in July 2009 (charge mode) from c) F2 (cold well). Numbers indicate carbon number of fatty acids (*i* and *ai*, *iso* and *anteiso* branching positions; ISTD, internal standard). Note that there is a shift in retention time by 1.6 min between the discharge and charge sample due to the time delay between the measurements of these samples of more than 1 year.

PLFA Inventory in Filter Systems during Different Operating Modes

During the discharge operating mode in April 2008, when water was pumped from the warm well to the cold well and energy was extracted via the heat exchanger for district heating, PLFA distribution in filter F1 (warm side) was dominated by saturated FAs (Figs. 6.2 and 6.3a) with a proportion of 54 % (Table 6.1). One monounsaturated FA, the 18:1 ω 9 cis , was detected at 6 %. Branched monounsaturated FAs could not be observed in this sample from F1. However, branched saturated FAs, mainly *iso* and *anteiso*, represented the second dominant group at ca. 40 % (Fig. 6.3a and Table 6.1). This PLFA pattern in F1 during the discharge mode was interpreted to represent the microbial community from the warm water affected aquifer (Fig. 6.3a and c).

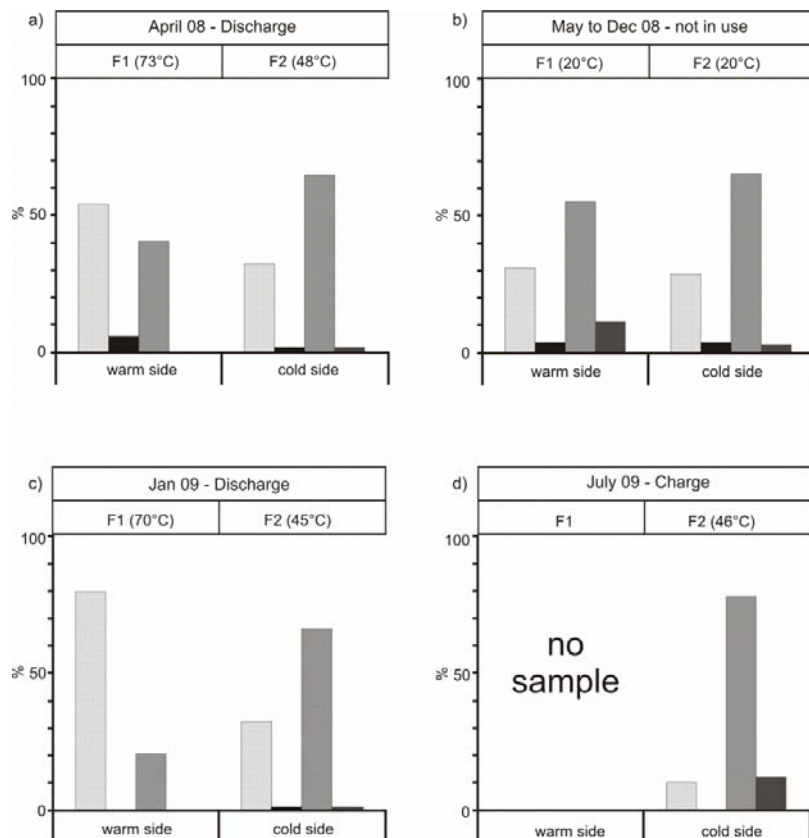


Fig. 6.3: PLFA composition in filters (F1 warm side, F2 cold side) before and after heat exchanger. Light grey, saturated FAs; black, unsaturated FAs; grey, saturated branched FAs; dark grey, monounsaturated branched FAs. Between May and December 2008 the heat storage was not in use, but the filters remained in the plant. For filter positions see Figure 6.1.

Interestingly, the PLFA profile from the downstream filter F2 on the cold side in April 2008 (Fig. 6.3a) changed substantially, although it was the same fluid that had passed F1 on the

warm side. In F2 the saturated FAs significantly decreased to 32 % and the branched FAs, mainly the *ai*-15:0 and *i*-15:0, became dominant (ca. 53 %, Fig. 6.2b). Thus, the PLFA signal in the downstream filter F2 seemed to represent a microbial community adapted to the conditions on the cold side.

A higher proportion of saturated FAs, as detected in F1 on the warm side, counterbalanced the effect of increased membrane fluidity caused by higher ambient temperature (F1, 73°C) (Russell & Fukunaga, 1990; Russell *et al.*, 1995). This is due to the fact that saturated FAs have a higher melting temperature, leading to a higher phase transition temperature of the cell membrane than the corresponding branched or unsaturated FAs. Thus, the dominance of saturated FAs on the warm side most likely reflects membrane adaptation to higher temperatures. Besides the decrease in saturated FA on the cold side, the dominance of branched FAs is an indication of an adaptation of the prokaryotic community to cooler ambient temperature conditions, as incorporation of branched FAs lowers the phase transition temperature (Kaneda, 1991). The ratio of branched to saturated FAs during different discharge modes (Table 6.1) ranged between 2.1 and 2.7 on the cold side but only between 0.3 and 0.7 on the warm side, clearly revealing the changes in the microbial PL composition on the different temperature sides of the energy storage. These changes seem to reflect different dominating microorganisms on both sides of the HE being better adapted to the corresponding temperature conditions.

Another often observed adaptation to cooler temperature conditions is an increase in the proportion of unsaturated FAs (Russell *et al.*, 1990). However, such an increase was not observed on the cold side, indicating that changes in the degree of unsaturation played only a minor role in the adaptation process in the indigenous microbial communities of the Neubrandenburg heat storage. In general, unsaturated FAs were not or little biosynthesized.

From May to December 2008 the heat storage was not in use due to problems with the pumping system in the cold well. During this time, the above ground installation such as the filter systems F1 and F2 cooled to 20°C. During the plant downtime, the PLFA inventory of the microbial community in F1 became comparable to that of F2 with a predominance of branched FAs (Fig. 6.3b), suggesting that the microbial communities in the filters on both sides became more similar. Also the monoenoic branched FAs usually only occurring in F2,

were present in F1 (Table 6.1). Some differences in the microbial population might still be indicated by the variability of some specific FAs.

In December 2008 filters were replaced and the plant operation was restarted to continue the discharge mode. With the warm water (70°C), the PLFA pattern dominated by the saturated FAs re-occurred in F1, while in F2 the branched FAs still prevailed (Fig. 6.3c). This indicated that on the warm side the microbial community being adapted to warmer conditions was quickly re-established.

During the charge mode in June and July 2009 (Table 6.1), when water was pumped up from the cold well, uploaded with heat and charged into the warm well, branched FAs exceeded 80 % of the PLFAs in samples from F2 (Fig. 6.3d and Table 6.1), while *i*-15:0 became dominant (Fig. 6.2c and Table 6.1). Saturated terminally branched FAs with 15 and 17 carbons are widely distributed in significant amount in many sulfate-reducing bacteria (SRB) (Parkes & Taylor, 1983; Edlund *et al.*, 1985; Dowling *et al.*, 1986; Dowling *et al.*, 1988; Ollivier *et al.*, 1991; Kohring *et al.*, 1994) and also in gram-positive bacteria (Lechevalier & Moss, 1977; Kaneda, 1991).

Tab. 6.1: Comparison of PLFA compositions obtained after alkaline hydrolysis of intact phospholipids from the warm and cold well during different operating modes (*i*, *iso*; *ai*, *antiso*; Me = methyl branch; br, branched FA; Cx:y, carbon number of PLFA and number of double bonds; DC, discharge mode, C, charge mode).

Sampling time	Feb 08	Apr 08		May to Dec 08		Jan 09		Mar 09	Jun 09	Jul 09
Operating mode	DC	DC		not in use		DC		DC	C	C
Filter installation side	F2 - cold	F1 - warm	F2 - cold	F1 - warm	F2 - cold	F1 - warm	F2 - cold	F2 - cold	F2 - cold	F2 - cold
Filter operating time	2 months	2 months	4 months	8 months	8 months	1 month	1 month	3 months	1 day	1 month
Temperature	46°C	73°C	48°C	73-20°C	48-20°C	70°C	45°C	48°C	47°C	46°C
PLFA (%)										
12:0	-	-	-	0.3	0.1	-	0.1	-	-	-
<i>i</i> -13:0	0.1	-	0.1	-	-	-	0.1	-	-	-
13:0	-	-	-	-	-	-	0.1	0.3	-	-
<i>i</i> -14:0	1.8	2.6	2.1	1.0	1.3	-	1.7	3.9	-	-
14:0	0.5	2.2	0.8	1.1	0.6	8.8	0.6	1.1	-	-
<i>i</i> -15:1	-	-	-	0.8	-	-	-	-	1.3	1.5
<i>i</i> -15:0	26.4	12.6	18.4	30.0	24.0	10.2	20.0	24.2	51.5	53.1
<i>ai</i> -15:0	36.8	16.5	34.8	5.1	27.9	4.8	34.1	28.2	9.2	3.0
15:0	16.4	9.3	15.0	2.4	10.5	7.2	12.7	11.1	3.4	1.2
<i>i</i> -16:0	2.4	2.5	3.0	2.7	3.3	-	3.1	4.1	2.3	2.2
<i>ai</i> -16:0	0.2	-	-	1.4	-	-	-	-	-	-
16:0	7.3	24.4	10.1	12.3	9.0	35.1	10.9	12.7	4.5	4.1
10-Me-16:0	0.3	-	-	-	-	-	-	0.9	-	-
<i>i</i> -17:1	-	-	1.3	6.2	1.7	-	1.1	-	4.3	7.3
<i>i</i> -17:0	2.9	4.1	4.1	12.0	5.8	5.6	4.2	5.5	12.3	17.7
<i>ai</i> -17:0	0.9	2.1	1.9	2.8	2.0	-	1.7	1.7	3.2	1.7
17:0	1.3	-	2.0	2.4	2.0	-	1.7	2.6	2.3	2.0
<i>i</i> -18:1	0.3	-	0.4	4.1	0.9	-	-	-	2.5	3.2
18:2	-	-	-	-	0.6	-	-	-	-	-
18:1ω9	0.4	5.8	1.4	3.3	2.4	-	0.9	0.9	-	-
18:1ω7	-	-	0.2	-	0.4	-	-	-	-	-
18:0	1.3	18.0	4.1	11.3	6.2	28.4	5.1	3.0	3.2	2.8
<i>i</i> -19:0	0.3	-	-	-	1.0	-	1.1	-	-	-
<i>ai</i> -19:0	0.1	-	-	-	-	-	-	-	-	-
19:0	0.1	-	-	0.4	0.2	-	-	-	-	-
20:0	-	-	-	0.3	-	-	0.9	-	-	-
Σ <i>n</i> -saturated FA	27.0	53.9	32.0	30.5	28.7	79.5	32.1	30.7	13.4	10.2
Σ <i>n</i> -unsaturated FA	0.4	5.8	1.7	3.3	3.4	-	0.9	0.9	-	-
Σ br-saturated FA	72.3	40.4	64.5	55.1	65.3	20.5	65.9	68.4	78.4	77.8
Σ br-unsaturated FA	0.3	0.0	1.8	11.1	2.6	0.0	1.1	0.0	8.2	12.1
ratio br-FA/saturated FA	2.7	0.7	2.1	2.2	2.4	0.3	2.1	2.2	6.5	8.8

Branched monoenoic FAs such as *i*-15:1, *i*-17:1 and *i*-18:1 increased in total to 8-12 % with the *i*-17:1 dominant. Since branched FAs have similar behaviour to unsaturated FAs in lowering the phase transition temperature of the cell membrane (Silvius & McElhaney, 1979; Silvius *et al.*, 1979), it is assumed that the branched monoenoic FAs also have a strong effect on the membrane phase transition temperature and represent an adaptation to cooler ambient temperature conditions. The branched monoenoic FAs, especially the *i*-17:1 ω 7, are described to originate from SRB (Boon *et al.*, 1977; Parkes & Taylor, 1983; Edlund *et al.*, 1985; Dowling *et al.*, 1986; Dowling *et al.*, 1988; Ollivier *et al.*, 1991; Kohring *et al.*, 1994) and some actinomycetes (White *et al.*, 1996). However, actinomycetes also contain mid-chain branched FAs (White *et al.* 1996 and references therein), which were not observed in our samples. In addition, mineralogical investigation of the filter particles from the cold side during charge mode showed mainly Fe sulfides. This observation confirms the interpretation that SRB were present on the cold side, since the release of their metabolic product H₂S might have caused increased precipitation of iron sulfides. Thus, SRB might at least represent a part of the microbial community on the cold side. Branched monoenoic FAs were not detected on the warm side during normal operation of the plant, indicating that there were at least partly different microbial communities on both sides. The PLFA pattern in F2 (cold side) during the charge mode was interpreted to represent the microbial signal pumped up from the cold side aquifer with the branched FAs (saturated and monoenoic) dominating.

When comparing the PLFA composition from F2 during discharge (Fig. 6.3a and c) and charge mode (Fig. 6.3d), it became obvious that the PLFA patterns differed mainly in the relative proportion of saturated and branched FAs. This is also expressed in the ratio of branched FAs to saturated FAs (Table 6.1). The ratio increased to 8.8 during charge mode vs. discharge mode, with values up to 2.7 for the cold side. Since the temperature was more or less the same in F2 during charge and discharge mode (ca. 46°C), the higher relative abundance of saturated FAs indicated that the microbial community in F2 during the discharge mode appeared to be a mixture of microorganisms coming with the fluid from the warm side (dominated by saturated FAs) and microorganisms prevailing on the cold side (dominated by branched and monoenoic branched FAs).

During the downtime of the plant, the PLFA signal and, therefore, the microbial community on the cold side did not change much, compared with the lipid signal during the discharge

mode (Fig. 6.3 and Table 6.1). Only a slight increase in unsaturated FAs, namely 18:1 ω 9 and 18:1 ω 7 at 2.4 and 0.4 %, and 18:2 FA at 0.6 % were observed.

6.5.2 Characteristics of the Brine: Energy and Carbon Supply for the Indigenous Microbial Biota

The NaCl dominated (average Cl^- concentration 75 g/L; Fig. 6.4) brine is characterized by total dissolved solids at 134 g/L (Kühn *et al.*, 1998; Seibt & Kellner, 2003). Such a high salt content is in accord with the assumption that salinity increases by 10-15 g/L per 100 m depth in the study area (Wolfgramm & Seibt, 2006; Obst & Wolfgramm, 2010). The fluid is slightly acidic with pH values between 5.9 and 6.1 and shows reducing conditions of < -50 mV (standard hydrogen electrode, SHE). The major cation in the fluid is Na^+ , followed by Ca^{2+} , Mg^{2+} and K^+ ; the major anion is Cl^- , followed by SO_4^{2-} and HCO_3^- . Minor and trace components are NH_4^+ , Li^+ , Sr^{2+} , Ba^{2+} , Fe, Mn^{2+} , Zn^{2+} , Pb^{2+} , SiO_4 , H_3BO_3 , as well as I^- , Br^- and F^- .

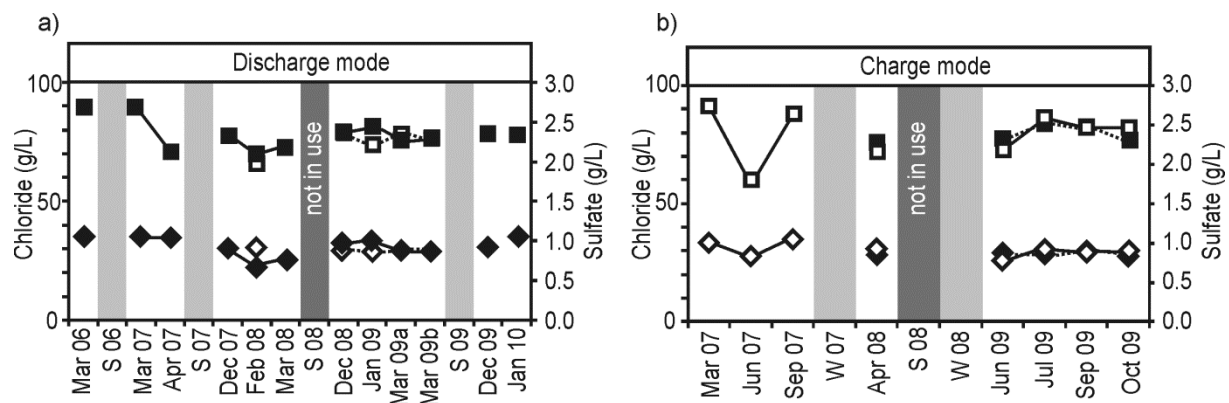


Fig. 6.4: Sulfate (diamonds) and chloride (squares) concentration in the fluid of the heat storage during a) discharge and b) charge mode. The filled symbols represent the fluid from the warm well (tapping point B, see Fig. 6.1), the open symbols the fluid from the cold well (tapping point C, see Fig. 6.1). Between May and December 2008 the heat storage was not in use (summer 08). March 07a, sampling date 6.3.2007; March 07b, sampling date 29.3.2007; March 09a, sampling date 10.3.2009; March 09b, sampling date 31.3.2009; S, summer (charge mode), W, winter (discharge mode).

DOC, a complex mixture of dissolved organic compounds which can be potential electron donors for microbial metabolism, had a wide concentration range from 0.1 to 19 mg C/L in the fluid (Fig. 6.5). SO_4^{2-} , a potential electron acceptor for SRB occurred in sufficient amount (0.7-1.1 g/L) in the fluid (Fig. 6.4). Before the plant was renewed to a heat storage facility in

2003 Möller *et al.* (2008) measured the isotope composition of sulfate ($\delta^{34}\text{S}$ 32.1‰, $\delta^{18}\text{O}$ 20.5‰) at the original aquifer temperature of 54°C and concluded that microbial sulfate reduction occurs at the study site. Since the plant works as a heat storage, isotope measurements showed similar results for SO_4^{2-} on the cold side ($\delta^{34}\text{S}$ 32.2‰, $\delta^{18}\text{O}$ 14.5‰), also confirming the interpretation from the PLFAs that SRB occur on the cold side. Bacterial sulfate reduction to H_2S is reported to occur at a temperatures up to 110°C in hydrothermal vents (Jørgensen *et al.*, 1992). However, in common geological settings bacterial sulfate reduction is observed at temperatures between 0°C and 80°C (Machel, 2001), within the temperature range observed in the heat storage (45°C cold side to 85°C warm side).

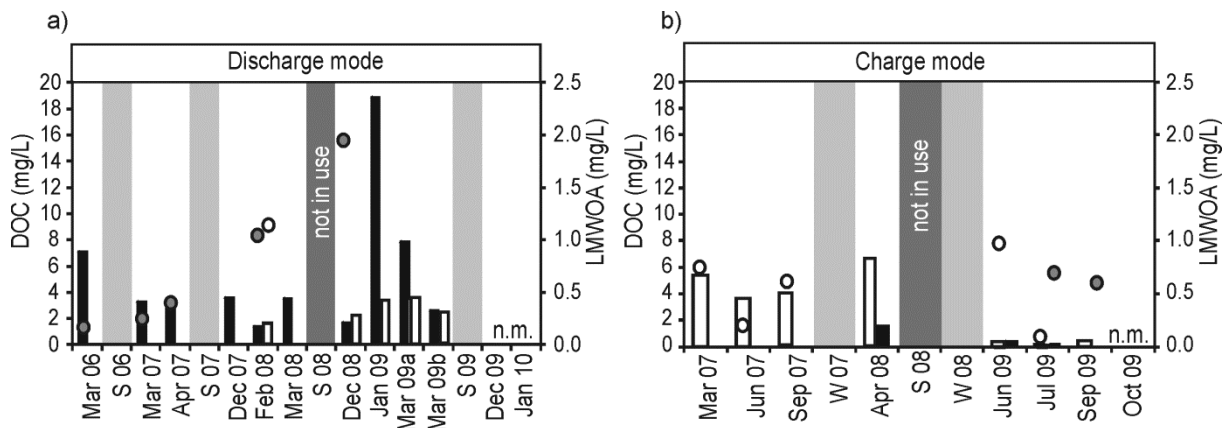


Fig. 6.5: DOC and LMWOAs sum of acetate and formate concentration over the monitoring time during a) discharge and b) charge mode. Black bars give DOC concentration in water from warm well (tapping point B), the open bars DOC in water from the cold well (tapping point C). Grey circles represent sum of formate and acetate from warm side and open circles sum of formate and acetate from cold side. Between May and December 2008 the heat storage was not in use. March 07a, sampling date 6.3.2007; March 07b, sampling date 29.3.2007; March 09a, sampling date 10.3.2009; March 09b, sampling date 31.3.2009; S, summer (charge mode); W, winter (discharge mode). Note, that from October 2009 until January 2010, DOC content was not measured and LMWOAs were not detected.

The total natural gas concentration of the formation water is a maximum 8 ml per 100 ml (8 Vol%). The relative composition of the gas is: CO_2 (86.2 Vol%), N_2 (13.8 Vol%), H_2 (<0.01 Vol%), and CH_4 (0.02 Vol%). In addition to these gases, H_2S was often detected by odour but was below detection limit (Seibt & Thorwart, 2011). Nonetheless, after the downtime of the plant in summer 2008, when the plant was in charge mode, a low H_2S concentration of 210 $\mu\text{g/L}$ was detected on the cold side. With ongoing operation, the concentration of H_2S decreased to 120 $\mu\text{g/L}$. The in-situ measurement of dissolved H_2S was

conducted using the Multi-Sensor measuring instrument MS 08 (AMT Analysenmesstechnik GmbH, Germany).

During the monitoring time the sulfate concentration varied around an average 1 g/L. In general, a significant difference in sulfate concentration between the warm and cold side or charge and discharge mode was not observed (Fig. 6.4). Thus, the suggested higher abundance of SRB on the cold side was not supported in the fluid chemistry. However, the amount of sulfate in the fluid (1 g/L) might have been too large for the indigenous SRB to cause a significant change in sulfate concentration.

A potential carbon source for microbial communities in the geothermal heat storage is DOC. From March 2006 to January 2010, DOC and some low molecular weight organic acids (LMWOAs) in the fluid were monitored. As shown in Fig. 6.5, LMWOAs detected as formate and acetate are occasionally present in the fluid, with an average concentration of 0.5 mg/L. The highest concentration of LMWOAs occurred in December 2008 at 1.9 mg/L after the plant not been in use for 8 months. This high amount might be the result of the stagnating water, where the LMWOAs could accumulate. LMWOAs were not detected in all samples and the highest DOC concentration (19 mg C/L) in January 2009 did not correlate with the highest LMWOA concentration. In general, DOC concentration was on average 3.5 mg C/L. Neither DOC concentration nor LMWOA content showed a correlation with operating modes (charge/discharge) and the related temperatures or the dominating microbial community composition inferred from PLFA analysis.

It might have been expected that the concentration of LMWOAs would be higher on the warm side, since it has been reported that the release of LMWOAs, especially acetate, can increase with rising temperature either to an temperature activation of the organic matter to microbial attack (Wellsbury *et al.*, 1997; Parkes *et al.*, 2007) or by an abiotic thermogenic breakdown of organic matter (Cooles *et al.*, 1987), although for the latter process temperatures in the heat storage might not been high enough. A reason why such effects might not be seen in the Neubrandenburg plant could be that the heat storage represents a highly active and turbulent system (compared with unaffected aquifers), where water is pumped from one side to the other, preventing the establishment of detectable concentration differences.

In summary, unlike the results from PLFA analysis, there was no clear difference in fluid chemistry between the cold and the warm side of the system. Nevertheless, electron donors

(DOC) and acceptors (SO_4^{2-}) for SRB and other heterotrophic microorganisms, e.g. fermentative microorganisms, were available in sufficient amounts.

6.6 Summary and Conclusions

The fluid chemistry and the phospholipid composition of viable microorganisms in the Neubrandenburg heat storage were monitored from March 2006 until January 2010 to obtain a deeper insight into the biogeochemistry of the system and its seasonal and operational variations. Our results suggest that viable microorganisms exist above-ground on both the cold and warm side of the heat storage. Depending on temperature and operating modes the composition of the microbial communities varied.

During the discharge phase of hot water the PLFA pattern on the warm side showed an adaptation to warm temperature conditions (70-73°C), with a dominance of saturated FAs. After the heat exchanger, on the cold side, the PLFA pattern showed a dominance of branched FAs and therefore, an adaptation to cooler temperature (45-48°C). These shifts in the PLFA composition might be a result of adaptation processes and/or different dominating microorganisms on both sides of the heat exchanger being better adapted to the prevailing temperature conditions.

During the downtime of the plant, when the temperature in the above-ground installations dropped to around 20°C, the microbial PLFA composition in the filters suggested that the microbial communities became more equal on both sides, showing a predominance of branched FAs. The dominance of saturated FAs on the warm side was rapidly re-established after continuation of the discharge mode.

During the charge mode, the PLFA pattern on the cold side was interpreted to represent the microbial community from the cold side aquifer. The PLFA signal was distinctly dominated by branched FAs and additionally contained the highest amount of branched monoenoic FAs. Both are known to occur in sulfate-reducing bacteria, which seem to be part of the microbial community on the cold side.

The monitored fluid chemistry (sulfate, DOC) showed no dependence on temperature or operating modes and can therefore not be used to trace variation in the microbial communities in the heat storage. Nevertheless, for microorganisms, there are sufficient nutrients available to serve as carbon and energy sources.

6.7 Acknowledgement

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7 Conclusions

The failure-free operation of geothermal plants is compulsory for an efficient use of this renewable energy technology. Thus, it is important to know the factors influencing the working reliability of geothermal plants. The goal of this study was the evaluation of the contribution of microbial communities on impairments of geothermal plants and, vice versa, investigation of the influence of the operation modes on the indigenous microbial community. In addition, fluid composition in consideration of electron acceptors and donors for microorganisms has been evaluated. To reach this goal, 7 geothermal plants with different applications (heat/cold storage, district heating, power production, and balneology) have been monitored over three years. Here, 65 fluid and 26 filter samples from the North German Basin and 33 fluid samples from the Molasse Basin have been analysed. Filters represent the enrichment of the indigenous microbial communities transported with the fluid throughout the geothermal plants over time of exposition, fluids have been analysed with emphasis on potential electron acceptors and donors and it has been tested if these parameters are indicative for changing plant conditions.

Adaptation Capabilities of the Indigenous Microbial Communities

Temperature

The most influencing factors on the microbial ecosystem in aquifer thermal energy storages are temperature, operation mode, and nutrient availability.

Viable microbial communities have been observed by the detection of intact phospholipids in all investigated ATES systems operating with different temperature regimes. The PLFA inventory was dominated by saturated and monounsaturated FAs in the shallow heat storage Rostock (operation temperature 8-50°C) and by saturated and branched FAs in the deep heat storage Neubrandenburg (operation temperature 45-80°C). Temperature adaptation capabilities were adjusted by these two biosynthetic pathways. The saturated/unsaturated and saturated/branched biosynthetic pathways can only be adjusted by the *de novo* pathway from new produced chains if cells grow and divide (Russell *et al.*, 1995). Thus, it can be concluded that the microbial communities are active in both heat storage systems. In the investigated

shallow cold storage (German Parliament) with operation temperatures of 5-30°C no temperature adaptation of the microbial community could be observed. This could be due to the low temperature range of this cold storage. In comparison, the heat storages cover broader temperature ranges and therefore also a broader range of microorganisms namely mesophilic and thermophilic bacteria. These findings should be taken into account when the working reliability of the plant is deteriorated by biotic mechanisms and cleaning processes should involve a short-time increase in operation temperature to kill the microbial biota. Because, these two categories of bacteria (mesophilic/thermophilic) will presumably have beside their optimum growth temperature a much higher maximum growth temperature as it is provided by the plant conditions.

Operation Mode

In addition to temperature adaptation of the microbial community, changes of the microbial community due to changes in the operation modes in the terms of changes of the fluid flow direction (charge/discharge) has been observed in the deep heat storage in Neubrandenburg. For the cold side with operation temperatures of around 45°C variabilities in PLFA composition occurred. These variations were related to charge and discharge mode of the fluid. PLFA profiles from filters of the warm and cold side indicate a temperature adaptation during discharge mode when water is pumped from the warm side to the cold side. However, during charge mode when the water is pumped from the cold side, the PLFA inventory significantly changed. Specific biomarkers indicate the dominance of sulfate-reducing bacteria (SRB) in these charge mode samples from the cold side. Due to similar temperatures of the aquifer from the cold side with around 45°C and the aquifer temperature before it was used as a heat storage (54°C) it could be likely that these SRB are indigenous from the deep aquifer. This finding has to be taken into account when this stratigraphical unit is used for further geothermal applications because the produced metabolic by-product of SRB is H₂S and this is known to be corrosive for many materials of plant equipments.

Nutrient Status

PLFA patterns of the cold storage (German Parliament) showed a correlation between favourable and unfavourable conditions of certain microorganisms that are associated to iron and sulfur oxidizers. This correlation is expressed in the *trans/cis* ratio of monounsaturated

fatty acids. It has been shown that the *trans/cis* ratio during favourable conditions for the oxidizers is below 0.1 that reflects a healthy population whereas in times of unfavourable conditions the ratio exceeds 0.1. The conversion of *cis* into *trans* fatty acids by isomerization (Heipieper *et al.*, 1994) is a possibility for microorganisms to regulate the membrane fluidity when cells are inhibited for growth and *de novo* biosynthesis of fatty acids. This *cis* into *trans* conversion leads to a more tight compaction of the membrane that prevents the cell from harmful environmental influences (starvation, toxic substances, etc). This adaptation mechanism provides the possibility for microorganism to survive environmental stress. As a consequence, it has to be taken into account that cleaning mechanisms after plant deterioration show maybe not a long-standing effect due to the resistance and protection mechanism of microorganisms.

Participation of Microorganisms in Processes Causing Operation Failures of Plant Installations

Two investigated geothermal energy storages showed deteriorations of the plants during the long-term monitoring. In the cold storage (German Parliament) two clogging events have occurred leading to a reduced injection. Using biomarker analysis it has been shown that a variety of microorganisms contributes to the microbial community including iron- or sulfur-oxidizers and sulfate-reducing bacteria. Furthermore, it has been shown that there is a distinct shift in the microbial community composition in times of reduced injection compared to times of normal plant operation. Hence, the shift in the microbial community composition can be assumed as an evidence of the contribution of microbes in plant deterioration. Therefore, this variation has been expressed in a new biomarker ratio referring to iron- and sulfur-oxidizers by including monounsaturated fatty acids with 16 and 18 carbon atoms as well as sulfate-reducing bacteria by including branched fatty acids with 15, 16, and 17 carbon atoms. This ratio can be a helpful tool to distinguish between biotic and abiotic clogging processes for the investigated cold storage and it supports the decision of selected cleaning mechanisms (e.g. mechanical cleaning vs chemical disinfection).

For the investigated deep heat storage corrosion phenomena have occurred on the pump equipment of the cold side. According to biomarker analysis, PLFA profiles from the cold side during charge mode indicate the presence of SRB. Anaerobic microbiologically

influenced corrosion on iron and steel is a known phenomenon and SRB has been recognised as the most significant contributors to biocorrosion (Coetser & Cloete, 2005). Thus, it can be assumed that the corrosion phenomena can be associated with the presence of SRB in the brine.

General Fluid Chemistry: changes with Depth, over Time, and with Operation Mode.

North German Basin-with Depth

Fluid chemistry of electron acceptors and donors were specific to the investigated study sites. Sulfate and DOC concentration of the shallow aquifers (Rostock, German Parliament) are surely influenced by the overlaying till units rich in sulfate and carbon (Keller & Van der Kamp, 1988; Wassenaar *et al.*, 1990b). Hence, the results of the shallow heat storage in depth down to 30 m showed a higher sulfate concentration compared to the cold storage down to 60 m because of the overlaying till with thickness of around 15 m (Seibt, 2002). In contrast, it has been shown that DOC concentration was around 2 mg C/L higher at the study site of the cold storage compared to the shallow heat storage. These findings for the deeper cold storage could be due to the thin and/or absent overlaying till layer. Therefore recharge of groundwater should be higher (although it is still low) and a constant influx of natural organic carbon could be the reason for the higher observed DOC. Interestingly isotope composition of the DOC seemed to be relative similar of these two energy storages and no correlation with depth is observed. The deep heat storage (Neubrandenburg) exhibits the highest sulfate and the most variable DOC content compared to the fluids of the shallow energy storages. The sulfate and DOC concentrations in the deep aquifer are mainly influenced by water-rock interactions (e.g. dissolution of sulfate salts), seawater evaporation (trapped fossil water) and oxidation of sedimentary organic matter respectively.

Therefore, fluid composition can be used for site declaration and give indications for the prevailing redox-reaction providing energy for microorganisms. All investigated study sites contained as most important electron acceptor sulfate and DOC as most important electron donor to sustain microbial life. This creates favourable conditions for sustaining life of SRB in subsurface and associated plants as shown that biomarkers for SRB were present in all investigated sites. The turnover of the available substrates by microorganisms can lead to deteriorate plant conditions.

Molasse Basin-with Depth

It has been shown that the concentration of DOC increased with increasing depth of the Malm aquifer. Additionally, the composition of DOC changed from dominating LMWNS in shallower depth to building blocks, as a transition zone, to LMWOA in the deeper parts of the Malm aquifer. Below depths of 2200 m the dominant organic acid is acetate. In general, with increasing burial depth the sedimentary organic matter becomes more recalcitrant and with this, the water-amenable organic structures will change. With the transition of organic matter structures towards expulsion of hydrocarbons, the generation of organic acids is assumed to occur before, at temperatures above 50°C (Seewald, 2003). In addition, crude oil has been described and observed to occur at the investigated deep sites of the Malm aquifer. Thus, the presence of buried sedimentary organic matter as well as crude oil, explain the occurrence of LMWOA in formation waters of the Malm aquifer.

Operation Mode and Time

The applied parameters showed variabilities or dependencies to the individual operating modes in the investigated geothermal plants. In the shallow heat storage system, the microbial community responded to temperature changes within the cycles. In the deep heat storage every operation cycle with its individual temperature ranges inhabits well adapted microbial communities that were specific to the operation modes and temperature sides. Variations in fluid chemistry have been observed in the deep geothermal plants of the Molasse Basin that were different for the start-up phase, initial phase, and regular plant operation. Additionally, changes in fluid chemistry with flow throughout the heat exchanger indicate biotic or abiotic processes that are related to changes in temperature.

Indicative Tools for the Assessment of the Working Reliability of Geothermal Plants

In the scope of this thesis it has been shown that certain chemical and biological parameters are useful tools for the assessment of the operational state of the plant. Long-term monitoring of PLFA composition was very helpful for the differentiation of biotic and abiotic clogging processes in the shallow cold storage. In addition, PLFA composition in the deep heat storage revealed the presence and dominance of sulfate-reducing bacteria within the microbial community. The activity of SRB can lead to the observed corrosion processes. Fluid

composition in the North German Basin as concluded above is valuable for general site description and provides information for the possible redox-reactions by microorganisms that can but not must change the fluid equilibrium. However, evaluation of electron acceptors and donors during the long-term monitoring on individual sites revealed no clear correlation to plant failures. Slight variations occurred during the clogging event in the German Parliament but only at the moment where clogging already happened. While at site Neubrandenburg no correlation to fluid composition and corrosion phenomena has been observed. This is maybe due to the continuous input of nutrients by pumping high volumes of water through the plant, hence, variabilities in fluid chemistry is not visible. Therefore, fluid composition seemed to be not suitable as indicators for changing plant conditions.

Fluid composition of the Molasse Basin showed variabilities especially changes in the DOC concentration and composition on individual plants. This provides us information about the operational state of such plants and can be helpful as indicator in consideration of the steady state conditions of the plant.

Concerning fluid and microbial community composition of the investigated geothermal plants, individual parameters were developed for assessment of the working reliability. These investigations represent a solid base for the improved understanding of biogeochemical processes in geothermal plants.

8 References

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Appendix – Geothermal Plants

Study sites in the Molasse Basin

All tables include selected fluid chemistry data.

Study site	M1	M2	M2	
Sampling date	Mar 09	Mar 09	Oct 09	
Tapping point	production well	before HE	before HE	after HE
Cl ⁻ (mg/L)	2	72	93	87
SO ₄ ²⁻ (mg/L)	12	27	17	12
C1 (mg/L)	-	<0.1	<0.1	<0.1
C2 (mg/L)	-	-	-	-
DOC(mg C/L)	0.7	0.4	0.3	0.4
δ ¹³ C _{DOC} (‰)	n.m.	n.m	-31.5	-31.8
HOC (% of DOC)	9	23	17	24
LMWOA (% of DOC)	-	14	8	11
LMWNS (% of DOC)	76	33	38	39
BB (% of DOC)	6	27	36	25
Bio (% of DOC)	-	3	1	1
HS (% of DOC)	10	-	-	-

Study site	M3		M3	
Sampling date	Mar 09		Oct 09	
Tapping point	before HE	after HE	before HE	after HE
Cl ⁻ (mg/L)	167	165	177	189
SO ₄ ²⁻ (mg/L)	7	4	4	4
C1 (mg/L)	<0.1	<0.1	0.2	<0.1
C2 (mg/L)	0.4	0.3	0.7	0.7
DOC (mg C/L)	0.7	1.3	1.0	0.8
δ ¹³ C _{DOC} (‰)	n.m	n.m.	-31.2	-31.2
HOC (% of DOC)	59	n.m.	1	24
LMWOA (% of DOC)	15	n.m.	31	24
LMWNS (% of DOC)	9	n.m.	26	18
BB (% of DOC)	-	n.m.	41	34
Bio (% of DOC)	-	n.m.	0.2	0.2
HS (% of DOC)	-	n.m.	-	-

Appendix

Study site	M4							
Sampling date	Apr 08	Mar 08	Sep 08		Mar 09		Oct 09	
Tapping point	before HE	after HE	before HE	after HE	before HE	after HE	before HE	after HE
Cl ⁻ (mg/L)	280	276	262	262	293	306	231	264
SO ₄ ²⁻ (mg/L)	37	32	30	30	39	38	33	38
C1 (mg/L)	1.2	0.9	1.9	2.0	0.9	0.8	0.9	1.0
C2 (mg/L)	15.1	16.8	14.4	14.2	9.7	10.0	11.1	12.6
C3 (mg/L)	2.2	3.1	3.0	2.8	2.3	2.4	2.0	2.3
C4 (mg/L)	0.6	0.7	0.5	0.6	0.6	0.6	0.4	0.6
C5 (mg/L)	-	-	-	-	0.2	0.2	-	-
DOC (mg C/L)	12.3	19.3	10.3	11.8	11.9	11.6	7.3	6.4
δ ¹³ C _{DOC} (‰)	-22.5	-24.9	-16.0	-20.9	-24.9	-21.6	-25.7	-25.0
HOC (% of DOC)	n.m.	n.m.	n.m.	13	18	n.m.	7	-
LMWOA (% of DOC)	n.m.	n.m.	n.m.	83	50	n.m.	78	89
LMWNS (% of DOC)	n.m.	n.m.	n.m.	3	30	n.m.	10	7
BB (% of DOC)	n.m.	n.m.	n.m.	-	2	n.m.	6	6
Bio (% of DOC)	n.m.	n.m.	n.m.	-	0.4	n.m.	-	0.1
HS (% of DOC)	n.m.	n.m.	n.m.	-	-	n.m.	-	-

Study site	M 5 test of injection well		M 5 test of production well	
Sampling date	Jan 07		May 07	
Tapping point	injection well	downhole sample	before HE	after HE
Cl ⁻ (mg/L)	110	10970	113	100
SO ₄ ²⁻ (mg/L)	30	11	33	29
NO ₃ ⁻ (mg/L)	1.0	1.5	-	-
C1 (mg/L)	1.6	1.2	0.1	0.3
C2 (mg/L)	7.0	10.1	0.8	-
C3 (mg/L)	-	-	0.3	0.4
C4 (mg/L)	-	-	-	-
DOC (mg C/L)	10.0	9.5	3.5	4.9
δ ¹³ C _{DOC} (‰)	-24.5	-25.7	-28.3	-22.2
HOC (% of DOC)	n.m.	n.m.	n.m.	n.m.
LMWOA (% of DOC)	n.m.	n.m.	n.m.	n.m.
LMWNS (% of DOC)	n.m.	n.m.	n.m.	n.m.
BB (% of DOC)	n.m.	n.m.	n.m.	n.m.
Bio (% of DOC)	n.m.	n.m.	n.m.	n.m.
HS (% of DOC)	n.m.	n.m.	n.m.	n.m.

Study site	M 5					
Sampling date	Mar 09			Oct 09		
Tapping point	before HE	after HE	before injection	before HE	after HE	before injection
Cl ⁻ (mg/L)	99	100	99	104	112	104
SO ₄ ²⁻ (mg/L)	27	27	32	32	35	31
NO ₃ ⁻ (mg/L)	-	-	-	-	-	-
C1 (mg/L)	0.3	0.3	0.3	0.2	0.3	0.3
C2 (mg/L)	2.2	2.2	2.2	2.7	3.1	2.7
C3 (mg/L)	0.4	0.5	0.4	0.3	0.4	0.3
C4 (mg/L)	0.1	0.1	0.1	0.1	0.1	0.1
DOC (mg C/L)	2.3	1.7	2.4	1.9	1.9	2.0
δ ¹³ C _{DOC} (‰)	-23.1	-22.5	-21.1	-29.6	-29.6	-29.3
HOC (% of DOC)	n.m.	20	n.m.	17	17	19
LMWOA (% of DOC)	n.m.	66	n.m.	67	68	66
LMWNS (% of DOC)	n.m.	8	n.m.	8	9	9
BB (% of DOC)	n.m.	6	n.m.	8	6	7
Bio (% of DOC)	n.m.	0.1	n.m.	-	0.1	0.3
HS (% of DOC)	n.m.	-	n.m.	-	-	-

Study site	M 5						
Sampling date	Nov 07	July08	Sep 08	Oct 08	Dec 08	Jan 09	Feb 09
Tapping point	after HE						
Cl ⁻ (mg/L)	96	98	99	97	106	99	93
SO ₄ ²⁻ (mg/L)	23	27	23	24	29	23	25
C1 (mg/L)	-	0.5	0.6	0.2	0.2	0.3	0.2
C2 (mg/L)	2.5	2.8	4.4	3.2	2.4	2.5	2.1
C3 (mg/L)	0.3	0.5	0.7	0.5	0.4	0.5	0.3
C4 (mg/L)	-	0.2	-	0.1	0.2	0.1	-
C5 (mg/L)	-	<0.1	-	-	-	-	-
DOC (mg C/L)	7.6	2.4	1.8	2.4	1.8	2.3	2.3
δ ¹³ C _{DOC} (‰)	-25.9	-23.8	-23.9	-22.3	-22.0	-22.6	-22.3
HOC (% of DOC)	5	44	21	n.m.	16	n.m.	n.m.
LMWOA (% of DOC)	62	45	65	n.m.	72	n.m.	n.m.
LMWNS (% of DOC)	34	9	14	n.m.	11	n.m.	n.m.
BB (% of DOC)	-	-	-	n.m.	-	n.m.	n.m.
Bio (% of DOC)	-	-	-	n.m.	1	n.m.	n.m.
HS (% of DOC)	-	-	-	n.m.	-	n.m.	n.m.

Appendix – Energy Storages

Heat storage - Rostock:

Sampling time	Operating mode	T (°C)	Cl ⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	NO ₃ ⁻ (mg/L)	DOC (mg C/L)	Formate (mg/L)	Acetate (mg/L)	δ ¹³ C _{DOC} (‰)
July 2007	charge	11.7	70	271	0.8	1.2	<0.5	<0.5	-27.9
Sep 2007	charge	10.9	53	233	2.9	2.6	-	-	-25.4
Nov 2007	depth sample	n.m.	60	225	0.1	5.0	0.2	0.2	-27.3
Mar 2008	discharge	13.7	71	253	-	3.3	-	-	-27.9
May 2008	charge	10.2	65	226	-	2.4	-	-	-27.4
Nov 2008	discharge	20.9	58	206	-	2.9	-	-	-27.2
Dec 2008	discharge	15.9	40	129	-	1.2	-	-	-27.5

Table E1: Selected hydrochemical data of Rostock.

PLFA (%)	Discharge	
	50.0-20.9°C	20.0-13.7°C
C _{12:0}	0.5	-
<i>br</i> -C _{13:0}	1.1	-
C _{13:0}	0.5	1.8
<i>i</i> -C _{14:0}	2.0	-
C _{14:0}	6.3	2.0
<i>i</i> -C _{15:0}	12.0	5.2
<i>ai</i> -C _{15:0}	13.6	6.1
C _{15:1}	1.1	-
C _{15:0}	2.5	2.5
<i>i</i> -C _{16:0}	2.1	-
C _{16:1}	10.2	20.5
C _{16:0}	16.6	21.8
<i>mcb</i> -C _{17:0}	6.6	18.4
<i>i</i> -C _{17:0}	1.5	0.7
<i>ai</i> -C _{17:0}	2.0	1.8
C _{17:0}	1.9	2.4
C _{18:1}	3.1	7.3
C _{18:0}	14.1	6.7
C _{19:0}	-	2.9
C _{20:0}	2.3	-

Table E2: Relative proportion (%) of the phospholipid fatty acid (PLFA) composition of Rostock.

IPL compound classes		PG	PG	PG	PE	PE	PE	PC	PC	PC
Number of carbon and double bonds of linked FA	Linked FA combinations	20.9°C (8.9%)	13.7°C (6.2%)	10.2°C (2.9%)	20.9°C (66.4%)	13.7°C (88.3%)	10.2°C (89.1%)	20.9°C (24.7%)	13.7°C (5.4%)	10.2°C (8.0%)
C _{29:0}	15:0/14:0	-	-	-	-	X	-	-	-	-
C _{30:2}	15:1/15:1	-	-	-	-	-	-	X	-	-
	16:1/14:1	X	-	-	-	-	-	-	-	-
C _{30:0}	15:0/15:0	X	-	-	X	X	X	X	-	-
C _{31:2}	16:1/15:1	-	-	-	-	X	X	-	-	-
C _{31:1}	16:1/15:0	-	-	-	X	X	X	-	-	-
	16:0/15:1	-	-	-	-	X	X	-	-	-
C _{31:0}	15:0/16:0	-	-	-	-	X	-	-	-	-
C _{32:2}	16:1/16:1	X	X	X	X	X	X	X	-	-
C _{32:1}	16:1/16:0	-	-	-	X	X	X	-	-	-
	15:0/17:1	-	-	-	X	-	X	-	-	-
C _{32:0}	16:0/16:0	-	X	-	X	X	X	X	-	-
	17:0/15:0	-	-	-	X	X	-	X	-	-
C _{33:2}	16:1/17:1	-	-	-	X	X	X	-	-	-
C _{33:1}	17:0/16:1	-	-	-	X	X	X	-	-	-
	17:1/16:0	-	-	-	X	X	X	-	-	-
	15:0/18:1	-	-	-	X	-	-	-	-	-
C _{33:0}	17:0/16:0	X	-	-	X	X	X	X	-	-
	15:0/18:0	-	-	-	-	X	-	-	-	-
C _{34:2}	16:1/18:1	X	X	-	X	X	X	-	-	-
	17:1/17:1	-	X	-	X	X	X	X	-	-
C _{34:1}	16:0/18:1	-	-	-	X	X	-	-	-	-
	17:0/17:1	X	-	-	X	X	-	-	-	-
	16:1/18:0	-	-	-	X	-	-	-	-	-
C _{34:0}	17:0/17:0	X	X	-	X	X	X	X	-	-
	15:0/19:0	-	-	-	X	-	X	-	-	-
C _{35:2}	16:1/19:1	-	-	-	-	X	-	-	-	-
	17:1/18:1	-	-	-	-	X	-	-	-	-
C _{36:2}	18:1/18:1	-	X	-	X	X	X	-	-	X
	19:1/17:1	-	-	-	-	X	-	-	-	-
C _{36:0}	17:0/19:0	-	-	-	-	X	-	X	X	-
	18:0/18:0	-	-	-	X	-	-	-	X	-
C _{37:1}	19:0/18:1	-	-	-	-	X	-	-	-	-
C _{38:2}	19:1/19:1	-	-	-	X	-	-	-	X	-
C _{38:0}	19:0/19:0	-	-	-	-	X	-	-	-	-

Table E3: Intact phospholipids (IPLs) and fatty acid side chains of Rostock.

	M+	Compound	20.9°C (%)	13.7°C (%)	10.2°C (%)
			EDM	LDM	CM
archaeal etherlipids	1302	Caldarchaeol	37.2	30.8	36.6
	1300	I	13.2	12.1	10.9
	1298	II	9.9	12.9	11.2
	1296	III	5.7	5.8	6.2
	1294	IV	12.1	13.0	12.2
	1292	Crenarchaeol	21.9	25.4	23.0
bacterial etherlipids	1050	V	17.9	25.1	14.9
	1036	VI	15.1	19.1	18.8
	1022	VII	38.7	40.4	51.3
	1020	VIII	24.4	11.6	8.1
	1018	IX	2.1	2.1	2.1
	1046	X	0.2	0.6	1.4
	1034	XI	1.5	1.1	3.4
	1032	XII	0.1	-	-

Table E4: Etherlipids of archaeal and bacterial origin of Rostock.

Cold storage - German Parliament:

Sampling time	Operating mode	T (°C)	Cl ⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	NO ₃ ⁻ (mg/L)	DOC (mg C/L)	Formate (mg/L)	Acetate (mg/L)	δ ¹³ C _{DOC} (‰)
May 07	discharge	8	63	221	-	3.8	-	-	-26.7
Aug 07	discharge	10	57	208	1	5.9	0.1	-	-26.7
Mar 08	charge	16	57	194	-	3.7	-	0.2	-27.3
May 08	discharge	9	59	196	-	5.2	-	-	-27.2
Jun 08	discharge	11	59	198	-	4.8	-	-	-27.0
Jul 08	discharge	10	59	202	-	4.9	-	-	-26.9
Aug 08	discharge	10	59	201	-	6.2	-	-	-26.8
Sep 08	discharge	13	56	169	-	3.9	-	-	-26.6
Oct 08	discharge	10	59	185	-	3.9	-	-	-27.2
Nov 08	discharge	n.m.	50	164	-	4.1	-	-	-27.4
Dec 08	charge	16	60	195	-	4.0	-	-	-27.4
Jan 09	charge	16	56	189	-	3.6	-	-	-27.0
Feb 09	charge	15	59	202	<0.1	4.0	-	-	-27.3
Mar 09	charge	14	60	210	-	3.8	<0.1	-	-27.4
Apr 09	charge	n.m.	59	208	-	3.9	-	-	-28.0

Table E5: Selected hydrochemical data of the plant fluid of the German Parliament.

Wells	Aquifer	Operating mode	Cl ⁻ (mg/L)	SO ₄ ²⁻ (mg/L)	NO ₃ ⁻ (mg/L)	DOC (mg C/L)	Formate (mg/L)	Acetate (mg/L)	δ ¹³ C (‰)
warm side	semi-confined								
KS 1		charge	64	197	0.2	4.3	-	-	-27.8
KS 3		discharge	62	215	-	3.9	-	-	-27.3
KS 5		discharge	61	206	1.6	4.0	-	-	-27.3
KS 7		discharge	62	210	<0.5	3.6	-	-	-27.2
KS 9		charge	60	197	0.2	4.3	-	-	-27.0
on average			62	205	0.7	4.0	-	-	-27.3
cold side	phreatic								
KS 2		discharge	58	274	-	4.2	<0.5	<0.5	-27.2
KS 4		discharge	56	248	-	3.9	<0.5	<0.5	-26.8
KS 6		discharge	57	243	1.6	3.8	<0.5	<0.5	-26.9
KS 8		discharge	62	214	-	3.7	-	-	-28.3
KS 10		discharge	58	265	1.0	3.5	<0.5	<0.5	-28.3
on average			58	249	1.3	3.8	<0.5	<0.5	-27.5
observation wells									
P 1	semi-confined	discharge	57	194	<0.1	4.3	<0.1	<0.1	-27.3
P 2	semi-confined	discharge	48	138	<0.1	4.9	<0.1	<0.1	-27.4
P 3	phreatic	discharge	57	164	<0.1	4.2	<0.1	<0.1	-27.4
P 4	phreatic	discharge	54	138	1.3	5.4	<0.1	<0.1	-27.0
on average			54	159	0.4	4.7	<0.1	<0.1	-27.3

Table E6: Selected hydrochemical data from wells of the German Parliament.

Sampling time	Operating mode	DOC (mg C/L)	δ ¹³ C (‰)	HS (%)	BB (%)	NC (%)	HOC (%)	LMWOA (%)	Bio (%)
Jan 06	charge - NOOP	4.0	-	55	21	11	6	1.4	0.1
Sep 08	discharge - RI	3.9	-26.6	66	17	13	2	0.6	0.6
Jan 09	charge - NOOP	3.6	-27.0	69	16	14	0.5	-	-
Apr 09	discharge - NOOP	3.9	-28.0	64	20	13	2	0.4	0.3

Table E7: Concentration, isotope composition, and size fraction of DOC.

Sampling time	Operating mode	PG (%)	PE (%)	PC (%)
Aug 06	discharge - RI	9.1	88.2	2.7
Sep 06	discharge - RI	8.5	88.3	3.2
Oct 06	discharge - RI	13.8	84.6	1.7
Nov 06	discharge - NOOP	18.6	78.8	2.6
Jul 07	discharge - NOOP	18.9	74.6	6.4
Aug 07	discharge - NOOP	9.9	81.2	8.9
Nov 07	charge - NOOP	11.1	83.8	5.0
Jul 08	discharge - RI	10.9	86.6	2.5
Sep 08	discharge - RI	14.8	82.9	2.4
Oct 08	discharge - RI	19.9	77.1	3.0
Nov 08	discharge - NOOP	14.4	81.3	4.3
Feb 09	charge - NOOP	12.3	83.4	4.3
Aug 09	discharge - NOOP	22.5	71.7	5.8

Table E8: Intact phospholipid compound classes.

Appendix

IPL compound		PG	PG	PE	PE	PC	PC
Number of carbon and double bonds of linked FA	Linked FA combinations	Jul 07 (18.9%)	Aug 07 (9.9%)	Jul 07 (74.6%)	Aug 07 (81.2%)	Jul 07 (6.4%)	Aug 07 (8.9%)
C _{28:0}	14:0/14:0	-	-	X	X	X	-
	15:0/13:0	-	-	-	X	-	-
C _{29:1}	14:0/15:1	-	X	X	X	-	-
	14:1/15:0	-	-	X	X	-	-
	16:1/13:0	-	-	X	X	-	-
C _{29:0}	15:0/14:0	-	X	X	-	X	-
C _{30:2}	16:1/14:1	X	-	X	-	-	-
	15:1/15:1	-	-	X	-	-	-
C _{30:1}	16:1/14:0	X	X	X	X	-	-
	16:0/14:1	X	X	X	X	-	-
	15:1/15:0	-	X	X	X	-	-
C _{30:0}	15:0/15:0	X	X	X	X	-	-
	14:0/16:0	-	-	X	X	-	-
C _{31:2}	15:1/16:1	-	X	X	X	-	-
	17:1/14:1	-	-	X	-	-	-
C _{31:1}	16:1/15:0	-	X	X	X	-	X
	14:0/17:1	-	X	X	-	-	-
	16:0/15:1	-	-	X	X	-	-
C _{31:0}	16:0/15:0	X	-	X	X	-	-
	17:0/14:0	-	-	X	-	-	-
C _{32:2}	16:1/16:1	X	X	X	X	X	X
	16:0/16:2	X	-	-	-	-	-
	17:1/15:1	-	-	X	-	-	-
C _{32:1}	16:1/16:0	X	X	X	X	-	-
	15:0/17:1	-	-	X	X	-	-
C _{32:0}	16:0/16:0	-	X	X	X	-	-
	15:0/17:0	-	X	X	X	-	-
C _{33:2}	16:1/17:1	X	X	X	X	-	-
	18:1/15:1	X	-	-	-	-	-
C _{33:1}	17:0/16:1	X	X	X	X	-	-
	17:1/16:0	X	X	-	X	-	-
	18:1/15:0	X	-	X	-	-	-
C _{33:0}	16:0/17:0	X	-	X	X	-	X
	18:0/15:0	X	-	X	X	-	-
C _{34:2}	18:1/16:1	X	X	X	X	X	X
	17:1/17:1	X	-	X	X	-	-
	18:2/16:0	X	-	-	-	-	-
C _{34:1}	16:1/18:0	X	-	X	X	X	-
	18:1/16:0	X	X	X	X	-	X
	17:0/17:1	-	-	X	X	-	-
	15:0/19:1	-	-	-	X	-	-
C _{34:0}	17:0/17:0	X	-	X	X	-	-
	16:0/18:0	-	X	-	-	-	-
C _{35:2}	19:1/16:1	X	-	X	-	-	-
	18:1/17:1	X	-	X	X	-	-
C _{35:1}	19:1/16:0	-	-	X	X	-	-
	18:0/17:1	-	-	-	X	-	-
	20:1/15:0	-	-	-	X	-	-
C _{36:2}	18:1/18:1	X	X	X	X	X	X
C _{36:1}	17:1/19:0	-	-	X	-	-	-
	16:0/20:1	-	-	-	-	X	-
C _{36:0}	18:0/18:0	-	-	-	-	X	-
C _{37:2}	17:1/20:1	-	-	X	-	-	-
	18:1/19:1	-	-	-	X	-	-
C _{37:1}	19:0/18:1	X	-	-	-	-	-
C _{38:2}	19:1/19:1	-	-	X	-	X	X

Table E9: Intact phospholipids (IPLs) and fatty acid side chains of the German Parliament.

Table E10: Relative proportion (%) of the phospholipid fatty acid (PLFA) composition of the German Parliament.

sampling time	Aug 06	Sep 06	Oct 06	Nov 06	Jul 07	Aug 07	Nov 07	Jul 08	Sep 08	Oct 08	Nov 08	Feb 09	Aug 09
operating mode	DC	DC	DC	DC	DC	DC	C	DC	DC	DC	DC	C	DC
	RI	RI	RI	NOOP	NOOP	NOOP	NOOP	RI	RI	RI	NOOP	NOOP	NOOP
PLFA (%)													
br-13:0	-	-	-	-	-	-	-	-	0.1	-	0.1	0.3	-
13:0	-	0.1	-	-	-	0.6	-	-	0.1	0.1	0.2	0.1	-
br-14:0	-	0.1	-	-	-	1.2	0.8	0.1	0.1	0.4	0.7	0.5	-
14:1	-	0.2	-	-	-	-	-	0.3	0.2	-	-	0.8	-
14:0	0.7	0.6	-	2.4	0.7	1.6	1.0	0.6	0.6	0.7	1.2	1.8	3.6
meh-15:0	-	-	-	-	-	0.3	-	0.1	0.1	-	-	0.2	-
i-15:0	0.6	0.6	0.4	3.0	2.7	3.4	2.7	0.5	0.3	1.1	2.2	2.3	5.2
ai-15:0	1.1	0.8	0.6	7.1	2.7	6.2	2.7	0.9	0.6	0.9	3.3	3.6	5.6
15:1	0.2	-	-	0.9	0.7	-	-	-	0.1	-	-	0.8	-
15:0	0.2	0.3	-	2.0	0.5	0.2	0.8	0.1	0.2	0.3	0.4	0.8	2.6
i-16:0	-	0.2	-	2.2	0.6	0.9	0.7	0.1	0.1	0.3	0.9	0.6	-
16:1 ω 7c	64.3	68.0	64.7	19.2	42.1	33.9	29.0	67.6	66.2	55.7	44.1	31.3	38.1
16:1 ω 7t	2.9	3.5	8.0	2.7	4.8	4.1	5.5	2.3	1.7	3.4	5.5	3.6	-
16:1 ω 5c	10.5	5.5	3.5	10.0	15.2	12.6	10.4	2.6	2.0	3.9	5.2	14.7	16.7
16:0	15.9	11.3	12.2	33.0	17.1	17.2	21.4	13.9	11.9	14.0	13.8	16.3	28.3
10-Me-16:0	-	0.5	-	2.7	1.0	1.8	1.6	0.5	0.7	0.7	2.0	1.3	-
i-17:0	-	0.1	-	0.6	0.6	0.9	0.8	0.1	0.1	0.2	0.4	0.5	-
ai-17:0	-	0.1	-	1.7	0.6	0.8	0.9	0.1	0.1	0.2	0.6	0.7	-
17:1	-	0.5	-	1.9	1.6	1.8	2.0	0.8	0.5	1.3	2.3	3.6	-
17:0	0.6	0.1	-	1.1	0.8	0.5	0.7	0.1	0.1	0.1	0.4	0.6	-
br-18:0	-	-	-	-	-	-	1.2	-	-	-	-	-	-
18:1 ω 9c	-	-	-	-	-	-	1.6	0.1	0.1	-	-	0.8	-
18:1 ω 7c	2.9	7.0	9.0	4.3	7.4	8.6	6.7	9.0	13.7	14.1	12.1	12.4	-
18:0	0.1	0.3	1.6	2.3	1.0	3.2	9.5	0.1	0.4	2.6	3.5	1.4	-
19:1	-	-	-	1.7	-	-	-	0.1	-	-	0.8	0.2	-
19:0	-	-	-	1.0	-	-	-	-	0.1	-	-	-	-
20:0	-	-	-	-	-	-	-	-	-	0.1	0.2	0.2	-
\sum i-15:0 + ai-15:0 + 10-Me-16:0 + i-17:0 + ai-17:0	1.8	2.1	1.0	15.1	7.7	13.2	8.7	2.0	1.8	3.1	8.6	8.4	10.8
\sum 16:1 ω 7c + 16:1 ω 7t + 16:1 ω 5c + 18:1 ω 9c + 18:1 ω 7c	80.5	84.0	85.3	36.2	69.4	59.2	53.2	81.6	83.6	77.1	66.9	62.9	54.8
\sum n-saturated	17.5	12.7	13.8	41.9	20.0	22.8	33.5	14.9	13.3	17.8	19.5	21.1	34.4
ratio 16:1 ω 7t / 16:1 ω 7c	0.05	0.05	0.12	0.14	0.11	0.12	0.19	0.03	0.03	0.06	0.12	0.11	-
ratio (16:1 ω 7c + 16:1 ω 7t + 16:1 ω 5c + 18:1 ω 9c + 18:1 ω 7c) / (i-15:0 + ai-15:0 + 10-Me-16:0 + i-17:0 + ai-17:0)	45.8	39.4	88.9	2.4	9.1	4.5	6.1	40.5	47.5	25.0	7.8	7.5	5.1

Heat storage Neubrandenburg:

Sampling time	Operating mode	Tapping point	T (°C)	Cl ⁻ (g/L)	SO ₄ ²⁻ (g/L)	DOC (mg C/L)	Formate (mg/L)	Acetate (mg/L)
March 06	discharge	warm side	72	89.1	1.0	7.1	-	0.2
		cold side	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
March 07a	discharge	warm side	73	87.2	1.1	3.3	0.1	0.1
		cold side	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
March 07b	charge	warm side		n.m.	n.m.	n.m.	n.m.	n.m.
		cold side	47	91.3	1.0	5.4	0.1	0.6
Apr 07	discharge	warm side	54	71.0	1.0	3.3	0.1	0.3
		cold side	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
June 07	charge	warm side	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
		cold side	46	59.7	0.8	3.5	-	0.2
Sep 07	charge	warm side	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
		cold side	46	87.7	1.1	3.9	0.127	0.499
Dec 07	discharge	warm side	75	77.5	0.9	3.6	-	-
		cold side	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Feb 08	discharge	warm side	73	69.9	0.7	1.4	0.3	0.8
		cold side	46	65.6	0.9	1.7	0.4	0.8
March 08	discharge	warm side	73	72.8	0.8	3.5	-	-
		cold side	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Apr 08	charge	warm side	80	75.6	0.9	1.6	-	-
		cold side	47	71.9	0.9	6.6	-	-
May 08 - Dec 08	not in use							
Dec 08	discharge	warm side	71	78.5	0.9	1.7	1.9	-
		cold side	47	78.2	0.9	2.2	-	-
Jan 09	discharge	warm side	70	80.9	1.0	18.9	-	-
		cold side	45	73.5	0.9	3.1	-	-
March 09a	discharge	warm side	68	75.5	0.9	7.9	-	-
		cold side	48	77.3	0.9	3.4	-	-
March 09b	discharge	warm side	66	76.6	0.9	2.6	-	-
		cold side	51	76.8	0.9	2.3	-	-
June 09	charge	warm side	78	76.5	0.8	0.4	-	-
		cold side	48	72.7	0.9	0.1	0.4	0.6
July 09	charge	warm side	78	83.8	0.9	0.2	0.1	0.6
		cold side	46	85.8	0.9	0.1	<0.1	-
Sep 09	charge	warm side	78	82.7	0.9	-	-	-
		cold side	46	82.1	0.9	0.1	<0.6	-
Oct 09	charge	warm side	n.m.	76.0	0.9	<0.1	-	-
		cold side	46	81.9	0.8	<0.1	-	-
Dec 09	discharge	warm side	n.m.	78.2	0.9	<0.1	-	-
		cold side	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.
Jan 10	discharge	warm side	n.m.	78.0	1.0	<0.1	-	-
		cold side	n.m.	n.m.	n.m.	n.m.	n.m.	n.m.

Table E11: Selected hydrochemical data of the plant fluid of Neubrandenburg.

Table E12: Relative proportion (%) of phospholipid fatty acid (PLFA) composition of Neubrandenburg.

Sampling time	Feb 08	Apr 08	May to Dec 08	Jan 09	Mar 09	Jun 09	Jul 09
Operating mode	DC	DC	not in use	DC	DC	C	C
Filter installation side	F2 - cold 2 months	F1 - warm 2 months	F2 - cold 4 months	F1 - warm 2 months	F2 - cold 3 months	F2 - cold 1 day	F2 - cold 1 month
Filter operating time	46°C	73°C	48°C	70°C	48°C	47°C	46°C
Temperature							
12:0	-	-	0.3	0.1	-	-	-
<i>i</i> -13:0	0.1	0.1	-	-	-	-	-
13:0	-	-	-	-	0.3	-	-
<i>i</i> -14:0	1.8	2.6	2.1	1.3	3.9	-	-
14:0	0.5	2.2	0.8	0.6	1.1	-	-
<i>i</i> -15:1	-	-	0.8	-	-	1.3	1.5
<i>i</i> -15:0	26.4	12.6	18.4	24.0	24.2	51.5	53.1
<i>ai</i> -15:0	36.8	16.5	34.8	5.1	28.2	9.2	3.0
15:0	16.4	9.3	15.0	2.4	11.1	3.4	1.2
<i>i</i> -16:0	2.4	2.5	3.0	3.3	4.1	2.3	2.2
<i>ai</i> -16:0	0.2	-	1.4	-	-	-	-
16:0	7.3	24.4	10.1	35.1	12.7	4.5	4.1
10-Me-16:0	0.3	-	-	-	0.9	-	-
<i>i</i> -17:1	-	-	1.3	1.7	-	4.3	7.3
<i>i</i> -17:0	2.9	4.1	4.1	5.8	5.5	12.3	17.7
<i>ai</i> -17:0	0.9	2.1	1.9	2.0	1.7	3.2	1.7
17:0	1.3	-	2.0	2.0	2.6	2.3	2.0
<i>i</i> -18:1	0.3	-	0.4	0.9	-	2.5	3.2
18:2	-	-	-	0.6	-	-	-
18:1ω9	0.4	5.8	1.4	2.4	0.9	-	-
18:1ω7	-	-	0.2	0.4	-	-	-
18:0	1.3	18.0	4.1	28.4	3.0	3.2	2.8
<i>i</i> -19:0	0.3	-	-	1.0	-	-	-
<i>ai</i> -19:0	0.1	-	-	-	-	-	-
19:0	0.1	-	-	0.4	-	-	-
20:0	-	-	-	0.3	-	-	-
Σ <i>n</i> -saturated FA	27.0	53.9	32.0	28.7	30.7	13.4	10.2
Σ <i>n</i> -unsaturated FA	0.4	5.8	1.7	3.4	0.9	-	-
Σ br-saturated FA	72.3	40.4	64.5	65.3	68.4	78.4	77.8
Σ br-unsaturated FA	0.3	0.0	1.8	2.6	0.0	8.2	12.1
ratio br-FA/saturated FA	2.7	0.7	2.1	0.3	2.2	6.5	8.8