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

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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.

1 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). Søgaard

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 (Søgaard *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.

2 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. 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 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. 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. 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. 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. 1) to monitor the temperature regime and the groundwater table during the ATEs operation.

3 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.

4 Material and Methods

4.1 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. 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. 2). The process water of the cold storage is taken at the tapping point A (Fig. 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. 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. 6). The filters were taken directly from the filter system prior to the heat exchanger (Fig. 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.

4.2 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 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 %.

4.3 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 *Grüntzel* 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 %.

4.4 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 H₃PO₄, 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 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 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 ≤ 0.5‰.

4.5 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 Results and Discussion

5.1 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 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 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 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 2.

Table 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-} (mg/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. 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. 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. 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. 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. 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 $2/3$ 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. 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. 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 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 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 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 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 1 and 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.

5.2 Bacterial Variability during Normal and Disturbed Operating Modes in the ATES System

5.2.1 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; sample from August 2007). The dominating PLs were phosphatidylethanolamines (PEs), 72 – 89 %, followed by phosphatidylglycerols (PGs), 9 – 23 % and phosphatidylcholines (PCs), 2 – 9 % (Fig. 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: 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-glycero-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 %, while the PC distribution increased from 11 to 20 %.

Fig. 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. 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.

5.2.2 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 4 and Fig. 7) with 13 - 20 C atoms and showed clear changes during the monitoring (Table 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. 3, Table 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. 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. 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. 3).

Table 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, *mcb* = 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. 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	DC	NOOP	DC	RI	DC	RI	DC	RI	DC	NOOP	C	NOOP	DC	NOOP	
PLFA (%)																											
br-13:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	0.1	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	-	-	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	1.6	1.0	0.6	0.6	0.6	0.6	0.7	1.2	1.2	1.8	1.8	3.6	-	-	-	-	
mcb-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	-	-	-	1.1	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	0.9	0.6	0.9	3.3	3.6	5.6	-	-	-	0.9	3.3	3.6	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	-	-	-	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	0.1	0.3	0.9	0.6	-	-	-	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	-	-	-	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	-	-	-	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	-	-	-	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	-	-	-	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	-	-	-	-	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	0.1	0.2	0.4	0.5	-	-	-	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	0.1	0.2	0.6	0.7	-	-	-	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	2.3	3.6	-	-	-	-	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	0.7	0.1	0.6	-	-	-	0.1	0.1	0.7	0.4	0.6	-	-	-	-	-	
br-18:0	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
18:1 ω 9c	-	-	-	-	-	-	-	-	-	-	-	-	1.6	-	-	-	-	-	-	-	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	-	-	-	-	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	0.4	2.6	3.5	1.4	-	-	-	0.1	0.4	2.6	3.5	1.4	-	-	-	-	-	
19:1	-	-	-	1.7	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	-	0.8	0.2	-	-	-	-	-	
19:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	-	-	-	-	-	-	-	-	
20:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	0.2	-	-	-	-	-	
Σ i-15:0 + ai-15:0 + 10-Me-16:0	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	-	-	1.8	3.1	8.6	8.6	8.4	10.8	-	-	-	-	
Σ i-17:0 + ai-17:0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
Σ 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	-	-	-	83.6	77.1	66.9	62.9	62.9	54.8	-	-	-	-	
Σ 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	-	-	-	13.3	17.8	19.5	21.1	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	-	-	-	-	0.03	0.06	0.12	0.11	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	-	-	-	47.5	25.0	7.8	7.5	7.5	5.1	-	-	-	-	

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 4, Figs. 7 and 8).

Fig. 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. 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. 7 and Table 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. 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.

5.2.3 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. 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. 9 and Table 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. 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. 2).

A second ratio of interest is the *cis/trans* isomers of the 16:1ω7 FA detected in the filter samples of the cold storage. The main isomer is the *cis* 16:1ω7 FA, whereas the *trans* 16:1ω7

FA only occurs in lower abundance (Fig. 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. 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. 10: Ratio of *trans* and *cis* configuration of C_{16:1}ω₇ 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. 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. 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.

6 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.

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