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Comparison of the microbial community composition of pristine rock cores and technical influenced well fluids from the Ketzin pilot site for CO_2 storage

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ABSTRACT

Two geological formations at the CO_2 storage pilot site in Ketzin (Germany) were geochemically and microbiologically characterized to further evaluate changes resulting from CO_2 injection. Well fluids were collected from both Stuttgart (storage formation, ~650 m depth) and Exter Formations (~400 m depth, overlying the caprock) either through pump tests or downhole samplings. Rock samples were retrieved during a deep drilling into the Exter Formation and primarily comprised quartz, ferrous dolomite or ankerite, calcite, analcime, plagioclase and clay minerals, as determined through X-ray diffraction analyses. In the rocks, the total organic

carbon (TOC), which potentially contributes to microbial growth, was mostly below 1000 mg/kg. The geochemical characterization of fluids revealed significant differences in the ionic composition between both formations. The microbial characterization was performed through fluorescence *in situ* hybridization and 16S rRNA gene fingerprinting. In the fluids obtained from the Stuttgart Formation, the microbial activity was affected by the relatively high TOC, introduced by the organic drill mud. The total cell counts were approximately 10^6 cells mL^{-1}. The microbial community was characteristic of a saline deep biosphere environment enriched through increased carbon availability, with sulfate-reducing bacteria as the most abundant microorganisms (up to 60 % of total cells). Species belonging to halophilic/halotolerant Proteobacteria and Firmicutes were primarily detected. In Exter Formation rocks, Proteobacteria and Actinobacteria were detected. These data provide an explicit reference to further evaluate environmental changes and community shifts in the reservoir during CO_2 storage and provide information for evaluating the storage efficiency and reliability.

Keywords: CO_2 storage, molecular fingerprinting, FISH, microbial community

Introduction

Carbon dioxide capture and geological storage (CCS) might substantially contribute to the reduction of CO_2 greenhouse gas emissions into the atmosphere (Metz et al. 2005). With the advent of CO_2SINK and CO_2MAN projects, the first CO_2 onshore storage test site in Europe was established in 2004 as a research facility in Ketzin, Germany. The pilot project was intended to examine the feasibility and effects of carbon dioxide storage in a natural saline aquifer at a depth of approximately 650 m

(Schilling et al. 2009; Würdemann et al. 2010; Martens et al. 2012). The effect of gas storage in geological formations might include pressure increases, resident fluid migration, and geochemical and microbial reactions (Bauer at el. 2013). In 2007, one injection and two observation wells were established (Prevedel et al. 2009). In 2011, a shallow well (Wiese et al. 2013) was drilled into the Exter Formation, the aquifer overlying the CO_2 storage formation, for hydraulic and geochemical monitoring (Fig. 1).

Recent studies have revealed that saline aquifers are inhabited by elevated numbers of different groups of microorganisms (Goldscheider et al. 2006; Basso et al. 2009; Morozova et al. 2010; Lerm et al. 2013). The most important metabolic pathways in the deep biosphere are sulfate reduction, fermentation and in some cases, methanogenesis (Takai et al. 2001; Kotelnikova 2002; Baker et al. 2003; Basso et al. 2009). During CO_2 sequestration, interactions of native or introduced microbial populations with the geochemically and physically altered subsurface might promote geochemical processes that are beneficial or detrimental to the long-term storage of CO_2. Previous studies have demonstrated that after exposure to CO_2, the microbial abundance in formation water from saline and freshwater aquifers decreased (Gulliver et al. 2014a; Gulliver et al. 2014b). Nevertheless, bacteria that produce acids as a byproduct of metabolism might dissolve carbonate minerals and increase metal ion mobility (Gulliver et al. 2014a). The dissolution of feldspar (Welch et al. 1999) and Fe(III) minerals (Glasauer et al. 2003), and the precipitation of metal sulfides and silicate and carbonates (Schultze-Lam et al. 1996) are examples of how bacteria could influence mineralogical characteristics. In addition, microbial-induced precipitation and corrosion might occur, affecting the casing and casing cement of the wells. The growth of microorganisms on material surfaces (biofilms) could profoundly affect material performance (Beech et al. 2005). Therefore, when deep saline

aquifers are intended for geotechnical use, such as CO_2 storage, it is important to characterize the microbial community composition to predict the effectiveness and reliability of long-term utilization. Microbiological sampling opportunities in the deep biosphere are limited and costly, and differentiation between autochthonous and allochthonous microorganisms introduced through technical procedures remains a challenge. Little attention has been placed on comparing the populations present in well fluids to those inhabiting rock cores. To analyze deep biosphere diversity, subsurface rock and water samples were collected prior to CO_2 injection at the Ketzin site. A microbiological sampling of pristine rock cores and water samples from these wells requires specific techniques to avoid contamination (Pedersen et al. 1997; Basso et al. 2005; Kallmeyer et al. 2006). A fluorescent dye tracer, sodium fluorescein, was added to the drill mud to estimate the drill mud penetration depth into the rock cores and determine the amount of this mud in the collected fluid samples (Wandrey et al. 2010; Pellizzari et al. 2013).

In the current study, we present results obtained from the observation well CO_2 Ktzi 202/2007 (short name: Ktzi 202, hereafter referred to as Deep Observation Well or DOW), and the shallow monitoring well Hy Ktzi P300/2011 (short name: P300, hereafter referred to as Shallow Observation Well or SOW). Until recently, the Exter Formation has been poorly described, therefore a basic mineralogical characterization was performed. The geochemical characterization, through fluorescein, pH, dissolved organic carbon (DOC), total organic carbon (TOC), cations and anions measurements, was made in fluid samples collected during hydraulic tests (in DOW and SOW) and downhole sampling (in DOW only). Analyses of the microbial communities in fluid samples from both wells and in rock cores obtained during the coring campaign of the SOW were performed using 16S rRNA gene fingerprinting and fluorescence *in situ* hybridization (FISH).

Materials and methods

Study site

The storage site is located in the North German Basin near Ketzin. The target reservoir for CO_2 storage is the Stuttgart Formation sandstone of Triassic age, located at a depth approximately between 600 and 700 m, with an average thickness of approximately 80 m. The caprock of the storage formation comprises the playa-type mudstones of the Weser and Arnstadt formations (Förster et al. 2009). The Weser Formation consists mainly of mudstone and anhydrite and it is overlain by the Arnstadt Formation, which is composed by mudstones and carbonates (Norden et al. 2010). The high clay mineral content and the pore-space geometry confer sealing properties necessary for CO_2 storage. The two formations composing the caprock are, together, about 210 m thick (Norden et al. 2010). For the injection and monitoring of CO_2 in the natural saline aquifer, three holes, approximately 745 to 805 m deep, were drilled through mud rotary drilling from March to September 2007 (Fig. 1). The operation was performed using a water-based $CaCO_3$/bentonite/organic polymer drill mud containing carboxymethylcellulose (CMC), with the addition of biocide, comprising organic amines, to prevent microbial degradation and reduce the souring of the mud. Prior to CO_2 injection, pumping tests were performed on all wells (Wiese et al. 2010). To remove residual drill mud from the wells a so-called gas lift was performed. Therefore, N_2 was pumped in the injection well and in two observation wells a few days before the beginning of CO_2 injection, allowing the well fluid to flow out (Zettlitzer et al. 2010). The CO_2 injection into the Ktzi 201 well started at the end of June 2008 (Schilling et al. 2008).

In summer 2011 a shallow observation well was drilled approximately 25 m northwest of the DOW. This well was established to monitor and detect the hydraulic and geochemical impact of CO_2 on the groundwater of the first aquifer overlaying the caprock. The SOW reached a depth of approximately 450 m into the lowermost sandstone layer of the Exter Formation. The drill mud, employed during the coring of this shallow well, contained fresh water mixed with K_2CO_3 (60 g L^{-1}). No organic polymers were added to the drill fluid to avoid the stimulation of microbial activity. The rock samples of the Exter Formation retrieved during coring and analyzed in this work were sandstones to siltstones.

Rock core sampling

The drilled core diameter of the SOW was 85 mm. The samples were stored in aluminum bags in a nitrogen atmosphere and cooled during the transportation to the laboratory. To remove the outer and contaminated part of the samples, inner coring, parallel to the borehole orientation, was performed shortly after sampling. The details concerning this have been previously described elsewhere (Pellizzari et al. 2013). One portion of the resulting cylinder was stored at -20 °C until further molecular biological analysis, whereas another portion was used for long-term experiments (Pellizzari et al. subm.). Altogether six different rock cores, classified as sandstones to siltstones, were analyzed. The description of the rock samples retrieved during coring and analyzed in this study is summarized in Table 1.

Basic mineralogical characterization and TOC measurements of Exter Formation rock samples

Polished thin sections of the sandstones of the middle and lower part of the Exter Formation were petrographically analyzed using a Zeiss Axioplan polarization

microscope. Thin-section photographs were recorded with an AxioCam digital camera using the AxioVision 3.1 software. The pore space of the sandstones was quantified through an image analysis using the JMico Vision 1.2.5 software. Qualitative analyses of the mineral phases present in the rock cores were determined using X-ray diffraction (XRD). A Philips X'Pert (APD type) diffractometer with vertical goniometer PW 3020, equipped with curved graphite crystal monochromator and CuKα radiation, was used. Prior to analyses, approximately 5 g of averaged rock fragment was ground to 0.2 mm using an agate mill and pressed into the analytical cuvettes. The qualitative analyses were performed in the analytical range between 4-64° 2Θ, step 0.02°, and 1 s/step. The phase identification was obtained using Philips X'Pert Graphics and Identify software.

To determine mineral morphology and chemical composition, field emission scanning electron microscopy (FE-SEM; Hitachi S-4700) was performed. Rock fragments of ca. 1 cm were coated with carbon. The analyses were performed at 20 kV. The quantitative analysis of the chemical composition was performed using an energy dispersive spectrometry (EDS). The elements in the spectra were identified through the spot analysis of the chemical composition in the sample, with accordance to the standardless method using Thermo Scientific NSS analytical software. These analyses were based on the patterns of pure elements provided by the software. Correctness of the match was controlled by Chi-squared parameter. The results were recalculated to 100 % EDS. For TOC measurements, 10 g of each rock core sample were ground using a laboratory mill (Mixer Mill MM 400 from RETSCH GmbH, Germany). The measurements were performed according to the DIN ISO 10694 procedure.

Fluid sampling

Fluid samples were collected during hydraulic tests (DOW, SOW) and downhole samplings (DOW). The hydraulic pumping tests were conducted as open-hole tests, and the production rates were held at the maximal achievable rate. The fluids were sampled directly from the wellhead, collected in sterilized glass bottles, cooled and transferred to the laboratory for further analyses. During the pump test of the DOW, 93 m³ of fluid was produced (details are given in Wiese et al. 2010). Fluids were sampled after 4, 27, 30, 40, 55, 61, 79, 93 m³ of production. The samples collected at the beginning (4 m³) and end (93 m³) of the pump test, with highest and lowest DOC and fluorescein concentrations respectively, were selected for FISH and 16S rRNA fingerprinting analyses. Moreover, one mid-range sample (30 m³) was selected for fingerprinting. The contamination control in the fluids collected during pump tests have been previously described elsewhere (Wandrey et al. 2010). After the pumping test, downhole samplings were performed in the DOW at perforation depths using double ball-lining. Two sampling campaigns were conducted before the N_2 lift, at 40 days apart. Four days after the N_2 lift, another downhole sampling was performed. Each sampler was cleaned in the laboratory and washed with ethanol and sterilized-deionized water shortly before sampling. The pH, conductivity, temperature and other parameters were directly measured during the fluid production. The fluid samples intended for FISH and fingerprinting analyses were aseptically transferred into sterilized 500 to 1000 mL glass vials, whereas the samples for chemical analysis were collected in 100 and 250 mL polyethylene and glass vessels. The flasks were cooled to 4 °C and immediately transferred to the laboratory for molecular biological and chemical analyses. The pump test performed in the SOW have been previously described elsewhere (Wiese et al. 2013). During the pump test in the SOW, six samples were collected after 3, 5, 7, 10, 11 and 14 m^3 of fluid production to

characterize the microbial community composition. The drill fluid contamination control is described in Pellizzari et al. (2013).

Chemical analyses of fluid samples

During every sampling step of the pump tests and during the downhole samplings (DOW), approximately 100 mL of fluid were collected in glass bottles for total and/or dissolved organic carbon analyses. The measurements were performed using the DIN EN 1484-H3 procedure. The fluorescein concentration in the fluid samples obtained from the DOW was detected using a mobile light fluorometer (Hermes Messtechnik, Stuttgart, RS232), whereas for the SOW fluid samples, the tracer was measured using 96-well plates processed through a filter fluorometer (FLUOstar OPTIMA, BMG LABTECH, Germany). The samples collected via downhole sampling (DOW) and pump test (SOW) were filtered (0.45 µm), and the concentrations of cations and anions were measured using inductively coupled plasma – optical emission spectroscopy (ICP-OES) according to DIN 38402-21. Quality control included both accuracy (SRM) and precision (triplicates). The average recovery of elements from the SRM was 94-101 %, and the uncertainty of the method was below 8 %. The relative standard deviation (RSD) values were < 3 % for most of the analyzed samples. The electrical conductivity, pH and fluid temperature were measured during the sampling process using a portable pH/mV/Temperature meter (WTW, Weilheim, Germany).

Total cell count determination by FISH analyses

Molecular biological analyses were performed on Stuttgart and Exter Formation samples obtained from DOW and SOW, respectively. The fixation of the samples for FISH analyses was performed according to Pernthaler et al. (2001). Approximately

5 g of rock samples were fixed with paraformaldehyde (PFA) (1 % final concentration), whereas freshly collected fluid samples were concentrated to a volume of approximately 0.5 mL through centrifugation (Heraeus Biofuge Pico, Sigma 6K15, 9600 x-g for 1 h at 4 °C) and fixed with PFA (4 % final concentration). After cell extraction using Nycodenz (Morozova et al. 2013), FISH and DAPI (4',6-diamidino-2-phenylindole) staining were performed according to Morozova et al. (2010). The 16S rRNA-targeted oligonucleotide probes (Thermo Scientific, Ulm, Germany), labeled with the cyanine dye Cy3 and FLUOS, were used to identify the different phylogenetic groups of the domain Bacteria and Archaea (Table 2). The slides were examined under a Zeiss Axio Imager M2, equipped with a mercury-arc lamp and an AxioCam digital camera. The cell counting was manually conducted. The number of DAPI stained cells per mL of fluid was calculated as described in Morozova et al. (2010). The relative abundance of the different phylogenetic groups was calculated considering probe specific signals referred to total DAPI counts.

DNA extraction and PCR-amplification

For DNA extraction, the microbial cells present in fluid samples were concentrated through the filtration of approximately 500 mL well fluid on 0.2 µm filter units (Millipore). Total genomic DNA was extracted from the preserved filters using UltraClean™ Soil and Power Soil™ DNA Isolation Kits (Mo Bio Laboratories, USA), according to manufacturer's instructions. The rock core samples obtained from the Exter Formation were prepared for DNA extraction through cryogenic grinding using a RETSCH laboratory mill. To extract the total genomic DNA from rock samples, the FastDNA® SPIN Kit for Soil (MP Biomedicals, OH, USA) was used according to the manufacturer´s instructions. Bacterial 16S rRNA subunits were amplified through polymerase chain reaction (PCR) (iCycler, BioRad, USA and TPersonal, LABRepCo,

USA) using the primer set 341F-GC (5` CGC CCG CCG CGC CCC GCG CCC GTC CCG CCG CCC CCG CCC GCC TAC GGG AGG CAG CAG 3`; Muyzer et al. 1993) and 907R (5` CCG TCA ATT CMT TTR AGT TT 3`; Muyzer et al. 1993). The PCR reaction contained 2.5 µL 10X PCR reaction buffer (Genecraft), 3 µL of deoxynucleosidase-triphosphate mix (dNTPs, 2.5 mM each, Thermo Scientific), 3 µL of $MgCl_2$ (25 mM, Genecraft), 0.2 µL of bovine serum albumin (BSA, Thermo Scientific), 1.5 µL of each primer (10 mM), 0.2 µL of Biotherm D-Taq Polymerase (Genecraft, Cologne, Germany), 1.5 µL of DNA template and RNA/DNA-free water (Thermo Scientific) to a final volume of 25 µL. The amplification conditions included an initial denaturation at 94 °C for 2.5 min, followed by 35 cycles at 94 °C for 45 s, 55 °C for 45 s and 72 °C for 50 s, with a final elongation step for 5 min at 72 °C. For the rock core samples, due to low cell numbers, the execution of a nested PCR was necessary, using the universal 16S rRNA primer set 27F (5` AGA GTT TGA TCM TGG CTC AG 3`; Lane et al 1991) and 1492R (5` TAC GGY TAC CTT GTT ACG ACT T 3`; Weisburg et al 1991) (96 °C for 5 min, 30 cycles at 96 °C for 45 s, 55 °C for 45 s and 72 °C for 60 s, with a final cycle at 72 °C for 10 min) followed by a run with the primer set 341F-GC and 907R. For the second reaction, 1 µL of the long fragment PCR product was used as a template. The PCR products were assessed through electrophoresis in a 1.5 % (w/v) agarose gel (ethidium bromide staining). The PCR products were purified using the GeneJET PCR Purification Kit (Thermo Scientific, USA).

16S rRNA gene fingerprinting analyses: SSCP and DGGE

The genetic profiling of amplified 16S rRNA genes was performed to characterize the microbial community through Single-Strand-Conformation Polymorphism (SSCP) or Denaturing Gradient Gel Electrophoresis (DGGE). The SSCP analyses were

performed with four fluid samples from the DOW. Three samples were collected during the pump test (after 4, 30 and 93 m^3 of production) and one sample was collected during downhole sampling (second sampling, 647 m). The analyses were conducted by AMODIA Bioservice GmbH (Braunschweig, Germany), according to the description of Dohrmann and Tebbe (2004). The gels were silver stained according to the procedure of Bassam et al. (1991). The dominant bands were excised, re-amplified and sequenced.

DGGE analyses were performed with SOW well fluid obtained from pump test and rock samples using the Bio-Rad DCode System (Hercules, CA, USA). The purified PCR products were loaded onto a 6 % polyacrylamide gel with a denaturing gradient ranging from 35 to 70 %. The electrophoresis was performed at 60 °C with 100 V for 17 h. After electrophoresis, the gel was silver stained. The bands were excised, placed in clean 1.5 mL Eppendorf tubes and the DNA was extracted using the “crush and soak” method (Sambrook and Russell 2001; Czarnetzki and Tebbe 2004). The DNA was re-amplified using the primer set 341F and 907R, purified using the GeneJET PCR Purification Kit and subjected to sequencing (GATC Biotech AG, Konstanz, Germany). For the taxonomic assignment, the sequences were compared to the available sequences in the GenBank database using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Results

Basic mineralogical characterization and TOC measurements of Exter Formation rock core samples (SOW)

Basic studies of the mineralogical composition of Exter Formation rock samples were performed. The lithology and mineral composition of the studied rocks collected from

the SOW were variable with depth (Table 1). The petrographic analyses through polarization microscopy indicated that the deltaic to shallow marine sandstones were characterized by a mature composition dominated by quartz and dolomite. Some minor amounts of feldspar and pyrite were also present. The lower sandstones (~441 m) exhibited a considerable amount of well-rounded carbonatic peloids with a highly variable grain size (~20-1900 µm). The middle sandstones (~418 m) peloids were not observed, although carbonatic matrix and ooids were detected. The thin sections possessed a visual porosity of 8-22 % vol. Furthermore, XRD analyses confirmed the presence of main mineral phases (Fig. 2): quartz (Qz), ferrous dolomite or ankerite (Ank), calcite (Cal) analcime (Anl), plagioclase (PL), and clay minerals (Ill/Sme). A representative SEM image of the surrounding clay minerals is shown in Fig. 3. EDS analyses of Mg-Fe carbonates indicated the presence of solid solution between dolomite and ankerite end-members; however, no pure end-member was detected. The content of Fe and Mg indicated the presence of ferroan dolomite, with a Fe content of no higher than 20 % (Fig. 4). The SEM-EDS studies indicated the additional presence of halite, calcium chloride (precipitated form the brine), barite, pyrite, Fe (hydr)oxides and titanium oxides. For all rock cores, the TOC values were lower than 1000 mg/kg (0.1 %).

Fluid geochemistry

Deep observation well

The concentration of DOC and fluorescein strongly decreased during the production of 93 m^3 of fluid through the pumping test in the DOW (Fig. 5). The DOC values decreased from 755 to 4 mg L^{-1}. Fluorescein diminished from 0.005 to 0.0008 mg L^{-1}, with a peak of 0.13 mg L^{-1} after 9 m^3 of production. Detailed analyses of the fluid samples obtained from the well via downhole sampling prior to CO_2 injection were

performed with regard to the concentrations of anions, cations, pH, conductivity, salinity and TOC (Table 3). The pH, conductivity and salinity values only slightly shifted. The potassium concentration decreased from 561 mg L^{-1} (first sampling) to 331 mg L^{-1} after fluid production with N_2 lift. Nitrate and phosphate were consistently less than 5 mg L^{-1} (data not shown). The TOC ranged between 18.4 and 134.7 mg L^{-1}. The sulfate concentrations showed values from 4071 to up to 4409 mg L^{-1}, whereas the concentrations of sodium, calcium, magnesium and chloride varied only slightly.

Shallow observation well

Analyses of the fluids collected during the pumping test of the SOW revealed average fluorescein concentrations of 0.2 mg L^{-1} whereas the TOC and DOC concentrations ranged between 4 and 16 mg L^{-1}. The fluorescein concentration slightly decreased during the test, whereas the TOC fluctuated around the mean value. The sulfate concentration ranged between 1725 and 1935 mg L^{-1} (Table 4). The concentration of potassium significantly decreased during the pumping test from 3017 mg L^{-1} to 1644 mg L^{-1} at the end of the procedure. Additional results concerning the fluid geochemistry are summarized in Table 4.

Quantification of the microbial communities

Deep observation well – Pump test

At the beginning of the pump test (1st sampling, after production of 4 m^3) approximately $6x10^6$ cells mL^{-1} were enumerated (Table 5). Approximately 95 % of those cells were attributed to members of the domain Bacteria and 3 % of the cells were attributed to the Archaea. Members of Proteobacteria and Firmicutes contributed 12 % and 6 %, respectively, of the cell population. The low abundance of

Sulfate-Reducing Bacteria (SRB), with up to 6 % Desulfovibrionales (with many Desulfuromonales) and Desulfobacteriaceae groups, were enumerated, whereas the Desulfovibrionales and *Desulfotomaculum* groups each contributed 3 %. At the end of the pump test (93 m³ of fluid produced) approximately 10^6 cells mL^{-1} were enumerated. The fraction of Bacteria detected through FISH was approximately 50 %. Up to 5 % of the enumerated cells were attributed to Archaea. Members of Proteobacteria and Firmicutes groups contributed 33 % and 26 %, respectively. The detection of SRB revealed a high abundance of Desulfovibrionales, contributing up to 45 % of the total cell counts. The quantification of the *Desulfotomaculum* cluster I and Desulfobacteriaceae revealed 4 % and 9 %, respectively (Table 5).

Deep observation well – Downhole sampling

The cell numbers detected in all fluid samples obtained via fluid downhole sampling from the DOW are summarized in Table 5. DAPI staining revealed total cell numbers of approximately $5x10^6$ cells mL^{-1}. The fraction of cells detected using the probe specific for Bacteria was around 85 %. Members of Proteobacteria (Alphaproteobacteria, Betaproteobacteria, and Gammaproteobacteria) and bacteria with low G+C content (Firmicutes) were highly abundant in the fluid samples and contributed up to 35 % and 34 % of the DAPI cell counts.

A high abundance of SRB, with up to 60 % for the Desulfovibrionales group, detected together with many Desulfuromonales, was quantified. The Desulfovibrionales group, with other bacteria, resulted in 33 % of all detected cells, whereas *Desulfotomaculum* cluster I and other Firmicutes contributed to 24 % of all detected cells. The quantification of *Archaea* in the fluid samples resulted in approximately 28 % of total cell counts. It has to be considered that the cell enumeration through staining techniques may be influenced by particles in the well fluid sample. This may entail

small inaccuracy in the detection method, leading to a slight overestimation cell counts as in the case of the downhole sample, where the sum of bacterial and archaeal counts is higher than the total number of DAPI stained cells.

Shallow observation well

Low or absent DAPI signals were observed for microorganisms in fluid samples of the SOW collected during pump test. Notably, a high fluorescein (0.2 mg L^{-1}) and therefore drill mud concentration was detected in these fluids (Pellizzari et al. 2013), which interfered with the fluorescent signal of the cells. Quantification of the bacteria inhabiting the rock samples from the SOW with positive signals relative to DAPI counts revealed low cell numbers, below 10^2 cells mL^{-1} (data not shown).

Characterization of the microbial community composition in rock cores and fluid samples via fingerprinting analyses

The diversity of the microbial communities in rock and fluid samples was determined through SSCP and DGGE 16S rRNA gene fingerprinting. Sequence analyses revealed the presence of microorganisms related to Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Deltaproteobacteria, Firmicutes, Bacteroidetes and Actinobacteria (Tables 6, 7 and 8). 16S rRNA sequences obtained in this study are deposited under the GenBank accession numbers KR336949-KR336967.

Deep observation well, fluid samples

The SSCP genetic profile of fluids from the DOW collected at the beginning of the pump test (Fig. 6a) showed a different microbial community composition with respect to the samples obtained at the middle and end of the test. The sequences obtained

from genetic fingerprinting of the fluids collected after production of 4 m³ fluid were assigned to *Bacteroides graminisolvens* (band 1), *Proteiniclasticum ruminis* (band 2), *Flavobacterium* spp. (band 3, 4) and *Clostridium sticklandii* (band 5). After 30 m³ of fluid production and until the end of the pump test (93 m^3 fluid produced), the analyses revealed the predominance of halophilic fermenting bacteria related to *Halanaerobium* spp. (30 m^3: band 6, and 93 m^3: bands 7 and 8), corresponding to the fluid salinity (Table 6). The SSCP profiles of the fluids collected via downhole sampling from a depth of 647 m (Fig. 6b) revealed a band pattern different to that for the samples collected at the end of the pump test. The sequences were assigned to the halophilic fermenting bacteria *Halanaerobiaceae* (bands 1), *Halanaerobium* spp. (band 3) and an uncultured bacterium, whose closest relative is a SRB of the genus *Desulfohalobium* (band 2) (Table 7).

Shallow observation well, rock and fluid samples

The DGGE profile of six rock cores taken from the SOW showed one to three dominant bands (Fig. 7a). From samples ExF 1 and ExF 10, insufficient DNA for sequencing was amplified. In ExF 3, two bands were most intensive. The sequences had highest similarities to *Variovorax paradoxus* (band 1) and *Hymenobacter psychrophilus* (band 2), respectively. In ExF 4, only one dominant band with a sequence affiliated to *Pseudomonas* spp. (band 3) was observed. The sequences of the two dominant bands in sample ExF 6 were attributed to *Ochrobactrum* spp. (band 4) and *Propionicimonas paludicola* (band 5). In ExF 9, microorganisms related to *Rhizobium* spp. (band 6) and *Pelomonas* spp. (band 7) were detected.

The DGGE profile of the fluids collected during the pump test from P300 is shown in Fig. 7b. Altogether, 5 different bands were detected in the genetic fingerprinting of fluids after 7 m³ and 14 m³ of production. Different members of Firmicutes and

Alphaproteobacteria were found (Table 6). The sequences were assigned to *Dethiobacter alkaliphilus* (7 m^3, bands 1 and 2) and *Alkalibacter saccharofermentans* (14 m^3, band 3). The sequence analyses also revealed the presence of chloroplasts (14 m^3, band 4) in the samples.

Discussion

Petrological and mineralogical characterization of Stuttgart and Exter Formation

Because it is the target reservoir for CO_2 storage, the Stuttgart Formation was largely investigated in recent years (Norden et al. 2010; Fischer et al. 2010; Scherf et al. 2011). The drilling of deep well in the Ketzin area offered numerous opportunities to obtain Stuttgart Formation samples, which together with the lower part of the caprock, were the only formations consistently cored (Prevedel et al. 2009). In contrast, the sandstone aquifer overlaying the storage formation caprock was rarely sampled and therefore has been poorly characterized. The Exter Formation was cored only during the drilling of the SOW (Martens et al. 2013). The rock formation primarily comprises fine- to medium-grained sandstones, siltstones, claystones, marly mudstones and marlstones (Norden and Klapperer 2011). The mudstones show dark reddish brown to dark red colors, but the sandstones are more various in color, ranging from dark grayish to light grayish, grayish blue-green and reddish-orange. The main mineral phase is quartz, and the second most abundant mineral group is carbonate minerals (ferrous dolomite or calcite) in sandstone and analcime in siltstones, accompanied by plagioclases and clay minerals. The siltstone fractions comprise clay minerals (illite/smectite). Clay minerals were not observed in the sandstones, except a low intensity peak in one sample, indicating the presence of

kaolinite. Although information about the mixed-layered illite/smectite and kaolinite was obtained, information concerning the composition based on only standard XRD data is not possible. In the Exter Formation, carbonatic peloids were abundant in the lower sandstone (~440 m), but these grains were not detected in the middle sandstone (~418 m), where ooids were detected instead, indicating the origin in a low energy environment, such as shallow water.

The lithologically heterogeneous Stuttgart Formation comprises alternating intervals of sandstone, siltstone and mudstone, the latter interbedded with minor layers of anhydrite and coal (Norden et al. 2010). The channel sandstones are predominantly fine-grained with sub-rounded grains and an overall fining-upward trend (Norden et al. 2010). The storage formation sandstone, similarly to the Exter Formation, primarily comprises quartz. The other mineral phases include, in decreasing order, feldspar, illite, analcime, anhydrite and amorphous phases, as indicated through XRD and thin-section analysis. In contrast, the mudstone intervals are mainly laminated, comprising illite, dolomite, anhydrite, quartz, feldspar and chlorite (Förster et al. 2009). Scanning Electron Microscopy (SEM) and Electron Microprobe Analysis (EMPA) revealed variable amounts of authigenic poikilitic dolomite as the only carbonate species (Bock et al. 2013).

The total porosities of the Stuttgart Formation (5 to >35 vol. %), which are variable depending on variations in grain size, facies and cementation (Norden et al. 2010), and the thin-section porosities of the Exter Formation (from 8 to 22 vol. %) were similar.

Drill mud contamination control

For highly sensitive molecular biological analyses, it is important to assess the drill mud content in well fluid and rock samples (Wandrey et al. 2010; Pellizzari et al.

2013) because microorganisms might be introduced with the mud. Moreover, organic polymers can serve as nutrients for microorganisms, as occurred in the injection well, where these polymers stimulated microbial activity, affecting the injectivity (Zettlitzer et al. 2010; Morozova et al. 2010). The tracer monitoring of the DOW revealed that most of the drill mud was removed during the pump test within the first 10-20 m^3 of production (Wandrey et al. 2010). At the beginning of the pump test (4 m^3), a portion of 0.33 % of drill mud was observed. After 10 m^3 of production, the fluorescein reached a maximum of 0.79 % and decreased to 0.05 % at the end of the hydraulic tests (93 m^3 of production). Together with the fluorescein concentration, the DOC also decreased during the test, indicating that the concentration of organic polymer-based drill mud diminished in the well fluid. During the downhole sampling, the higher TOC (up to 135 mg L^{-1}) of the DOW fluids resulted from residues of the organic technical fluids. After the N_2 lift, the TOC value of the well fluid decreased to 18 mg L^{-1} but was still influenced by technical fluid, as in the formation fluid from the injection well (Ktzi 201), after the N_2 lift, the concentration of TOC decreased to 3 mg L^{-1} (Zettlitzer et al. 2010). Other geochemical parameters were measured during downhole samplings of the DOW. The ionic composition (sulfate, calcium, iron) and conductivity are similar to the formation fluid analyzed from the deep observation well Ktzi 200 by Morozova (2010). Furthermore, between the first and the other two sampling campaigns (one before and one after the N_2 lift) only slight differences in the geochemical parameters were observed, indicating that residual drill mud influenced mainly the organic carbon concentration. However, the microbial community detected in the well fluids sampled at the end of the pump test and by downhole sampling was characteristic of deep biosphere environment (Fry et al. 1997; Sahl et al. 2008) and can therefore be considered representative of the

formation fluid in the near well area and only influenced by low concentrations of the drill mud.

The fluorescein concentration decreased during the pump test of the SOW, but approximately 8 % of drill mud was still detected until the end of the test (Wiese et al. 2013). The hydraulic test produced only 14 m^3 of fluid, which was obviously not sufficient to remove the drill mud. In conclusion, the tracer monitoring revealed that fluid from the SOW was significantly influenced by drill mud. The fingerprinting and FISH analyses indicated that the autochthonous microbial community was mixed with atypical species. The DOC and TOC values (in average 6 and 7 mg L^{-1}, respectively) only slightly fluctuated during the test and were presumably influenced by the freshwater used as drill mud. However, no particularly high values of DOC and TOC were expected because no organic compounds were used for the drill mud. Due to the K_2CO_3 mixed with freshwater, the potassium concentration in the SOW fluid was significantly higher (in average nearly 6 times) than in the DOW fluids. During the pump test the potassium concentration considerably decreased, but at the end of the test the potassium concentration remained anomalously high, confirming that the pump test did not sufficiently remove technical fluids, as previously discussed. Accordingly, the geochemical parameters measured during the pump test of the SOW must be processed with caution. Regarding the contamination degree of the Exter Formation rocks, the penetration depth of drill mud, labeled with fluorescein, varied in the cores. Some rock fragments were contaminated, but in several cores, fluorescein was observed only up to 3 mm from the surface (Pellizzari et al. 2013).

Microbiological characterization of the well fluids

The DOW fluids collected at the beginning and the end of the hydraulic test and by downhole sampling (647 m depth, performed before the N_2 lift), counted

approximately 10^6 cells mL^{-1}. Morozova et al. (2010) observed similar cell numbers in the fluids obtained from a nearby deep observation well (Ktzi 200, Fig. 1), prior to CO_2 injection. In the DOW fluids analyzed in the present study, the enumeration of cells revealed the predominance of Bacteria. FISH and fingerprinting analyses indicated significant differences in the bacterial community structure between the beginning and the end of the DOW pump test. These differences reflect the strong influence of drill mud at the beginning of the test, where the fraction of Bacteria detected through FISH was approximately 95 %. Considering the fingerprinting analyses, at the beginning of the pump test Bacteroidetes presumably made up a large proportion of cells which were detected through the Bacteria specific probes. To the end of the pump test, the Bacteroidetes decreased with the drill mud concentration in the fluid, indicating less influence of the technical procedures on the microbial community. Interestingly, the portion of cells related to the bacterial phyla, namely the Proteobacteria, Firmicutes and several SRB (affiliated to Deltaproteobacteria) was only 36 %, indicating the presence of additional groups of bacteria such as Bacteroidetes. At the end of the test only 50 % of the cells were bacterial, although Proteobacteria, Firmicutes and SRB (affiliated to Deltaproteobacteria) were detected in high abundances (33, 26 and 45 %, respectively) using specific probes. A reduced number of detectable Bacteria when applying the EUB probes set compared to the more group specific probes was observed. In this context, it should be further noted that the amount of Proteobacteria reported in Table 5 was determined using a mix of Alphaproteobacteria, Betaproteobacteria and Gammaproteobacteria specific probes, whereby the probes SRB385, DSS658 and DSV687 were used to detect, together with other bacteria, the SRB of the class Deltaproteobacteria. As described above, the fingerprinting analyses indicated that the DOW fluid collected at the beginning of the pump test was

strongly influenced by technical fluids. The microorganisms affiliated to *Bacteroides graminisolvens*, *Proteiniclasticum ruminis*, *Flavobacterium* spp. and *Clostridium sticklandii* were observed only at the beginning of the pump test and did not represent a site characteristic community. In contrast, during and at the end of the pump test, only sequences related to the typical obligate anaerobic and halophilic genus *Halanaerobium* (99 % similarity) (Oren 2008) were detected. The relatively high TOC of the well fluids (Table 3), due to the presence of technical fluids, might facilitate bacteria that metabolize organic compounds. Moreover, a relative of *Halanaerobium* was also detected in the well fluid collected via downhole sampling, together with halophilic microorganisms related to the family of Halanaerobiaceae and the genus *Desulfohalobium* (Table 6). Considering the high salinity of the aquifer, a predominance of halophilic microorganisms is not surprising. The next cultivated relative of the *Desulfohalobium* organism was the moderately halophilic, sulfate-reducing bacterium *Desulfohalobium utahense*, isolated from anoxic hypersaline sediment of the Great Salt Lake, USA (Jakobsen et al. 2006). High concentrations of sulfate, sodium and magnesium, which favor the growth of *Desulfohalobium utahense,* were measured in well fluids. FISH analyses revealed that in well fluids collected during downhole sampling, up to 60 % of the total cell number were SRB.

In general, both FISH and fingerprinting analyses indicated the predominance of Firmicutes-species and SRB (affiliated to Deltaproteobacteria) in the DOW fluids. Similar observations have been previously reported in other deep microbial ecosystems (Fry et al. 1997; Sahl et al. 2008). Basso and colleagues (2009) analyzed a deep subsurface gas storage aquifer and observed a microbial community primarily comprising Firmicutes and Deltaproteobacteria. Shimizu et al.

(2007) investigated groundwater from a 900 m deep aquifer, in Japan. The microbial community comprised 40 % Firmicutes and 27 % Deltaproteobacteria.

In fluids of the SOW collected during the pump test, the evaluation of the cell numbers was not successful due to the high portion of drill mud remained in the fluids, which also explains strong difference of the microbial community composition between fluid and rock samples. Accordingly, no substantial differences in the community structure of fluids sampled at the beginning and the end of the pump test were observed. The results of the fingerprinting analyses revealed sequences affiliated to two different Firmicutes and one Alphaproteobacteria species. Members of the genus *Dethiobacter* are anaerobic halotolerant bacteria, which have also been detected in other alkaline habitats (Sorokin et al. 2008; Whittleston et al. 2013). Moreover, sequences assigned to plant chloroplasts were detected. These findings reflect the influence of shallow ground water used together with K_2CO_3 as drill mud.

Microbiological characterization of the rocks

The microbial community in Stuttgart Formation sandstone samples collected from the same DOW detected by Wandrey et al. (2011) differed from the discussed population of the well fluids. In DOW rocks, sequences affiliated to the phylotypes Alphaproteobacteria (*Agrobacterum tumefaciens* and *Rhizobium* spp.), Betaproteobacteria (*Hydrogenophaga* spp. and *Burkholderia fungorum*) and Actinobacteria (*Propionibacterium acnes*) were observed (Wandrey et al. 2011). Although the microorganisms, related to Alphaproteobacteria and Betaproteobacteria, have previously been detected in subsurface samples, *Propionibacterium acnes* was formerly observed in the human skin flora, therefore the presence of this bacteria might be related to contamination (Wandrey et al. 2011).

Nevertheless, differences in the well fluid- and rock-associated microbial population have been previously observed (Hazen et al. 1991; Fry et al. 1997).

In the Exter Formation rocks, the sequence analyses revealed the presence of species from the Alphaproteobacteria and Betaproteobacteria, as already detected in the Stuttgart Formation. In particular, in both rock formations sequences affiliated to the genus *Rhizobium* spp. and to the family Comamonadaceae were detected. For the Stuttgart Formation the Comamonadaceae member was related to the genus *Hydrogenophaga*, whereas for the Exter Formation, the organism of the family Comamonadaceae was related to the genus *Pelomonas*. Furthermore, in Exter Formation rocks, relatives of the Gammaproteobacteria subclass (*Pseudomonas* spp.) and the Actinobacteria member *Propionicimonas paludicola* were also detected. The typical habitats for these microorganisms are soil and fresh water; however, relatives of the genus *Pelomonas* have also been previously identified in deep subsurface habitats (Boivin-Jahns et al. 1996; Kouduka et al. 2012). Using PCR specific primers, Archaea were not detected in the rock core samples obtained from the Exter Formation. Archaea were also not found in rock cores of the Stuttgart Formation (Wandrey et al. 2011), but they were detected in fluids from the deep Ketzin wells through FISH (Morozova et al. 2010; Morozova et al. 2013). These microbes might not be detected as the number of cells in the sandstone was extremely low, and these organisms are often underrepresented. Particularly with respect to the study of low biomass samples, it should be considered that contaminating DNA is ubiquitous in extraction kits and could influence DNA analyses (Salter et al. 2014). However, in all analyses performed in the present study, negative controls were considered during DNA extraction, PCR and fingerprint analyses, and the results did not show any indication of contamination.

Interaction between microorganisms, minerals and fluids

To characterize the microbial habitat and evaluate how the mineralogical composition influences the microbial diversity and abundance, a basic mineralogical characterization of the Exter Formation rock was performed. During microscopic observation, it was not possible to observe microorganisms or biofilms directly on mineral surfaces. However, the microbial diversity might be influenced through the mineralogical composition as differences were detected in the rock community. Changes in the community were likely influenced through fluid-microorganism interactions (Morozova et al. 2011). Moreover, some naturally occurring processes, such as the formation of secondary minerals (e.g., pyrite or ferro(hydr)oxides), might be triggered through microorganisms (Berner 1970; Schoonen 2004), even though the relative timing of formation of those minerals remains uncertain. A high total iron content with up to 2-4 wt % reactive Fe was measured in the Stuttgart Formation rock (Förster et al. 2010). Iron is associated with the goethite/hematite fraction as a result of the coating of some silicate minerals, and some iron is present as alteration products within silicate minerals (for example iron oxides and oxyhydroxides), although most of the iron present is detected in the fractions of silicate materials, such as illite and chlorite (Kasina et al. subm.). Because oxidized iron was observed, the presence of aerobic or facultative anaerobic bacteria is likely.

Carbon availability and environmental conditions control microbial growth in the Deep Biosphere (Brockman et al. 1992; Fry et al. 1997; Whitman et al. 1998; Aldén et al. 2001; Fredrickson and Balkwill 2006; Goldscheider et al. 2006; Jørgensen and Boetius 2007), therefore in the Exter Formation rock cores, where the carbon concentration is particularly low, the number of microorganisms is comparatively low. TOC in rocks of Exter Formation were low, with less than 1000 mg/kg. In the Stuttgart

Formation rock samples, the TOC was between 74 and 3316 mg/kg (Wandrey et al. 2010; Scherf et al. 2011). The TOC in the well fluid of the SOW samples obtained during the pump test showed an average of 7 mg L^{-1}, whereas after the N_2 lift, in the DOW fluids, the TOC was 18 mg L^{-1}. These values were influenced through technical fluids containing easily degradable organic carbon, that stimulated the microbial activity and iron sulfide precipitation in the DOW. During the N_2 lift, the TOC in the Stuttgart Formation well fluid contained acetate as the major constituent (Morozova et al. 2010) and favored the growth and activity of microorganisms inhabiting the reservoir aquifer or introduced together with drill mud.

The identification of SRB in fluids and rocks could be important for the reliable operation of CO_2 storage facilities. Many industries employing buried pipelines and interred structures made a great effort on study the microbial induced corrosion (Iverson 1987; Ford and Mitchell 1990; Lavania et al. 2011; Enning and Garrelfs 2014), where SRB play an important role (Crombie et al. 1980; Pankhania 1988; Lee et al. 1995; Hao et al. 1996). Moreover, recent investigations showed that SRB rapidly and massively change the injectivity in the near well bore area (Morozova et al. 2010; Zettlitzer et al. 2010).

Conclusions

Before CO_2 injection, the microorganisms inhabiting the storage formation well fluid consisted of species well adapted to high salinities. The microbial community predominantly comprised fermentative halophilic anaerobic bacteria and especially SRB. The interaction between SRB, reservoir rock-fluid system and injected CO_2 might induce changes in structure and chemical composition of the formations, provoking corrosion at the well casing, favored by organic carbon, which is mobilized

through supercritical CO_2 (Scherf et al. 2011), affecting the well integrity on a long-term basis.

The data presented here provide an explicit reference to further evaluate community shifts in the subsurface reservoir during CO_2 storage. Future comparative analyses of the environmental parameters and the microbiology will be useful to obtain further insights into the complex geo-bio interactions that might occur under the influence of elevated CO_2 concentrations in deep reservoirs. The present study gives also a benchmark to detect potential microbial and mineralogical changes in the first aquifer overlying the storage formation, as a consequence of caprock leakage. Thus, these data are important for evaluating the efficiency and reliability of the long-term CO_2 storage techniques.

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Table 1 Shallow Observation Well core sample description

Sample	Top depth [m]	Core length [m]	Lithology	Main minerals phases
ExF 1	407.30	0.14	ss	Qz, Ank, Kln, Ms, Pl, Kfs
ExF 3	419.80	0.20	si, fg, ss	Qz, Anl, Fe Dol, Pl, Ilt/Mnt
ExF 4	420.20	0.15	fg, ss, si	Qz, Anl, Pl, Sep, Ilt, Py
ExF 6	428.34	0.13	ss	Qz, Ank, Ms, Anl, Pl, Kfs
ExF 9	436.71	0.13	fg–mg, ss	Qz, Fe Dol, Pl, Kfs, Ilt, Anl
ExF 10	438.09	0.13	fg, ss	Qz, Pl, Ank, Ms, Anl

Ss: sandstone, sandy; si: siltstone, silty; fg: fine-grained; mg: middle-grained
Mineral abbreviations. Qz: quartz; Ank: ankerite; Anl: analcime; Pl: plagioclase; Kfs: K-feldspar; Fe Dol: ferroan dolomite; Ms: muscovite; Kln: kaolinite; Ilt: illite; Mnt: montmorillonite; Sep: sepiolite; Py: pyrite

Table 2 rRNA-targeting oligonucleotide probes used for FISH

Probe	Target group	Sequence (5′–3′) of probe	Target site[a]	FA[b] (%)	Ref.
EUB338 I	Domain *Bacteria*	GCTGCCTCCCGTAGGAGT	16S rRNA (338)	0-35	Amann et al., 1990
EUB338 II	Domain *Bacteria*	GCAGCCACCCGTAGGTGT	16S rRNA (338)	0-35	Daims et al., 1999
EUB338 III	Domain *Bacteria*	GCTGCCACCCGTAGGTGT	16S rRNA (338)	0-35	Daims et al., 1999
NON338	Complementary to EUB338	ACTCCTACGGGAGGCAGC	16S rRNA	0-35	Wallner et al., 1993
ALF968	*Alphaproteobacteria*	GGTAAGGTTCTGCGCGTT	16S rRNA(968)	35	Neef, 1997
Bet42a	*Betaproteobacteria*	GCCTTCCCACTTCGTTT	23S rRNA (1027)	35	Manz et al., 1992
Gam42a	*Gammaproteobacteria*	GCCTTCCCACATCGTT	23S rRNA (1027)	35	Manz et al., 1992
LGC354a	Gram-positive bacteria with low G+C content	TATAGTTACCACCGCCGT	16S rRNA (354)	25	Meier et al., 1999
LGC354b	Same as LGC354a	CGGAAGATTCCCTACTGC	16S rRNA (354)	35	Meier et al., 1999
LGC354c	Same as LGC354a	CCGAAGATTCCCTACTGC	16S rRNA (354)	35	Meier et al., 1999
Arc915	Archaea	GTGCTCCCCCGCCAATTCCT	16S rRNA (915)	n.d.	Stahl and Amann, 1991
SRB385	Most *Desulfovibrionales* and other Bacteria	CGGCGTCGCTGCGTCAGG	16S rRNA(385)	35	Amann et al., 1990
DEM1164r	*Desulfotomaculum* cluster I and other *Firmicutes*	CCTTCCTCCGTTTTGTCA	16S rRNA(1164)	10	Stubner and Meuser, 2000
DSS658	*Desulfobacteraceae* and other Bacteria	TCCACTTCCCTCTCCCAT	16S rRNA(658)	60	Manz et al., 1998
DSV687	*Desulfovibrionales* and many *Desulfuromonales*	TACGGATTTCACTCCT	16S rRNA(687)	15	Ramsing et al., 1996

n.d.: not determined

[a] : E. coli numbering

[b] : Percentage (vol/vol) of formamide in the hybridization buffer

Table 3 Concentrations of selected anions, cations, conductivity and TOC explored through fluid analyses of samples obtained from the Deep Observation Well during downhole sampling

Fluid samples	Depth [m]	Conductivity [µS cm^{-1}]	pH	Salinity [g L^{-1}]	TOC [mg L^{-1}]	Sulfate [mg L^{-1}]	Ca [mg L^{-1}]	Fe [mg L^{-1}]	Mg [mg L^{-1}]	K [mg L^{-1}]	Cl [mg L^{-1}]	Na [mg L^{-1}]
sampling 1	625	224	7	229.2	55.6	4138	2025	13.7	764	455	134636	87074
sampling 2		219	7	218.1	124.6	4194	1944	n.d.	732	337	130311	84715
After N2 lift		225	7.1	n.d.	18.4	4183	1968	n.d.	752	331	137806	88238
sampling 2	635	219	7	217.4	63.4	4180	1936	n.d.	730	339	130417	83920
sampling 1	647	221	7.1	227.7	122.4	4122	2062	24.4	759	534	134028	86068
sampling 2		224	6.9	231.3	107.1	4409	2019	n.d.	769	348	136481	87356
sampling 1	675	222	7.2	226.2	134.7	4071	2036	24.9	746	561	132854	85765
sampling 2		225	7	228.9	22.8	4371	2055	n.d.	772	357	137184	88532

n.d.: not determined

Table 4 Concentrations of selected anions, cations, TOC and DOC explored through fluid analyses of the samples obtained from the Shallow Observation Well during the pump test

Production [m^3]	TOC [mg L^{-1}]	DOC [mg L^{-1}]	Fluorescein [mg L^{-1}]	Sulfate [mg L^{-1}]	Ba [mg L^{-1}]	Ca [mg L^{-1}]	K [mg L^{-1}]	Li [mg L^{-1}]	Mg [mg L^{-1}]	Na [mg L^{-1}]	Sr [mg L^{-1}]
3	5.8	5.3	0.21	1851.8	0.3	429.5	3017.4	0.8	361.0	17612.7	12.4
5	6.6	5.5	0.24	1724.8	0.4	604.2	3410.3	0.7	356.3	16492.3	17.1
7	4.3	4.1	0.25	1839.5	0.0	620.1	2432.4	0.8	363.6	17932.4	17.1
9	5.1	4.8	0.23	1879.7	0.3	648.0	1938.4	0.8	380.2	18632.0	15.4
11	15.7	14.1	0.22	1903.1	0.4	584.2	1818.1	0.8	384.1	18441.7	15.3
14	4.6	4.1	0.20	1935.1	0.3	785.8	1644.3	0.8	400.4	18724.7	21.5

Table 5 Total bacterial cell counts and relative percentage of cells hybridized with specific probes in the fluid sampled during pump test and downhole sampling from the Deep Observation Well prior to CO_2 injection

Fluid samples		Total cell counts[a] (10^6 cells mL^{-1}) (mean ± SD)	Relative percentages of phylogenetically defined groups [% of DAPI]							
			Bacteria[b]	Proteo-bacteria[c]	Low GC[d] (Firmicutes)	SRB				Arc
						SRB385	DEM1164r	DSS658	DSV687	
Pump test	4 m³	6 ± 3	95 ± 3	12 ± 0	6 ± 1	3 ± 0	3 ± 1	6 ± 1	6 ± 1	3 ± 0
	93 m³	1 ± 0	50 ± 5	33 ± 3	26 ± 3	33 ± 6	4 ± 1	9 ± 1	45 ± 2	5 ± 1
Downhole s.	647 m	5 ± 2	85 ± 3	35 ± 3	34 ± 3	33 ± 2	24 ± 7	n.d.	60 ± 3	28 ± 2

n.d.: not determined

[a] : obtained by DAPI staining

[b] : probe mix of EUB I, II, III. Numbers have been corrected by subtracting NON338 counts

[c] : probe mix of ALF968, Bet42a, Gam42a

[d] : probe mix of LGC354a, LGC354b, LGC354c

Table 6 Taxonomic assignment of sequenced SSCP/DGGE bands (16S rRNA gene) in fluid samples collected during pump tests in the Deep and Shallow Observation Wells

Well	Formation	Produced volume [m^3]	Detection method	Band	GenBank accession	Phylogenetic group	Closest relative	Similarity [%]
DOW	Stuttgart	4	SSCP	1	KR336962	Bacteroidetes	*Bacteroides* graminisolvens	100
				2	KR336963	Firmicutes	*Proteiniclasticum ruminis* D3RC-2	99
				3	KR336964	Bacteroidetes	*Flavobacterium* spp.	99
				4	KR336965	Bacteroidetes	*Flavobacterium* spp.	99
				5	KR336966	Firmicutes	*Clostridium sticklandii* DSM519	99
		30		6	KR336967	Firmicutes	*Halanaerobium* spp.	99
		93		7, 8	KR336967	Firmicutes	*Halanaerobium* spp.	99
SOW	Exter	7	DGGE	1	KR336956	Firmicutes	*Dethiobacter alkaliphilus*	99
				2	KR336957	Firmicutes	*Dethiobacter alkaliphilus*	99
		14		3	KR336958	Firmicutes	*Alkalibacter saccharofermentans*	98
				4	KR336959	Chloroplast	*Chloroplast*	99

Table 7 Taxonomic assignment of sequenced SSCP bands (16S rRNA gene) in fluid samples collected during downhole sampling in the Deep Observation Well

Well	Formation	Depth [m]	Band	GenBank accession	Phylogenetic group	Closest relative	Similarity [%]
DOW	Stuttgart	647	1	KR336960	Firmicutes	*Halanaerobium* spp.	90
			2	KR336961	Deltaproteobacteria	*Desulfohalobium utahense*	92
			3	KR336967	Firmicutes	*Halanaerobium* spp.	99

Table 8 Taxonomic assignment of sequenced DGGE bands (16S rRNA gene) in rock samples from the Shallow Observation Wells

Rock sample	Well	Formation	Band	GenBank accession	Phylogenetic group	Closest relative	Similarity [%]
ExF 3	SOW	Exter	1	KR336949	Betaproteobacteria	*Variovorax paradoxus*	100
ExF 3			2	KR336950	Alphaproteobacteria	*Hymenobacter psychrophilus*	100
ExF 4			3	KR336951	Gammaproteobacteria	*Pseudomonas* spp.	99
ExF 6			4	KR336954	Alphaproteobacteria	*Ochrobactrum* spp.	100
ExF 6			5	KR336955	Actinobacteria	*Propionicimonas paludicola*	99
ExF 9			6	KR336952	Alphaproteobacteria	*Rhizobium* spp.	100
ExF 9			7	KR336953	Betaproteobacteria	*Pelomonas* spp.	100

Fig. 1 Geologic Cross-section showing the wells system in Ketzin (from www.co2ketzin.de)

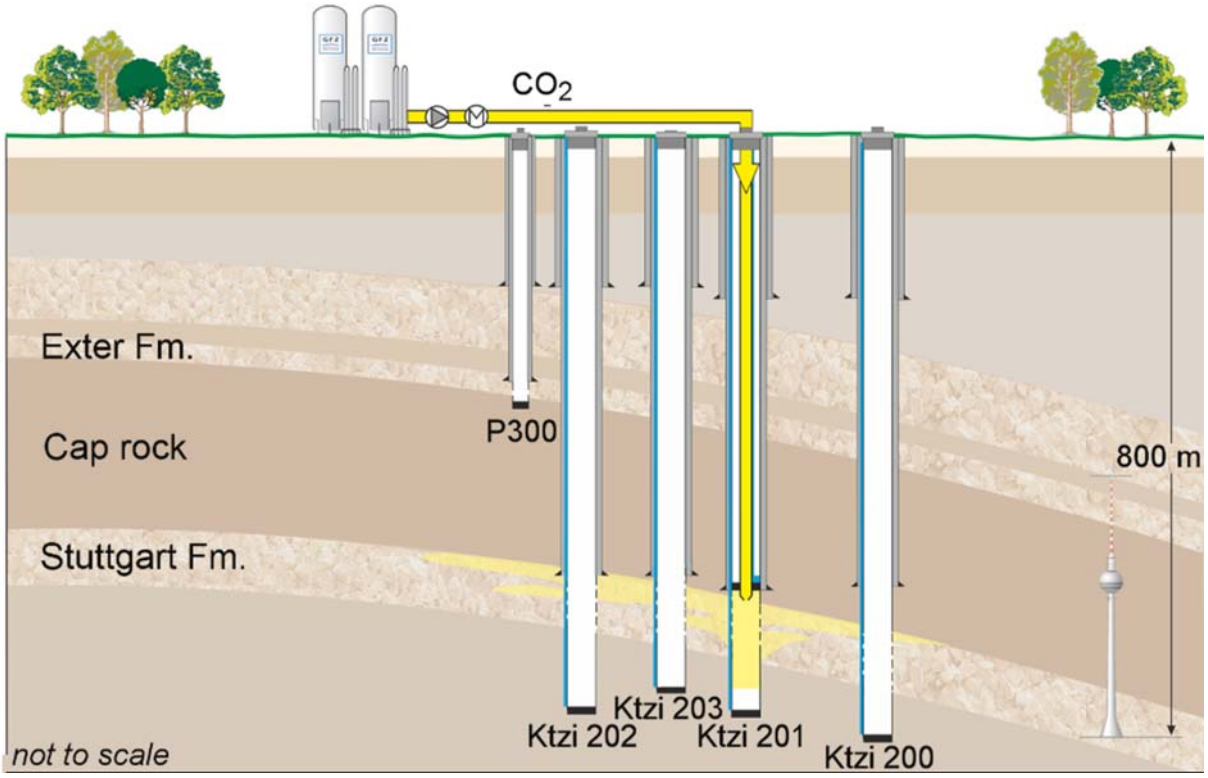

Fig. 2 The X-ray diffraction patterns of the studied Exter Formation sandstones (ExF 1, ExF 6, ExF 9, ExF 10) and siltstones (ExF 3, ExF 4). The vertical scale in counts per second is relative. Abbreviations: Qz-quartz, Pl- plagioclase, Ank - ferroan dolomite or ankerite, Anl- analcime, Cal- calcite, Ill/Sme - illite smectite mixed layered silicates, Kln – kaolinite

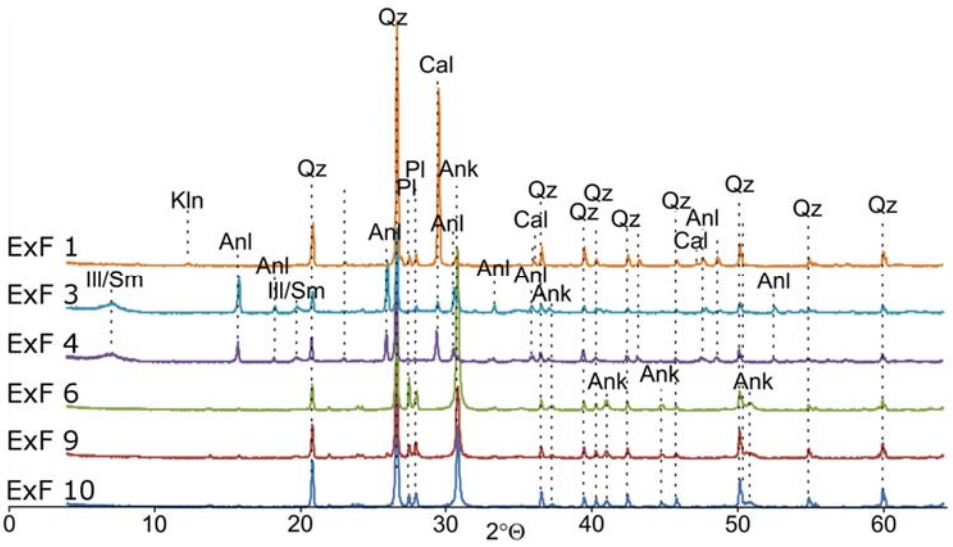

Fig. 3 SEM picture of sample ExF 9 showing ferrous dolomite crystals surrounded by clay minerals filling the pore space

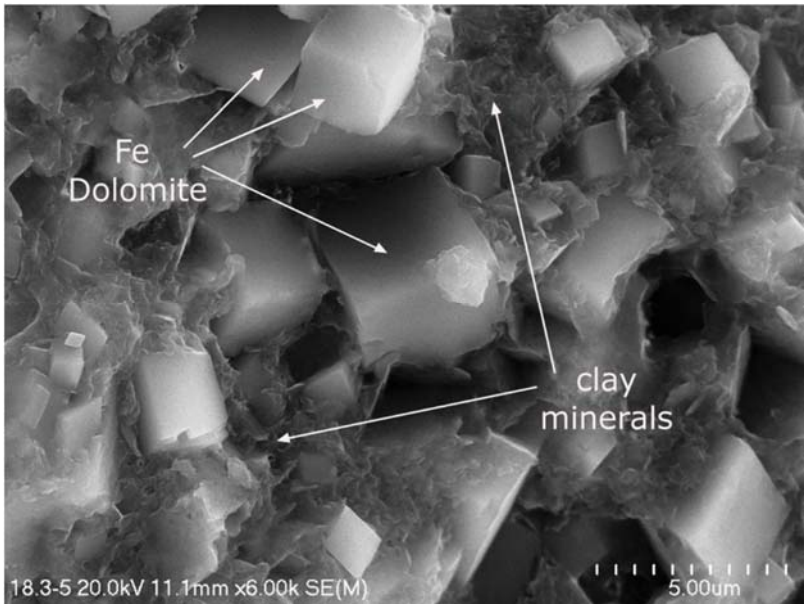

Fig. 4 Chemical composition of the carbonate in Exter Formation rock samples, determined through energy dispersive spectrometry analyses

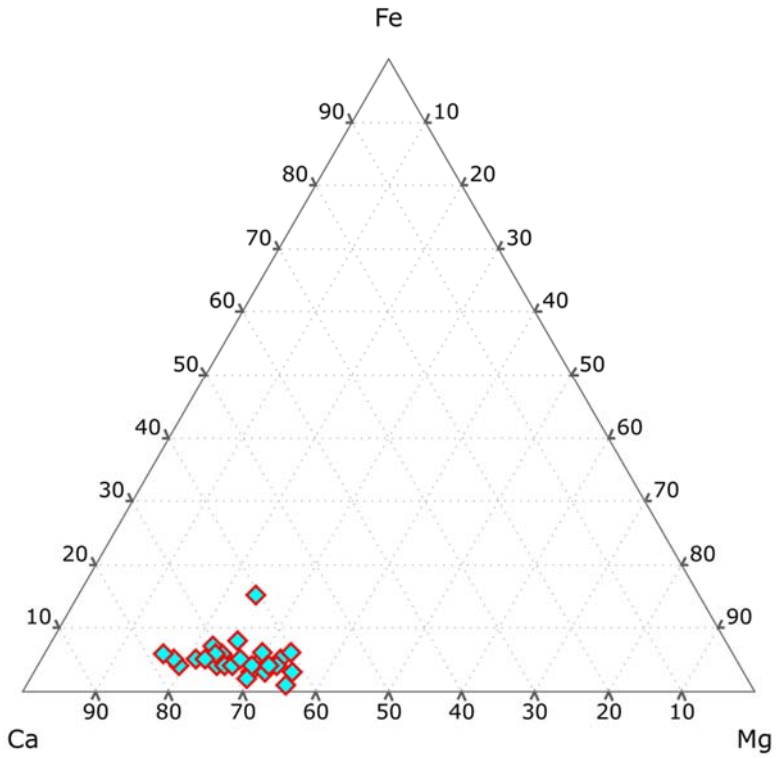

Fig. 5 DOC and fluorescein concentrations during pump test in the Deep Observation Well

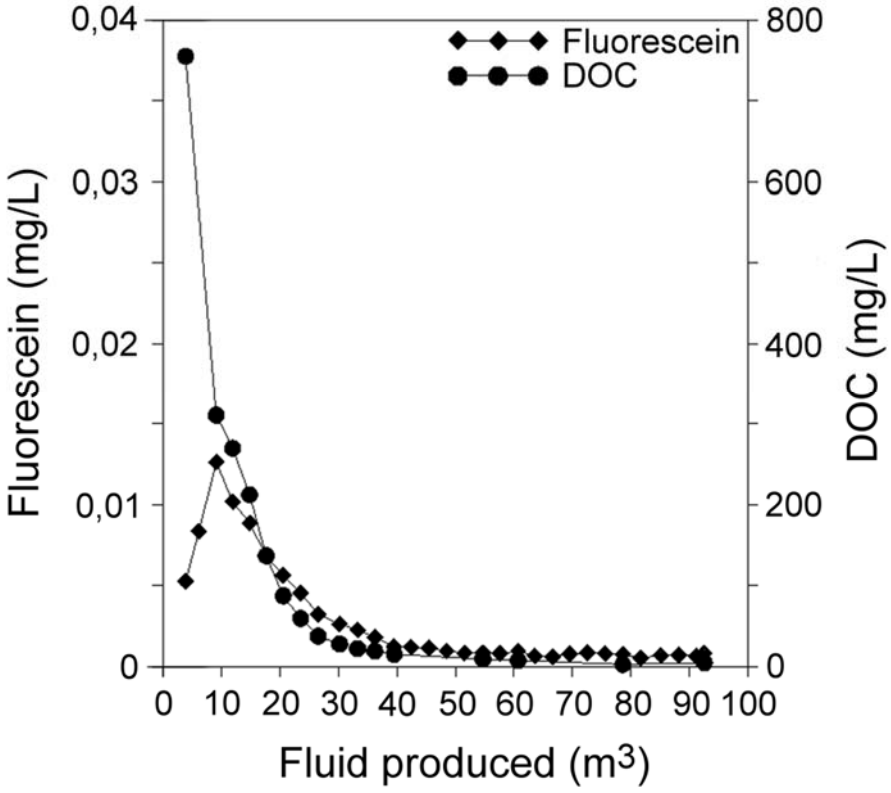

Fig. 6 Comparative PCR-SSCP analysis of bacterial 16S rRNA gene fragments of Deep Observation Well fluids collected during pump test (a) and downhole sampling (b). Sequenced bands are marked with numbers

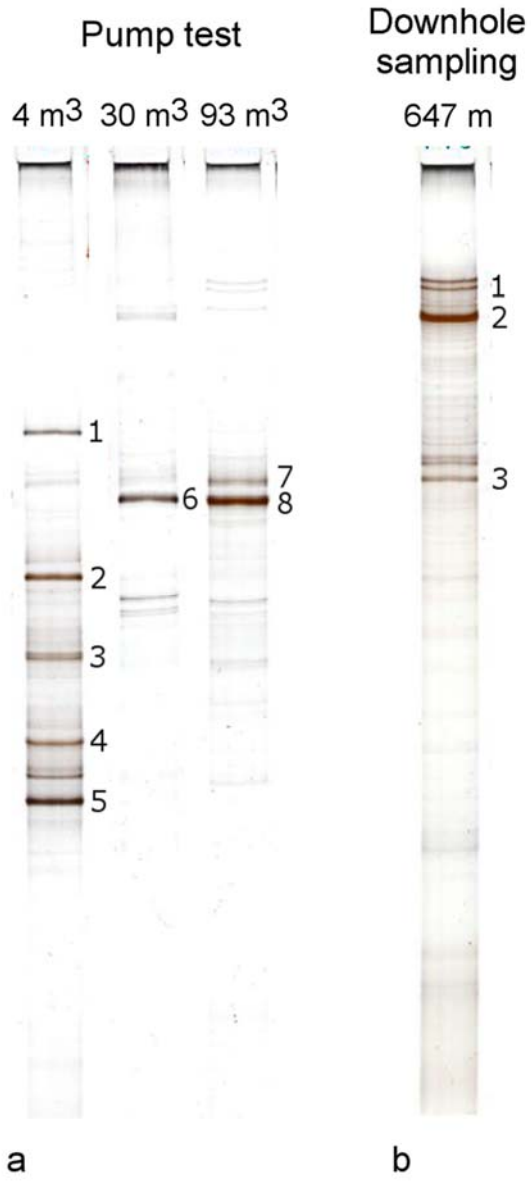

Fig. 7 Comparative DGGE analysis of bacterial 16S rRNA gene fragments of Shallow Observation Well samples: rock cores (a) and fluids collected during hydraulic test (b). Sequenced bands are marked with numbers