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Microbial community and inorganic fluid analysis during CO_2 storage within the frame of CO_2SINK – Long-term experiments under *in situ* conditions

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Abstract

Microorganisms play an important role in the transformation of material within the earth's crust. The storage of CO_2 could affect the composition of inorganic and organic components in the reservoir, consequently influencing microbial activities. To study the microbial induced processes together with geochemical, petrophysical and mineralogical changes, occurring during CO_2 storage, long-term laboratory experiments under simulated reservoir P-T conditions were carried out. Clean inner core sections, obtained from the reservoir region at the CO_2 storage site in Ketzin (Germany) from a depth of about 650 m, were incubated in high pressure vessels together with sterile synthetic formation brine under *in situ* P-T conditions of 5.5 MPa and 40 °C. A 16S rDNA based fingerprinting method was used to identify the dominant species in DNA extracts of pristine sandstone samples. Members of the α- and β-subdivisions of *Proteobacteria* and the *Actinobacteria* were identified. So far sequences belonging to facultative anaerobic, chemoheterotrophic bacteria (*Burkholderia fungorum*, *Agrobacterium tumefaciens*) gaining their energy from the oxidation of organic molecules and a genus also capable of chemolithoautotrophic growth (*Hydrogenophaga*) was identified. During CO_2 incubation minor changes in the microbial community composition were observed. The majority of microbes were able to adapt to the changed conditions.

During CO_2 exposure increased concentrations of Ca^{2+}, K^+, Mg^{2+} and SO_4^{2-} were observed. Partially, concentration rises are (i) due to equilibration between rock pore water and synthetic brine, and (ii) between rock and brine, and are thus independent on CO_2 exposure. However, observed concentrations of Ca^{2+}, K^+, Mg^{2+} are even higher than in the original reservoir fluid and therefore indicate mineral dissolution due to CO_2 exposure.

Keywords: CO_2 storage; Ketzin; long-term CO_2 exposure experiments; *in situ* P-T conditions, microbial and geochemical analysis

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1. Introduction

Carbon dioxide capture and storage (CCS) is considered as an option for the mitigation of CO_2 emissions for stabilization of greenhouse gas concentrations [1]. With the CO_2SINK project, the first onshore storage site in Europe was established as a research facility in Ketzin, Germany, in 2004 to examine the feasibility and effects of carbon dioxide storage in a natural saline aquifer at a depth of approximately 650 m [2].

Saline aquifers are investigated as possible storage sites for large volumes of anthropogenic CO_2 emissions [3]. Sub-terrestrial regions such as saline aquifers also represent microbial habitats [4]. Microorganisms play an important role in the transformation of materials within the earth's crust. The dissolution of feldspar [5] and Fe(III) minerals [6], and the precipitation of metal sulfides, and silicate and carbonate phases [7] are examples on how bacteria can determine mineralogical characteristics.

During CO_2 storage changes in the availability of inorganic and organic components in the reservoir system may occur. Thus, the acidification of the brine, caused by CO_2 dissolution, leads to alterations of mineral phases and to a concentration increase of inorganic fluid components, which may affect microbial activities. Furthermore, mineral dissolution produces activated surfaces, which may support microbial attachment and biofilm formation. An increased mobilization of organic molecules, caused by the mobilization by supercritical CO_2, as observed for the Frio storage site [8], may also influence bacterial growth. The complex geochemical changes may affect microbial activities, subsequently influencing physical reservoir properties like porosity and permeability.

Within the frame of the CO_2SINK project, effects of CO_2 on brine saturated sandstone cores from the test site in Ketzin are investigated. To specify and estimate the microbial impact on physical reservoir characteristics, long term laboratory CO_2 exposure experiments under *in situ* P-T conditions were performed. Microbial analyses were carried out together with geochemical, mineralogical [9, 10] and petrophysical studies [11]. Here the microbial and geochemical results from one experiment (B4-2) are reported.

2. Materials and methods

2.1 Experimental setup

Long term laboratory CO_2 exposure experiments were set up as described in [12]. Fresh formation water-filled reservoir sandstone material from the well Ktzi 202 was used for the experiment. During the coring of sandstone samples in Ketzin, the fluorescent dye tracer fluorescein was used to label the drilling mud [13]. Fluorescein analysis and carbon concentrations showed that drilling mud filtrate penetrated the outer 15 to 20 mm of sandstone cores. Inner regions were not affected by drilling mud. The results of these studies were proved by laboratory experiments, using a pressure vessel and a replication of the drilling mud, to simulate different pressures and incubation times that could occur during coring operations. In order to avoid contamination with drilling mud, outer core sections were removed and inner cores sections were incubated together with sterile synthetic brine (172.8 g/l NaCl, 0.62 g/l KCl, 8.0 g/l $MgCl_2 \cdot 6H_2O$, 4.9 g/l $CaCl_2 \cdot 2H_2O$) in a high pressure vessel under high CO_2 partial pressure and simulated reservoir P-T conditions of 5.5 MPa and 40 °C for about 24.5 months.

Because no pristine formation water was available at the time of the experimental setup, synthetic brine was used for the experiment. The chemical composition differed from that of Ketzin formation water. In the formation water higher concentrations were found for Na^+ (+18%), Cl^- (+17%) and Ca^{2+} (+35%) than in the synthetic brine. Lower concentrations were detected for Mg^{2+} (-31%) and K^+ (-33%). The largest difference was found for SO_4^{2-} (+95%). Because the rock material used for the long term experiments contained pore water, a chemical equilibrium (brine-brine equilibrium) between both fluids (synthetic brine and formation water trapped in the rock pores) will be formed in the vessel fluid. The brine-brine equilibrium concentrations of elements were calculated based on the amount of pore water, deduced from porosity, and the amount of synthetic brine. After 15, 21, and 24.5 months of CO_2 exposure, vessels were opened (pressure was released) and rock and fluid samples were analyzed for geochemistry, microbial community, and mineralogical and petrophysical characteristics.

2.2 Inorganic fluid component analysis

Concentrations of anions and cations in fluid samples were determined in 1:100 dilutions by ion chromatography and inductively coupled plasma optical emission spectrometry, respectively, according to DIN 38402-21. Sodium chloride concentrations were determined via potentiometric titration of chloride.

2.3 DNA extraction and DGGE analysis

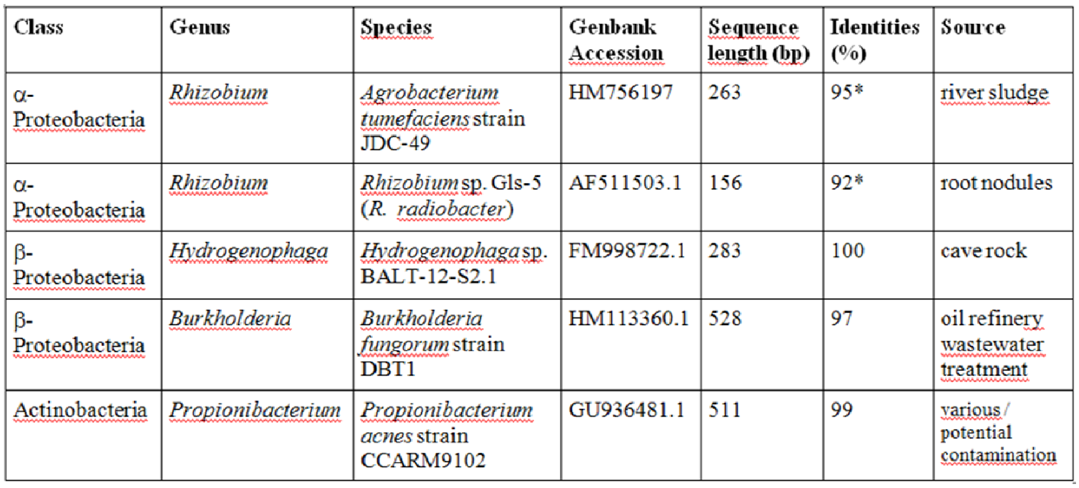
DNA was extracted from about 15-20 g of CO_2 exposed and non-exposed sandstone material (B4-2, 633.6 m) using the Ultra Clean Soil DNA extraction kit (MoBio, Carlsbad, CA, USA). Bacterial 16S rRNA genes were amplified using universal primers [14] and separated by denaturing gradient gel electrophoresis (DGGE) according to [15]. For the taxonomic assignment, sequences were compared to the nr/nt database at NCBI using blastn (www.blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results and discussion

3.1 Microbial community profiles

To detect changes in the microbial community, bacterial 16S rRNA genes from sandstone material, exposed 0, 15, 21 and 24.5 months to CO_2 were analyzed using DGGE. The 16S rDNA profiles revealed only minor changes in the microbial community composition during CO_2 exposure. Sequences could be assigned to the subspecies listed in Table 1. Thus, bands corresponding to a *Rhizobium* subspecies, to *Burkholderia fungorum* and to *Propionibacterium acnes* were detected in DNA extracts from non-exposed and from exposed sandstone. An *Agrobacterium tumefaciens* and a *Hydrogenophaga* corresponding band were detected in non-exposed material, exclusively.

Table 1 Microorganisms, identified with DGGE analysis. 16S rDNA sequences, obtained from sequencing of DGGE bands, were assigned to the corresponding microorganisms. The taxonomic assignment and alignment details of the nearest match are shown.



Class	**Genus**	**Species**	**Genbank Accession**	**Sequence length (bp)**	**Identities (%)**	**Source**
α-Proteobacteria	*Rhizobium*	*Agrobacterium tumefaciens* strain JDC-49	HM756197	263	95*	river sludge
α-Proteobacteria	*Rhizobium*	*Rhizobium* sp. Gls-5 (*R. radiobacter*)	AF511503.1	156	92*	root nodules
β-Proteobacteria	*Hydrogenophaga*	*Hydrogenophaga* sp. BALT-12-S2.1	FM998722.1	283	100	cave rock
β-Proteobacteria	*Burkholderia*	*Burkholderia fungorum* strain DBT1	HM113360.1	528	97	oil refinery wastewater treatment
Actinobacteria	*Propionibacterium*	*Propionibacterium acnes* strain CCARM9102	GU936481.1	511	99	various / potential contamination

* Identity values may be influenced by low sequence quality.

3.2 Changes in inorganic fluid component concentrations

To determine the influence of mineral dissolution and precipitation on the fluid chemistry, fluid samples, taken after 15, 21, and 24.5 months of CO_2 incubation, were analyzed for inorganic geochemistry. It was observed that concentrations of the main anions and cations increased during CO_2 exposure (Fig. 2).

Na^+ and Cl^- concentrations increased and reached their brine-brine equilibrium concentrations.

The Ca^{2+}, Mg^{2+} K^+ and SO_4^{2-} concentrations exceeded their brine-brine equilibrium concentration, which reflects mineral dissolution. But the mineral dissolution can only partly be attributed to CO_2 exposure, because a proportion of the concentration increase is due to the equilibration between rock and brine. However, concentrations, exceeding Ketzin formation water concentrations, as in the case for Ca^{2+}, Mg^{2+} and K^+, can be attributed to mineral dissolution in response to CO_2 exposure.

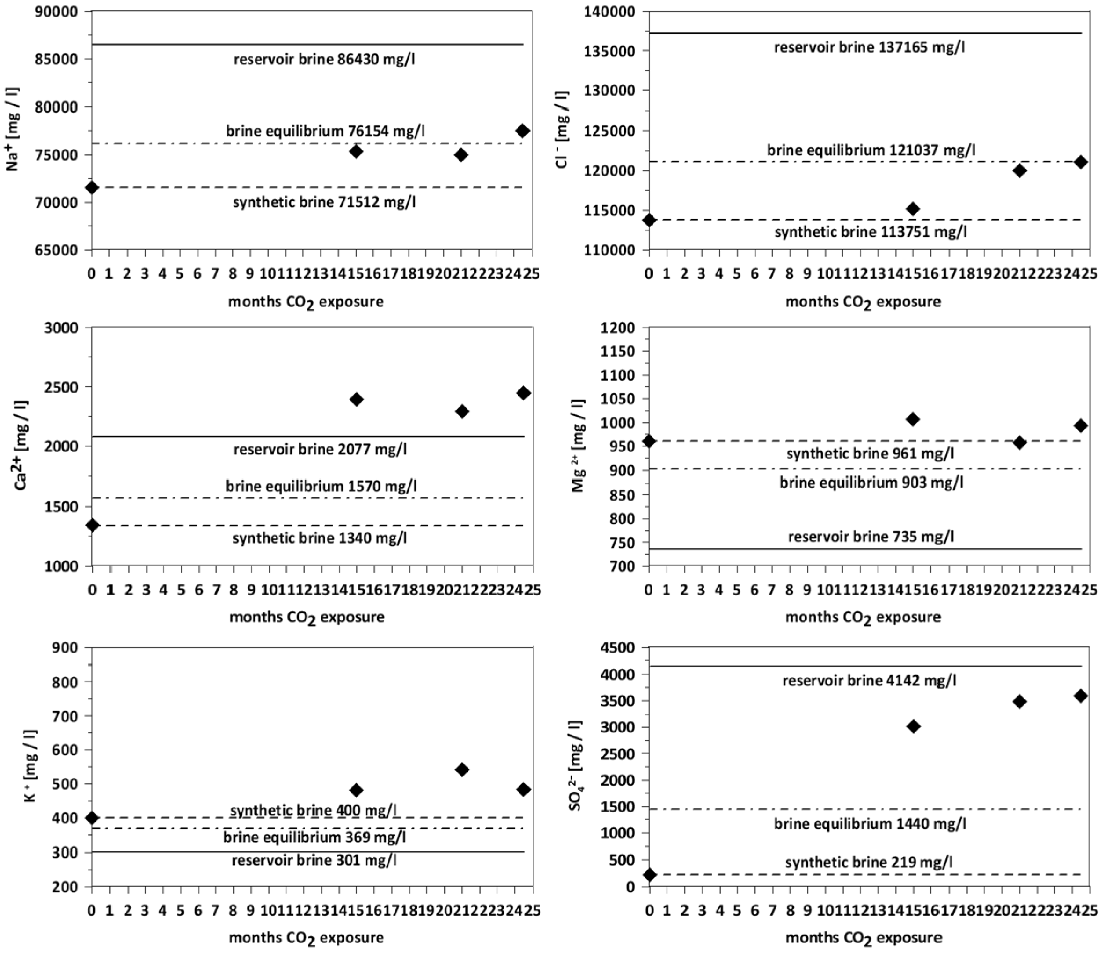


Figure 2 Course of inorganic fluid component concentrations during a long-term CO_2 exposure experiment. Fluid samples were analyzed after 0, 15, 21 and 24.5 months of exposure.

Furthermore, Na^+, Ca^{2+} and Mg^{2+} concentrations showed fluctuations, with decreasing values after 21 months of incubation, increasing again after 24.5 months. This could be a consequence of the interplay of dissolution and secondary precipitation processes. Secondary mineral precipitation may also be the reason for the decreasing K^+ concentration after 24.5 months of exposure.

The sulfate concentration showed the most drastic change and was increased by 135% (brine-brine equilibrium: 1440 mg/l, 24.5 months: 3400 mg/l), but did not exceed the Ketzin formation water concentration. Thus, dissolution of SO_4^{2-} bearing minerals has occurred, but may be a consequence of the low initial SO_4^{2-} concentration of the synthetic brine.

4. Discussion

In the sandstone samples, obtained from the Ketzin reservoir horizon, microorganisms belonging to the phylotypes *α-Proteobacteria*, *β-Proteobacteria* and *Actinobacteria* were detected. The organisms have a chemoheterotrophic energy metabolism and a facultative anaerobic respiration in common [16]. Although some DGGE derived sequences were short and of low quality, the sequences could clearly be assigned at the genus level. Identified organisms are typical for surface habitats like soil and fresh water, but have been found in subsurface environments before [17-19]. The ability to grow anaerobically with nitrate (nitrate respiration) has been shown for species of the *Rhizobium*, *Burkholderia* and *Hydrogenophaga* genera.

The indigenous occurrence of *Propionibacterium acnes* in environmental samples is still matter of debate. It has been isolated from a number of subsurface samples, e.g. hydrothermal vent samples [20], petroleum crude oil [21] and deep-granitic-fracture water [22]. However, *P. acnes* is a commensal bacterium of human skin [23] and its finding could also be a product of contamination during sampling or DNA extraction.

Low molecular weight organic acids like acetate and format are constituents of the organic material in the reservoir sandstone. Although the concentrations (total organic carbon concentration < 0.1% [13, 24]) are very low, they may be utilized as substrates for microbial growth. *Hydrogenophaga*, as a facultative lithoautotrophic bacterium, has the ability to oxidize hydrogen. Traces of hydrogen were detected in the Ketzin reservoir fluid (0.2%, personal communication Martin Zimmer, GFZ, Germany). After CO_2 exposure *Hydrogenophaga* sequences were not detected, which may be the consequence of the lack of hydrogen in the high pressure vessel. In general, the microbes were able to adapt to the changed conditions. However, microbiological results have to be verified by analyzing material from additional vessels [12]. Because little physiological information about the organisms is available, more evaluation is needed to estimate to microbial impact on mineral dissolution and precipitation during CO_2 storage.

Archaea and sulfate reducing bacteria (SRB) could not be identified in the sandstone samples using specific primers, although members of the Archaea and SRB were detected in downhole samples from the Ketzin wells [25]. Because, the number of cells in the reservoir sandstone samples is very low and these organisms are often underrepresented, they may be therefore not detected. Cells from reservoir sandstone samples need to be concentrated *via* density centrifugation for counting and microscopical studies like fluorescence *in situ* hybridization or electron microscopicy. Also an optimization of the DNA extraction method and cloning may deliver further insight into the indigenous microbial community.

During CO_2 exposure the concentrations of Ca^{2+}, Mg^{2+}, K^+ and SO_4^{2-} in solution increased, exceeding brine-brine equilibrium concentrations. This is in consistence with mineralogical studies, were dissolution of the corresponding minerals phases like anorthitic plagioclase, K-feldspar and anhydrite was observed [9, 10]. However, mineral dissolution may also be a consequence of rock-brine equilibration. But concentrations, exceeding Ketzin formation water level, as in the case for Ca^{2+}, Mg^{2+} and K^+ can be attributed to CO_2 exposure. Mineral dissolution was also reflected in increased porosities, as observed in [12]. The reverse trend to decreasing porosities after 24 months of exposure, decreasing fluid concentrations and the precipitation of albite grains [10], may be caused by secondary mineral precipitation.

With the combined analysis of biological, chemical and physical parameters, the experiments will help to estimate long-term effects during geological CO_2 storage. However, more evaluation is needed to identify significant mineralogical changes related to CO_2 exposure, also taking into account fluid dynamics and different brine/gas saturations.

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