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Monitoring of the microbial community composition in deep subsurface saline aquifers during CO_2 storage in Ketzin, Germany

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Abstract

This study characterized the composition and activity of the autochthonous microbial community in formation fluids of a saline CO_2 storage aquifer during CO_2 injection and during an N_2 lift. The clean-up of the wells prior CO_2 injection by N_2 lift decreased the total microbial cell numbers, and the number of sulphate reducing bacteria (SRB) was reduced by at least two orders of magnitude. Fluorescence *in situ* Hybridisation (FISH) and molecular fingerprinting demonstrated that the microbial community was strongly influenced by the CO_2 injection. Before CO_2 arrival, up to 10^6 cells ml^{-1} were detected by DAPI-staining at a depth of 647 m below the surface. The microbial community was dominated by fermentative halophilic bacteria and sulphate reducing bacteria. Both the FISH and fingerprinting analyses revealed quantitative and qualitative changes after CO_2 arrival. An enhanced activity and quantity of the microbial population after five months of CO_2 storage indicated that the community was able to adapt to the extreme conditions of the deep biosphere and to the extreme changes of these anthropogenically modified conditions.

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CO_2 storage, microbial monitoring, Fluorescence *in situ* Hybridisation, genetic fingerprinting

1. Introduction

CO_2 capture and storage in saline aquifers is a promising method to dispose of CO_2 that would be otherwise emitted into the atmosphere. Microbiological monitoring of these systems is essential when reservoirs are favourable to microbial life, as microbes can influence storage by lowering injectivity, or precipitating carbonate and/or other minerals. This study reports the development of efficient microbiological monitoring procedures at the CO_2SINK project, located near Ketzin, west of Berlin, Germany. CO2SINK is a pilot project for testing and monitoring of CO_2 storage in a saline aquifer (Fig. 1). A complete understanding of subsurface processes, including

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the effects of microbes, is essential for successful wide-scale implementation of CCS and for guaranteeing public acceptance of this relatively new technology.

The potential influence of injection and long-term storage of CO_2 in saline aquifers on the subsurface microbial communities is presently unexplored. Changes in microbial community composition and activity, should result from the injection of CO_2 into the reservoir. The decreased pH value and other geochemical change induced by CO_2 injection has an influence on the metabolism of the both heterotrophic and lithoautotrophic microorganisms [1]. Therefore, injection of the CO_2 in the supercritical state (temperature above 31.1 °C, pressure above 72.9 atm) may induce metabolic shifts in the microbial communities. Furthermore, microbial populations and activity can be strongly influenced by changes in the pH value, pressure, temperature, salinity and other abiotic factors. Therefore, it is important to characterise the microbial community of the deep subsurface before and during the injection of CO_2.

Thorough studies of samples from deep boreholes, using a variety of molecular techniques, have shown an active biosphere composed of diverse groups of microorganisms [2], [3], [4]. In addition, numerous studies of microbial communities in the deep biosphere have revealed that the most important metabolic pathways in the deep subsurface are sulphate reduction, fermentation and methanogenesis [5], [6], [7], [8], [9]. A description of microbial communities that originated from varied deep terrestrial settings has shown that those subsurface microbial communities could represent the greatest mass of living organisms on our planet [3], [10]. Furthermore, analyses of the composition of microbial communities will contribute to the understanding of biogeochemical processes in the deep subsurface and will enable better prediction of CO_2 behaviour in saline aquifers. The interactions between microorganisms and the minerals of both the reservoir and the cap rock may cause major changes to the porosity and permeability of the reservoir [11], [12], [13]. In addition, microbiologically enhanced precipitation and corrosion may occur around the well affecting the casing and the cement. Moreover, the growth of microorganisms on the metal surface (biofilms) can have a profound effect on metal deterioration, known as microbially-influenced corrosion (MIC) [14].

In this study we concentrated on the results obtained from the observation well 1 (Ktzi 200), where CO_2 arrived after two weeks of injection. We were able to study the effects of the ten months CO_2 storage on the microbial community after arrival. The main objective was to characterise the microbial community of a deep saline aquifer and to assess the influence of CO_2 exposure on the composition of the microbial community by using fingerprinting methods and FISH technique without previous incubation. The completed analyses provide fundamental data on the predominant microbial processes and changes in those processes during the CO_2 storage monitoring.

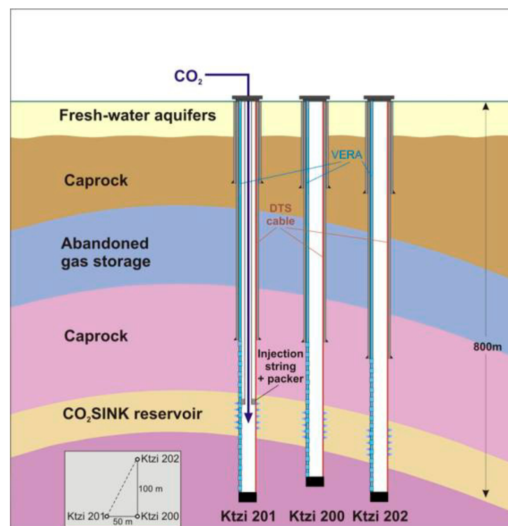

Figure 1 The concept of CO2SINK CO_2 storage monitoring

2. Methodology

2.1 Study site

The storage site is located in the north eastern Germany near Ketzin. The target reservoir for CO_2 storage is the Triassic Stuttgart Formation [15], consists of siltstones and sandstones interbedded by mudstones deposited in a fluvial environment [16]. For the injection and monitoring of the CO_2 in a natural saline aquifer, three 700 to 850 m deep holes were drilled by mud rotary drilling in March and April 2007 (Fig. 1). The temperature and pressure of the formation fluid were approximately 35 °C and 62 bar, and the salinity was roughly 235 g l^{-1}.

Prior to CO_2 injection, a number of hydraulic tests were performed on all wells. The so-called N_2 lift was performed in the injection well and the two observation wells two days prior to the CO_2 injection to “clean up” the wells from the rest of the drill mud [12]. The injection of CO_2 into the Ktzi 201 well was started on the end of June 2008 [17]. The CO_2 arrival in the Ktzi 200 well was observed about two weeks later, after 500 t of CO_2 have been injected. The CO_2 arrival in the observation well 1 (Ktzi 200) was detected by the downhole measurements of the amount and nature of the dissolved gases in the fluid samples. Notably, detailed physical, geochemical and microbiological monitoring was performed in all three wells [18]. This study concentrates on the results obtained from the observation well 1 (Ktzi 200), where it was possible to monitor the effects of the CO_2 exposure during the five months.

2.2 Sample collection

Fluid samples during N_2 lift were collected directly from the well head. Fluid samples were collected from the reservoir using downhole sampling (Erdöl-Erdgas Workover GmbH) at a depth of 647 m (perforation depth) using double ball-lining (Doppelkugelbüchse, DKB) and flow-through (PNL64) samplers. Both samplers were sterilized flushed with sterilised deionised water and ethanol immediate before sampling. The pH, conductivity, temperature and other parameters were measured directly after the sampling process. The fluids were transferred aseptically into sterilised 100 to 1000 ml glass vials, refrigerated to 4 °C and immediately transferred to the laboratory for microbiological analyses. Contamination control during sampling is described by Wandrey and co-authors [19].

2.3 Geochemical analysis

The total organic carbon content was determined using a TOC-analyser (Dimatec GmbH) according to DIN EN 1484-H3. The concentrations of cations and anions were measured after filtration of the sample (0.45 µm) using an ion chromatograph with an emission spectrometry (ICP-OES: inductively coupled plasma-optical emission spectrometry) according to DIN 38402-21. Quantification of the dissolved low molecular weight organic acids (e.g. acetate) has been done by ion chromatography with conductivity detection (ICS 3000, Dionex Corp.). Electrical conductivity, pH and fluid temperature were measured during the sampling process using a portable pH/mV/Temperature meter (WTW).

2.4 Molecular approaches applied

2.4.1 Fluorescence *in situ* hybridisation

FISH coupled with rRNA-targeted oligonucleotide probes was applied for direct visualisation, identification and localisation of bacterial cells from selected phylogenetic groups in environmental samples. To obtain sufficient biomass for FISH, freshly collected fluid samples (500 to 1000 ml) were concentrated to a volume of approximately 0.5 ml by centrifugation (Heraeus Biofuge Pico, Sigma 6K15) and were then fixed as described previously by Pernthaler et al. [20]. The probes for the domains *Bacteria* and *Archaea* and specific probes for the sulphate reducing bacteria, labelled with the cyanine dye Cy3 and FLUOS (Thermo Scientific) were used. Detection limit is 10^3 cells ml^{-1}. Details of the procedures are given in Morozova et al. [21].

2.4.2 PCR–Single-Strand-Conformation Polymorphism and Denaturing Gradient Gel Electrophoresis

Genetic profiling of amplified 16S rRNA genes were applied for characterization of the microbial community by Single-Strand-Conformation Polymorphism (PCR-SSCP) and Denaturing Gradient Gel Electrophoresis (DGGE). For DNA extraction, microbial cells were concentrated by filtration of the reservoir fluids on 0.2 µm filter units (Millipore). Nucleic acids were extracted from preserved filters with Ultra Clean Power Soil DNA Isolation Kit (Mo

Bio Laboratories) according to manufacturer's suggested protocols. 16S rRNA subunits were amplified by polymerase chain reaction (PCR) using different *Bacteria,* sulphate-reducing bacteria and *Archaea*-specific primers. PCR products were analysed by electrophoresis via SSCP [22] and DGGE methods [23]. The obtained 16S rRNA gene sequences were compared with the sequences available in the GenBank database using BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Microbial monitoring during CO_2 storage

Saline aquifers could be characterised as an extreme habitat for microorganisms due to reduced geochemical conditions, high pressure and salinity, a high number of microorganisms were found in all fluid samples. A total of 10^6 cells ml^{-1} were measured in samples from a depth of 647 m (Fig. 2). The observed cell numbers are at least one order of magnitude higher than the values identified by other analyses of anaerobic aquifers and enumerated by acridine orange direct microscopic counts [24], [25]. Fingerprinting analyses revealed that fermentative halophilic bacteria (*Halanaerobium sp*., *Halobacteroidaceae*) and sulphate reducing bacteria (*Desulfohalobium sp., Desulfotomaculum sp*.) are dominant microorganisms in this microbial community. FISH analyses with specific probes that targeted phylogenetic groups of the SRB confirm those results. Previous microbiological and molecular analyses of deep saline groundwater revealed a sulphate-reducing microbial population belonging to *Firmicutes* [26]. Several other studies have discovered evidence of *Desulfotomaculum* spp. and other sulphate-reducing bacteria in the deep subsurface [8], [27], [28], [29].

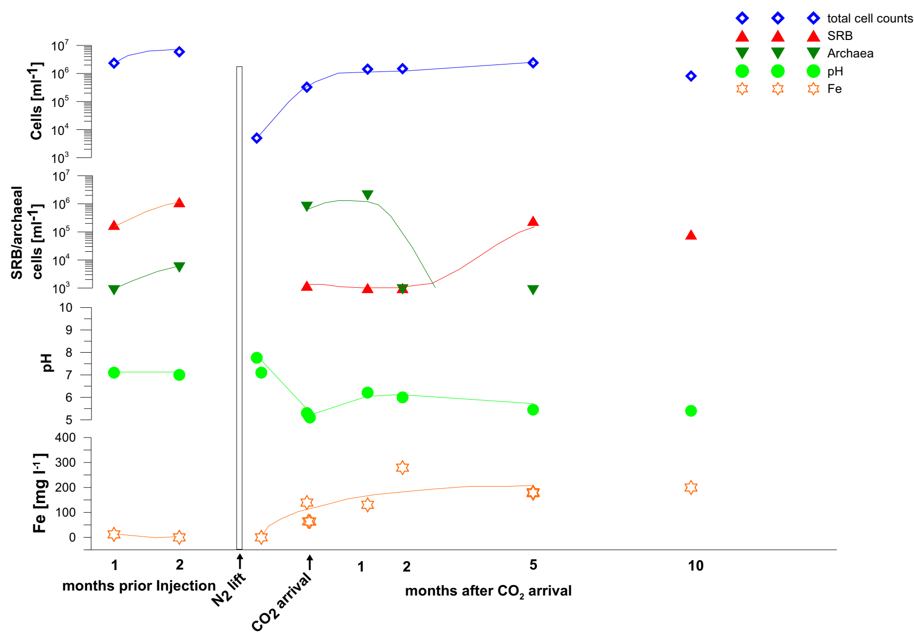

Figure 2 Iron concentration, pH, SRB and archaeal cell counts (FISH), and total cell counts (DAPI) during CO_2 monitoring. Last sampling (10 months after CO_2 arrival) was performed at a 692 m depth because the fluid level was lowered to the bottom of the filter screens

The N_2 lift prior to CO_2 injection removed remaining organics from the drill mud [19]. Furthermore, N_2 lift resulted in decreasing of the TOC and acetate concentrations [21] and drastic reduction of the numbers of bacteria after clean up the well (Fig. 2). Similar results were reported for the injection well Ktzi 201 after N_2 lift [11], [12]. The decrease in organic carbon in the well by N_2 lift contributed to the reduction of the SRB cell numbers and activity. Thus, the cell activity was too low to be detected using FISH (Fig. 2). Also both the PCR-SSCP analyses

and DGGE with specific primer for SRB were not able to detect the presence of SRB in the fluid samples taken after N_2 lift (Fig. 3A, 3B; Tab. 1, 2).

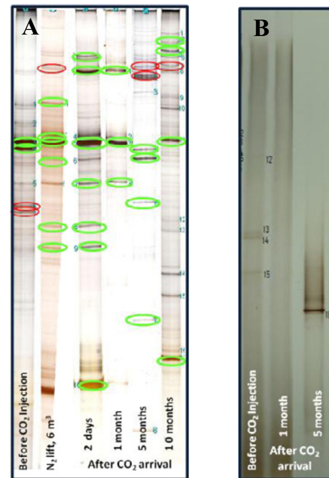

Figure 3 Molecular fingerprinting analyses. A: PCR-SSCP analyses of the microbial community in the fluids taken before CO_2 injection, during N_2 lift and after CO_2 arrival in observation well. SRB are marked with red circles, fermenting bacteria are marked with green circles. B: DGGE analyses of the sulphate-reducing community in the fluids taken before CO_2 injection, and after CO_2 arrival in observation well.

Table 1 Affiliation of the PCR-SSCP fragments

SSCP Fragments			Similarity [%]	Organism	References
Before CO_2 injection		3, 4	90 - 100	*Halanaerobium sp.*	Cayol, J.L. et al. Haloanaerobium lacusroseus sp. nov., an extremely halophilic fermentative bacterium from the sediments of a hypersaline lake, 1995 Rainey, F.A. et al. The Taxonomic Status of the Fermentative Halophilic Anaerobic Bacteria: Description of Haloanaerobiales ord. nov., Halobacteroidaceae fam. nov., Orenia gen. nov. and further Taxonomic Rearrangements at the Genus and Species Level, 1995 Wu, X.Y. et al. A strictly anaerobic halophilic organism isolated from the sediment of northeast Pacific, submitted
N_2 lift		1, 2, 3, 4, 6, 7			
After CO_2 arrival	2 days	1, 4, 5, 6, 8, 9			
	1 month	2, 3, 4			
	5 months	4, 5, 7			
	10months	2, 5, 6, 11			
After CO_2 arrival	2 days	7, 10	95	*Halobacteroidaceae*	Gales, G. et al. A new Gram-positive halophilic fermentative bacterium isolated from a deep hypersaline subsurface environment, unpublished
	1 month	4			
	10months	6, 17			
Before CO_2 injection		6,7	97	*Desulfohalobium utahense*	Jakobsen, T.F. et al. Desulfohalobium utahense sp. nov., a moderately halophilic, sulfate-reducing bacterium isolated from Great Salt Lake, 2006
N_2 lift		0			
After CO_2 arrival	5 months	1, 2, 3			
	10months	7			
After CO_2 arrival	2 days	1, 2, 3	91 - 98	*Bacteroidetes*	Wang, L. et al. Gene diversity of CYP153A and Alk B alkane hydroxylases in oil-degrading bacteria isolated from the Atlantic Ocean, 2010 Wang, J.J. et al. Diversity of free-living bacteria along with a salinity gradient, unpublished
	1 month	1			
After CO_2 arrival	10months	4	94	*Comamonas aquatica*	Liang, B. and Li, S.P. Isolation and characterization of a phenanthrene-degradation strain from a polluted farmland, unpublished
After CO_2 arrival	10months	12, 13	95	*Empedobacter sp.*	Cao, J. Empedobacter brevis strain Y7D isolated from a petroleum-oil contaminated soil, unpublished

Table 2 Affiliation of the DGGE fragments

DGGE Fragments			Similarity [%]	Organism	References
Before CO_2 injection		12, 13, 14	100	*Desulfotomaculum halophilum*	Zverlov, V. et al. Lateral gene transfer of dissimilatory (bi)sulfite reductase, 2005
After CO_2 arrival	5 months	16	99	uncultured	Kjeldsen, K.U. et al. Diversity of sulfate-reducing bacteria from an extreme hypersaline sediment, Great Salt Lake (Utah), 2007

The CO_2 arrival resulted in a pH decrease from 7.5 to 5.5 after degassing of CO_2 and decrease of the number of bacteria from 10^6 to 10^3 (Fig. 2). A change of the environmental parameter, among others the pH decrease, is known to have deleterious effect on the cell function and biochemistry, affecting bacterial activity and composition [30], [31]. The bacterial population recovered in the following months after CO_2 arrival as indicated by the total cell counts (Fig. 2) and PCR-SSCP analyses (Fig. 3A). Furthermore, our previous study revealed the increase of the active cells proportion from ½ to ¾ in the microbial community five months after CO_2 arrival [21]. This strengthened the assumption that the microorganisms are capable of adapting to the extreme shifts of environmental condition in the deep biosphere and to their active involvement in reservoir biogeochemical cycling.

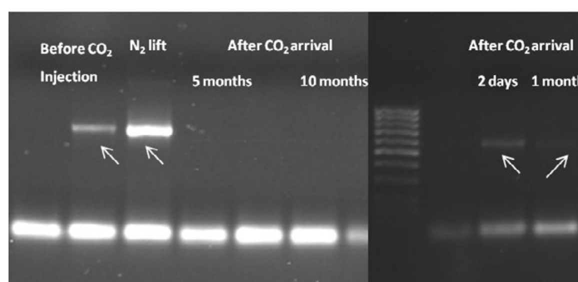

Figure 4 PCR with specific primers for domain *Archaea.* Arrows show PCR-products.

After CO_2 arrival, an increase of the archaeal cell numbers was detected (Fig. 2). PCR with specific primers for domain *Archaea* revealed presence of archaeal communities in the fluid samples taken during N_2 lift and after CO_2 arrival (Fig. 4). Further analyses by DGGE and special primer for methanogenic archaea are in progress. It could be taken into consideration that CO_2 arrival and pH decrease contributed to the temporal outcompetition of sulphate reducing bacteria by methanogenic archaea. The previously described groups of microorganisms are important terminal oxidisers in the anaerobic mineralisation of organic matter and can be observed as ecological equivalents, mineralising organic matter to CO_2 or to CO_2 and CH_4 in high-sulphate and low-sulphate environments, respectively [5]. Under acidic conditions, the outcome of competition between these trophic groups was shown to be regulated by their pH susceptibility rather than by their thermodynamic energy yield [32]. At pH below 7 methanogenic archaea have better growth properties than SRB and would be expected to outcompete the SRB [33]. Interestingly, certain strains of methanogenic archaea have been shown to grow at low pH [34]. This correlates well with the observed increase in the archaea community for the samples where no or only a methodically insignificant number of SRB was detected (Fig. 2). Importantly, methanogenic archaea are highly resistant microorganisms [35] that may use only hydrogen and CO_2 as energy and carbon sources and do not require other organic substrates.

Further monitoring revealed that the SRB populations increased from 10^4 to 10^6 cells ml^{-1} after 5 months of CO_2 supply (Fig. 2). PCR-SSCP analyses and DGGE with specific primers for SRB also showed the presence of SRB in the samples taken after 5 and 10 months CO_2 supply (Fig. 3A, 3B). Notably, recent studies revealed that in acidic environments addition of organic substrates increased sulphate reduction only after a lag phase [36], [37]. Furthermore, both FISH and PCR with archaeal primers revealed, that no archaea could be found in the fluid samples taken 5 months after CO_2 arrival (Fig. 2, 4). Those results suggested that SRB outcompeted the

methanogens due to a reduction in sulphate, which is a major catabolic process due to the higher affinity of sulphate reducers for hydrogen and acetate and a higher energy yield of sulphate reduction [38], [39]. Under these conditions, methanogenesis occurs very slowly, and the cell activity is too low to be detected using FISH, as is the case for the samples obtained after five months of the CO_2 arrival (Fig. 2).

The identification of the SRB, which are known to be involved in corrosion [40], [41], [42], [43], [44], could be of great importance for the technical progress of the long-term CO_2 storage technique. The increase of iron concentration (Fig. 2) followed by increasing of SRB in the observation well could be addressed to the biologically simulated corrosion processes, since their activity tends to pit the iron [14], [45], [46], [47]. Furthermore, our recent investigations showed that members of this group were able to rapidly and quantifiably change the permeability of the injectivity in the near well bore area [11], [12]. Further microbial monitoring should provide insight into microbial activity and composition and technical reliability of the CO_2 storage technique.

4. Conclusions

The first results of the fluid samples analyses revealed high diversity of the saline aquifer inhabitants. Although saline aquifers could be characterised as an extreme habitat for microorganisms due to reduced conditions, high pressure and salinity, a high number of microorganisms were found. The microbial community was dominated by fermentative halophilic anaerobic bacteria, and sulphate reducing bacteria. Microbial monitoring during CO_2 injection has shown that the microbial community was strongly influenced by the CO_2 injection. Both the fingerprinting analyses as well as FISH analyses revealed quantitative and qualitative changes after CO_2 arrival. Our study revealed temporal shifts in the microbial community from chemoorganotrophic to chemolithotrophic populations, as evidenced by the temporarily outcompetition of sulphate reducing bacteria by methanogenic archaea. Of great importance was the identification of the sulphate reducing bacteria, which are known to be involved in corrosion processes. The reactions between the microorganisms and the minerals of both the reservoir rock and the cap rock may cause major changes in the structure and chemical composition of the rock formations, corrosion at the casing and the casing cement around the well, affecting the well integrity on the long-term basis. Analyses of microbial community composition and its changes provide information about the efficiency and reliability of the long-term CO_2 storage technique.

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