INFLUENCE OF DRILLING FLUIDS AND LONG-TERM CO_2 EXPOSURE ON THE MICROBIAL COMMUNITY INHABITING ROCK FORMATIONS – A CASE STUDY FROM THE KETZIN SITE

Zur Erlangung des akademischen Grades einer
Doktorin der Naturwissenschaften (Dr. rer. nat.)

von der Fakultät für Bauingenieur-, Geo- und Umweltwissenschaften des
Karlsruher Instituts für Technologie (KIT)

genehmigte Dissertation

von

Dott.ssa Mag. Linda Pellizzari
aus Vicenza, Italien

Hauptreferent: Prof. Dr. T. Neumann
Korreferentin: Prof. Dr. Ing. H. Würdemann
Tag der mündlichen Prüfung: 13.01.2017

Erklärung

Hiermit erkläre ich, dass ich die vorliegende Dissertation selbstständig verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt habe. Die Stellen der Arbeit, welche anderen Quellen im Wortlaut oder dem Sinn nach entnommen wurden, sind durch Zitation kenntlich gemacht. Dies gilt auch für bildliche Darstellungen, Tabellen sowie für Quellen aus dem Internet. Die Grundsätze des Karlsruher Instituts für Technologie zur Sicherung guter wissenschaftlicher Praxis in ihrer aktuell gültigen Fassung habe ich beachtet.

Declaration

I hereby affirm that I have written the present dissertation on my own, relying on no other means than the ones stated and followed the rules of good scientific practice of the Karlsruhe Institute for Technology, in the current valid version. Those sections of this work, which were taken from other sources, cited directly or indirectly, are quoted as such. This also applies to illustrated figures, tables as well as internet quotations.

Potsdam, October 17th, 2016

Abstract

The geotechnical use of the subsurface entails changes of the complex biogeochemical equilibrium of the geological formations (Bauer et al. 2013). When gases or fluids are injected into a reservoir as in the case of gas storage (e.g., CO_2, H_2) or geothermal energy, favorable injectivity properties must be ensured. Therefore, reservoir characteristics need to be maintained or optimized. It is assumed that microbial processes have the potential to influence the geotechnical utilization of the underground by reducing the permeability of the reservoir rock (Jaiswal at al. 2014; Zettlitzer et al. 2010). These influences were already observed, as a consequence of drilling, before any specific utilization of the underground. Drill mud and other technical fluids are sources of biochemical contamination for the near-well area and also for rock or fluid samples (Zettlitzer et al. 2010; Struchtemeyer et al. 2011).

The present study is focused on microbiological processes in the deep subsurface of Europe's first CO_2 onshore storage test site, located in Ketzin (Brandenburg, Germany). To obtain pristine rock samples from drilling operations, laboratory experiments were performed evaluating different tracers (i.e., fluorescein, microspheres and DAPI-stained cells) suitable to assess the drill mud penetration depth into rock cores. The investigations indicated that fluorescein is the most suitable tracer and thus it was used during the coring of two observation wells. An improvement of the tracer quantification method speeded up the analysis and enhanced the detection limit. Additionally, the use of fluorescein helped to determine the concentration of drill mud in well fluid samples acquired during subsequent pump tests and downhole samplings.

The microbiological characterization of formation rocks and fluids performed by genetic fingerprinting analyses (16S rRNA gene), provided insights into the autochthonous microbial community. Well fluid of the storage reservoir, the Stuttgart Formation, was characterized by a relatively high total organic carbon (TOC) content derived from residual drill mud. The formation was inhabited by a microbial population typical of a saline deep biosphere environment. The microbial community was dominated by halophilic/halotolerant *Proteobacteria* and sulfate reducing bacteria (SRB). In rocks of the Exter Formation, the sandstone layer above the caprock, *Proteobacteria* and *Actinobacteria* were identified. Database analyses indicated that the

detected microorganisms are typically found in soil, fresh water and deep subsurface habitats.

The coring of another deep well provided Stuttgart Formation rock cores that had been partly in contact with the injected CO_2 for more than four years. Furthermore, well fluid from the injection well, which was obtained through downhole sampling after five years of injection, was analyzed. The microbial community was relatively poor in diversity and differed from the population that was described before CO_2 injection. In addition, the response of the autochthonous microbial community to organic drill mud exposure was investigated under simulated *in-situ* conditions. The influence of acetate as a biodegradation product of drill mud polymers and the effectiveness of biocide were analyzed as well. In all setups, autochthonous microorganisms were stimulated in growth, confirming the biodegradability of drill mud and its influence on the microbial community.

The results permitted to get insights into the causal chain which induced an injectivity loss observed in the near-well area of a well intended for injection in Ketzin. Most likely, microorganisms (e.g., *Burkholderia* spp., *Variovorax* spp.) biodegraded organic components of the drill mud (e.g., cellulose polymers) and produced a significant amount of low molecular weight acids such as acetate. As demonstrated through the laboratory experiments, the addition of acetate supported the growth of SRB (i.e., *Desulfotomaculum* spp.). Subsequently, the microbial induced precipitation of amorphous iron sulfide caused a reduction of permeability in the near-well area (Zettlitzer et al. 2010). The injectivity loss incident at the CO_2 storage site demonstrated the immense influence that microbial-mediated processes can have on the productivity and reliability of geotechnical installations.

Kurzfassung

Die geotechnische Nutzung des Untergrunds bedingt Veränderungen im komplexen biogeochemischen Equilibrium der geologischen Formation (Bauer et al. 2013). Bei der Injektion von Gasen und Fluiden, wie bei der Gasspeicherung (z.B. CO_2, H_2) oder Geothermie, muss eine hohe Injektionsrate gewährleistet werden. Daher, müssen die Reservoireigenschaften erhalten oder auch optimiert werden. Es wird angenommen, dass mikrobiologische Prozesse die geotechnische Nutzung des Untergrunds aufgrund einer Reduzierung der Permeabilität des Reservoirgesteins beeinflussen können (Jaiswal at al. 2014; Zettlitzer et al. 2010). Diese mikrobiell bedingten Prozesse können bereits bei der Bohrung und damit vor der eigentlichen Nutzung des Untergrunds ausgelöst werden. Technische Fluide, wie beispielsweise Bohrspülungen, können den bohrlochnahen Bereich, aber auch Kernmaterial und Fluidproben biochemisch beeinflussen (Zettlitzer et al. 2010; Struchtemeyer et al. 2011).

Im Fokus der vorliegenden Studie stehen mikrobiologische Prozesse im tiefen Untergrund von Europas erstem Pilotstandort (Ketzin, Brandenburg) zur geologischen Speicherung von CO_2. Um unkontaminiertes Probenmaterial von den Kernbohrungen zu erhalten, wurden Laborexperimente zur Evaluierung verschiedener Tracer (Fluoreszein, Microspheres und DAPI-markierte Bakterien) durchgeführt. Die Experimente dienten der Bestimmung der Eindringtiefe von Bohrspülungen in erbohrtes Kernmaterial. Die Untersuchungen ergaben, dass Fluoreszein am besten geeignet ist. Daher wurde es anschließend beim Kernen zweier Beobachtungsbohrungen eingesetzt. Die Nachweismethode von Fluoreszein wurde optimiert und ermöglichte die schnelle und einfache Messung mit einer verbesserten Nachweisgrenze von markierter Bohrspülung in Kernmaterial. Darüber hinaus wurde Fluoreszein eingesetzt, um die Konzentration der Bohrspülung während Pump-Tests und Tiefenprobenahmen von Fluiden zu bestimmen.

Die Charakterisierung der autochthonen mikrobiellen Gemeinschaft der Formationsgesteine und Fluide wurde mittels DNA-Fingerprinting-Methoden (16S rRNA Gen) durchgeführt. Die Brunnenfluide der Stuttgart Formation, der Speicherformation, wiesen bedingt durch Bohrspülungsreste, relativ hohe TOC (organischer Gesamtkohlenstoff) Konzentrationen auf. Es wurde eine mikrobielle

Gemeinschaft vorgefunden, die typisch für saline Habitate in der Tiefen Biosphäre ist. Die Gemeinschaft bestand maßgeblich aus halophilen und halotoleranten *Proteobacteria* und Sulfat-reduzierenden Bakterien (SRB). In Gesteinen der Exter Formation, dem Sandstein über dem Deckgebirge, wurden *Proteobacteria* und *Actinobacteria* nachgewiesen. DNA-Datenbankabgleiche weisen darauf hin, dass ähnliche Mikroorganismen sowohl in Böden, Süßwasser aber auch in der Tiefen Biosphäre vorkommen.

Kernmaterial und Fluid, das während einer weiteren Bohrung in die Stuttgart Formation gewonnen wurde, war dem eingeleiteten CO_2 mehr als vier, beziehungsweise fünf Jahre lang ausgesetzt. Die in diesen Proben nachgewiesenen mikrobiellen Gemeinschaften unterschieden sich von denen vor der CO_2 Injektion. Insbesondere die genetische Diversität war vergleichsweise niedrig. Darüber hinaus wurde der Einfluss der organischen Bohrspülung auf die Struktur der mikrobiellen Gemeinschaft unter simulierten *in-situ* Bedingungen untersucht. Im Speziellen wurden die Auswirkungen von Acetat, als Abbauprodukt der in der Bohrspülung beigesetzten Polymere, und die Effektivität von Biozid untersucht. In allen Inkubationsansätzen wurde das Wachstum autochthoner Mikroorganismen stimuliert. Die Ergebnisse bestätigen die mikrobielle Abbaubarkeit der eingesetzten Bohrspülung und verdeutlichen die Auswirkungen auf die mikrobielle Gemeinschaft.

Die Untersuchungen erlauben detailliertere Einblicke in die Kausalkette, welche zur Injektivitätsabnahme einer der Bohrungen in Ketzin beitrug. Einige der nachgewiesenen Mikroorganismen sind in der Lage, Zellulose-Polymere zu hydrolysieren (z.B., *Burkholderia* spp. *Variovorax* spp.) und eine signifikante Menge niedermolekularer Säuren, wie beispielsweise Acetat, zu produzieren. Wie durch die Laborexperimente aufgezeigt, nahm das Wachstum von SRB (*Desulfotomaculum* spp.) durch die Zugabe von Acetat zu. Die daraus resultierende Präzipitation von amorphem Eisensulfid bewirkte vermutlich die Permeabilitätsabnahme im bohrlochnahen Bereich (Zettlitzer et al. 2010). Der beobachtete Injektivitätsverlust an der Pilotanlage zur CO_2-Speicherung verdeutlicht, welche Auswirkungen mikrobielle Prozesse auf die Produktivität und Funktionsfähigkeit geotechnischer Installationen haben können.

Acknowledgements

I would like to take the opportunity to express my thanks to everyone who contributed to the success of this work.
Firstly, I would like to thank Prof. Dr.-Ing. Hilke Würdemann (University of Applied Sciences, Department of Engineering and Natural Sciences, Merseburg) for the possibility to perform fascinating studies and for the support of my Ph.D study. I would like to thank Prof. Dr. Thomas Neumann (Karlsruhe Institute of Technology KIT) for his supervisorship, support and interest in my work. Best thanks to the commission of the dissertation.

I am grateful to all my colleagues of the Microbial GeoEngineering working group at the German Research Centre for Geosciences GFZ, Potsdam, headed by Prof. Dr.-Ing. Hilke Würdemann, for the nice time spent together. Dr. Daria Morozova and Dr. Monika Kasina are respectively acknowledged for the fluorescence *in-situ* hybridization analyses and the mineralogical analyses discussed in this thesis. Dr. Tobias Lienen, Dr. Dominik Neumann, Dr. Mashal Alawi and Dr. Hannah Halm are acknowledged for precious discussions and collaborations. Thanks to Sebastian Teitz, Heiko Baschek und Jennifer Weigt for their support in the field and in the lab. Anke Westphal, Dr.-Ing. Anne Kleyböcker, Dr. Stephanie Lerm and Dr. Marietta Liebrich are acknowledged for always being ready to assist me. I would like to acknowledge Dr. Ben Norden, Dr. Stefan Klapperer and the entire Ketzin group for their valuable help during sampling. Thanks to Dr.-Ing. Hans-Dieter Voigt (Institute of Drilling Technology and Fluid Mining, TU Bergakademie Freiberg) for his help during the tracer experiments.

Sincere thanks to my family and my friends for supporting and encouraging me despite the distance. In particular I thank my mother Giancarla and my father Claudio for all the unconditional love and for support every my decisions. And for the same reasons I thank my sisters Laura and Elisa. Special thanks to Mashal, for his encouragement.

Preface

This dissertation is written in English and submitted at the Department of Civil Engineering, Geo and Environmental Sciences at the Karlsruhe Institute of Technology (KIT).

The following scientific publications reporting parts of the here presented results, has been published or submitted:

Pellizzari L, Neumann D, Alawi M, Voigt D, Norden B, Würdemann H (2013) Use of tracers to assess drill mud penetration depth into sandstone rock cores during deep drilling: method development and application. Environ Earth Sci 70:3727–3738. doi:10.1007/s12665-013-2715-2;

Pellizzari L, Morozova D, Neumann D, Kasina M, Klapperer S, Zettliter M, Würdemann H (2016) Comparison of the microbial community composition of pristine rock cores and technical influenced well fluids from the Ketzin pilot site for CO_2 storage, Environ Earth Sci 75: 1323. doi:10.1007/s12665-016-6111-6;

Pellizzari L, Lienen T, Kasina M, Würdemann H (2017) Influence of drill mud on the microbial communities of sandstone rocks and well fluids at the Ketzin pilot site for CO_2 storage. Environ Earth Sci 76: 77. doi:10.1007/s12665-016-6381-z;

The laboratory work was performed at the Microbial GeoEngineering working group of the GFZ German Research Centre for Geosciences, Potsdam. The laboratory experiments with tracers were performed at the Institute of Drilling Technology and Fluid Mining, TU Bergakademie Freiberg. The fluorescence *in-situ* hybridization analyses discussed in the present work were performed by Dr. Daria Morozova, while the mineralogical analyses (field emission scanning electron microscope) were conducted by Dr. Monika Kasina.

This study was supported by the GRASP (Greenhouse-gas Removal Apprenticeship and Student Program) project (MRTN-CT-2006-035868), funded under the European commission's 6th Framework Program, by the Federal Ministry for Education and Research, within the GEOTECHNOLOGIEN Program in the framework of the CO2MAN (CO_2 Reservoir Management) project (03G0760A-F) and within the Energiespeicher program in the framework of the H2STORE (Hydrogen to Store) project (03SF0434B).

Table of contents

List of abbreviations

BSA	Bovine serum albumin
$CaCO_3$	Calcium carbonate
CMC	Carboxymethylcellulose
CO_2	Carbon dioxide
DAPI	4’,6-diaminodino-2-phenylindole
DGGE	Denaturing Gradient Gel Electrophoresis
DNA	Deoxyribonucleic acid
dNTPs	deoxynucleosidase-triphosphate mix
DsrB	Dissimilatory Sulfite Reductase
DOC	Dissolved organic carbon
e.g.	exempli gratia
FeS	Iron sulfide
FISH	Fluorescence *in-situ* hybridization
i.e.	id est
$MgCl_2$	Magnesium chloride
N_2	Nitrogen
NCBI	National Center for Biotechnology Information
PCR	Polymerase chain reaction
RDP	Ribosomal Database Project
rRNA	Ribosomal ribonucleic acid
SRB	Sulfate-reducing bacteria
SSCP	Single-Strand-Conformation Polymorphism
DFS-Taq	DNA Free Sensitive polymerase from Thermus aquaticus
TOC	Total organic carbon
UV light	Ultraviolet light
-BL	Rock core before long-term incubation
-S	Incubation with synthetic brine
-W	Incubation with well fluid

List of figures

List of tables

1. INTRODUCTION

Geological reservoirs are composed by a complex biogeochemical system. The interaction between minerals, brines and microorganisms has been subject to little research and is largely still not understood. Microorganisms drive many important processes in the geological subsurface (Lovely & Chapelle, 1995). A geotechnical use of the reservoir inevitably changes the natural conditions, such as substrate availability, pH, temperature and pressure, influencing the microbial activity. Depending on the kind of geotechnical use, the quantity of microorganisms as well as community composition can be influenced. In turn, the microbial activity may influence the chemistry of the reservoir and the productivity of the geotechnical installation.

A key issue for many geotechnical applications is the reduction of permeability in the vicinity of drillings and the related decreased injectivity. It is hypothesized that microbes play a critical role in the reduction of injectivity, as it was discussed by other authors (Zettlitzer et al. 2010; Morozova et al. 2010). The present study aims to better understand the microbial response to changed environmental conditions in the deep subsurface.

The scientific concept includes an optimization of the sampling procedure through the application of tracers to assess contamination by drill mud. The coring of two wells at a pilot site for CO_2 storage in Ketzin (Germany) gave the opportunity to obtain sample material from two different saline aquifers. The storage formation was partly in contact with the injected CO_2 and thus allowed a comparison of the microbial communities before and after CO_2 exposure. Furthermore, long-term laboratory experiments were carried out to characterize the response of the microbial community structure to organic polymers and biocides which are often constituents in the formulation of drill mud. DNA-databank analyses were performed to identify potential microbial processes that contribute to the reduction of permeability in the near-well area and storage formation. The results are useful for the development of countermeasures which make geotechnical installations more efficient and reliable.

1.1. Key questions and approaches

The aim of the present study is to enhance the knowledge about the changes that autochthonous microbial communities undergo in response to geotechnical operations and, conversely, about the influence that those microbial communities may have on the performance of geotechnical operations.

The study is structured according to three subtopics: (i.) use of tracers to obtain pristine rock samples intended for geochemical and microbiological analyses; (ii.) characterization of the microbial community in reservoir rocks and well fluids, before and after CO_2 storage; and (iii.) influence of organic drill mud on the autochthonous microorganisms.

The present work aims to answer the following key questions:

- *Which approach is the most efficient to assess drill mud penetration depth into rock cores during drilling? Which tracer is the most suitable?*

When sampling rock cores or well fluid for microbiological or geochemical analyses, it is of fundamental importance to detect whether the samples obtained are influenced by drill mud or other technical fluids. To assess the penetration depth of contaminants into the rock cores it is necessary to label the drill mud with a suitable tracer. Besides, the tracer also helps to verify the drill mud discharge through hydraulic tests and cleaning procedures.

The most suitable tracer should be non-hazardous for the environment, easy to handle in the field, quickly detectable in the samples and should be affordable. As a part of the present investigation, three different tracers (i.e. fluorescein, microspheres and DAPI-stained cells) were evaluated by laboratory experiments. In addition, a new approach for the detection of the selected tracer, namely fluorescein, was assessed. Beside the optical assessment, a more sophisticated procedure to extract and quantify the tracer fluorescein in rock cores was evaluated.

- *Which microorganisms inhabit the two geological formations sampled in Ketzin? Is the microbial community of the storage formation changing after exposure to CO_2?*

In the frame of the microbiological baseline characterization, the CO_2 storage formation in Ketzin (the Stuttgart Formation) and the sandstone overlaying its caprock (the Exter Formation) were analyzed. A comparison of the microbial communities of the two different formations may help to identify formation leakages in a long-term perspective. Qualitative microbiological analyses were conducted by means of 16S rRNA gene fingerprinting. In order to evaluate the impact of the CO_2 storage on the microorganisms, rock cores retrieved during the drilling of a well into the CO_2 plume and fluid collected from the injection well after five years of injection were microbiologically analyzed. Finally, the microbial community before and after CO_2 exposure were compared to each other.

- *Which microbial-mediated processes induced the injectivity loss in Ketzin? Do the autochthonous microbial communities of Exter and Stuttgart Formations react differently to an increment of substrate availability?*

The field experience showed that if residual drill mud remains in the rock formation, its organic components can be used by microorganisms as energy and carbon sources, provoking serious consequences on the reliability of the geotechnical installations. In this investigation, long-term laboratory experiments were performed incubating Stuttgart and Exter Formation rock cores in high-pressure vessels, at simulated reservoir pressure and temperature conditions. To analyze the response of autochthonous microorganisms to substrate stimulation, the pristine rock samples were incubated with sterile synthetic brine or well fluid, together with drill mud or acetate, as a biodegradation product. Moreover, to evaluate inhibition efficiency, experiments were conducted with and without the addition of biocide. The outcomes of the experiments provide insights into the reaction chain of the interacting processes observed in the field.

2. STATE OF THE ART

2.1. Sampling the Deep Biosphere: application of tracers

Scientific drilling is an important tool to investigate the lithosphere, to understand its chemistry, physics and biology and to answer open questions about bio-geo interactions. The extensive comprehension of the complex Earth system is of crucial importance not only for water, mining and other natural resource industries, but also for the geotechnical use of the subsurface, as the geological storage of CO_2.

In addition to the geological and geochemical interest, rock cores are important to acquiring knowledge about the Deep Biosphere and analyze microbial-mediated processes. Examples for the importance of the understanding of microbial-mediated processes are provided by studies focusing on drinking water reservoirs (Balkwill and Ghiorse, 1985), mines (Edwards et al. 2012), hydrocarbon reservoirs (Youssef et al. 2009), or waste repositories (Herman and Frankenberger, 1998; Lièvremont et al. 2009).

The opportunity to obtain Deep Biosphere samples for microbiological analyses is limited and costly. Frequently, rock cores are collected during drilling of boreholes where the recovery of pristine samples for biogeochemical analyses is not in the main focus. For geochemical and microbiological analyses it is of major concern to retrieve uncontaminated fluid or rock samples. Recent studies revealed that several microorganisms normally attributed to marine and terrestrial formations were also detected in drill muds (Struchtemeyer et al. 2011). Therefore, special effort has to be put on differentiating between the autochthonous microbial community and the microorganisms introduced in the rock formation during drilling and technical procedures. Drill mud and technical fluids are necessary during coring to lubricate the drill bit, to transport cuttings to the surface and to stabilize the borehole. To assess the contamination degree of rock cores and well fluid samples, dissolved (e.g., perfluorocarbons, fluorescein) or particle-based (e.g., polyethylene fluorescent microspheres) tracers are used (McKinley and Colwell 1996; Wilkins et al. 2014). One of the first and most common fluorescent dye tracer is the water-soluble disodium salt fluorescein (synonym Uranine), synthesized by Adolf von Bayer in 1871 (Duan et al. 2009). Fluorescein is highly soluble, inexpensive and toxicologically safe; therefore, it

is commonly used in hydrology and hydrogeology to investigate the fluid flow and the permeability of formations (Goldscheider et al. 2008). Fluorescein has been used to trace drill mud and identify the contamination degree of formation fluid (Thordsen et al. 2005). In 2007, during the coring of three wells at the Ketzin storage site (Germany), fluorescein was used to detect the penetration depth of drill mud in rock cores and to observe the discharge of technical fluid during the following pump tests (Wandrey et al. 2010). To verify the tracer results, Wandrey et al. (2010) measured also the TOC concentration in the core samples, as an indication of the carboxymethyl cellulose (CMC) used for the drill mud formulation. Fluorescein and TOC data indicated a drill mud penetration into the rock cores of around 20 mm. Due to its susceptibility to photodecomposition (Diehl and Horchak-Morris 1987) fluorescein potentially loses fluorescence when exposed to sunlight in the drill mud storage tanks. However, the tracer concentration can be monitored and adjusted. Spectrofluorometry is the standard method to analyze fluorescent dyes in fluid samples (Goldscheider et al. 2008). In rock samples the tracer fluorescein can be observed through fluorescence microscopy, as described by Wandrey et al. (2010).

Two other tracers also suitable to track the drill mud penetration depth into rock cores are microspheres and DAPI- (4’,6-diaminodino-2-phenylindole) stained cells. Microspheres are a particle tracer, having diameter (0.45 µm) similar to bacterial size. They are internally dyed and therefore visible using a fluorescence microscope. However, in many cases the high costs restrict the use of microsphere in the field. Microspheres were employed to detect drill mud intrusion in cores and obtain samples for microbiological studies (Smith et al. 2000; House et al. 2003; Kallmeyer et al. 2006; Cardace et al. 2013; Yanagawa et al. 2013). During an Integrated Ocean Drilling Program (IODP) expedition, together with microspheres, DAPI- stained cells were used as tracer, thereby making it possible to evaluate the feasibility of their utilization (Fisher et al. 2011).

2.2. Microbial communities in storage reservoirs

To predict the response of the formation to geological storage of CO_2 or to a geotechnical use of the subsurface in general, an extensive knowledge of the rock and

fluid composition and biogeochemical processes is of primary importance (Gluyas and Mathias 2013). In particular, the knowledge about the microorganisms inhabiting the rock formations is a fundamental prerequisite to monitor microbiological changes due to the geotechnical use of the subsurface. Possible storage formations are in general different from each other in many physical and geochemical parameters. Likewise, the microbial communities inhabiting them vary. In the aquifers, a distance of only few centimeters may be sufficient to detect different groundwater composition as well as microbial diversity (Pedersen 2014).

The microbial communities inhabiting the Deep Biosphere include species which are well adapted to the subsurface conditions and play an important role in biogeochemical cycling. Different phylogenetic and metabolic groups of microorganisms were detected in saline aquifers (Basso et al. 2009; Morozova et al. 2010; Lerm et al. 2013). Sulfate reduction, fermentation, acetogenesis and methanogenesis are the most significant metabolic types in marine and terrestrial Deep Biosphere (Takai et al. 2001; Kotelnikova 2002; Baker et al. 2003; Basso et al. 2009). In the frame of geological storage of CO_2, besides chemoheterotrophic microorganisms which gain energy by oxidizing organic compounds, also chemolithoautotrophs are of particular interest. This group of microorganisms obtains energy by oxidizing inorganic compounds (e.g., H_2, sulfur/iron/nitrogen compounds) and uses CO_2 as an alternative electron acceptor and as sole carbon source. In groundwater ecosystems, the total cell number of bacteria varies over several orders of magnitude in both fluids and sediments (from 10^2 to 10^6 and from 10^4 to 10^8 cells per cm^3, respectively) (Griebler and Lueders, 2009). Further insights into the microbial community structure of saline aquifers are provided by studies of well fluids from geothermal plants. In these environments, a predominance of microorganisms involved in the metabolic iron- and sulfur-cycling was observed (Alawi et al. 2011; Lerm et al. 2013; Würdemann et al. 2014). In aquifers, many microorganisms are attached to mineral surfaces, sediment particles or detritus, forming microcolonies or biofilms (Weaver et al. 2015). However, most of the microbiological analyses of aquifers are performed on pumped groundwater, overlooking the important contribution of microorganisms attached to the sediments. Moreover, in the absence of substrates, microorganisms can survive long periods of starvation, reducing their

metabolism by forming spores (Smith et al. 1952; Balkwill and Ghiorse, 1985; Goldscheider et al. 2006).

2.3. Interaction between geo-engineered systems and microbial-mediated processes

As a consequence of the geotechnical use of the subsurface, autochthonous microorganisms interact with a geochemically and physically modified subsurface. The resulting bio-geochemical processes may be advantageous or disadvantageous for the underground utilization. Bacteria can influence the mineralogy, causing the dissolution of feldspar (Welch et al. 1999) and Fe(III) minerals (Glasauer et al. 2003) or inducing the precipitation of clay minerals, silicates and calcium carbonate phases (Schultze-Lam et al. 1996; Douglas and Beveridge 1998; Deng et al. 2010). Sulfate-reducing bacteria (SRB), largely identified in deep saline aquifers, may enhance the precipitation of insoluble metal sulfides such as pyrite (Giangiacorno and Dennis 1997; Spark 2000). The dissolution of carbonate minerals and the increased metal ion mobility may be incremented by the activity of acids generated as a secondary product by bacterial metabolisms. Furthermore, microbial-induced precipitation and corrosion affects materials (Beech et al. 2005) of the near-well area and the casing of the well. In this case, chemolithoautotrophs and especially SRB play an important role. Microcolonies and biofilms can deteriorate the casing material and decrease the geotechnical performance. Besides, they can damage the rock formation (Lappan and Fogler, 1992) and lower the injection rates by blocking the pore throats (Rosnes et al. 1991; Jaiswal at al. 2014) or by encouraging mineral precipitation (Zettlitzer et al. 2010). Further examples on microbial-mediated problems are given by studies in the field of oil production (Hill et al. 1987; Gieg et al. 2011) and geothermal energy (Alawi et al. 2011; Lerm et al. 2013; Würdemann et al. 2014). The problem of biocorrosion in geothermal installations is common, but it is site-specific, since it is largely influenced by the fluid temperature, the geochemical conditions and the microbial abundance (Lerm et al. 2013).

In the specific case of underground storage of CO_2, laboratory experiments indicated minor changes in the composition of the microbial community, which was able to adapt to the changed conditions (Wandrey et al. 2011*a*; *b*). Other studies suggested that the

microbial abundance in formation water from saline and freshwater aquifers decreased after exposure to CO_2 (Gulliver et al. 2014*a*; *b*). Moreover, a recent study showed that communities will change in response to differences in the ability of cells to tolerate elevated CO_2 levels as well as shifts in the balance of microbial reactions (Kirk et al. 2016). Microorganisms may influence the hydrodynamics of reservoirs during the storage of CO_2, affecting fluid flow and trapping of CO_2 (capillary, solubility and mineral trapping) (Kirk et al. 2016). Paul et al. (2017) demonstrated the ability of halotolerant SRB to induce the precipitation of carbonate minerals during long-term CO_2 storage in saline aquifers. Mineral trapping of CO_2 in the reservoir rock is the most stable of the storage procedures (Oelkers et al. 2008*b*). Over a long time, the reaction of the acid formed by the dissolution of CO_2 in water with the surrounding rock can form solid carbonate minerals, reducing the permeability of the formation. However, mineral trapping is an extremely slow process. Numerical simulations regarding the Ketzin pilot site, estimated that after 10,000 years CO_2 dissolution will be the dominating trapping mechanism and mineralization will occur between 10 % to 25 % with only small changes in porosity and permeability (Kempka et al. 2014). Mitchell et al. (2010) tested in laboratory experiments the use of hydrolyzed urea to microbially enhance the CO_2 trapping. As a consequence of microbial ureolysis, the concentrations of CO_3^{2-}, HCO_3^-, as well as the pH increased and the formation of biofilms resistant to supercritical CO_2 was enhanced. Through experiments under varying conditions, Mitchell et al. (2009*a*; *b*) demonstrated that the growth of biofilms reduced the permeability of rock cores by 95-99 %. The formed biofilms were stable also to the mechanical shear stress induced by CO_2 injection.

2.4. Influence of residual drill mud on microbial processes and consequent damages on the storage formation: the injectivity loss at the Ketzin pilot site for CO_2 storage

To limit the invasive effect of drilling in the environment, drill mud components are normally biodegradable and can be decayed by microorganisms (Cadmus et al. 1982; Ezzat et al. 1997). Organic polymers, originating from plants or microbes, are required during drilling because they reduce the loss of drill mud into the formation, promote the

formation of thin filter cakes and stabilize clay (Roscoe Moss, 1990). The employed organic components may additionally serve as energy and carbon sources for native or introduced microbial populations, enhancing their growth (Zettlitzer et al. 2010; Struchtemeyer et al. 2011). During a first drilling campaign, performed in 2007, three wells were drilled into the targeted saline aquifer for the CO_2 storage, the Stuttgart Formation (Prevedel et al. 2009). The wells were drilled with a water-based $CaCO_3$/ bentonite/ organic polymer drill mud, which contained CMC. The drill mud was labeled with the tracer fluorescein (Wandrey at al. 2010). Additionally, xanthan gum, which is a concentrated biopolymer, was used during the completion phase, to facilitate the staged cementation (Würdemann et al. 2010). Hydraulic tests were performed in the three wells twelve weeks after completion, before starting the CO_2 injection operations (Wiese et al. 2010). For the well intended for injection, the permeability obtained through the injection test was much lower than expected (Wiese et al. 2010; Würdemann et al. 2010). The cleaning procedures did not completely removed drill mud, xanthan gum and technical fluid from the well, which remained behind the filter screens, enhancing the microbial activity (Zettlitzer at al. 2010). Even though a biocide was used in the drill mud formulation, the microbial community proliferated, acquiring an energy source through the organic polymers of the drill mud. Additionally, the microbial activity was facilitated through the fresh water pumped into the formation during the well completion, which reduced the salinity of the rock formation. Encouraged by these favorable conditions, SRB formed hydrogen sulfide as a product of their metabolism. The hydrogen sulfide reacted with dissolved iron, inducing the precipitation of amorphous iron sulfide (FeS) which caused the injectivity loss. In consequence, a gas lift was performed to remove the blocking material and re-establish the permeability properties. The injection of gas reduces the density of the fluids in the tubing, lowering the pressure at the bottom of the well and allowing the well fluid to flow out (Fleshman and Obren-Likic 1999). Nitrogen gas was chosen over air to be used for the lift because the oxygen content in the air would lead to corrosion and oxidation of dissolved ferrous ions (Zettlitzer et al. 2010). The removal of the amorphous precipitant and the residual drill mud was substantiated by the decrease of the suspended solids content, mostly FeS, and by the diminution of fluorescein and TOC in the fluid produced during the lift (Zettlitzer et al. 2010; Wandrey et al. 2010). High

values of TOC were associated with the organic component of the drill mud. The highest TOC measured during the lift was 380 mg L^{-1} (Zettlitzer et al. 2010). Acetate contributed half of this amount, suggesting that the organic drill mud was microbiologically degraded. The SRB cell number drastically decreased in the fluid obtained during a downhole sampling, performed after the rigorous cleaning procedure (Morozova et al. 2010).

3. STUDY AREA

As a consequence of the global warming effect, the emission of CO_2 into the atmosphere must be substantially reduced. CO_2 is the primary greenhouse gas emitted through human activities and most of the emissions are connected to the combustion of fossil fuels (coal, natural gas, and oil) for energy generation and transportation. Carbon dioxide capture and storage (CCS) is one of the possible technologies to effectively reduce anthropogenic greenhouse gas emissions (IPCC, 2005). The CCS process involves the capture of CO_2 from point sources (e.g., coal-fossil fuel power plants or industrial plants), transportation to the storage site and injection through boreholes into the subsurface, where it is geologically stored. CO_2 can be stored in saline formations, in coal seams, basalts, or depleted oil and gas reservoirs (IPCC, 2005; Oelkers and Cole 2008*a*). The Ketzin pilot site for geological storage of CO_2 in a saline aquifer is Europe's longest-operating on-shore CO_2 storage facility (Schilling et al. 2009; Würdemann et al. 2010; Martens et al. 2012; Martens et al. 2015). The storage site is situated in a rural area near the city of Ketzin (52°29N, 12°52E), 25 km west of Berlin, in the federal state of Brandenburg, Germany (Fig.1). Here, from 1964 until 2000, a gas storage located at a depth of approximately 280 m was used for the seasonal storage of natural and city gas (www.co2ketzin.de). The industrial land and pre-existing infrastructure (e.g., roads, buildings, cores storage facilities) made the site suitable for testing CO_2 storage (Förster et al. 2006).

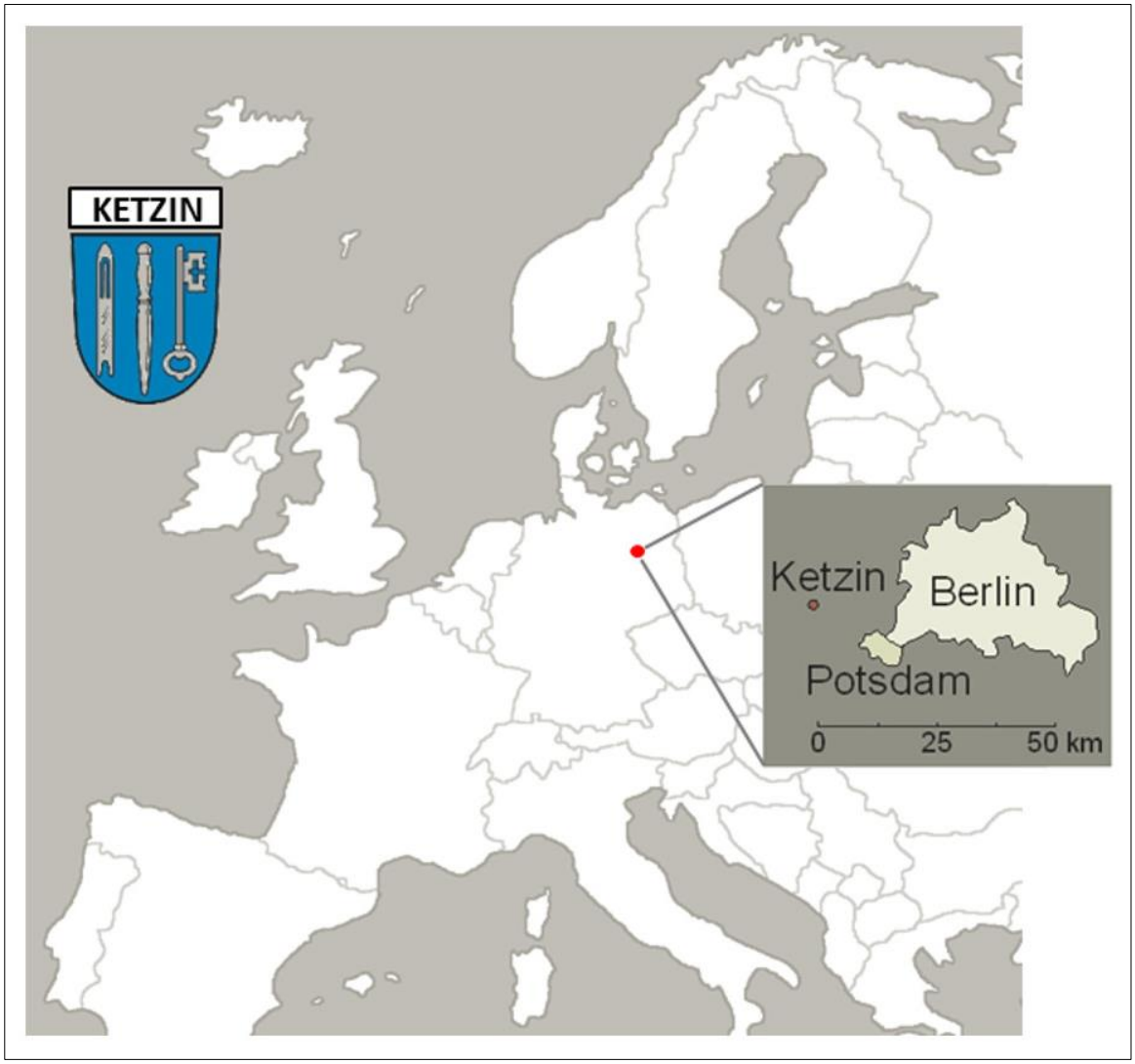

Fig.1: Location of the Ketzin pilot site. Image credits: www.co2ketzin.de.

3.1. Geological background

The storage site is located in the Northeast German Basin, a subbasin of the Central European Basin System. The sedimentary succession is several kilometers thick and contains geological formations of Permian to Quaternary age, including numerous deep saline aquifers. The CO_2 was injected into the Stuttgart Formation of Triassic (Middle Keuper) age, into the southern flank of a gently dipping double anticline (Fig.2).

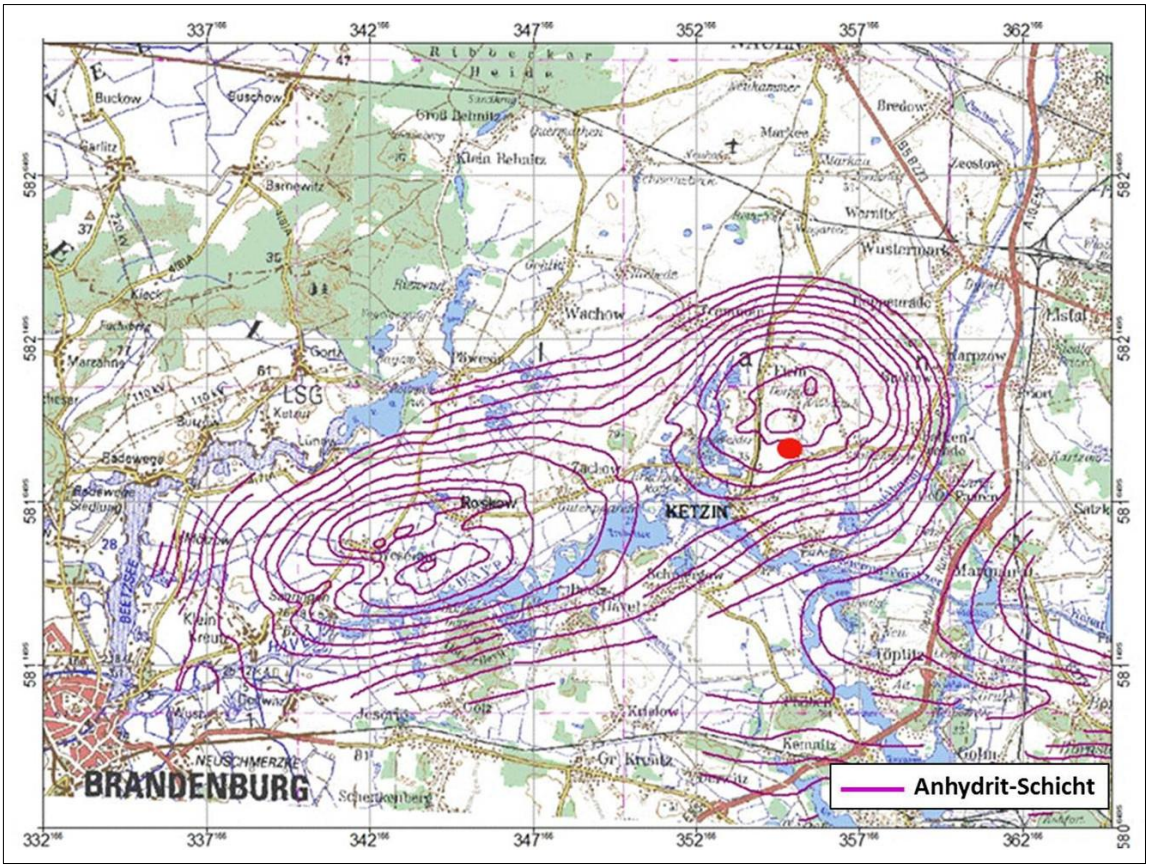

Fig.2: Roskow-Ketzin double anticline. The red dot locates the Ketzin storage site. Images credits: www.co2ketzin.de.

The anticline formed above an elongated salt pillow situated at a depth of 1500–2000 m. In Ketzin, the Triassic (Buntsandstein, Muschelkalk, and Keuper) and the Lower Jurassic geologic formations constitute the immediate overburden above the salt pillow. The saline aquifer is situated at approximately 630 to 710 m depth, and has a thickness of about 80 m in average (Förster et al. 2006; Prevedel et al. 2009: Norden et al. 2010) (Fig.3).

At a depth of 700 m, the formation pressure is in a range between 70 and 75 bar and the temperature is of about 38°C (Förster et al. 2009). The Stuttgart Formation in the larger Ketzin area is lithologically heterogeneous. Deposited in a fluvial environment, the Stuttgart Formation is made up of channel-(string)-facies composed by sandstones and siltstones which present good reservoir properties and which are interbedded with flood-plain-facies rocks, mostly mudstones, of poor reservoir quality. Furthermore, minor layers of anhydrite and coal are present in this formation. The sandstone interval may

reach several tens of meters (up to 30 m) in thickness, while the width is between several tens of meters and several hundreds of meters. In the central and western part of the Ketzin anticline, a channel string system 8 km width cause the Stuttgart Formation to be composed of more than 60 % of sandstone (Förster et al. 2006; Förster et al. 2009).

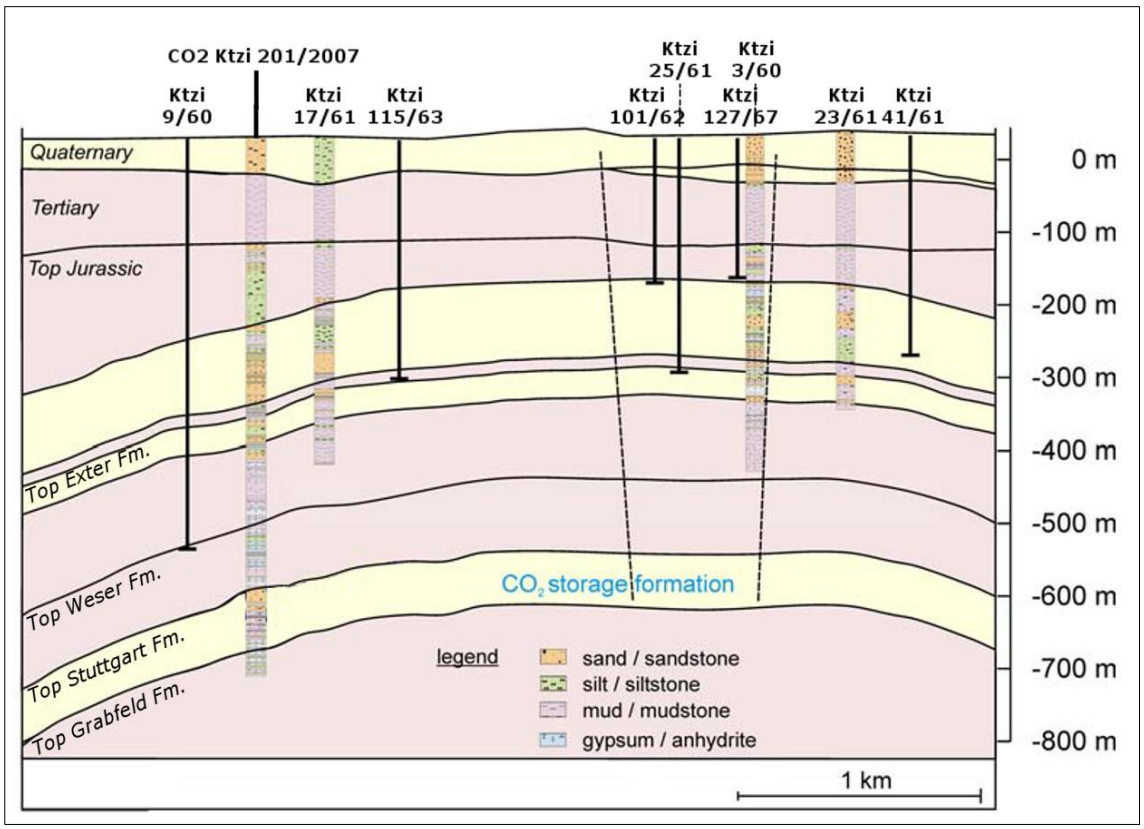

Fig.3: Simplified geology of the Ketzin anticline with aquifer (light yellow) and aquitard (pink) units. Detailed lithology is shown for selected boreholes. The location of major faults is indicated by dashed lines. (Modified after Förster et al. 2009)

The sandstone channels, predominantly fine-grained with sub-rounded grains, are mainly composed of quartz, plagioclase, illite, analcime, anhydrite, amorphous phases. Cement distribution is mostly characterized by local concentrations in spotted zones with high intergranular volume. Pore spaces show good connectivity, porosity and brine permeability range from 5 to >35 % and from 0.02 mD to >5000 mD, respectively. The mudstones consist mainly of illite, dolomite, anhydrite, quartz and plagioclase. The average permeability is 0.010 mD and the porosity ranges from 5 to 15 %, where the higher values were observed in finely fractured rocks. Pore bodies and pore throats are

small and the pore space is usually unconnected (Förster et al. 2009; Norden et al. 2010). 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 TOC content of the rock cores is low with less than 1000 mg kg^{-1} rock (0.1 % TOC) (Wandrey et al. 2010; Scherf et al. 2011). The lipid fatty acids found in the reservoir rock cores are of bacterial origin, indicating the presence of an active bacterial community (Scherf et al. 2011). Because of the high importance of a detailed characterization of the storage formation and of its sealing conditions, the Stuttgart Formation and the lower part of the overlaying caprock were the only materials always cored during the drilling of the first three deep wells in 2007 (Prevedel et al. 2009).

An important requirement for the feasibility of CO_2 storage is the presence of a reliable caprock, composed by layers of low permeability rock overlaying the storage formation. The caprock prevents the floating dense or vapor phase CO_2 from leaking into overlaying formations (IEAGHG 2011*a*). In addition the caprock must ensure leak tightness especially after the abandonment of the site (Schilling et al. 2015). Great effort is focused on developing technologies which ensure sustainability and safety standards for the short and long term. The overburden of the storage formation contains several aquifers and aquitards (Fig.4). The caprock of the Stuttgart Formation comprises the playa-type mudstones of the Weser and Arnstadt formations. The Weser Formation (Middle Keuper) consists mainly of fine grained clastics (e.g., clayey and sandy siltstone) alternated with thin-bedded lacustrine sediments (carbonates and evaporites). The high clay mineral content and the pore-space geometry confer sealing properties necessary for CO_2 storage. The Weser Formation is overlaid by the Arnstadt Formation (Middle Keuper), which is of lacustrine origin and presents similar sealing properties. Together, the two formations composing the caprock are about 210 m thick (Förster et al. 2009; Prevedel et al. 2009; Norden et al. 2010). The Exter Formation, which is the sandstone aquifer overlaying the storage formation caprock (Fig.3), was cored only during the drilling of the shallow observation well Hy Ktzi P300/2011 (short name: P300) and therefore it was, so far, poorly characterized (Martens et al. 2013). The Exter Formation primarily comprises fine- to medium-grained sandstones, siltstones and marly mudstones (Norden and Klapperer 2011). The carbonatic peloids, abundant in the

lower sandstone (~440 m), were not detected in the middle sandstone (~418 m). Here, instead, the presence of ooids was observed, indicating the origin in shallow water environment (Pellizzari et al. 2016). The mudstones show dark reddish brown to dark red colors, while 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. Clay minerals (illite/smectite) were observed in siltstones, while in the sandstone a small amount of kaolinite was detected (Pellizzari et al. 2016). Similarly to the sandstone channels of the Stuttgart Formation the thin-section porosities of the Exter Formation ranges from 8 to 22 %.

A 3D seismic survey conducted over the Ketzin anticline showed a normal fault system across the top of the anticline, called the Central Graben Fault Zone, which is composed mainly by NW–SE striking features. At least one EW striking fault zone was observed to extend into the Tertiary unit. Nevertheless, the fault presents a vertical displacement much smaller than the thickness of the caprock, demonstrating that the former gas storage formation and the CO_2 reservoir are actually disconnected (Würdemann et al. 2010).

3.2. Site setting and CO_2 injection operation

Since March 2007, through three drilling campaigns, five wells with a maximum distance of 112 m to each other were completed (Fig.4a/b).

During the first campaign, three wells were drilled to a depth of 750 to 800 m. One of these wells (CO2 Ktzi 201/2007, short name Ktzi 201) was used as a combined injection and observation well while the other two (CO2 Ktzi 200/2007, short name Ktzi 200 and CO2 Ktzi 202/2007, short name Ktzi 202) were designed as observations wells. Prior to CO_2 injection, pump tests were performed on all wells (Wiese et al. 2010). The CO_2 injection started on June 30, 2008. The primary source of injection at the pilot site was food grade CO_2, with a purity of 99.9 %. Additionally, 1.5 kt of CO_2 (purity 99.7 %) captured from the oxyfuel pilot plant Schwarze Pumpe (Vattenfall), were injected in 2011 (Martens et al. 2012).

In 2013, an experiment of co-injection with CO_2 and N_2 was performed, testing and demonstrating the technical feasibility of a continuous impure CO_2 injection. In total, 613 t of CO_2 were mixed with 32 t of N_2, for an average CO_2/N_2 mass ratio of approximately 95 to 5 (Martens et al. 2012; Martens et al. 2014). The CO_2 was delivered by trucks in a cooled, liquefied state to the injection facility, where it was stored in two intermediate storage tanks at approximately 18 bars and -18°C. Before injection, the CO_2 was gasified and heated to formation temperatures (45°C). The CO_2 was injected in a supercritical state (Schilling et al. 2009). The pressure in the downhole was continuously monitored by pressure/temperature sensors installed in the injection well (Würdemann et al. 2010). The injection facility was designed to allow a maximum injection rate of 78 t per day. The average injection rate was 45 t per day. At the end of the injection operations, in August 2013, a total amount of 67 kt of CO_2 was safely stored (Martens et al. 2014).

Between June and August 2011 a shallow observation well P300, reaching a depth of 446 m, was drilled into the Triassic Exter Formation, the first sandstone aquifer overlaying the CO_2 storage caprock. The aim of this borehole was to continuously monitor the integrity of the storage and to characterize potential hydraulic and geochemical impact of CO_2 injection on the aquifer above the storage formation, in the event of leakage. Between August and September 2012, the last observation well CO2 Ktzi 203/2012 (short name: Ktzi 203) was drilled to a depth of approximately 700 m. This well is located close to the injection well and was aimed at investigating the CO_2-fluid-rock interactions in the CO_2 storage formation (Fig.4a/b).

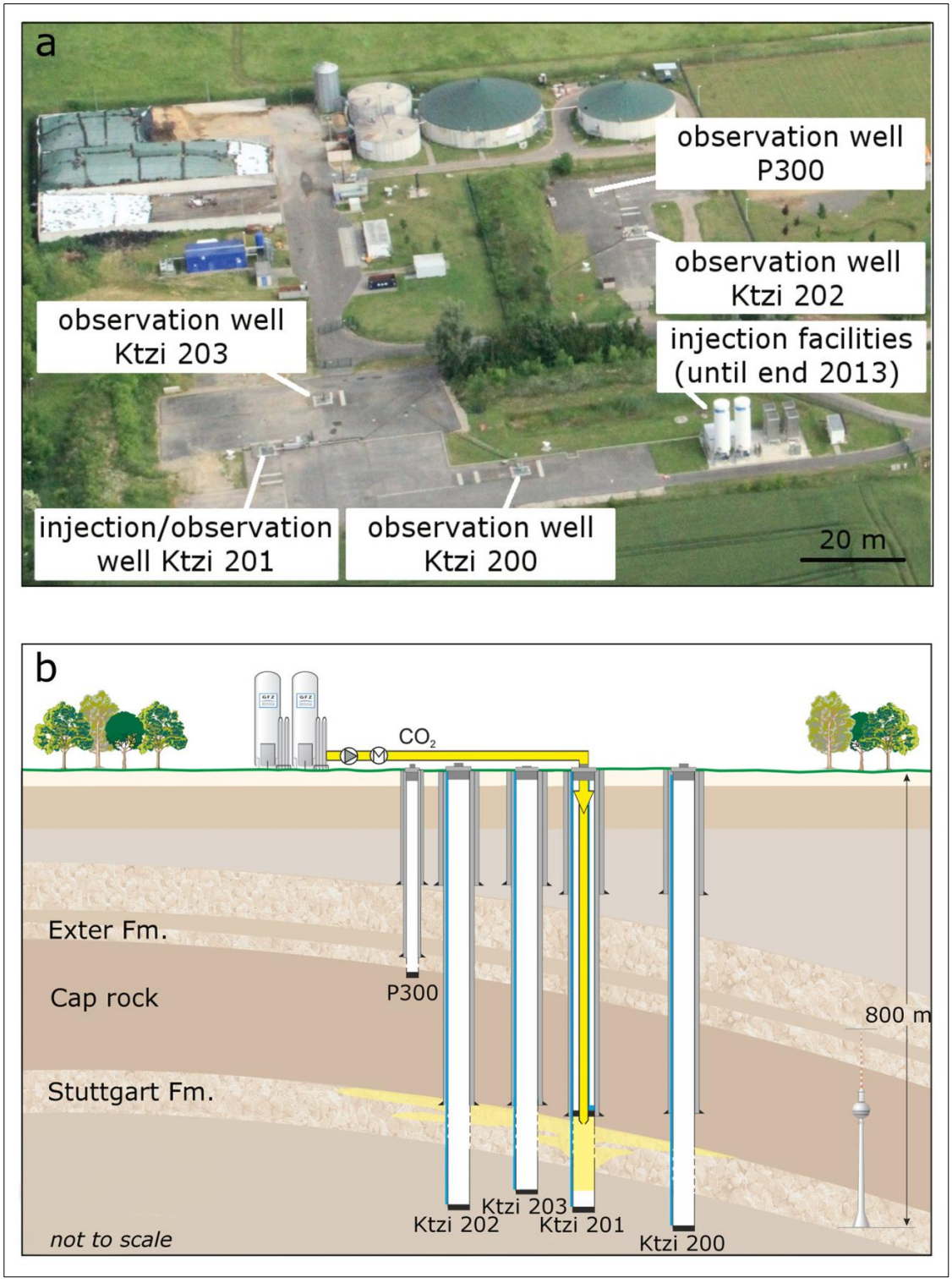

Fig.4: Ketzin pilot site. a: Aerial view (June 2013) with scientific infrastructure, northwest view (modified after Martens et al. 2014); b: Cross-section showing the wells system. Image credits: www.co2ketzin.de.

4. MATERIALS AND METHODS

4.1. Tracer experiments

Through laboratory experiments, fluorescein, microspheres and DAPI-stained bacteria were tested as tracers to label the drill mud. The aims of these investigations were to assess potential differences in penetration depth and evaluate the best tracer to be used in the field. The present section describes also an improvement of the detection method for the tracer fluorescein. Beside the microscopic observations of the penetration depth under UV light, a quantification of the tracer through the extraction from rock samples and subsequent fluorometric analysis is introduced.

4.1.1. Drill mud and tracers

A synthetic drill mud (Tab.1) following the composition of the KCl/$CaCO_3$/CMC-based mud used in Ketzin during the 2007 coring campaign, was employed for the experiments. Ingredients and formulation were provided by Mi SWACO Deutschland GmbH (Celle, Germany), the company responsible for the drill mud in the field.

Tab.1: Drill mud composition provided by Mi SWACO GmbH.

Product	Amount
Water	920 mL
CMC-LV	25 g
KCl	150 g
M-I Cal SL	60 g
M-I Cide	1 mL
XC-Polymer	2 g

CMC-LV: sodium carboxymethyl cellulose; KCl: potassium chloride; M-I Cal SL: calcium carbonate; M-I Cide: biocide; XC-Polymer: xanthan gum.

The sodium fluorescein salt (F6377, Sigma–Aldrich, Germany) shows a bright green fluorescence when observed under UV light (Fig.5a). To visually detect the tracer, the rock samples were observed using a reflected-light microscope, equipped with a

mercury-arc lamp (MZ10F, GFP2 filter set, Leica Microsystems, Wetzlar, Germany) and a digital microscope camera (Leica DFC 420 C).

For fluorescein extraction, core samples were ground using a ceramic mortar and pestle and homogenized. Afterwards, 0.25 g of ground material was extracted with 600 µL buffer (50 mM TRIS, pH 9) in a 2 mL reaction tube. The tubes were placed on a vortex and mixed for 30 min at maximum speed. The samples were then centrifuged at 20,800 x g for 10 min and the supernatant was transferred to a fresh 1.5 mL reaction tube. The extraction procedure was then repeated. The supernatants were combined, centrifuged and transferred to a clean tube. The fluorescein content was measured in triplicate using 96-well plates processed using a filter fluorometer (FLUOstar OPTIMA, BMG LABTECH, Germany).

The microspheres used were Fluoresbrite Polychromatic Red Microspheres (0.5 Micron, Polysciences, Warrington, PA, USA). The bacterial tracer, consisting of *Pseudomonas halophila*, was purchased from the DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen, Braunschweig) and cultivated in liquid media (300 mL Erlenmeyer flasks), as described by Fendrich (1988). Cell numbers were estimated by staining aliquots (1 mL) of the culture with 4 µL of DAPI (1 mg mL^{-1} stock solution) and counting with a Helber counting chamber using a Zeiss Axioskop 2. Cells were collected by centrifugation (10,000 x g, 20 min, 4°C) to reach a cell density of approximately $5x10^6$ cells mL^{-1}. For staining, the bacteria pellets were suspended in 50 mL fresh media and subjected to an ultrasonic treatment (mod. Sonopuls™, Bandelin, Berlin, Germany) for 30 s. Then, 35 µL DAPI was added and mixed for 5 s using a vortex. The cells were incubated for 20 min in the dark and added to the drill mud.

To detect microspheres and DAPI-stained cells in rock samples, 0.5 g of ground material was collected in a 2 mL reaction tube and diluted with 500 µL of a 150 g L^{-1} NaCl solution. Microspheres and DAPI-stained cells were microscopically analyzed using a UV light with the Zeiss Axioskop 2, equipped with filters 02 (DAPI), 10 (FLUOS) and 20 (Cy3), a mercury-arc lamp and an Axiocam digital camera (Fig.5b).

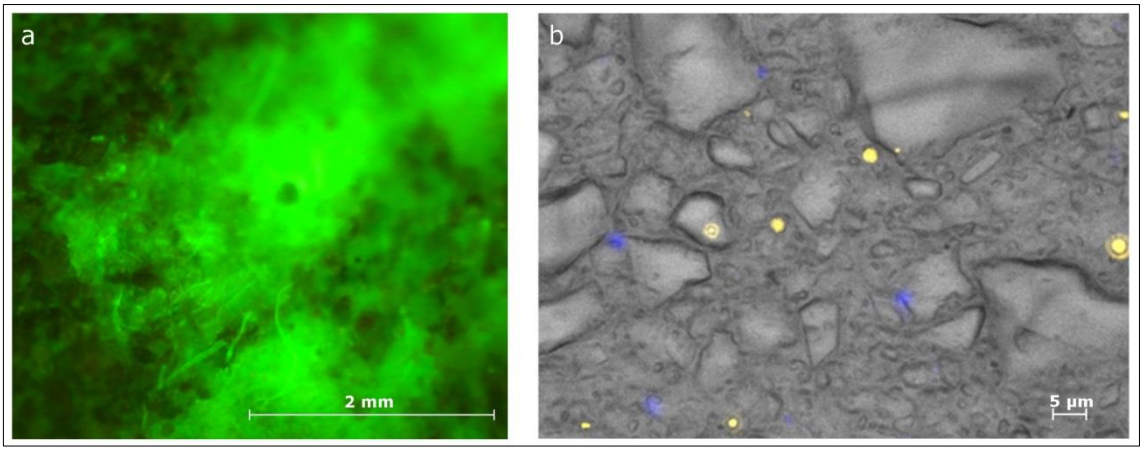

Fig.5: Tracers observed under UV light. a: Fluorescein shows a bright green fluorescence. b: Microspheres show a vivid orange fluorescence and a perfectly rounded shape. DAPI-stained cells (*Pseudomonas halophila*) are identified by blue fluorescence which is relatively difficult to distinguish from naturally fluorescent rock particles.

4.1.2. Incubation of rock samples with drill mud at ambient air pressure, applying uniform pressure or vacuum

Preliminary experiments were conducted employing Stuttgart Formation samples obtained from an outcrop at a clay open-pit mine near Erfurt (Gispersleben, Germany) (Pellizzari et al. 2013). The rocks were cut in parallelepipeds (approximately 3 to 4 cm) and saturated with synthetic brine. Afterwards, they were wrapped in Parafilm and one side of each block was left uncovered, so that the drill mud penetrated only from one direction into the rock sample. The samples were incubated in drill mud labeled with fluorescein, at room temperature and in the dark. Ambient air pressure (Fig.6a) was applied to simulate the field condition after sampling, and to assess whether the drill mud further penetrate the rock core due to gravity. In other setups, a uniform pressure from 40 to 90 bar perpendicular to each surface (Fig.6b) was applied to obtain different drill mud penetration depths and to observe the behavior of different tracers. For the same purpose, vacuum was applied in an exicator by the use of a pump (Fig.6c). The incubation time varied between 1 and 24 h. After every experiment, the sandstone was cut longitudinally and the fluorescein penetration was microscopically observed.

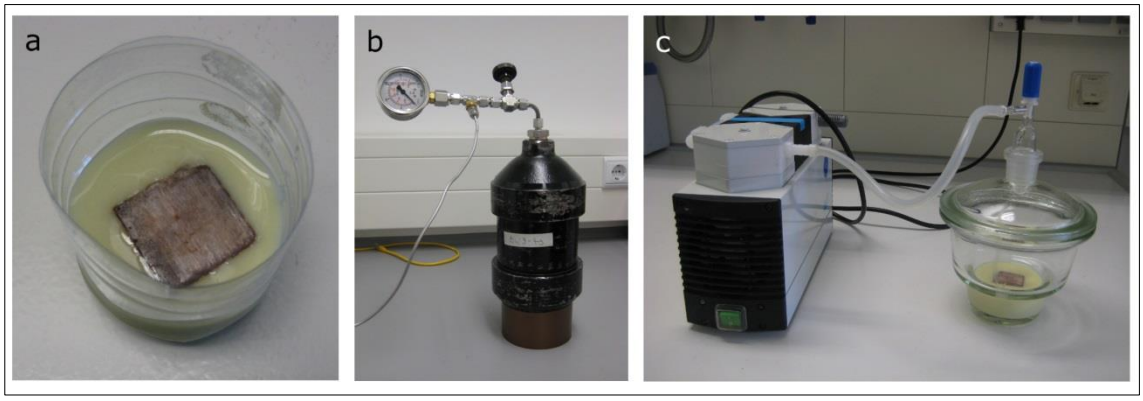

Fig.6: Experiments with Stuttgart Formation rock samples obtained from an outcrop in Gispersleben, Germany. a: Incubation at ambient air pressure. b: Incubation applying a uniform pressure of up to 90 bar. c: Vacuum incubation.

4.1.3. Incubation of rock samples with drill mud applying an uniaxial pressure difference

To simulate coring conditions and induce the flow of drill mud into the rock cores, a uniaxial pressure differential was applied (Pellizzari et al. 2013). Stuttgart Formation sandstones from the outcrop are strongly weathered, making the preparation of the experimental plugs difficult. Therefore, to perform the flow experiments, the more homogeneous Bentheimer sandstone was used. The Bentheimer sandstone (Lower Cretaceous) is a fine-to-medium grained homogeneous quartz sandstone. The selected samples, collected from an outcrop in Lower Saxony, showed porosities (approximately 25 %) and permeabilities (approximately 500 mD) similar to the Stuttgart Formation. The cylindrical samples were 48.6 mm in diameter and 50 mm in length.

Beside fluorescein, also microspheres and DAPI-stained bacteria were employed for these experiments. The experimental setups are presented in Tab.2. Fluorescein was used by itself (Exp. 1, 2 and 3) or combined with microspheres and bacteria. For experiment 1, the synthetic drill mud was labeled with fluorescein at a concentration of 1 mg L^{-1}, while 5 mg L^{-1} were used in the other setups, to increase visibility. Two tests (Exp. 4 and 5) were performed combining microspheres (concentration of 2 mL L^{-1}) with fluorescein. Moreover, in one experiment (Exp. 5), DAPI-stained cells were also added.

Tab.2: Setup of laboratory experiments using Bentheimer sandstone samples and applying a uniaxial pressure difference to analyze the dissimilarities in penetration depths of tracers used to label drill mud.

Exp.	Differential pressure between drill mud and water	Fluorescein concentration [mg L^{-1}]	Microspheres (2mL L^{-1})	DAPI-stained *Pseudomonas halophila* (10^5 cells L^{-1})
1	15 bar (95 – 80)	1	-	-
2	15 bar (95 – 80)	5	-	-
3	10 bar (95 – 85)	5	-	-
4	10 bar (95 – 85)	5	yes	-
5	10 bar (95 – 85)	5	yes	yes

The uniaxial pressure system and a scheme explaining the operating principle are shown in Fig.7a/b. The core sample, located in the core holder, was wrapped in a rubber membrane (Fig.7c/d) and placed in a vessel filled with oil. Each end of the sample holder was connected to a piston storage, one filled with water and one with drill mud. The sample was flushed and saturated with water from the piston storage. A containment pressure of approximately 100 bar was applied to the sample through the oil, and the tight membrane guaranteed that the drill mud penetrated only from one side of the rock core. The uniaxial pressure was simultaneously raised to force the water and drill mud into the core. The final pressure was 95 bar and 80-85 for drill mud and water piston storage, respectively, resulting in a pressure difference of 15 bar for experiments 1 and 2, and 10 bar for the other experiments. The magnitude of the pressure difference was simulating the pressure between rock and drill mud during the coring of wells (D. Voigt, personal communication). After 140 min under these pressure conditions, the sample was removed from the vessel (Fig.7d/e).

Using a hammer and a chisel, the sample was cut into two parts along the fluid flow direction. One part was observed through reflected-light microscope to visually detect the fluorescein. Cross section pictures were taken using the digital microscope camera. The photos were assembled using graphic software (Adobe Photoshop CS3 Extended), providing a complete view of the tracer penetration in the sample. The other half of the sample was cut into layers for further analyses (i.e., fluorescein extraction, microspheres and DAPI-stained cells observation). The 3-mm-thick layers were sawn using a multi-tool (Dremel^{TM}, model 285, Breda, The Netherlands) equipped with a diamond

blade. The sawing procedure started from the bottom, where no drill mud was expected, and after each cut the blade was cleaned and flamed with ethanol for sterilization. Each layer was then ground and homogenized. The detection accuracy of the method is limited to the size of the rock layers. As previously described, fluorescein was extracted and quantified, and DAPI-stained cells and microspheres were microscopically observed.

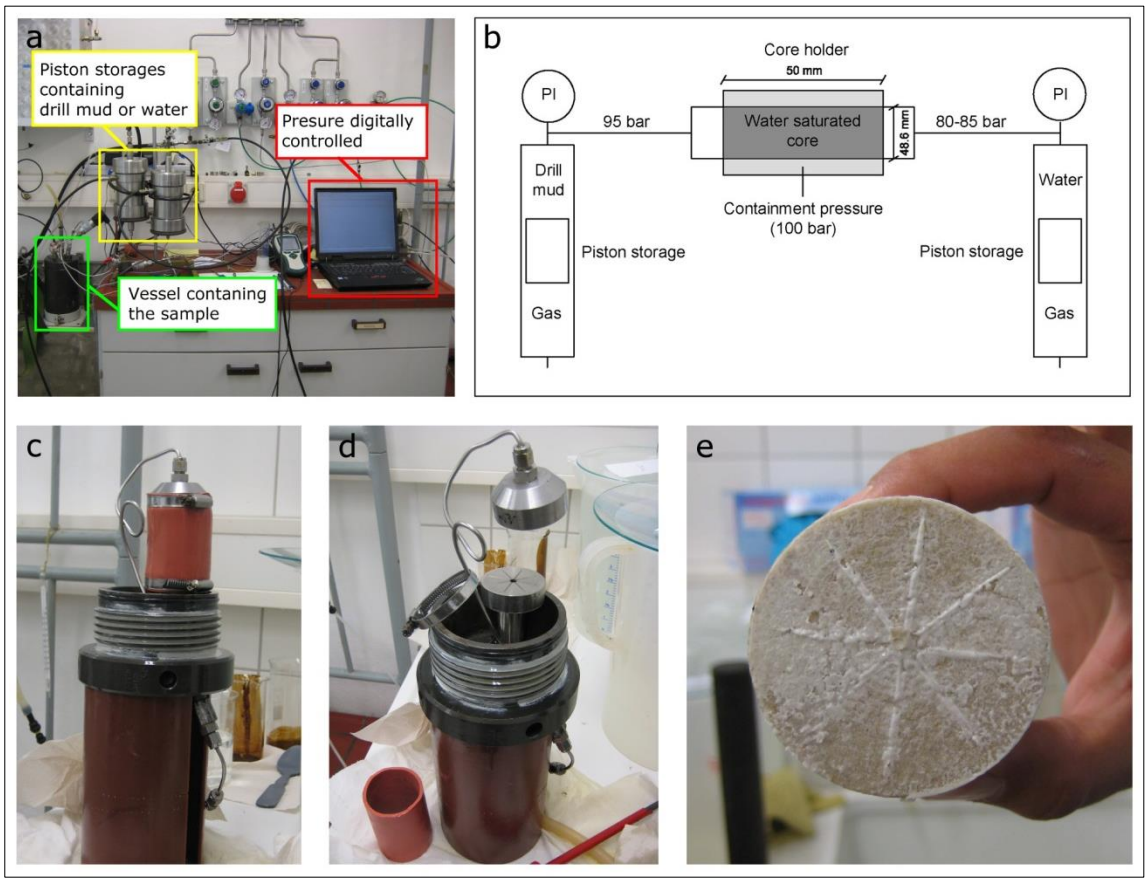

Fig.7: Tracer experiments. a: Uniaxial pressure system. The three vessels, containing respectively core sample, drill mud and water, are highlighted. The uniaxial pressures from the drill mud and water piston storages, as well as the containment pressure were digitally controlled. b: Operating principle scheme of the uniaxial pressure system. c: Core sample wrapped in the rubber membrane. d/e: Core sample removed from the core holder. Residual drill mud is visible on the core surface.

4.2. Coring and fluid sampling: use of the tracer fluorescein to assess drill mud contamination in rock cores and well fluids

The present section describes the application of the tracer fluorescein during two drilling campaigns at the CO_2 storage site in Ketzin (Pellizzari et al. 2013). Exter

Formation rock cores were obtained through the drilling of the shallow hydrogeological observation well (P300) in 2011. The employed drill mud consisted of fresh water mixed with K_2CO_3 (0.06 kg L^{-1}). To avoid the stimulation of SRB, no organic polymers were added to the drill fluid. Fluorescein dissolved in water was added to the mud tank during the drilling phase, three days before the coring started, allowing adequate homogenization (Fig.8a/b). A total of 40 g of fluorescein was added to 10 m^3 of fluid, resulting in a final concentration of 4 mg L^{-1}. Because the drill mud was exposed to sunlight (which causes photo-decomposition) and because of losses and subsequent replacement of drill mud during coring, the fluorescein concentration decreased with time and was on average 2.8 mg L^{-1}. The investigated rocks were sandstones to siltstones and one mudstone and were collected from a depth range of approximately 415 to 440 m below ground level. The diameter of the drilled core was 85 mm.

Fig.8: Use of fluorescein during the coring of the shallow observation well P300. a: Fluorescein addition into the drill mud tank. b: Green colored drill mud by the incorporation of fluorescein.

The Stuttgart Formation rock cores were retrieved in 2012 through the coring of the Ktzi 203 observation well, drilled into the injected CO_2 plume. Due to technical reasons (e.g., to reduce the risk of core loss), the use of polymers in the drill mud formulation was unavoidable. Cellulose-based polymers (CMC, PAC-polyanionic cellulose) and a natural polysaccharide-based polymer (Biolam) were employed. Due to the required density of the drill mud (1.21 kg L^{-1}) to safely drill the CO_2-bearing reservoir, K_2CO_3 was added together with bentonite. 380 g of dissolved fluorescein were added to the approximately 50 m^3 drill mud tank system at the beginning of the coring. For the

following six days, the concentration was on average 8.4 mg L^{-1}. The sandstones of the Stuttgart Formation are heterogeneous in composition. The six core samples investigated in this study were taken mainly from sandy intervals, covering a depth range from 641 to 670 m below ground level. In contrast to the cores from the P300 borehole, the drilled core diameter was 102 mm.

For both coring campaigns, as soon as the rock cores were retrieved, fragments of the samples were microscopically observed, in order to detect the penetration depths of labeled drill mud. In addition, for the Ktzi 203 rock cores, cross-section photographs were taken. The rock cores were immediately stored in aluminum bags under nitrogen atmosphere, and cooled during the transportation to the laboratory. The same day or maximum three days after the rock core recovery, an inner coring parallel to the borehole orientation was performed using a 50-mm-diameter drill bit (Fig.9a/c). For the inner coring of the Exter Formation samples, the use of fluid to cool down the drill bit could not be avoided, therefore autoclaved millipore water was used (Fig.9a/b). In the case of the Stuttgart Formation cores, Argon was used instead of water (Fig.9c). In both cases, before the use, the tools were cleaned with ethanol and flushed with autoclaved millipore water. Because of the different rock core diameter, the thickness of the outer core mantle resulting from the inner coring was approximately 17.5 mm and 26 mm for Exter and Stuttgart Formations cores, respectively (Fig.10a/b).

Fig.9: Inner coring operations. a/b: Autoclaved distilled water was used for the preparation of the Exter Formation rock cores. c: Argon was used to prepare the Stuttgart Formation inner core plugs. In this case, for security reasons, the operations were performed outdoor.

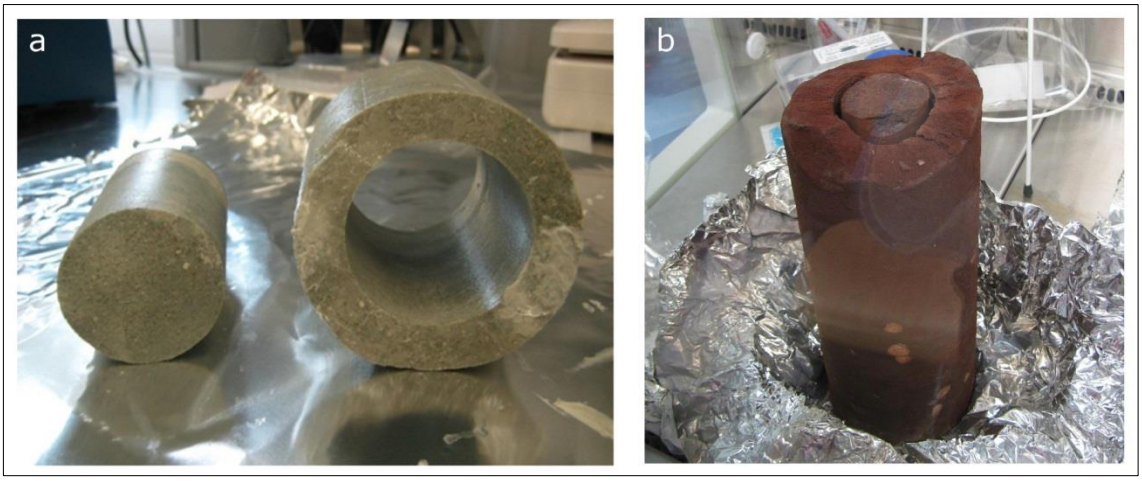

Fig.10: Inner core plugs and core mantels for a. Exter and b. Stuttgart Formations rock samples. The same drill bit (50 mm in diameter) was used for the inner coring of both Formations cores, having different core diameter. Consequently, the core plugs had all the same diameter, while the core mantle discarded from the Stuttgart Formation cores was thicker.

Each inner core plug was divided in three portions, which were used for tracer assessment, microbiological baseline characterization and long-term microbiological experiments. The portion of sample intended for tracer analyses was firstly observed at the microscope and afterwards fluorescein was extracted and quantified using a 96-well plate filter fluorometer.

Each portion of the inner core plug used for long-term microbiological experiments was incubated in a stainless steel high-pressure vessel, together with synthetic formation brine (Fig.11a/b). During the incubation, the drill mud which remained in the pore space of the rock cores got solved in the fluid. The concentration of the fluorescein in the brine after incubation served as additional drill mud contamination control.

Fluid samples were drawn from the P300 and Ktzi 203 setups after six and one month of incubation, respectively. A flask containing a small aliquot of the drill mud used in the field was stored together with the high-pressure setups, to control possible fluorescein decomposition.

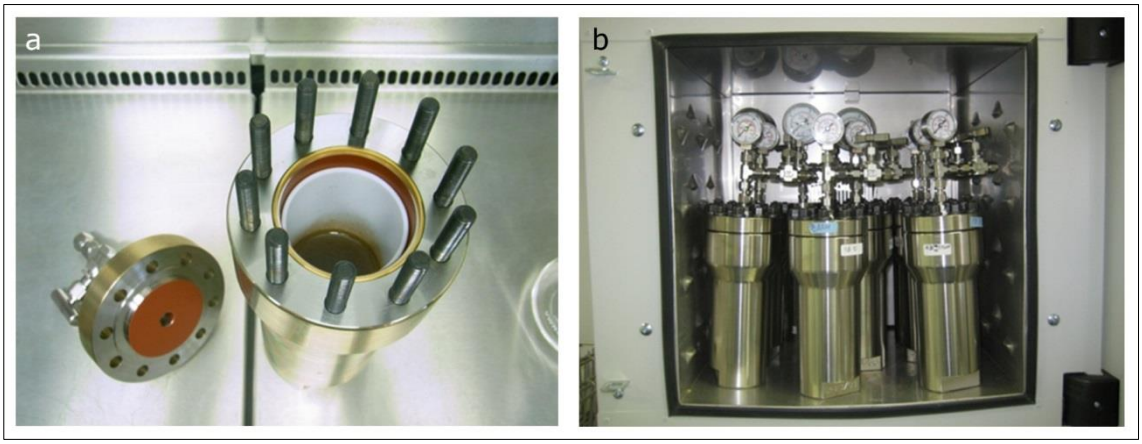

Fig.11 a: High-pressure setups. b: The vessels were stored at *in-situ* conditions.

An open-hole pump test was performed in the P300 borehole in January 2012, five months after well completion. The pumping phase started with a flow rate of 20 L min^{-1} and after approximately two hours, the production rate was increased to 24 L min^{-1}. At the end of the test a total volume of approximately 14 m^3 of fluid was produced. To monitor the discharge of drill fluids from the well into the formation, six fluid samples were collected after 3, 5, 7, 10, 11 and 14 m^3 of fluid production and the fluorescein concentration was measured using a 96-well plate filter fluorometer.

The shallow observation well P300 was equipped with a U-tube sampling system which allows deep borehole fluid sampling (Wiese et al. 2013). For 28 months after well completion, fluid samples were regularly collected and the fluorescein concentration measured.

4.3. Well fluids and rock cores employed for microbiological analyses

The present section introduces the environmental samples (well fluids and rock cores) employed for microbiological analyses (Tab.3). The microbial community in Stuttgart Formation well fluid before CO_2 injection was characterized by analyzing fluid samples obtained through a pump test and downhole sampling of a deep observation well (Ktzi 202). The hydraulic pump test was conducted in January 2008 as an open-hole test, and the production rates were held at the maximal achievable rate. Details about the hydraulic test have been previously described in Wiese et al. (2010). Through pumping, 93 m^3 of fluid was produced. Samples collected at the beginning (4 m^3) and end (93 m^3)

of the test, with highest and lowest dissolved organic carbon (DOC) and fluorescein concentrations respectively, and a mid-range sample (30 m^3), were selected for microbiological analyses. The contamination control in the fluids collected through the pump tests has been already described by Wandrey et al. (2010). After the test and before of CO_2 injection, different downhole sampling campaigns were performed in the perforation depths using a double ball-lining. The fluid retrieved during one of those downhole samplings was used for fingerprinting analyses.

For each sampling step of the pump tests, samples were collected directly from the wellhead into sterilized glass bottles, for the downhole samplings the fluids were aseptically transferred into the flasks. The samples were cooled to 4°C and immediately transferred to the laboratory for molecular biological analyses.

With the same downhole sampling procedure, a fluid sample from the injection well (Ktzi 201) was obtained after five years of injection. The formation fluid was collected in October 2013 at a depth of 675 m. The sample was microbiologically analyzed and used for further experiments (as later described).

Moreover, rock cores of the storage formation were collected through the coring of the last deep observation well Ktzi 203, drilled into the CO_2 injection plume. Beside Stuttgart Formation samples, six rock cores of Exter Formation, collected through the coring of the shallow observation well (P300), were analyzed. The rock core sampling procedure is described in section 4.2. and the microbiological analyses are described in section 4.5.

Tab.3: Overview of the environmental samples (well fluids and rock cores) employed for microbiological analyses

Formation	Source (well)	Sample	Depth [m]	Sampling time	Sample type	Sampling procedure
Exter	observation well P300	ExF 1-BL	407.30 (top)	Aug 2011	rock	coring - inner coring
		ExF 3-BL	419.80 (top)			
		ExF 4-BL	420.20 (top)			
		ExF 6-BL	428.34 (top)			
		ExF 9-BL	436.71 (top)			
		ExF 10-BL	438.09 (top)			
Stuttgart	observation well Ktzi 202	4 m^3	-	Jan 2008 (before CO_2 injection)	fluid	pump test
		30 m^3	-			
		93 m^3	-			
		647 m	647	Mar 2008 (before CO_2 injection)	fluid	downhole sampling
	observation well Ktzi 203	StF 1-BL	641.92 (top)	Sep 2012 (after CO_2 injection)	rock	coring - inner coring
		StF 3-BL	654.94 (top)			
		StF4-BL	656.97 (top)			
	injection well Ktzi 201	well fluid	675	Oct 2013 (after CO_2 injection)	fluid	downhole sampling

4.4. Long-term experiments of pristine rock cores incubated with drill mud and acetate

The aim of the long-term incubation experiments was to characterize potential changes in autochthonous microbial communities as a consequence of drill mud and acetate exposure. The following section describes the setup of the experiments performed with pristine rock cores and synthetic brines or well fluid.

4.4.1. Prearrangements of the high-pressure setups

Only uncontaminated rock core samples retrieved during the coring of the P300 and Ktzi 203 observation wells were employed in the long-term experiments. Immediately after the inner coring, each rock samples was incubated in a high-pressure vessel.

Since no fresh well fluid was available directly after the coring campaigns, two synthetic brines were prepared, sterilized by autoclaving and incubated with the rock cores in the vessels. The Exter Formation brine (Tab.4) was synthetized according to the chemical measurements of fluid sample retrieved from the Ktzi 117-63 observation well in the Ketzin area (Pchalek et al. 1964). It later turned out that the salinity of the Exter Formation brine employed in this work was underestimated. After incubation of the synthetic brine with Exter Formation rock cores in the frame of other experiments, the salinity of the fluid increased of about 109% in average, as a consequence of rock-fluid equilibrium (H2STORE, Abschlussbericht). The setups containing the Exter Formation sample were stored under *in-situ* pressure and temperature conditions (45 bar, 25°C). After an equilibrium phase of six months in a N_2 atmosphere, the gas was changed to CO_2. The Stuttgart Formation brine (Tab.5) was synthetized after Wandrey et al. (2011*a*). The *in-situ* pressure of the Stuttgart Formation, which at a depth of 700 m is 70-75 bar, could not be reproduced due to technical reasons. Therefore the vessels were incubated at 50 bar and at the *in-situ* temperature (40°C). The Stuttgart Formation samples were directly incubated in a pure CO_2 atmosphere because at the time of coring, the plume of injected CO_2 likely already reached the rock formation. In all setups, a constant pressure was maintained. A small portion (5 mL) of the headspace was regularly sampled, and the gas composition was analyzed using a gas chromatograph (SRI 8610C; SRI Instruments, Torrance, CA, USA). After one year of

incubation under the described conditions, the laboratory experiments with drill mud or acetate were established.

Tab.4: Composition of the Exter Formation synthetic brine (after Pchalek et al. 1964).

Compound	Conc. [g L^{-1}]
NaCl	53.15
K_2SO_4	0.22
$CaCl_2$	2.81
$MgSO_4$	1.53
KCl	0.18
K_2HPO_4	0.01

Tab.5: Composition of the Stuttgart Formation synthetic brine (after Wandrey et al. 2011*a*)

Compound	Conc. [g L^{-1}]
NaCl	216.56
KCl	0.66
$MgCl_2$, $6H_2O$	6.19
$CaCl_2$, $2H_2O$	7.27
$SrCl_2$, $6H_2O$	0.13
Na_2SO4, $10H_2O$	14.41
NaBr	0.05

4.4.2. Experimental setup

Six high-pressure vessels, each containing one rock sample (three for Stuttgart and three for Exter Formation) and synthetic brine, were employed for the incubation experiments. Ten different setups were established. An overview, including mixing ratio and incubation time, is provided in Tab.6. The incubation time was chosen according to the field observation, where the injectivity problem was recognized during an injection test performed twelve weeks after well completion. Since the blockage of the pore throats may have developed before the injectivity loss observation, different incubation times were considered. The first five experiments with the Stuttgart and Exter

Formations rock cores involved the addition of laboratory-made drill mud to the synthetic brine. The drill mud was produced following a standard formulation (composition in Tab.1, subsection 4.1.1.) (StF 4-S; ExF 4-S; ExF 6-S) or a formulation that excluded the biocide component (StF 3-S; ExF 9-S). The biocide (M-I Cide, ingredient name: 1,3,5-Triazine-1,3,5(2H,4H,6H)-triethanol) used in this study is non-bio accumulating and completely biodegradable, according to the OECD 306 test (Biodegradability in Seawater) (www.oilfield-biocides.com), which was performed under aerobic conditions (www.oecd-ilibrary.org). Beside the drill mud experiments, an approach incorporating acetate to the synthetic brine of a vessel containing a Stuttgart Formation rock core was set up (StF 1-S). The acetate concentration was chosen based on the *in-situ* conditions at the beginning of the N_2 lift which was performed to reestablish the permeability of the near injection well area. The maximum observed TOC content accounted for 380 mg L^{-1} (Zettlitzer et al. 2010), with 50 % thereof being acetate. Consequently, a final acetate concentration of 190 mg L^{-1} was used for the experiment. Drill mud and acetate solution were sterilized by autoclaving before being mixed with the synthetic brines and added into the setups. After 8 or 16 weeks of incubation, the vessels were opened, and a small portion of rock (~10-20 g) was sampled. The mixture of synthetic brine and drill mud or acetate was removed and analyzed.

Four additional experiments with fresh well fluid collected from the injection well were performed with rock cores and vessels that had been previously incubated with acetate or with drill mud containing biocide. Therefore, the synthetic brine mixture was replaced with well fluid and mixed with acetate (StF 1-W) or drill mud with biocide (StF 4-W; ExF 4-W; ExF 6-W), respectively. Whereas the synthetic brines only contained the main chemical components, the fresh sampled well fluids should have provided more realistic *in-situ* conditions. The incubation with well fluid also permitted to analyze the effect of carbon sources on the combined rock and fluid associated microbial community. For one experiment (ExF 6-W) the well fluid was sterile filtered (0.2 µm) twice, before being added to the setup. In this way, the microorganisms were removed from the fluid to specifically analyze the influence of well fluid on the microbial community inhabiting the rock. After 15 or 20 weeks, rock and fluid samples were collected. After sampling from the vessels, the rock cores were immediately

frozen in liquid nitrogen and stored at -20°C until further analyses, while the DNA of the fluid samples was immediately extracted.

Tab.6: Setups of long-term experiments.

	Incubation with synthetic brine					Incubation with well fluid				
Formation	Sample	Rock [g]	Drill mud /brine	Fluid [mL]	Duration [weeks]	Sample	Rock [g]	Drill mud /brine	Fluid [mL]	Duration [weeks]
	Addition of drill mud without biocide									
Stuttgart	StF 3-S	120	1:3	75	8					
Exter	ExF 9-S	95	1:3	60	8					
	Addition of drill mud					Addition of drill mud				
Stuttgart	StF 4-S	240	1:2	150	16	StF 4-W	225	1:3	140	20
Exter	ExF 4-S	130	1:2	80	16	ExF 4-W	120	1:3	75	15
Exter	ExF 6-S	140	1:2	85	16	ExF 6-W	120	1:3	75*	20
	Addition of acetate (190mg L^{-1})					Addition of acetate (190mg L^{-1})				
Stuttgart	StF 1-S	280	-	170	16	StF 1-W	260	-	160	20

*fluid was filtered

4.5. DNA extraction, PCR and fingerprinting analysis

Single-strand-conformation polymorphism (SSCP) or denaturing gradient gel electrophoresis (DGGE) was performed to characterize amplified partial16S rRNA and DsrB genes. The two applied electrophoresis-based fingerprinting methods (SSCP and DGGE) separate the variants of PCR amplified DNA fragments of all species inherent in a sample. In SSCP analyses the nucleic acids are separated as single-stranded nucleotide sequences which are fold into a preferred secondary structure (Dohrmann and Tebbe 2004). During DGGE, double stranded DNA partially denatures passing through a chemical gradient. The banding patterns of both methods generally provide a profile of the populations in that the relative intensity of each band and its position represents the relative abundance of a particular species in the population (Muyzer et al. 1993).

The baseline characterization of the microbial community inhabiting the Stuttgart Formation well fluid was performed analyzing four fluid samples from the deep observation well Ktzi 202, prior to CO_2 injection. Three samples were collected through

pump test (after 4, 30 and 93 m^3 of production) and one sample was obtained through a downhole sampling (647 m). Approximately 500 mL of each well fluid sample were filtered on 0.2 µm filter units (Millipore). After filtration, which was performed using a stainless steel filter holder (Sartorius AG, Göttingen, Germany), the filters were sent to AMODIA Bioservice GmbH (Braunschweig, Germany) for fingerprinting analyses. SSCP analyses were performed 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.

The DNA extraction, amplification and the fingerprinting analyses (DGGE) of all the other samples were performed in the GFZ laboratory. The well fluid (250 mL) obtained from the injection well Ktzi 201 through downhole sampling was filtered (50 mm, 0.3 µm PC - filter, WhatmanTM, GE Healthcare Europe GmbH, Freiburg, Germany) using the stainless steel filter holder. The filter was stored at -20°C until DNA extraction.

The fluid which was collected from the setups after the long-term experiments could not be filtrated because of the high viscosity induced by the drill mud. Therefore the complete fluid (75 to 170 mL) of each setup was progressively centrifuged in 50 mL sterile falcon tubes at 14.000 x g at 4°C for 1 h. After every first centrifugation, the supernatant was pipetted off and centrifuged in a second falcon tube under the same conditions to ensure that all cells were pelleted. Therefore, for each fluid sample, two pellets resulting from two centrifugation steps were produced and separately analyzed by fingerprinting analyses.

The rock core samples were crushed into small pieces using hammer and chisel. Finally, the samples were subjected to cryogenic grinding using a laboratory mill (Mixer Mill MM 400 from RETSCH GmbH, Germany). All tools were previously sterilized by autoclaving.

The rock samples, pellets and filter were stored at -20°C until DNA extraction with the FastDNA® SPIN Kit for Soil (MP Biomedicals, OH, USA), according to the manufacturer's protocol. After extraction the DNA concentration was measured fluorometrically (BMG Labtech FLUOstar OPTIMA) by labeling the DNA with

Quant-iT PicoGreen (Invitrogen). For all samples the amount of extracted genomic DNA was lower than the detection limit of the fluorometer (0.02 µg mL^{-1}).

The partial 16S rRNA gene from bacteria and archaea as well as the protein-coding gene from SRB, the Dissimilatory Sulfite Reductase (DsrB), were amplified by polymerase chain reaction (PCR). Due to the low cell number in the samples, nested PCR was performed to acquire enough products for the DGGE analyses. Salter et al. (2014) demonstrated how contaminating DNA is ubiquitous in extraction kits and therefore, particularly for low biomass samples, it may influence the DNA analyses. However, the negative controls counted during DNA extraction, PCR and fingerprint analyses, did not show any indication of contamination. The universal 16S rRNA primer pairs 27F (5` AGA GTT TGA TCM TGG CTC AG 3`; Lane 1991) and 1492R (5` TAC GGY TAC CTT GTT ACG ACT T 3`; Weisburg et al 1991) were used to amplify a long DNA fragment, followed by 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) for the short fragment. The amplification conditions (Thermocycler, TPersonal, Biometra GmbH, Goettingen, Germany) consisted of (first/second PCR) an initial denaturation at 95°C for 5 min, followed by 25/28 cycles of 95°C for 45/50 s, 55/54°C for 45/50 s and 72°C for 50 s, with a final elongation step of 10/15 min at 72°C. The primer pairs used for the amplification of the SRB gene were DSR1F/DSR4R (Wagner et al. 1998, Karkhoff-Schweizer et al. 1995) for the long fragment and DSRp2060F-GC/DSR4R (Geets et al. 2006) for the short one. The amplification conditions were (first/second PCR) 95°C for 3 min, followed by 24/29 cycles of 95°C for 35 s, 54/55°C for 35 s, and 72°C for 50 s, followed by a final elongation of 72°C for 5 min. The primer pairs used for the amplification of the 16S rRNA-gene of Archaea were 340F/1000R (Gantner et al. 2010) for the long DNA fragment and Arch346aF-nd/GC2-Arch934b for the short (Kemnitz et al. 2007; Hoshino et al. 2011). The PCRs (first/second PCR) started with a 2 min initial denaturation at 98°C, followed by 25/28 cycles of 95°C for 30 s, 57/60°C for 30 s, and 72°C for 90 s and a final 7 min elongation at 72°C. For all of the described PCRs, the reaction tubes contained 2.5 µL of 10xPCR reaction buffer COMPLETE II (Bioron GmbH, Ludwigshafen, Germany), 2.5 µL of dNTPs (2.5 mM each, Thermo Scientific), 0.5 µL of $MgCl_2$ (50 mM, Bioron), 1 µL of

each 10 mM primer stock, 0.25 µL of DFS-Taq DNA Polymerase (Bioron), 1 µL of DNA template and DNAse/RNAse-free water (Thermo Scientific) for a final volume of 25 µL.

The PCR products were assessed through electrophoresis in a 1.5 % (w/v) agarose gel (ethidium bromide staining) and purified using the GeneJET PCR Purification Kit (Thermo Scientific, USA).

DGGE was performed using the DCode System (Bio-Rad Laboratories, Hercules, CA, USA). PCR products were loaded onto 6 % - 9 % (w/v) polyacrylamide gels in TAE buffer. Gels had a denaturing gradient ranging from 35 to 70 % urea for the general bacterial samples and from 40 % to 70 % urea for archaea and SRB samples. Electrophoresis was carried out at 60°C at 110 V for 17 h. The gel was silver stained and photographed. Bands were excised and the DNA was extracted through the "crush and soak" procedure (Sambrook and Russell 2001; Czarnetzki and Tebbe 2004). The DNA was re-amplified using primer pairs and protocols mentioned above. The PCR products were purified using the GeneJET PCR Purification Kit. The concentrations of the amplicons were measured fluorometrically as described before. The PCR products were sequenced by GATC Biotech AG. Sequence homologies were analyzed using BLAST (Basic Local Alignment Search Tool) (Altschul et al. 1990).

With respect to the high contamination risk during extraction and sequential PCRs, blank controls were included at each extraction and amplification step. After the nested PCR, those blanks occasionally showed weak product, but did not indicated any relation to the DGGE band patterns of the samples.

5. RESULTS

5.1. The use of tracer to assess drill mud contamination in Deep Biosphere samples

In the following section, the outcomes of laboratory experiments to evaluate three different tracers, namely fluorescein, DAPI stained cell, and microspheres, are presented. Furthermore results from the field application of fluorescein during two coring campaigns are presented, indicating the degree of drill mud contamination in each sample.

5.1.1. Laboratory experiments to evaluate different tracers

Laboratory experiments were performed to determine the detection limit of three tracers suitable to track the drill mud contamination in environmental samples and to evaluate differences in penetration depth. After the incubation of sandstone samples with drill mud at ambient air pressure, fluorescein was detected only where the rock had direct contact with the drill mud, i.e., at the sample surface. A slightly deeper penetration depth, reaching 4 mm from the surface, was observed when applying uniform pressure (from 40 to 90 bar) perpendicular to the whole surface. A similar penetration depth of 3-4 mm was obtained when vacuum was applied, by the use of a pump and an exicator.

The application of a uniaxial pressure difference induced a reproducible drill mud penetration depth which was analyzed in detail in five experiments with three tracers under different conditions (Tab.7). The concentration of all tracers decreased from the surface to the inner part of the sandstone (Fig.12, for fluorescein).

The fluorescein penetration depths detected fluorometrically ranged from 15 to 33 mm, with an average value of 25 mm. The penetration depth observed by microscope, ranged between 16 to 28 mm, with an average of 20 mm. Cross-section pictures (Fig.13) provide an overview of the fluorescein penetration into the rock samples. In experiments 2 to 5 (5 mg L^{-1} fluorescein), when observed with the microscope, the fluorescence signal appeared much more intense than in experiment 1 (1 mg L^{-1} fluorescein). The penetration depth was on average 5 mm deeper (20 %) when

measuring the extracted fluorescein by fluorometer. In experiment 2, both detection methods revealed similar values. The penetration depths of microspheres were 18 to 36 mm while of DAPI-stained *Pseudomonas halophila* cells 33 mm (Tab.7). The penetration depths were similar to the ones of fluorescein when fluorometrically measured.

Tab.7: Results of the drill mud experiments applying uniaxial pressure difference using Bentheimer sandstone.

Exp.	Difference between drill mud and water pressure	Fluorescein concentration in drill mud [mg L^{-1}]	Penetration depth [mm] as detected by			
			Fluorescein (microscope)	Fluorescein (fluorometer)	Microspheres (2 mL L^{-1})	DAPI-stained *Pseudomonas halophila* (10^5 cells L^{-1})
1	15 bars (95 – 80)	1	16	27	n.d.	n.d.
2	15 bars (95 – 80)	5	17	15	n.d.	n.d.
3	10 bars (95 – 85)	5	20	27	n.d.	n.d.
4	10 bars (95 – 85)	5	19	21	18	n.d.
5	10 bars (95 – 85)	5	28	33	36	33
1-5	Average		20	25		

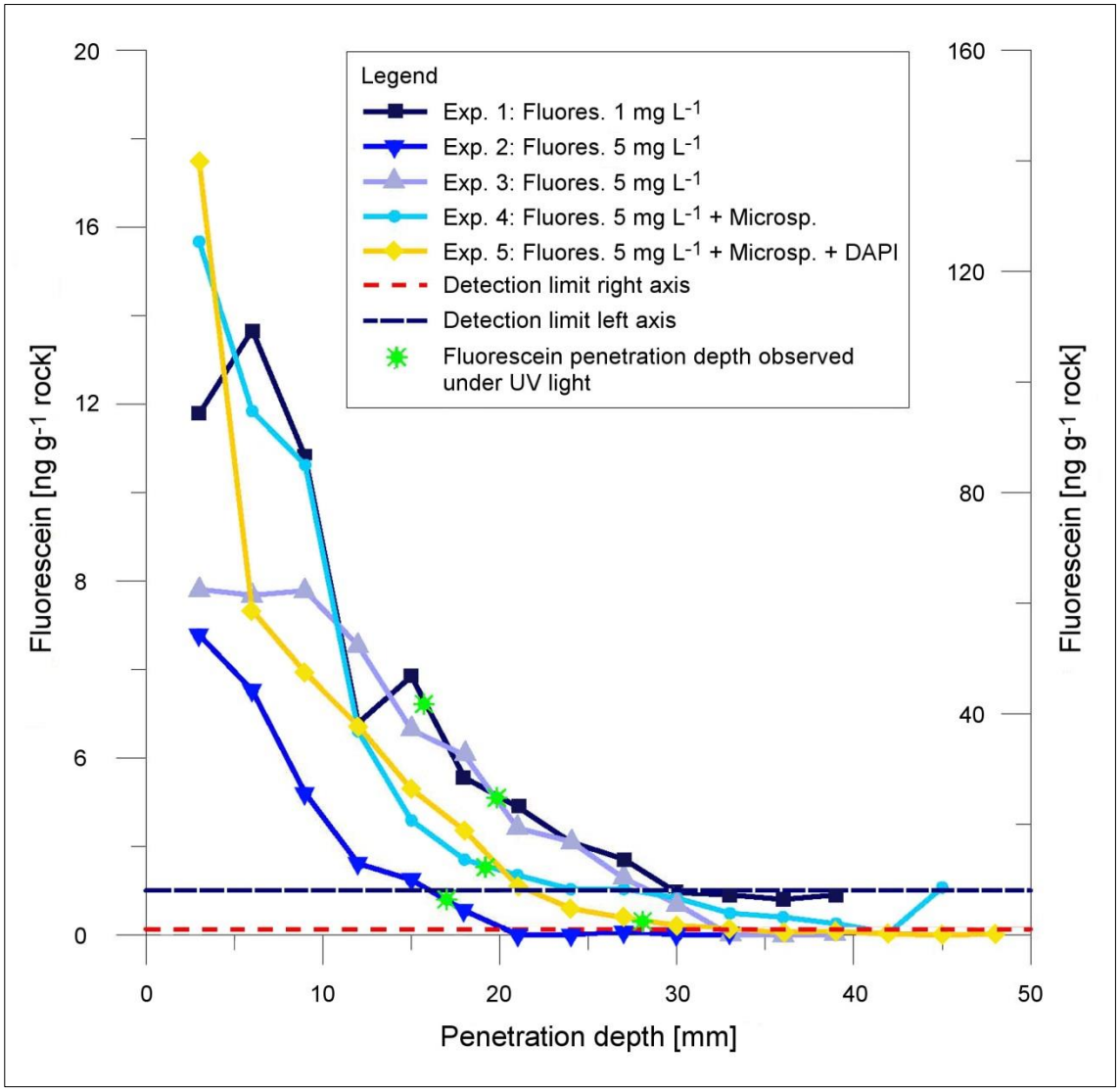

Fig.12: Fluorescein concentration measured fluorometrically in each sample layer. The horizontal lines represent the detection limit. Exp 1, Exp 2, Exp 3 and Exp 4 refer to the left axis, Exp 5 refers to the right axis.

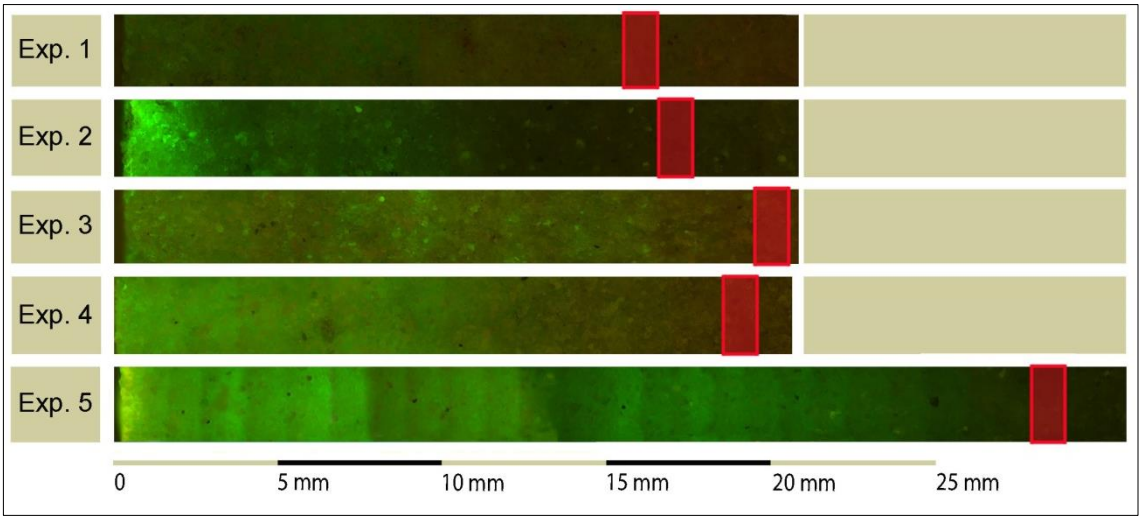

Fig. 13: Cross-section experiments 1-5. On the left side the surface of the samples is visible, while the inner part is shown on the right. The red rectangles indicate the fluorescein penetration depth observed by binocular. The border between contaminated and not contaminated zone is shown as interval of 1 mm (±0.5 mm).

5.1.2. Field application: drill mud contamination control in rock cores and well fluids using the tracer fluorescein

The use of fluorescein to label the drill mud allowed to determine the contamination degree of the rock cores retrieved from two coring campaigns in Ketzin. The fluorescein penetration depth in five samples retrieved during the coring of Ktzi 203 is shown in Fig.14.

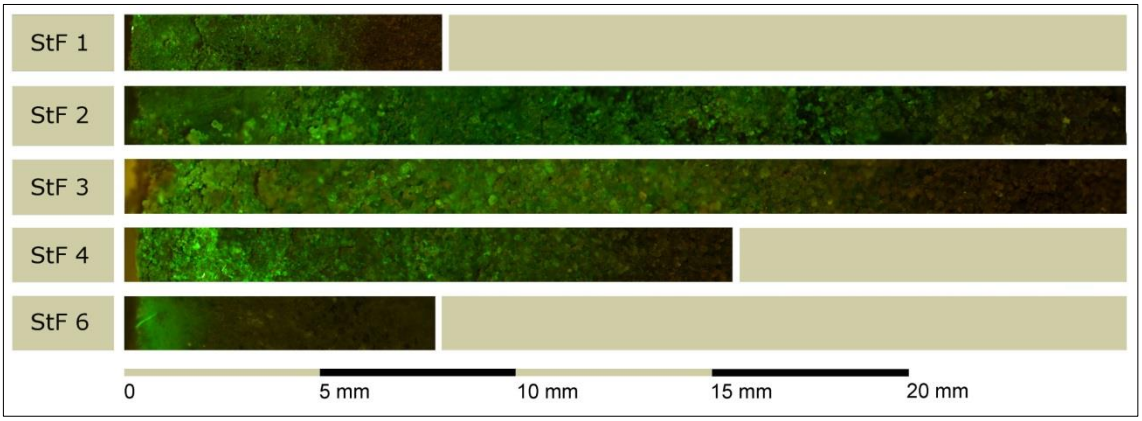

Fig.14: Cross-sections showing the penetration depth of the drill mud labeled with fluorescein for five samples retrieved during the coring of Ktzi 203. Fluorescein intensity decreases from the surface (on the left side) to the inner part (on the right) of the sample.

The penetration depth of the labeled drill mud varied in the cores retrieved from the two coring campaigns. Some rock fragments were strongly contaminated, whereas in several mudstone rocks, fluorescein was only detected 2 to 3 mm from the surface, while the formation brine was stored in the inner part of the core. The results of fluorescein measured by fluorometer in the P300 (Exter Formation) and Ktzi 203 (Stuttgart Formation) samples after coring and incubation with synthetic brine are shown in Tab.8a/b.

Tab.8: Estimation of drill mud contamination in inner core plugs retrieved during the coring of two observation wells and in the fluid after incubation in high-pressure setups. a: P300. b: Ktzi 203.

a

P300 Sample	Lithology	In rock samples [ng fluorescein/g rock]	In fluid samples [% of drill mud]	Sample contaminated
ExF 1	ss	< LOD	< LOD	No
ExF 2	ms	< LOD	< LOD	No
ExF 3	si, fg, ss	< LOD	< LOD	No
ExF 4	fg, ss, si	< LOD	< LOD	No
ExF 5	ss	2	9	Yes
ExF 6	ss	< LOD	0.02	Yes
ExF 7	fg-mg, ss	9	3	Yes
ExF 8	fg-mg, ss	2	1	Yes
ExF 9	fg-mg, ss	< LOD	< LOD	No
ExF 10	fg, ss	< LOD	0.01	Yes
ExF 11	fg, ss	40	16	Yes

b

Ktzi 203 sample	Lithology	In rock samples [ng fluorescein/g rock]	In fluid samples [% of drill mud]	Sample contaminated
StF1	ss	< LOD	< LOD	No
StF2	ss,si	8	0.001	Yes
StF3	ss, ms-si	< LOD	< LOD	No
StF4	ss, ms-si	< LOD	< LOD	No
StF5	ss	< LOD	< LOD	No
StF6	si, ms	< LOD	< LOD	No

<LOD: below limit of detection indicates un-contaminated samples;
ss: sandstone, sandy; ms: mudstone; muddy; si: siltstone, silty; fg: fine-grained; mg: middle-grained.

Five out of eleven (45 %) P300 core plugs were not contaminated. In those samples, fluorescein was below detection limit in rock and fluid. Very low fluorescein

concentrations (0.02 and 0.01 % of drill mud working concentration) were detected in the fluid of two samples (ExF 6 and ExF 10, respectively), while in the corresponding rock cores the concentration was below the detection limit of 1 ng fluorescein per gram of rock.

A 40 cm sandstone rock core was cut into three equal parts (ExF 7, ExF 8 and ExF 9) to perform the inner coring. Although the three subsamples originated from the same core, the fluorescein concentrations were remarkably different from each other. The concentration was high in the shallowest part (ExF 7 and ExF 8), while no fluorescein was detected in the bottom of the core fragment (ExF 9).

Regarding the Ktzi 203 cores, five out of the six incubated samples were free of fluorescein. Only the fluid sampled from one high-pressure setup (StF 2) had a very low fluorescein concentration (0.001 % of drill mud).

The control flask containing labeled drill mud and stored in the oven together with the high-pressure setups, indicated no significant loss of fluorescein over six months.

For the shallow observation well P300, the fluorescein concentration was measured also in the fluid produced through the pump test (Fig.15). At the end of the test (after the production of $14\,m^3$ of fluid) fluorescein in a concentration of $0.2\,mg\,L^{-1}$, corresponding approximately to 7.2 % of the original concentration, was still detected.

The fluorescein concentration measured in the fluid collected via a U-tube sampling system (Fig.16) fluctuated around an average of $0.03\,mg\,L^{-1}$, corresponding to approximately 1 % of the original fluorescein concentration in the drill mud.

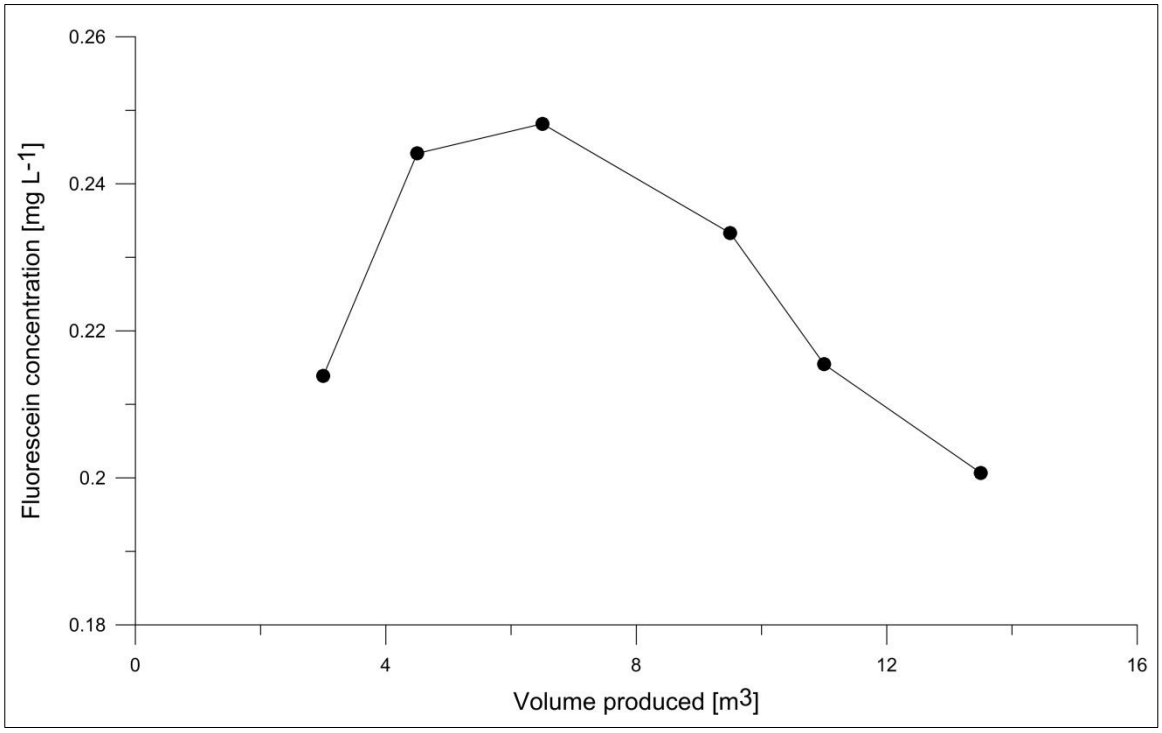

Fig.15: Fluorescein concentration in the fluid produced during the pump test performed at the shallow observation well P300.

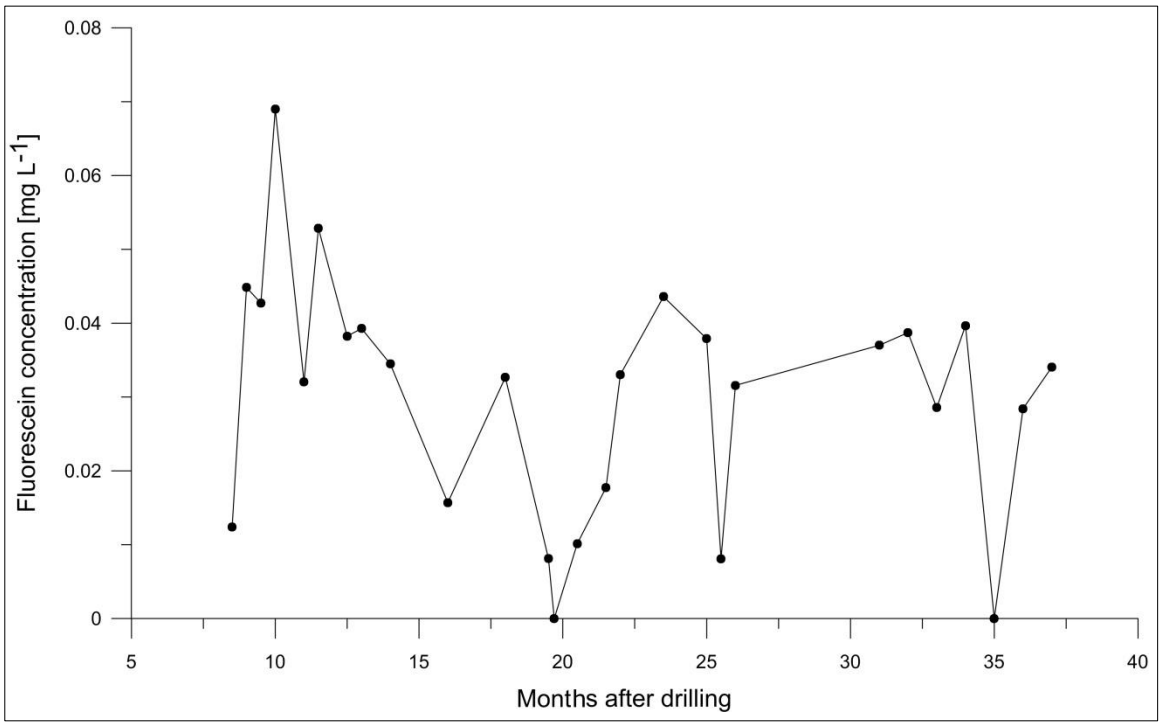

Fig.16: Fluorescein concentration in the fluid sampled through the U-tube installed into the shallow observation well P300.

5.2. Microbiological analyses of rocks and fluids sampled from the site

The use of the tracer fluorescein was a decisive tool for selecting pristine samples intended for microbiological analyses. The examination of the microbial community of the Stuttgart Formation before and after *in-situ* CO_2 exposure was from special importance for the characterization of potential changes in the community as a consequence of elevated CO_2 concentrations. The sandstone overlaying its caprock (Exter Formation) was also in the focus of the study because on a long-term perspective changes in the community structure might be indicative for CO_2 leakages. The results of the 16S rRNA gene fingerprinting of the microbial communities of the Exter as well as Stuttgart Formation are presented in this section.

5.2.1. Microbiological baseline characterization

5.2.1.1. Exter Formation

The comparative DGGE-analyses of the baseline characterization of the Exter Formation rock samples are presented in Fig.17. The retrieved 16S rRNA sequence data are given in Tab.9. Sequences are deposited at GenBank under the accession numbers KR336949- KR336955 (Appendix I).

The DGGE profile of the six rock cores showed one to three dominant bands (Fig.17). From samples ExF 1-BL and ExF 10-BL, no DNA sequences were retrieved. In ExF 3-BL, two bands were most intensive. The corresponding sequences had highest similarities to *Variovorax paradoxus* (band 12) and *Hymenobacter psychrophilus* (band 13), respectively. In ExF 4-BL, only one dominant band which sequence was affiliated to *Pseudomonas* spp. (band 14) was observed. The sequences corresponding to the two dominant bands observed in ExF 6-BL were attributed to *Ochrobactrum* spp. (band 15) and *Propionicimonas paludicola* (band 16). In ExF 9-BL microorganisms related to *Rhizobium* spp. (band 17) and *Pelomonas* spp. (band 18) were identified (Tab.9). For each rock sample, the number of DGGE bands is indicated in Tab.10.

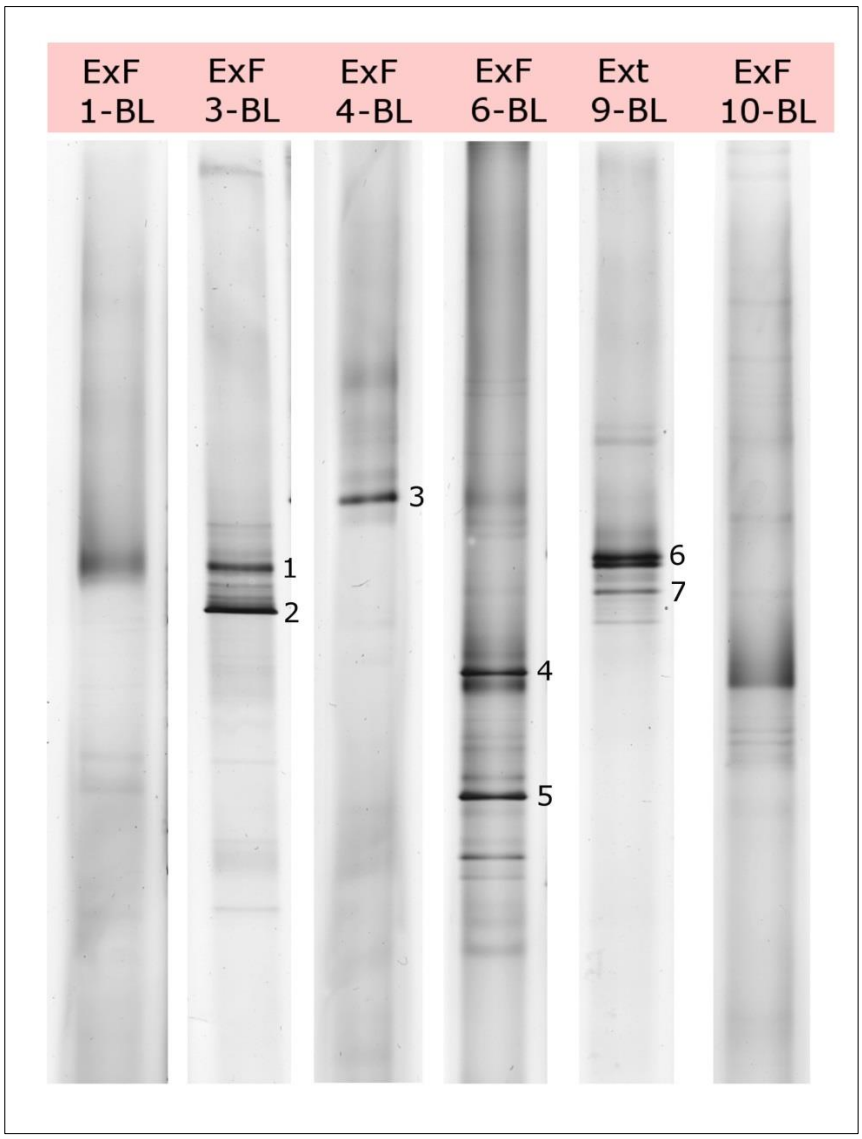

Fig.17: Comparative DGGE-analysis of bacterial 16S rRNA gene fragments of rock cores retrieved during the coring of the shallow observation well P300. Sequenced bands are marked with numbers.

Tab.9: Taxonomic assignment of sequenced DGGE bands (partial 16S rRNA) in rock core samples collected during the coring of the shallow observation well P300.

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

Tab.10: Number of DGGE bands in each Exter Formation rock core.

Sample	Number of DGGE bands
ExF 1-BL	3
ExF 3-BL	8
ExF 4-BL	3
ExF 6-BL	14
ExF 9-BL	8
ExF 10-BL	9

5.2.1.2. Stuttgart Formation

The comparative SSCP-analyses performed in the fluid samples collected through pump test and downhole sampling from the Ktzi 202 observation well before CO_2 injection, are shown in Fig.18a/b. The retrieved 16S rRNA sequence data for the baseline characterization of the fluid samples are given in Tab.11 and Tab.12. Sequences are deposited at GenBank under the accession numbers KR336960- KR336967 (Appendix I).

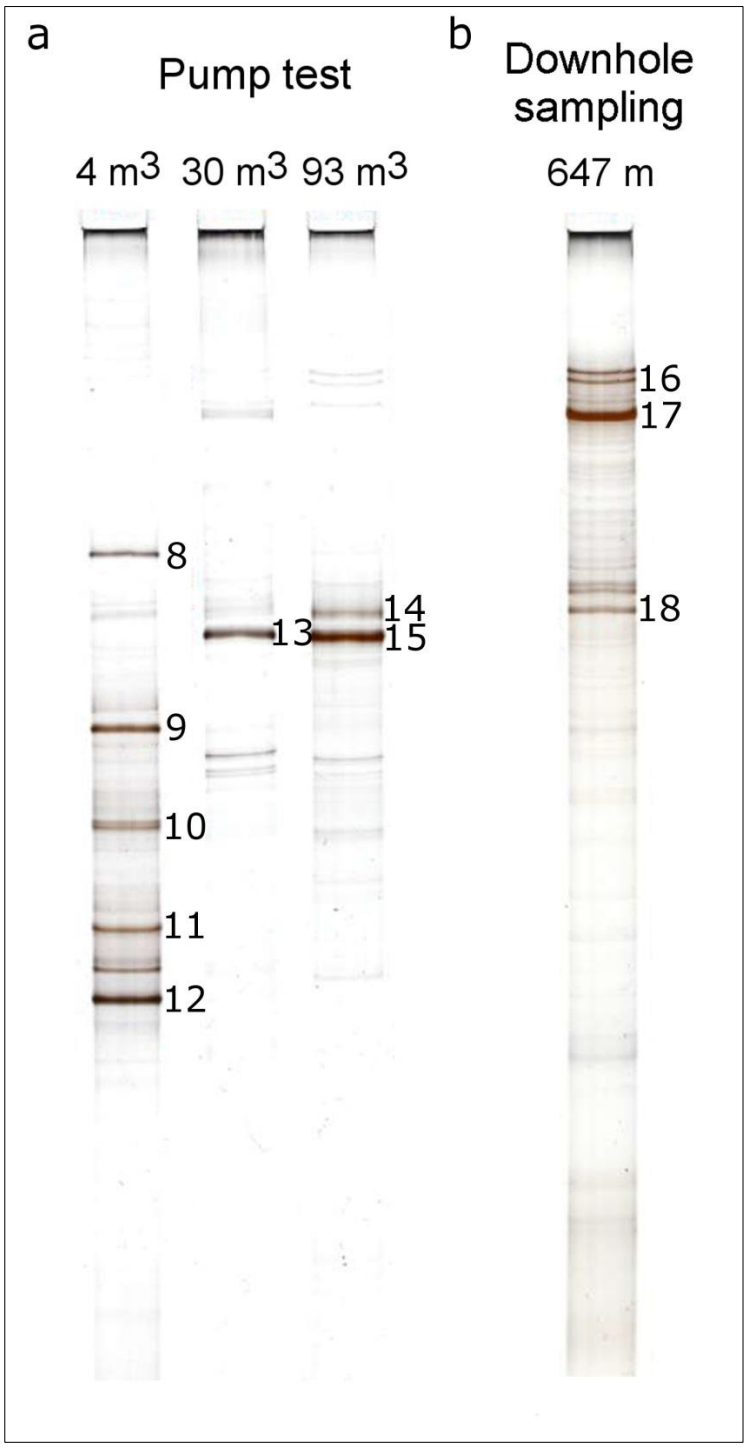

Fig.18: Comparative SSCP-analysis of bacterial 16S rRNA gene fragments of fluid retrieved from the Ktzi 202 observation well before CO_2 injection. a: Samples collected during pump test. b: Sample collected during downhole sampling. Sequenced bands are marked with numbers.

The SSCP genetic profile of the fluid samples (Fig.18a) revealed that the microbial community changed during the pump test. In the sample collected at the beginning of

the test, after production of 4 m³ fluid, the sequences 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; 93 m^3: bands 7 and 8) (Tab.11). The SSCP profile of the fluid collected via downhole sampling from a depth of 647 m (Fig.18b) was different to the band pattern of the fluid collected at the end of the pump test. The sequences were assigned to the halophilic fermenting bacteria Halanaerobiaceae (bands 1), to *Halanaerobium* spp. (band 11) and to an uncultured bacterium, distantly related to the SRB of the genus *Desulfohalobium* (band 10) (Tab.12).

Tab.11: Taxonomic assignment of sequenced SSCP bands (partial 16S rRNA) in fluid samples retrieved during the pump test performed in the Ktzi 202 observation well, drilled into the Stuttgart Formation.

Produced volume [m3]	Band	GenBank accession	Phylogenetic group	Closest relative	Similarity [%]
4	8	KR336962	Bacteroidetes	*Bacteroides graminisolvens*	100
	9	KR336963	Firmicutes	*Proteiniclasticum ruminis* D3RC-2	99
	10	KR336964	Bacteroidetes	*Flavobacterium* spp.	99
	11	KR336965	Bacteroidetes	*Flavobacterium* spp.	99
	12	KR336966	Firmicutes	*Clostridium sticklandii* DSM519	99
30	13	KR336967	Firmicutes	*Haloanaerobium* spp.	99
93	14, 15	KR336967	Firmicutes	*Halanaerobium* spp.	99

Tab.12: Taxonomic assignment of sequenced SSCP bands (partial 16S rRNA) in fluid samples retrieved during a downhole sampling performed in the Ktzi 202 observation well, before CO_2 injection.

Depth [m]	Band	GenBank accession	Phylogenetic group	Closest relative	Similarity [%]
647	16	KR336960	Firmicutes	*Halanaerobium* spp.	90
	17	KR336961	Deltaproteobacteria	*Desulfohalobium utahense*	92
	18	KR336967	Firmicutes	*Haloanaerobium* spp.	99

5.2.2. Microbial community in Stuttgart Formation rock cores and well fluid after in-situ CO_2 exposure

The DGGE-profiles of rock cores collected during the coring of the Ktzi 203 observation well and well fluid collected from the injection well (Ktzi 201) are presented in Fig.19a/b.

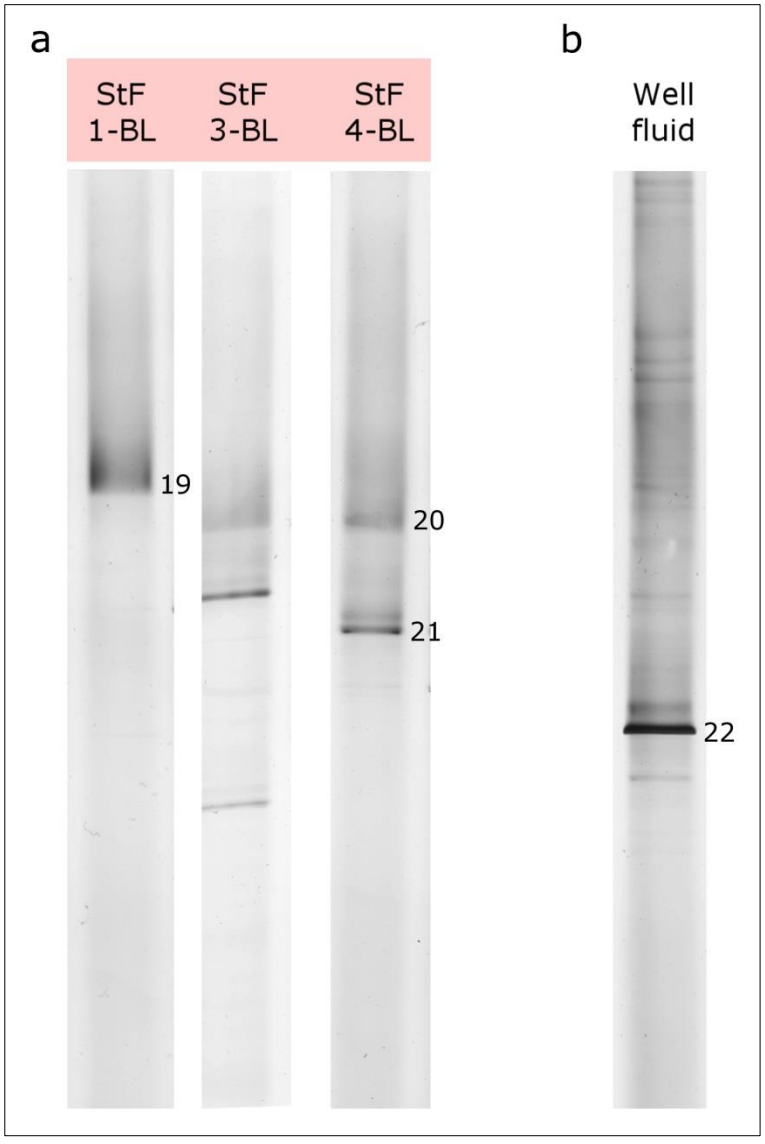

Fig.19: Comparative DGGE-analysis of bacterial 16S rRNA gene fragments of Stuttgart Formation samples after *in-situ* CO_2 exposure. a: Rock cores retrieved during the coring of the observation well Ktzi 203, after four years of CO_2 injection (red). b: Fluid obtained through downhole sampling in the injection well after five years of CO_2 injection. Sequenced bands are marked with numbers.

The retrieved 16S rRNA sequence data are given in Tab.13. Sequences are deposited at GenBank under the accession numbers KU603732, KU603736, KU603742 and KU603743 (Appendix I). For each sample, the number of DGGE bands is indicated in Tab.14. The DGGE analyses of the three rock cores (StF 3-BL; StF 4-BL; StF 1-BL), retrieved through the drilling of the deep observation well Ktzi 203 into the injected CO_2 plume, indicated the presence of microorganisms affiliated to *Sphingomonas* spp. (StF 4-BL, band 21), *Undibacterium* spp. (StF 1-BL, band 19) and to an uncultured Alphaproteobacterium (StF 4-BL, band 20). In the rock samples, SRB and archaea were not detected using specific primers. The genetic fingerprinting analysis of the fluid collected from the injection well after five years of CO_2 injection counted 17 bands (Fig.20b), one particularly dominant and others much weaker. The sequence of the dominant band was affiliated to an uncultured *Desulfotomaculum* spp. (band 22). The SRB was noticed also through the application of specific primer pairs. Archaea related to *Methanoculleus* spp. were identified as well.

Tab.13: Taxonomic assignment of sequenced DGGE bands (partial 16S rRNA) in rock cores collected during the coring of the deep observation well Ktzi 203 and in well fluid gained through downhole sampling from the injection well Ktzi 201.

Sample	Band	GenBank accession	Phylogenetic group	Closest relative	Similarity [%]
StF 1-BL	19	KU603732	Betaproteobacteria	*Undibacterium* spp.	100
StF 4-BL	20	KU603742	Alphaproteobacteria	uncultured Alphaproteobacterium	98
StF 4-BL	21	KU603736	Alphaproteobacteria	*Sphingomonas* spp.	100
Well fluid	22	KU603743	Firmicutes	uncultured *Desulfotomaculum* spp.	91

Tab.14: Number of DGGE bands in the three Stuttgart Formation rock cores and in the well fluid after *in-situ* CO_2 exposure.

Sample	Number of DGGE bands
StF 1-BL	2
StF 3-BL	5
StF 4-BL	4
Well fluid	17

5.3. Long-term experiments: incubation of pristine rock cores with drill mud and acetate

Drill mud components are potential carbon and energy sources for the autochthonous microbial community of storage reservoirs, leading to enhanced activity and subsequent growth. An increment of microbial activity may influence the aquifer characteristics and consequently the reliability of the geotechnical installation. In the following section the results of laboratory experiments performed under simulated *in-situ* conditions are presented. Samples were incubated with synthetic brine and drill mud excluding biocide (StF 3-S and ExF 9-S), with synthetic brine and drill mud containing biocide (ExF 4-S, ExF 6-S and StF 4-S), with well fluid and drill mud containing biocide (ExF 4-W, ExF 6-W and StF 4-W) and with acetate and synthetic brine (StF 1-S) or well fluid (StF 1-W) (Tab.6).

The 16S rRNA sequences data retrieved after every incubation experiment are shown at the end of the present section (Tab.19). Sequences are deposited at GenBank under the accession numbers KU603730- KU603731; KU603733- KU603741 and KU603743- KU603764 (Appendix I).

5.3.1. Incubation with synthetic brine and drill mud

5.3.1.1. Experiments excluding biocide

The comparative DGGE-analyses of 16S rRNA gene fragments of the bacterial community resulting from the pristine rock cores incubation with synthetic brine and drill mud excluding biocide (StF 3-S and ExF 9-S) are shown in Fig.20.

Compared with pristine rock core analyses (StF 3-BL and ExF 9-BL), the eight weeks of incubation produced a significant increase of microbial diversity in both Stuttgart (StF 3-S) and Exter (ExF 9-S) Formations setups (Tab.15).

For each fluid sample, the DGGE profiles of the two consecutive centrifugations were consistent (Fig.19: F 1ex and F 2ex). Very similar DGGE patterns, with only minor differences in the intensities of the bands, were observed between the fluid samples of Exter and Stuttgart Formations. The dominant microorganisms that were detected in the

rock and fluid samples of both setups were affiliated to members of the class Betaproteobacteria. The sequences were assigned to *Ralstonia* spp. (bands 23, 25, 28, 29, 30) and *Burkholderia tuberum* (bands 24, 31). Beside those microorganisms, in the Exter Formation setup, sequences related to *Variovorax* (band 26) and to uncultured *Acidobacteria* (band 32) were identified in the rock and fluid, respectively (Tab.19). Using specific primer pairs, SRB were not detected. One sequence, attributed to an unclassified archaeon was observed in the fluid samples of both setups.

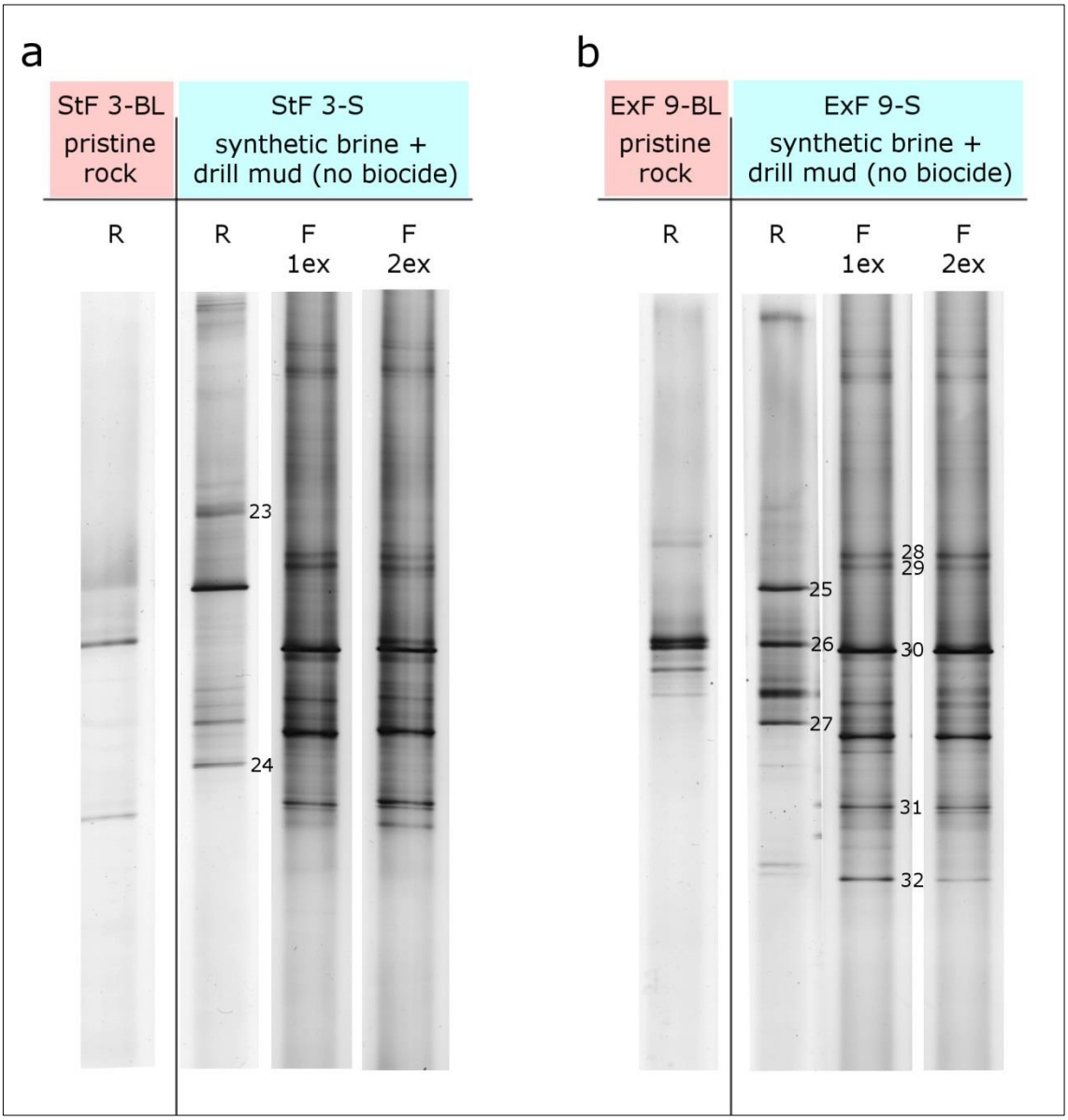

Fig.20: Comparative DGGE-analysis of bacterial 16S rRNA gene fragments of pristine rock cores (red) incubation with synthetic brine and drill mud excluding biocide (blue). a: Setup with a Stuttgart Formation sample. b: Exter Formation sample. Sequenced bands are marked with numbers. R: rock; F: fluid; 1ex: extraction after first centrifugation cycle; 2ex: extraction after second centrifugation cycle.

Tab.15: Number of DGGE bands in each sample after pristine rock core (red) incubation with synthetic brine and drill mud excluding biocide (blue).

		Addition of drill mud without biocide			
Sample	Pristine rock	Sample	Rock	Fluid 1ex	Fluid 2ex
StF 3-BL	5	StF 3-S	17	20	20
ExF 9-BL	8	ExF 9-S	14	22	22

5.3.1.2. Experiments including biocide

The DGGE profile of 16S rRNA gene fragments resulting from the incubation of pristine rock cores with synthetic brine and drill mud containing biocide is shown in Fig.21. Compared with the pristine rock analyses (StF 4-BL and ExF 4-BL), an increase of microbial diversity was observed in StF 4-S and ExF 4-S, after 16 weeks of incubation (Tab.16). In ExF 6-S the total number of bands decreased compared to the pristine rock (ExF 6-BL); however, the number of bands with higher intensities increased.

After the incubation, bacteria affiliated to Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria and Actinobacteria dominated the community (Tab.19). Microorganisms related to *Variovorax* spp. (ExF 4-S: band 39; ExF 6-S: band 43 and StF 4-S: band 33) were detected in all setups. Furthermore, bacteria related to *Burkholderia tuberum* (band 45); *Dyella* spp. (band 44) and *Corynebacterium vitaeruminis* (band 46) were detected in the ExF 6-S setup, together with *Isoptericola variabilis* (band 41) and an uncultured Gammaproteobacterium (band 42). In ExF 4-S, microorganisms affiliated to *Novosphingobium* spp. (band 38) were also detected. Relatives of *Propionibacterium acnes* were identified in ExF 4-S (band 40) and StF 4-S (band 36). Moreover, bacteria related to *Ralstonia* spp. (band 34), *Kosakonia* spp. (band 37) and *Phyllobacterium myrsinacearum* (band 35) were observed in StF 4-S.

SRB were not detected using specific primers. The unclassified archaeal sequence observed after incubation with drill mud without biocide (ExF 9-S and StF 3-S), was detected also in the fluid of setup ExF 4-S.

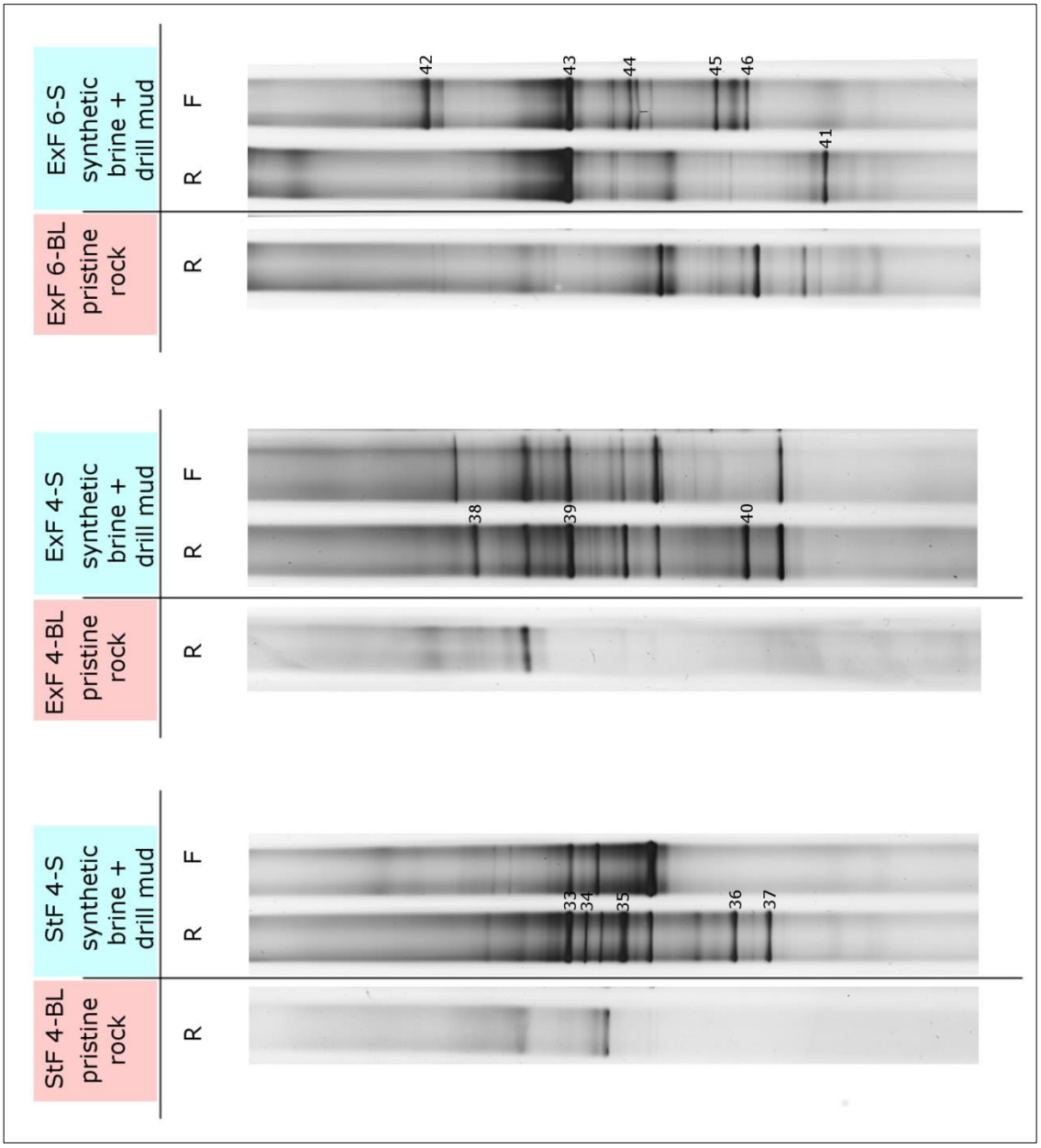

Fig.21: Comparative DGGE-analysis of bacterial 16S rRNA gene fragments of pristine rock cores (red) incubation with drill mud containing biocide and synthetic brine (blue). Sequenced bands are marked with numbers. R: rock; F: fluid.

Tab.16: Number of DGGE bands in each experimental setup of pristine rock cores (red) incubation with synthetic brine and drill mud containing biocide (blue).

		Addition of drill mud with biocide		
Sample	Pristine rock	Sample	Rock	Fluid
StF 4-BL	4	StF 4-S	16	9
ExF 4-BL	3	ExF 4-S	18	15
ExF 6-BL	14	ExF 6-S	11	13

5.3.2. Incubation with well fluid and drill mud containing biocide

The DGGE profiles of 16S rRNA gene fragments amplified from samples taken after the incubation with fresh well fluid and an energy source are shown in Fig.22. As previously described, the synthetic brine and an energy source mixture was replaced with the well fluid mixed with acetate (StF 1-W) or drill mud including a biocide (StF 4-W; ExF 4-W; ExF 6-W), respectively. For the Stuttgart Formation setup, the 20 week incubation with drill mud and un-filtered well fluid (StF 4-W) reduced the microbial diversity (Tab.17) compared to the outcomes obtained before of the fluid replacement (StF 4-S). For the Exter Formation setup, after 15 weeks of incubation (ExF 4-W) the microbial diversity increased in the rock but decreased in the fluid, compared to the results obtained before of the fluid exchange (ExF 4-S).

In both setups, sequences related to *Acinetobacter* (ExF 4-W, bands 60: *Acinetobacter. junii* and 59: *Acinetobacter* sp; StF 4-W, band 47: *Acinetobacter* spp.), *Burkholderia* (ExF 4-W, bands 62: *Burkholderia heleia* and 57: *Burkholderia tuberum*; StF 4-W, band 51: *Burkholderia* spp.) and to an uncultured Acidobacterium (ExF 4-W, band 58; StF 4-W, band 52) were detected. In ExF 4-W, microorganisms attributed to *Ralstonia* (band 61) and *Curvibacter* spp. (band 54), and to an uncultured bacterium (band 63) were also identified. Bacteria ascribed to *Sphingomonas* spp. (band 50) and *Brevibacillus* spp. (band 49) were observed in StF 4-W. SRB were not detected. The sequence of the unclassified archaeon detected in setup ExF 4-S, was identified also in ExF 4-W.

In ExF 6-W, the well fluid was filtered before the 20 weeks of incubation to separate the microbial community from the fluid. The filtrated well fluid was added to investigate the specific influence of well fluid geochemistry on the microbial community of the rock. After the incubation, the microbial diversity increased compared to the diversity before of the fluid replacement (Tab.17). Microorganisms that were stimulated by the incubation were related to the genera *Mesorhizobium* (band 66), *Sphingomonas* (band 68), to an uncultured Acidobacterium (band 67), and to the organism *Curvibacter lanceolatus* (band 64). Additionally, two sequences related to two different species of *Desulfotomaculum* were identified (bands 65 and 70). However, SRB (as well as archaea) were not detected using specific primer pairs.

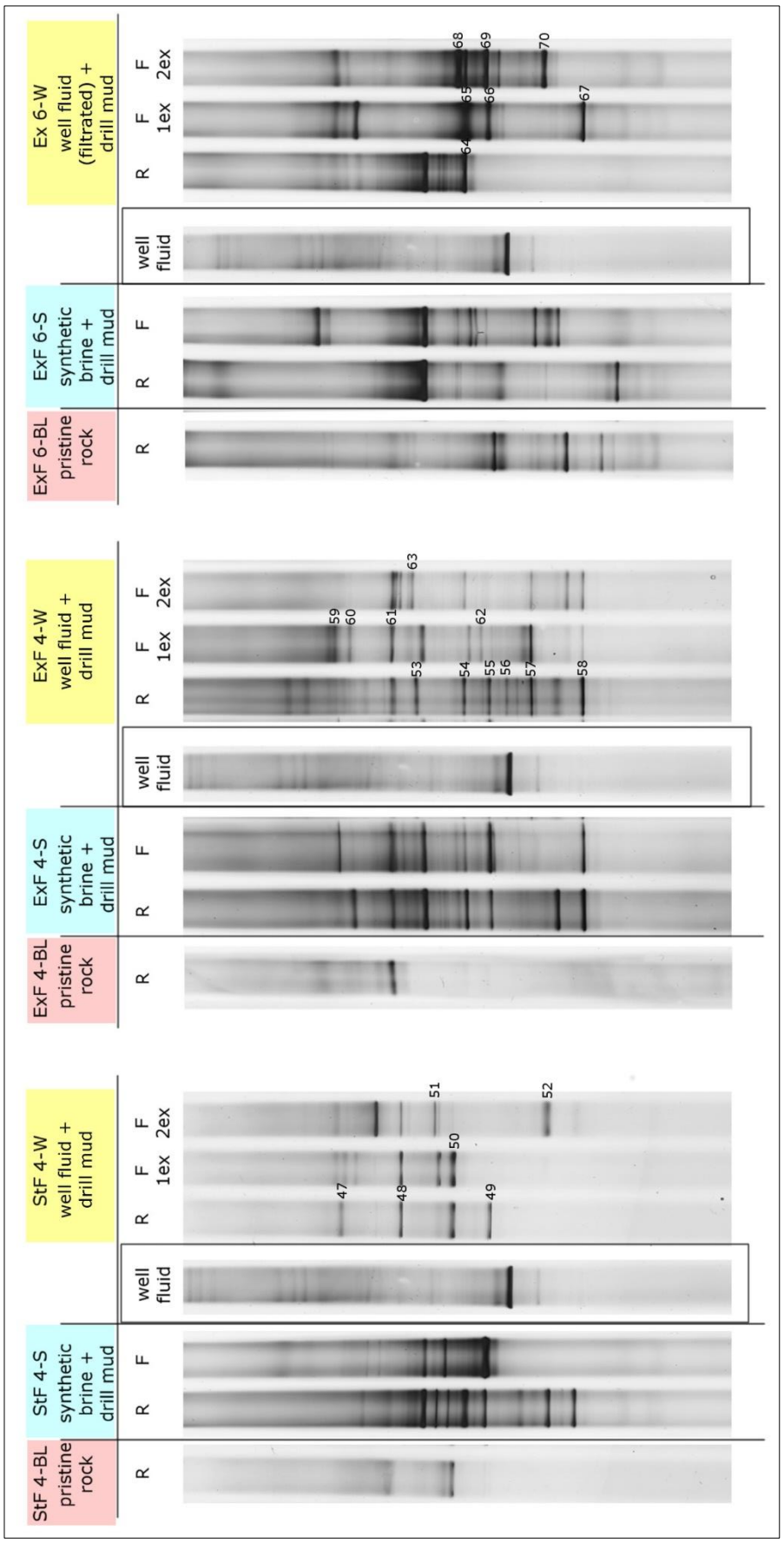

Fig.22: Comparative DGGE-analysis of bacterial 16S rRNA gene fragments of pristine rock cores (red) incubation with drill mud containing biocide and synthetic brine (blue, already shown in Fig.21) or well fluid (yellow). Sequenced bands are marked with numbers. R: rock; F: fluid; 1ex: extraction after first centrifugation cycle; 2ex: extraction after second centrifugation cycle.

Tab.17: Number of DGGE bands after well fluid experiments (yellow). The numbers of bands observed in the pristine rock (red) and before the fluid replacement (blue) are shown on the left and in the center of the table, respectively. *: the well fluid was filtered before being added to the setup.

		Addition of drill mud with biocide							
		Synthetic brine			Baseline fluid	Well fluid			
Sample	Pristine rock	Sample	Rock	Fluid		Sample	Rock	Fluid 1ex	Fluid 2ex
StF 4-BL	4	StF 4-S	16	9	17	StF 4-W	6	7	10
ExF 4-BL	3	ExtF4-S	18	15	17	ExF 4-W	20	13	10
ExF 6-BL	14	ExF 6-S	11	13	17*	ExF 6-W	15	11	17

*: the well fluid was filtered before being added to the setup.

5.3.3. Incubation with synthetic brine or well fluid and acetate

The comparative DGGE-analysis from the incubation with acetate and synthetic brine (StF 1-S) and afterwards with acetate and well fluid (StF 1-W) is shown in Fig.23.

After 16 weeks of incubation with acetate and synthetic brine, the microbial diversity increased compared to the pristine rock (Tab.18). Microorganisms related to *Variovorax* (bands 75 and 76), *Ralstonia* (band 71), *Kosakonia* (band 74) and uncultured *Acidobacteria* (band 73) were detected (Tab.19).

After the synthetic brine-acetate mixture was removed and the rock sample was incubated for 20 weeks with fresh collected well fluid and acetate, the number of dominant bands decreased in both, the rock and the fluid. A DNA sequence affiliated to the genus *Pelomonas* (bands 77) was detected together with the same sequence (band 79) that was detected in fresh well fluid and was related to an uncultured *Desulfotomaculum* spp.. SRB were identified also with specific primers. Archaea were not detected.

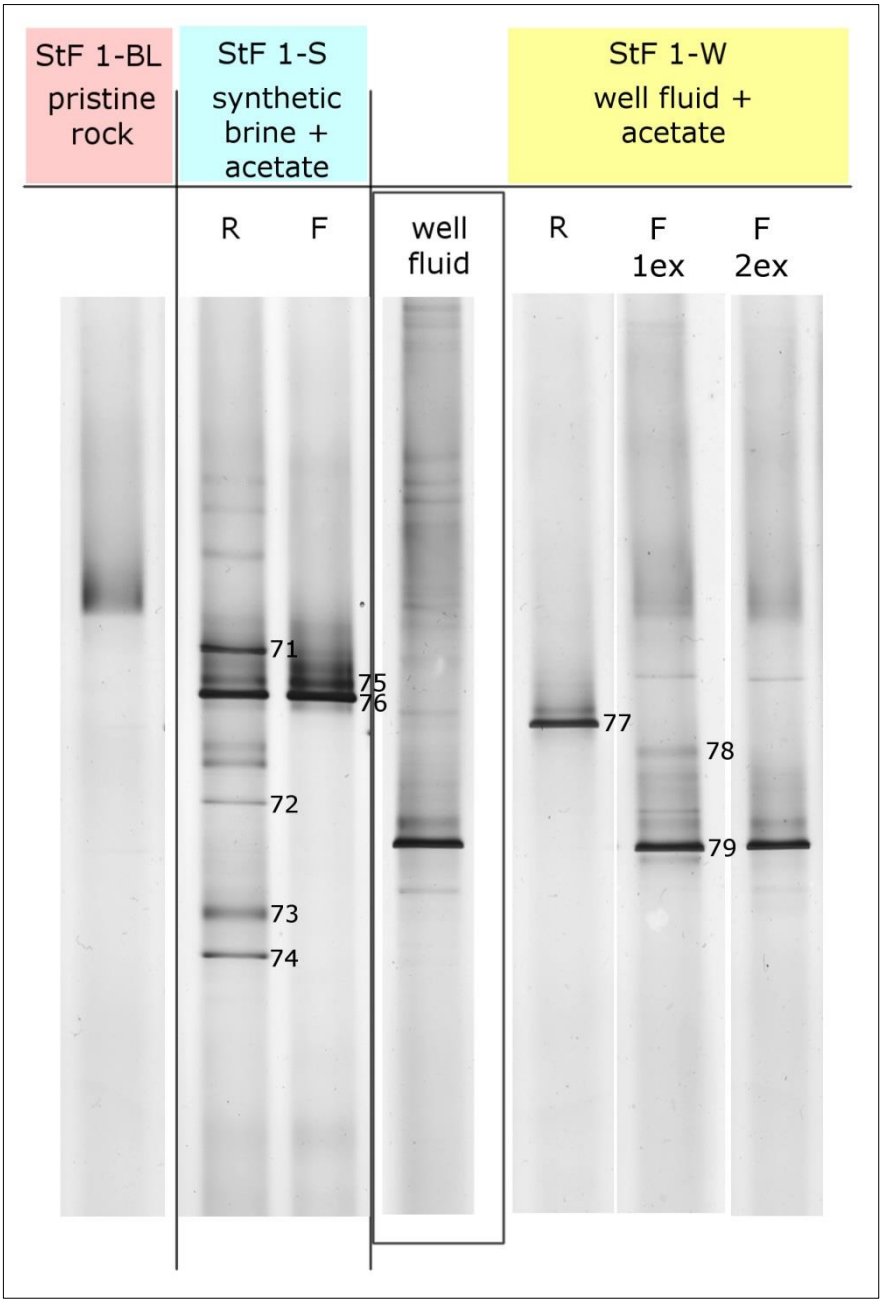

Fig.23: Comparative DGGE-analysis of bacterial 16S rRNA gene fragments of pristine rock core (red) incubations with synthetic brine and acetate (blue) and subsequently with well fluid and acetate (yellow). Sequenced bands are marked with numbers. R: rock; F: fluid

Tab.18: Number of DGGE bands after pristine rock core (red) incubation with synthetic brine and acetate (blue) and well fluid and acetate (yellow).

		Addition of acetate							
		Synthetic brine			Baseline fluid	Well fluid			
Sample	Pristine rock	Sample	Rock	Fluid		Sample	Rock	Fluid 1ex	Fluid 2ex
StF 1-BL	2	StF 1-S	12	5	17	StF 1-W	2	10	10

The 16S rRNA sequences data retrieved after every incubation experiment are listed in Tab.19. An overview of all DGGE profiles performed for the long-term experiments is given in Fig.24. Tab.20 and Fig.25 give an overview of the microorganisms identified before and after incubation in each sample and the frequency of detection of each identified bacterial species, respectively.

Tab.19: Taxonomic assignment of sequenced DGGE bands (partial 16S rRNA) in samples after long-term experiments with drill mud and acetate.

Sample	Band	GeneBank accession	Phylum	Closest relative	Similarity [%]
ExF 4-S	38	KU603752	Alphaproteobacteria	*Novosphingobium* spp.	99
ExF 6-W	68	KU603736	Alphaproteobacteria	*Sphingomonas* spp.	100
StF 4-W	50				
ExF 6-W	66	KU603757	Alphaproteobacteria	*Mesorhizobium* spp.	100
StF 4-S	35	KU603754	Alphaproteobacteria	*Phyllobacterium myrsinacearum* (T)	100
StF 1-W	78	KU603730	Alphaproteobacteria	unclassified Rhizobiales	98
ExF 6-W	64	KU603759	Betaproteobacteria	*Curvibacter lanceolatus*	100
ExF 4-W	54	KU603764	Betaproteobacteria	*Curvibacter* spp.	100
ExF 9-S	26	KU603747	Betaproteobacteria	*Variovorax* spp.	100
ExF 4-S	39				
ExF 6-S	43				
StF 4-S	33				
StF 1-S	75				
	76				
StF 1-W	77	KU603731	Betaproteobacteria	*Pelomonas* spp.	100
ExF 9-S	29	KU603745	Betaproteobacteria	*Ralstonia* spp. seq.1	100
	28				
	25				
	30				
ExF 4-W	61				
StF 3-S	23				
StF 1-S	71				
StF 4-S	34	KU603755	Betaproteobacteria	*Ralstonia* spp. seq.2	99
ExF 4-W	62	KU603749	Betaproteobacteria	*Burkholderia heleia*	100
ExF 4-W	57	KU603744	Betaproteobacteria	*Burkholderia tuberum* (T)	100
ExF 9-S	31				
ExF 6-S	45				
StF 3-S	24				
StF 4-W	51	KU603734	Betaproteobacteria	*Burkholderia* spp.	100
ExF 4-W	56	KU603750	Betaproteobacteria	unclassified Burkholderiales	99
StF 4-S	37	KU603753	Gammaproteobact.	*Kosakonia* spp.	100
StF 1-S	74				

Continue to next page

Continuation of Tab. 19

Sample	Band	GeneBank accession	Phylum	Closest relative	Similarity [%]
ExF 6-S	44	KU603761	Gammaproteobact.	*Dyella* spp.	99
ExF 4-W	60	KU603740	Gammaproteobact.	*Acinetobacter junii* (T)	100
ExF 4-W	59	KU603741	Gammaproteobact.	*Acinetobacter* seq.1	100
StF 4-W	47	KU603739	Gammaproteobact.	*Acinetobacter* seq.2	99
ExF 6-S	42	KU603762	Gammaproteobact.	uncultured Gammaproteobacterium	99
ExF 9-S	32	KU603735	Acidobacteria	uncultured bacterium seq.1	100
ExF 4-W	58				
ExF 6-W	67				
StF 4-W	52	KU603733	Acidobacteria	uncultured bacterium seq.2	100
StF 1-S	73				
ExF 6-S	46	KU603760	Actinobacteria	*Corynebacterium vitaeruminis*	100
ExF 6-S	41	KU603763	Actinobacteria	*Isoptericola variabilis*	98
ExF 4-S	40	KU603751	Actinobacteria	*Propionibacterium acnes*	100
StF 4-S	36				
StF 4-W	49	KU603737	Firmicutes	*Brevibacillus* spp.	100
StF 1-W	79	KU603743	Firmicutes	uncultured *Desulfotomaculum* spp.seq.1	100
ExF 6-W	70	KU603756	Firmicutes	uncultured *Desulfotomaculum* spp.seq.2	100
ExF 6-W	65	KU603758	Firmicutes	*Desulfotomaculum intricatum*	99
ExF 4-W	63	KU603748	n.d.	uncultured bacterium	100
ExF 9-S	27	KU603746	n.d.	uncultured bacterium	100
ExF 4-W	55				
ExF 6-W	69				
StF 1-S	72				
ExF 4-W	53	KU603738	n.d.	uncultured bacterium	100
StF 4-W	30				

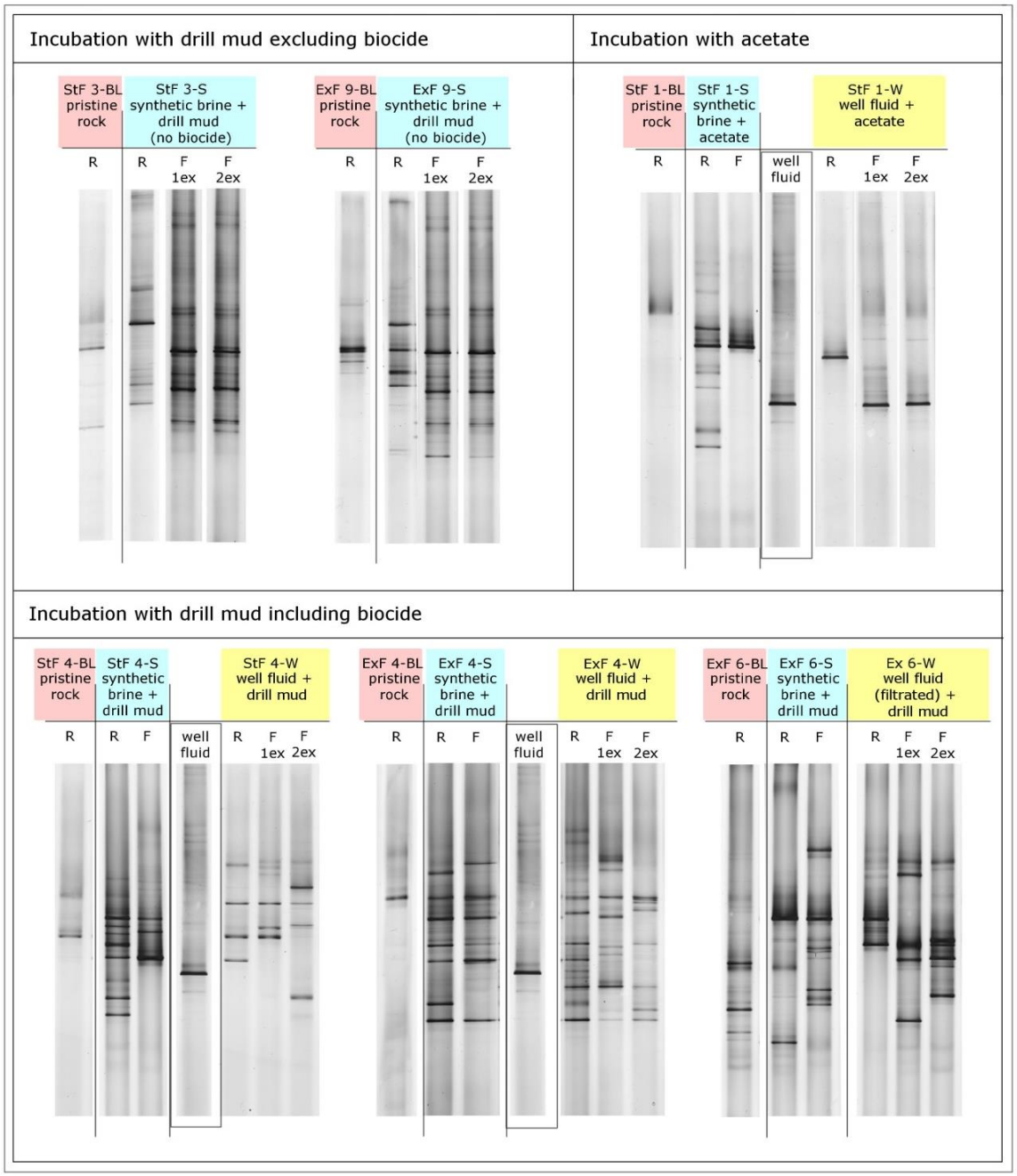

Fig.24: Overview of all DGGE profiles performed for the long-term experiments.

Tab.20: Overview of the microorganisms identified before and after incubation.

		Before incubation		After incubation with synthetic brine		After incubation with well fluid	
Formation	**Lithology**	**Sample**	**Microorganisms**	**Sample**	**Microorganisms**	**Sample**	**Microorganisms**
				Addition of drill mud without biocide			
Stuttgart	ss, ms–si	StF 3-BL	N.s.	StF 3-S	*Ralstonia* spp. seq.1; *Burkholderia tuberum*		
Exter	ss, ms–si	ExF 9-BL	*Rhizobium* spp.; *Pelomonas* spp.	ExF 9-S	*Ralstonia* spp. seq.1; *Burkholderia tuberum*; *Variovorax* spp.; uncultured bacterium (seq.1; seq.4)		
				Addition of drill mud		Addition of drill mud	
Stuttgart	fg, ss, si	StF 4-BL	*Sphingomonas* spp.; uncultured Alphaproteobacterium	StF 4-S	*Ralstonia* spp. seq.2; *Variovorax* spp.; *Phyllobacterium myrsinacearum; Kosakonia* spp.; *Propionibacterium acnes*	StF 4-W	*Sphingomonas* spp.; *Burkholderia* spp.; *Acinetobacter* spp. seq.2; *Brevibacillus* spp.; uncultured bacterium (seq.2; seq.5)
Exter	fg–mg, ss	ExF 4-BL	*Pseudomonas* spp.	ExF 4-S	*Variovorax* spp.; *Novosphingobium* spp.; *Propionibacterium acnes*	ExF 4-W	*Ralstonia* spp. seq.1; *Curvibacter* spp.; *Burkholderia heleia*; *Burkholderia tuberum*; *Acinetobacter junii*; *Acinetobacter* spp. seq.1; unclassified Burkholderiales; uncultured bacterium (seq.1; seq.3; seq.4; seq.5)
Exter	ss	ExF 6-BL	*Ochrobactrum* spp.; *Propionicimonas paludicola*	ExF 6-S	*Variovorax* spp.; *Burkholderia tuberum; Corynebacterium vitaeruminis*; *Isoptericola variabilis*; *Dyella* spp.; uncultured Gammaproteobacterium;	ExF 6-W*	*Sphingomonas* spp.; *Mesorhizobium* spp.; *Curvibacter lanceolatus*; Desulfotomaculum intricatum; uncultured *Desulfotomaculum* spp.seq.2; uncultured bacterium (seq.1; seq.4)
				Addition of acetate (190mg L^{-1})		Addition of acetate (190mg L^{-1})	
Stuttgart	ss	StF 1-BL	*Undibacterium* spp.	StF 1-S	*Ralstonia* spp. seq.1; *Variovorax* spp.; *Kosakonia* spp.; uncultured bacterium (seq.2; seq.4)	StF 1-W	*Pelomonas* spp.; unclassified Rhizobiales; uncultured *Desulfotomaculum* spp.seq.1;

ss: sandstone, sandy; ms: mudstone, muddy; si: siltstone, silty; fg: fine-grained; mg: middle-grained

N.s.: no suitable DNA for sequences was obtained for sequencing; *: the Ketzin well fluid was filtrated before being added to the vessel

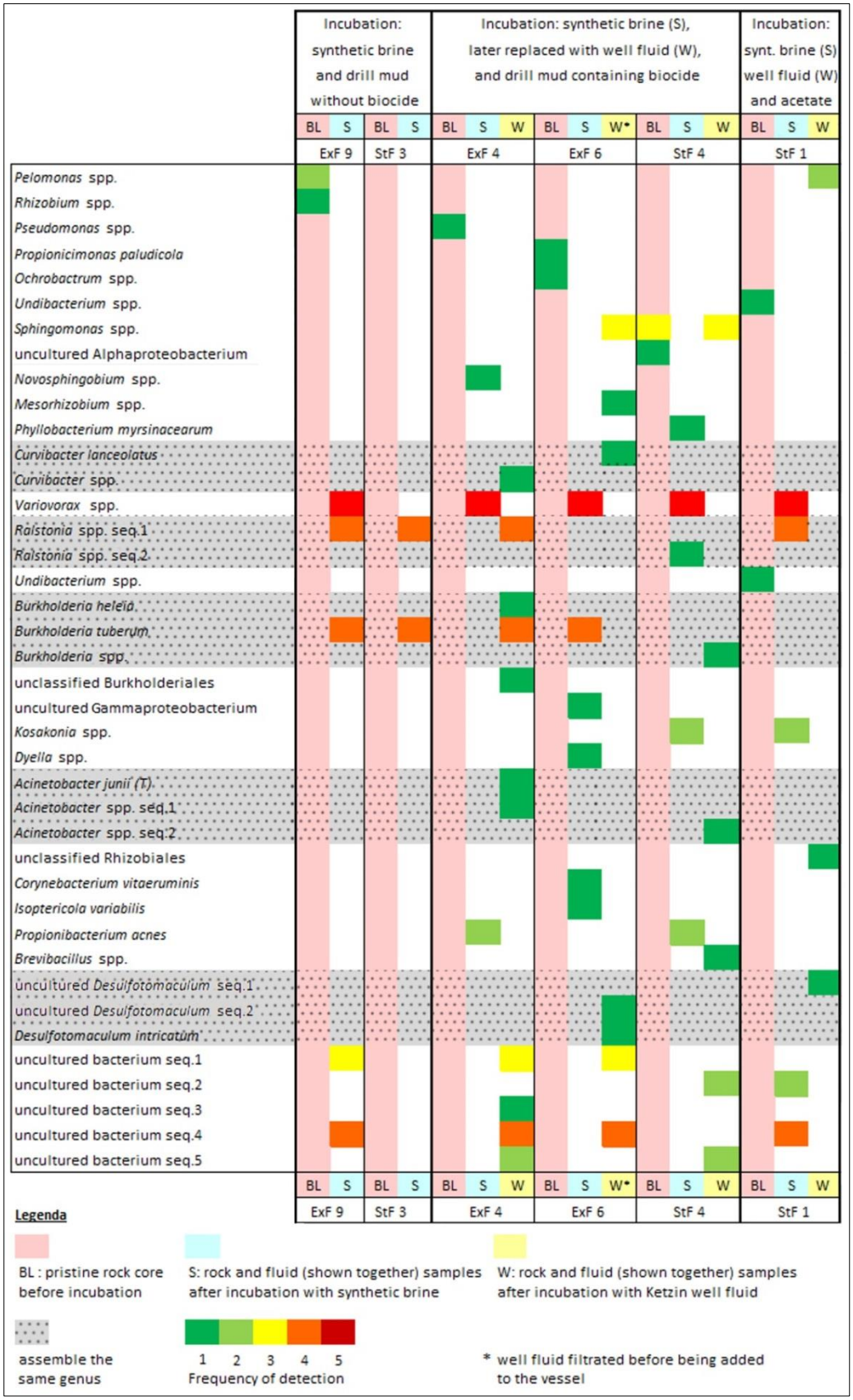

Fig.25: Identified bacterial species in the pristine rocks and incubation experiments. The different colors indicate the frequency of detection.

6. DISCUSSION

6.1. The use of tracers to assess drill mud contamination during drilling operations

The drill mud and the technical fluids applied during the drilling procedure are the major sources of biochemical contamination for both, the near-well area (Abrams 1977; Ladva et al. 2000) as well as the rock and fluid samples. Specifically, entailing changes to the geochemistry of the formation fluid and introducing allochthonous microorganisms to the near well area (Kieft 2010). Drilling techniques, drill mud composition and pressure (resulting in tensile fractures), and the characteristics of the drilled rock formation are major factors influencing the penetration depth of drill mud into rock samples (Darnadatu 2004). The main aim of the laboratory experiments studying the penetration of the drill mud into the rock cores was the evaluation of different tracers used to label drill mud in the field in regard to detectability and costs (Pellizzari et al. 2013), rather than the observation of the drill mud penetration depth in relation to the typology of sandstones. For this reason, the effect of weathering and fractures induced by pressure in the borehole were not considered in detail. Additionally, it has to be considered that the pressure employed during the coring is not the only pressure alteration which the rock cores are subjected to. The transport of the cores to the surface implies a decrease of the containment pressure on the sample, inducing a decrease of the pressure exerted on the fluid trapped in the pore spaces, which may also influence the penetration depth of the drill mud.

In the laboratory experiments, performed under ambient air pressure or under uniform pressure perpendicular to each surface, the drill mud labeled with fluorescein indicated a low penetration depth of maximum 4 mm. The applied pressure did not induce a flow through the saturated pores, as the brine could not be displaced. Therefore, the drill mud penetration depth was strictly related to the compressibility of the brine. In contrast to this, in the 2007 coring campaign in Ketzin, the drill mud labeled with fluorescein indicated a penetration depth into sandstone cores between 15 to 20 mm, indicating a significant flow during drilling procedures (Wandrey et al. 2010). Similarly, the application of a uniaxial pressure difference under laboratory conditions induced a fluid

flow through the cores (Pellizzari et al. 2013). Changes in the pressure difference slightly affected the penetration depths. Drill mud penetration depths similar to the field observations were necessary to study the transport behavior of the different tracers in plausible distances. In terms of microbial contamination, it is of special importance to understand the transport behavior of microorganisms introduced into the rock formations together with the drill mud and to compare their flow with the transit of other tracers.

By their nature, bacterial tracers propagate together with the non-autochthonous microbes, tracking the contamination (Hötzl et al. 1991). In the present investigation, DAPI-stained cells of *Pseudomonas halophila* were used in laboratory experiments as a bacterial tracer, since they are easy to produce, although bleaching over time and cell lysis caused by high salinity and pH or additives reduce the applicability. Even so, Ijzerman et al. (1993) showed that the cell numbers of DAPI-stained bacteria decreased by only 2–6 % within 48 h and the DAPI staining did not significantly affect the transport of bacteria through porous rock. Because of their surface characteristics, bacteria can easily bind to clay minerals (Kostka et al. 2002), iron-oxyhydroxides (Chan et al. 2009), and organic matter that constitute the rock, which could influence the penetration depth. The results of this study indicate that the penetration depths of DAPI-stained cells, fluorescein and microspheres labeled drill mud were in the same range. Despite the differences in physical and chemical properties of the tracers, separation due to different migration velocities in the cores was not observed. Apparently, the flow length of up to 40 mm through the rock core was not long enough to separate the tracers according to their different transport behavior. However, stained cells could be mistaken with naturally fluorescent rock fragments or minerals because of their color and shape, thus hampering the tracer detection.

Fluorescent microspheres were largely used as tracer during the drilling of deep wells (Kallmeyer et al. 2006; Cardace et al. 2013; Yanagawa et al. 2013). Because of their bright fluorescence and perfect circular shape they are easier to distinguish from the background than DAPI-stained cells. However, the analysis is time consuming and the high cost of the microspheres is a disadvantage, especially considering that drill mud has to be replaced during coring. Kallmeyer et al. (2006) reported a surprisingly high

loss of microspheres over time, due to loss of drill mud into various sinks e.g., trapping of microspheres in the surface scum.

Fluorescein is an inexpensive tracer, and the use of higher concentrations (>5 mg L^{-1}) in the drill mud does not considerably affect the total operation costs, but assures the detection of contamination in samples. The use of higher concentrations of fluorescein during coring assures detectable concentrations, even if unlabeled drill mud is added during operation. Wandrey et al. (2010) detected the tracer by microscopic observations under UV light. Through microscopic observations the samples were screened for the presence of fluorescein, but an exact quantification of the tracer concentration was impossible. In the present study, tracer detection was improved through the fluorescein extraction from rock samples and subsequent fluorometric analysis, which allowed the quantification of the tracer and increased the detection limit by 20 %. Through this procedure, the tracer is simultaneously extracted from up to 50 samples in less than two hours, while the fluorometer plate reader allowed a simultaneous quantification of fluorescein in up to 90 extracted samples within few seconds. With regard to the field application, the possibility to analyze a high number of samples at the same time is an enormous advantage compared to the other tested tracers (Pellizzari et al. 2013). In general, after rock cores are retrieved by drilling, quickness in sample proceeding is fundamental in order to avoid further contamination. Beside the penetration of drill mud, also the oxygen diffusion into the cores causes changes in the microbial communities and in the geochemical characteristics. For this reason it is necessary to select the most suitable samples and to remove the contaminated part (e.g., through inner coring) as soon as possible (Kieft 2010).

The initial evaluation of the contamination degree of each core by microscopic observation under UV light was of great support for choosing the rock cores for inner coring. The inner coring was performed not more than three days after core recovery. This enhanced work-flow helped to retrieve an adequate number of pristine rocks. For the deep well (Ktzi 203), a higher number of undisturbed samples had been collected in comparison to the shallow well (P300). The different number of pristine cores retrieved from the two wells was connected to several technical differences between the two coring campaigns. The most significant difference was the size of the wire-line coring system employed for coring, and consequently the rock cores diameter. The diameter of

the cores retrieved from the deep well was 17 mm wider than the one of the cores obtained from the shallow well. Since the same amount of material was required from each rock core, the same drill bit was employed for both inner corings. As a consequence, a greater amount of material was discharged for the deep well cores. Another important difference was the composition of the drill mud used during the two coring campaigns. For the shallow well only freshwater and K_2CO_3 were used, while for the deep well the use of polymers was necessary to reduce the risk of drill mud and core loss, making the mud more viscous. The much higher mud viscosity contributed to create a filter cake around the cores, which likely hampered the drill mud penetration into the rocks (Tibbitts et al, 1990). Moreover, plastic liners were used during the coring of the deep well, but were not employed for the shallow well operations. Also the inner coring procedure between the two sampling campaigns differed. For the Exter Formation, water was used as drilling fluid, possibly influencing the drill mud penetration depth, pushing and forcing the fluorescein labeled drill mud more deeply into the rock cores. For the inner coring of the Stuttgart Formation rocks, the use of Argon gas likely helped to keep the whole system cleaner. It is assumed that during the incubation in the high-pressure setups, the drill mud which remained in the pore space of the rock cores got partially solved in the synthetic brine. Therefore, a final contamination control by measuring the tracer concentration in the brine after one to six months of incubation served as final proof of the retrieval of pristine rock cores. For instance, the fluids incubated with two Exter Formation inner core plugs, which were supposed to be pristine after fluorescein extraction analyses, showed concentration of up to 0.02 % of drill mud, indicating slight contamination. Regarding the material retrieved during the coring of Ktzi 203, only one out of the six samples stored in high-pressure vessels was contaminated. A relatively high fluorescein penetration depth in this core was already detected through the first microscopic analyses performed in the field. After the inner coring, the microscopic analyses were repeated and drill mud contamination was still observed. Gas analyses revealed the highest concentration of CO_2 compared to all the other samples (personal communication Martin Zimmer, GFZ Potsdam, Germany). The higher compressibility of CO_2 compared to the pore-filling Stuttgart Formation brine may have allowed the drill mud to penetrate deeper into this core.

The results of this part of the study indicate that the penetration depth seems to be independent of the drilled core size diameter but the more outer material is removed through inner coring, the better the chances are to retrieve a pristine sample. Furthermore, the drill mud penetration depth is not only sensitive to lithology and petrophysical characteristics of the rocks, but also to the technical procedures. Concerning the sandstones obtained from the P300 borehole, three core samples, originating from a 40-cm sandstone core that was apparently free of fractures, indicate a remarkably different grade of contamination. Analyses of rock and fluid sampled from the setups indicated that one part was free of fluorescein, while in the other two parts the tracer was detected in both fluid and rock. This high variability of the measured drill mud penetration depth for one lithotype was described also by Kallmeyer et al. (2006). Because of the heterogeneity of rocks deriving from small differences in lithology (the sample was taken from an interbedded zone of fine-to-medium grained sandstones) or due to the invisible microfractures and drilling induced tensile fractures, the penetration depth may not be related directly to the average permeability. Therefore, every sample, even those from the same core section, should be checked for contamination by drill fluids, as also recommended by Wandrey et al. (2010).

Regarding the loss of drill mud in the near-well area, the fluorescein concentration can be monitored in the fluid samples collected through the well cleaning procedures or through the subsequent pump tests. At the end of a pump test (93 m^3), Wandrey et al. (2010) measured only 0.05 % of the total fluorescein concentration used for the coring. In the fluid collected from the shallow observation well (P300), 7.2 % of the initial fluorescein working concentration was measured. Obviously, the very limited fluid volume of 14 m^3 produced through the pump test was not sufficient to completely remove the drill mud left in the formation (Wiese et al. 2013). Because of the high costs of test procedures and fluid disposal, it was not possible to produce a higher amount of fluid from the shallow well. Beside the fluorescein concentration, further evidence of drill mud influence was the high potassium concentration measured in production fluid during the entire test (Pellizzari et al. 2016). The anomalous potassium concentration, nearly six times higher than in the deep well fluids, was due to the drill mud formulation, which consisted of freshwater mixed with K_2CO_3. Therefore, the geochemical parameters of the shallow well fluid collected via pump test should be

considered as not reliable (Pellizzari et al. 2016). Since no organic components were added, no particularly high concentrations of organic carbon were measured and the small DOC and TOC fluctuations (in average 6 and 7 mg L^{-1}, respectively) were presumably connected to the freshwater used as technical fluid.

Although fluorescein is susceptible to photodecomposition (Diehl and Horchak-Morris 1987), its concentration in the drill mud did not show a significant decrease for the entire duration of the coring campaigns of four to six days. In the subsurface, where photodecomposition can be excluded, fluorescein is stable (Smith and Pretorius 2002). Up to 1 % of the original fluorescein concentration in the drill mud was detected in the fluid collected from the U-tube sampling system, installed in the shallow observation well, three years after the drilling campaign. This underlines the suitability of fluorescein to monitor drill mud contamination in the field, even over longer time periods.

6.2. Microbiological characterization of Exter and Stuttgart Formations and potential changes resulting from CO_2 injection

For microbiological and geochemical analyses of the deep subsurface it is absolute crucial to ensure that rock cores and well fluids are pristine (uncontaminated). In the previous section it was shown that the applied tracer fluorescein was very useful for the detection of drill mud in fresh collected samples. Subsquently, this assessment allowed subsequently the characterization of the autochthonous microbial community in rocks retrieved from depths between 400 and 660 m.

The baseline characterization of the autochthonous microbial community is a prerequisite for the detection and prediction of community shifts resulting from the geotechnical use of the subsurface. Such changes in the microbial community structure may also provoke changes in the geochemistry of the rock formations. Even though the Exter Formation, at the study site, is not the target reservoir for CO_2 storage, a comparison of the microbial communities between both formations, may help to identify a potential interconnection of fluids in a long-term perspective. In Exter Formation rock cores the cell number was rather low (below 10^2 cells mL^{-1} DAPI-stained cells) (Pellizzari et al. 2016). Fingerprinting analyses indicated that each Exter

Formation rock sample presented a different microbial community. Those dissimilarities may be due to the mineralogical composition of the cores or may be linked to fluid-microorganisms-interaction (Morozova et al. 2011). The mineralogical characterization of a rock formation is of fundamental importance to evaluate the influence of single environmental parameters on the microbial community and vice versa. Microorganisms are known to drive various naturally occurring processes like the formation of secondary minerals (e.g., pyrite or ferro(hydr)oxides) (Berner 1970; Schoonen 2004). In the present study, no microorganisms or biofilms were observed through SEM observations directly on mineral surfaces (Pellizzari et al. 2016), but the more sensitive fingerprinting analysis was successful in identifying the microbial community.

As in the pristine Stuttgart Formation rocks (Wandrey et al. 2011*b*) also in the Exter Formation Alphaproteobacteria and Betaproteobacteria were identified. Specifically, in both formations sequences affiliated to the genus *Rhizobium* and 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.* The observation that the community structure of the two rock formations differed can be explained by the different depth (> 200 m difference), the age and the geochemical (e.g. salinity) as well as the mineralogical composition. In the Exter Formation, sequences related to *Pseudomonas* spp. and *Propionicimonas* spp. were detected. These microorganisms are known to inhabit soil and fresh water ecosystems. Relatives of the genus *Pelomonas* have also been identified in deep subsurface habitats (Boivin-Jahns et al. 1996; Kouduka, et al. 2012). However, it has to be taken into account that the assigned bacterial and archaeal taxa do not necessarily have identical physiological or metabolic characteristics to the organisms found in this study. So far uncharacterized species or genera could be well adapted to high salinities and a rather oligotrophic environment. As for the Stuttgart Formation (Wandrey et al. 2011*b*), also for the Exter Formation rock cores Archaea were not detected by genetic fingerprinting. Since the absolute microbial abundance in the rock cores was very low, Archaea may be present below the detection limit of the applied method.

Unfortunately, an appropriate comparison of the microbial community inhabiting rock cores and well fluid of Exter Formation cannot be formulated since the fluids collected through pump test (Wiese et al. 2013) carried a high concentration of fluorescein and consequently of drill mud. Here, beside of anaerobic halotolerant bacteria (i.e. *Dethiobacter alkaliphilus* and *Alkalibacter saccharofermentans*), sequences related to chloroplasts were detected, reflecting the influence of shallow groundwater used for the drill mud formulation (Pellizzari et al. 2016).

In the well fluid obtained from two observation wells that were drilled into the storage formation (Stuttgart Formation), prior to CO_2 injection, around 10^6 cells mL^{-1}, mostly belonging to the domain Bacteria were counted (Morozova et al. 2010; Pellizzari et al. 2016). The microbiological analyses of well fluid retrieved through the pump test, indicated that in the first cubic meters of produced fluid, the microbial community was influenced by drill mud. The Bacteroidetes fraction (i.e. *Bacteroides* spp. and *Flavobacterium* spp.) and Firmicutes related to *Proteiniclasticum ruminis* and *Clostridium sticklandii* were detected only at the beginning of the pump test. Here the drill mud content was still high (as confirmed through tracer analyses); therefore, those taxa had probably a non-autochthonous origin. On the other hand, they may be autochthonous and became dominant as a consequence of biodegradation of the organic drill mud. However, during the test a population more characteristic of the Deep Biosphere environment arose. The dominant microorganism detected during and at the end of the pump test was related to the genus *Halanaerobium*, typical obligate anaerobic and halophilic organisms (Oren 2008). The same microorganism was found in the well fluid retrieved through downhole sampling, together with sequences assigned to a halophilic microorganism of the family Halanaerobiaceae and the genus *Desulfohalobium*. The presence and predominance of halophilic microorganisms can be linked to the high salinity of the aquifer. The next cultivated relative of the detected *Desulfohalobium* organism was the moderately halophilic SRB *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 Ketzin well fluids (Pellizzari et al. 2016). Additionally, Fluorescence *in-situ* Hybridization (FISH) analyses revealed that in well fluids collected during downhole sampling, up to 60 % of

the total cell number were SRB. The microbial community of the well fluid was mainly composed by Firmicutes-species and SRB affiliated to Deltaproteobacteria (Pellizzari et al. 2016). Microbial communities composed by the same phylogenetic groups were described also in other deep microbial ecosystems such as deep subsurface gas storage aquifers (Fry et al. 1997; Shimizu et al. 2007; Sahl et al. 2008; Basso et al. 2009). The predominance of SRB was likely linked to the relatively high availability of organic carbon, derived from residual technical fluids. The TOC measured in the well fluid collected through downhole sampling (Pellizzari et al. 2016) was six times higher than the natural background level measured in another deep observation well (Ktzi 201) (Morozova et al. 2010). In the Deep Biosphere, microbial growth and activity are stimulated and controlled through the environmental conditions and specifically through carbon availability: a lower carbon concentration corresponds to a lower microbial diversity and activity (Brockman et al. 1992; Fry et al. 1997; Whitman et al. 1998; Aldén et al. 2001; Goldscheider et al. 2006; Fredrickson and Balkwill 2006; Jørgensen and Boetius 2007).

The TOC content measured in the Stuttgart Formation rock cores before of the CO_2 injection was between 74 and 3316 mg kg^{-1} (Wandrey et al. 2010; Scherf et al. 2011). Accordingly, the microbial activity in the rock cores was very low and the identified microorganisms (Wandrey et al. 2011*b*) differed from the population detected in well fluid. Disparities in the community inhabiting rock and well fluid were already observed in other studies (Hazen et al. 1991; Fry et al. 1997). In the Stuttgart Formation rock cores, microorganisms already detected in deep subsurface before and related to *Agrobacterium tumefaciens*, *Rhizobium* spp., *Hydrogenophaga* spp., *Burkholderia fungorum* and *Propionibacterium acnes* were abundant (Wandrey et al. 2011*b*). Although, *Propionibacterium acnes* was isolated from subsurface samples before, it is a commensal bacterium of human skin and therefore its presence may be due to contamination during sampling procedures or DNA extraction (Wandrey et al. 2011*b*). However, the identification of aerobic or facultative anaerobic bacteria is not surprising, since they were observed in numerous Deep Biosphere samples before (An et al. 2013) and in the Ketzin specific case, oxidized iron, as an indicator for the presence of oxygen in the formation, had been observed (Kasina et al. 2017).

In 2012, the Stuttgart Formation, partly in contact with the injected CO_2 (Bergmann et al. 2012) for more than four years, was cored (monitoring well Ktzi 203). The microbial community, relatively poor in diversity, differed compared to the initial population previously described by Wandrey et al. (2011*b*). In rock samples retrieved after CO_2 injection, one to two taxa were detected in each core and all over only three different species were identified. None of the organisms observed before of the CO_2 injection was detected again. *Sphingomonas*, common in the deep subsurface (Balkwill et al. 1997; Fredrickson et al. 1999), and *Undibacterium*, abundant in soil and fresh water (Kim et al. 2014) were detected. Moreover, a sequence related to an uncultured Alphaproteobacterium, already detected in oligotrophic ground water from a deep (290-324 m) monitoring well of a radioactive waste depository in Siberia, Russia (Nedelkova et al, 2005) was identified. The dissimilarities may be attributed to the CO_2 injection. Injection of CO_2 into a saline aquifer induces geochemical reactions in the system, previously regulated or buffered by the rock (Kaszuba and Janecky, 2009). As the injected CO_2 dissolves into the brine to form carbonic acid, acid-generating reactions are induced. Acid-base reactions and solubility of metals depends on interactions between CO_2, brine and rock, responding to new equilibrium pressures (Kaszuba and Janecky, 2009). These interactions include the dissolution and precipitation of minerals. The incubation of Stuttgart Formation rock cores in CO_2 atmosphere under simulated *in-situ* conditions induced the dissolution of calcium-rich plagioclase, K-feldspar and anhydrite, and the stabilization and precipitation of albite on mineral surfaces (Fischer et al. 2011). The interactions between CO_2, brine and rock affect not only the chemical, but also the physical properties of the reservoir and linked to that, the microbial diversity and activity. The CO_2, which fills pore spaces, can be reduced by microorganisms, mainly acetogens and methanogens. Acetogenic bacteria provide acetate, which is a relevant substrate for acetoclastic methanogens and for microorganisms reducing Fe^{3+}, $Mn^{3+,4+}$ and SO_4^{2-}. However, no typical acetogenic bacteria were detected in the formation before or after *in-situ* CO_2 exposure. In this context it should be mentioned that several sequences could not be assigned to characterized microorganisms and may therefore belong to so far unknown acetogenic bacteria. Kirk et al. (2016) indicated that microbial communities may change depending on differences in the ability of cells to tolerate elevated CO_2 concentrations as well as

alterations in the balance of microbial reactions. However, long-term experiments performed by Wandrey et al. (2011*b*) showed that after incubation in CO_2 atmosphere, only minor changes in the microbial community inhabiting the Stuttgart Formation rock cores occurred. It cannot be ruled out that the differences in the microbial communities are related to the distance between the two wells (ca. 100 m). Considering the high lithological heterogeneity of the Stuttgart Formation, differences in the mineralogical composition can occur, influencing the microbial diversity.

At the end of 2013, after five years of CO_2 injection, fresh well fluid was retrieved from the injection well (Ktzi 201) through downhole sampling. The microbial community was dominated by SRB as already observed before of CO_2 exposure. However, before of CO_2 exposure, the dominant SRB were distantly related to *Desulfohalobium*, and additionally the halophilic *Halanaerobium* spp. was detected. After *in-situ* exposure, the diversity decreased and the community was dominated by the genus *Desulfotomaculum*, which was the only bacterium observed. Because of the low 16S rRNA sequence similarity (91 %) to the next characterized microorganism, the sequence belongs likely to a novel species that can better cope with elevated CO_2 concentrations. Nevertheless, *Desulfotomaculum* species were already detected by FISH in the Ketzin storage reservoir after well completion, where the relatively high TOC content (134.7 mg L^{-1}; Morozova et al. 2013) was probably due to residual drill fluid in the well area. *Desulfotomaculum* species were observed in other Deep Biosphere environments as well (Detmers et al. 2004; Moser et al. 2005; Alawi et al. 2011). In addition, archaeal sequences related to the methanogenic genera *Methanoculleus* were identified. This genus is common in soil (Tian et al. 2010), formation water of a petroleum reservoir (Orphan et al. 2000) and in a saline aquifer, in conjunction with *Desulfotomaculum* spp. as well (Ehinger et al. 2009). After CO_2 injection, the presence of a methanogenic community in the Ketzin storage formation was confirmed through gas analyses of downhole samples. Methane was detected in the same samples where methanogenic archaea were observed by FISH analyses (Morozova et al. 2010).

6.3. Influence of cellulose-based drill mud on the autochthonous microbial community of both rock formations

The characterization of the microbial community of rock and fluid was the bases to conduct various laboratory experiments aimed to reveal which microbial-mediated processes induced the injectivity loss in Ketzin (Pellizzari et al. 2017). The injectivity decrease in the well intended for injection was indirectly associated with the deployed organic drill mud (Zettlitzer et al. 2010). Residual drill mud located behind the filter screens of the well served as nutrient for microbes. The detailed analyses involving pristine rock cores and well fluid performed in the present work showed that all types of microorganisms involved in the injectivity loss can be found in the natural community of the formation. However, this finding does not exclude that, additionally to those, also microorganisms potentially introduced into the formation together with the drill mud may have been involved. Geochemical parameters (e.g., acetate) and further drill mud components (e.g., biocide) that control microbial activity were evaluated in this investigation to help to prevent such incidents in future drilling operations. However, it is known that several microbial species are not growing under laboratory conditions and reaction kinetics may be shifted compared to *in-situ* conditions (Stewart 2012).

Incubation of pristine rocks with a complex carbon source in form of drill mud and the addition of acetate as the sole carbon source resulted in higher abundances of specific microorganisms. The addition of synthetic brine or well fluid mixed with drill mud or acetate resulted in a different community structure in each case. As a result of the altered environmental conditions, the microorganisms that were previously detected in the pristine rock were no longer dominant, but other microorganisms were observed with genetic fingerprinting (Pellizzari et al. 2017). Similar results were obtained in the experiments performed by Struchtemeyer et al. (2011), where the microbial community of drill water was almost completely changed after incubation with autoclaved drill mud. The present outcomes lead to the assumption that the autochthonous microbial community in the reservoir promptly responds to changes in substrate availability. Microorganisms living in such oligotrophic ecosystem developed special surviving strategies, showing small cell-sizes (Kallmeyer et al. 2012), low population densities and reduced activity (Goldscheider et al. 2006). Moreover, they may survive longer

periods, going in starvation state or forming spores (Smith et al. 1952), and then suddenly react and proliferate when growing conditions became more favorable.

6.3.1. Response of the microbial community to drill mud and biocide exposure

After long-term incubation of rock cores with synthetic brine and drill mud, microbial diversity and quantity increased considerably, even when biocide was included. The cellulose-based drill mud significantly enhanced the relative abundance of microorganisms affiliated to the genera *Burkholderia*, *Variovorax*, *Corynebacterium* and *Dyella*, species of which are able to hydrolyze cellulose polymers (Suihko and Skyttä 2009, Talia et al. 2012; Satola et al. 2013, Liang et al. 2014; Verastegui et al. 2014). This finding further substantiates the assumption by Zettlitzer et al. (2010) that cellulose-based drill mud is a valuable substrate for many soil- and rock-inhabiting microorganisms, even in the presence of biocide, and that it can cause remarkable growth acceleration (Pellizzari et al. 2017).

Species of the genus *Burkholderia* may play an important role in mineral alteration which is highly relevant to increase the safety of CO_2 storage. As discussed by Menez et al. (2007), these soil bacteria need Ca^{2+} and Mg^{2+} ions and acidify their medium during biomass synthesis likely by releasing organic acids. This acidification enhances the mineral dissolution/alteration and facilitates carbonate precipitation in the presence of CO_2, which is also relevant for the the long-term safety of the storage site (Fischer et al. 2010).

The Stuttgart Formation is characterized by a higher salinity and different microbial communities compared to the Exter Formation. Nevertheless, after incubation with drill mud (without biocide), dissimilarities in the community structures of the two formations were mainly observed in rocks, whereas only minor differences were observed in fluids. The incubation of a pristine Exter Formation rock under the same conditions, but in N_2 atmosphere produced similar results (data not shown). In this case, among all other environmental conditions, drill mud components obviously had the strongest influence on the microbial community structure. The comparison of the results after incubation in

N_2 and CO_2 atmosphere proved that the microbial community remains viable and active after long-term CO_2 exposure.

The microorganisms detected in all setups, including or excluding biocide, were related to the genera *Ralstonia*, *Burkholderia* and *Variovorax*. These microorganisms demonstrated to be well adaptable to the *in-situ* conditions as well as to the incubation in elevated CO_2 concentrations and organic drill mud. For instance, several species of the genera *Ralstonia* can use CO_2 and H_2 as carbon and energy sources respectively, but switch over to organotrophic metabolism in the presence of substrates such as acetate (Park et al. 2011). A conspicuous inhibitory effect on bacterial growth was expected when biocide was used. In contrast, microbial diversity increased compared with the pristine rock and the setups without biocide. One possible reason for the higher diversity might be the eight weeks longer incubation time of the setups including biocide, which probably allowed the bacterial community to further evolve. It is possible that the biocide decreased the quantity of cells from dominant species to an extent that less abundant microorganisms became detectable. An aspect that should be considered is whether the biodegradable biocide, which was added to the drill mud, can serve as a carbon and nitrogen source for microorganisms after a certain time and specific environmental conditions. The used biocide is known to be completely biodegradable under aerobic conditions. This biodegradability was demonstrated through laboratory experiments performed by the producer (www.oecd-ilibrary.org). As already discussed, although the storage formation is considered to be an anaerobic environment, the presence of oxygen cannot be excluded. Therefore, the contact between biocide and oxygen in the near-well area and subsequent biocide degradation cannot be ruled out. The biocide concentration is considered the most important factor that affects its efficacy (Russell and McDonnell 2000), and for this reason, it should always be optimized during treatments, even if it is rather difficult (Al Hashem et al. 2004). Moreover, it has to be considered that in the near-well area, the drill mud and therefore the biocide are diluted into the brine, creating a concentration gradient. The dilution affects the biocide efficiency since under minimum inhibitory concentrations the efficacy of the biocide is uncertain, as demonstrated by the manufacturer trough laboratory experiments. The manufacturers also showed that each bacterial species is affected by a specific concentration of biocide.

6.3.2. Changes of the microbial community structure after the replacement of synthetic brine for a mixture of well fluid and drill mud

In the experiments containing biocide, the microbial community of Exter and Stuttgart Formations responded differently to the synthetic brine replacement with fresh unfiltered well fluid. The community structure of the rock and fluid of the Exter Formation setups slightly varied after the addition of the unfiltered well fluid. Many microorganisms that were already detected after synthetic brine and drill mud exposure were also identified after the fluid replacement. In contrast, the community structure and diversity changed in the Stuttgart Formation samples after the fluid was replaced. Microorganisms related to *Variovorax, Phyllobacterium and Propionibacterium* that were detected before the fluid replacement were no longer dominant, but species belonging to *Ralstonia* and *Brevibacillus* were present after the fluid was changed and relatives of the genera *Sphingomonas*, *Burkholderia* and *Acinetobacter* became more abundant. It was shown that species of *Acinetobacter*, which are commonly detected in Deep Biosphere, use carboxymethylcellulose as a carbon source (Ekperigin 2007). Therefore, the relative abundance of this microorganism was likely enhanced through the organic polymers of the drill mud. An *Acinetobacter*-related organism, which was not detected after synthetic brine and drill mud incubation, was identified only in Exter and Stuttgart Formations setups after the fluid was replaced. Accordingly, it was probably added to the setup together with the well fluid and became detectable only after incubation, since it was not detected in the fresh collected fluid before.

The fresh well fluid was obtained from a depth corresponding to the Stuttgart Formation and therefore was similar in composition (especially in salinity) to the Stuttgart Formation synthetic brine, which was initially added to the incubations. In this context it is important to note that the well fluid had a higher salinity than the Exter Formation brine. The salinity of the Exter Formation was underestimated with 53 g L^{-1}. After interaction of the synthetic brine with the rock cores, the salinity increased to a maximum of 150 g L^{-1} (H2STORE, Abschlussbericht). Nevertheless, this maximum value was lower than the salinity of the Stuttgart Formation fluid, which was 235 g L^{-1}. Compared to the microbial community of Stuttgart Formation rocks, the population of

Exter Formation had probably more difficulties to adapt and evolve in consequence of the changed conditions, and therefore it varied only slightly. The depletion of important growth factors such as trace elements and low molecular weight acids might have played an important role in the microbial response to substrate availability before the fluid exchange. It is assumed that the simplified synthetic brine could not support the growth of several species inhabiting the Stuttgart Formation rock; therefore, the added well fluid had a strong impact on microbial growth through introducing a complex mixture of essential substances. The depletion of nutrients in the Stuttgart Formation approach was likely enhanced by a five weeks longer incubation time. This assumption is strengthened through the results of the setup with Exter Formation rock and filtered well fluid, which lasted as long as the Stuttgart Formation setup. The most abundant bacterium (related to *Variovorax* spp.) identified before the fluid exchange was also detected after the fluid replacement and at the same intensity. The other microorganisms which were detected after the fluid exchange, (i.e., next relatives to *Curvibacter lanceolatus* and *Mesorhizobium* spp.) are widely distributed and were previously detected in mineral water and soils (Falcone-Dias et al. 2012; Ghosh and Roy 2006; Lorite et al. 2010) and can be attributed to the autochthonous community of the rock, which was thus enhanced in growth. Besides, a sequence related to *Sphingomonas* spp. was detected. *Sphingomonas* has been detected in the deep subsurface before (Balkwill et al. 1997, Fredrickson et al. 1999). This microorganism may have been introduced with the drill mud because *Sphingomonas* carrying the *Xanthomonas campestris* genes secretes the polysaccharide xanthan gum, which is used for the drill mud synthesis (XC-Polymer) (Pollock 1993; Yamazaki et al. 1996). However, it is not likely that this organism was introduced since it was not detected in the drill mud. After incubation with filtered well fluid, two different sequences related to the genus *Desulfotomaculum* were also detected. These two SRB were both different from the *Desulfotomaculum* sequence identified in fresh well fluid. All three sequences are likely related to three different species. Therefore, it is assumed that these two SRB resided in low cell numbers as dormant cells or spores in the Exter Formation rock and then proliferated in response to the changed conditions and increased substrate availability during incubation. The sequence of an uncultured archaeon was identified in all setups after

incubation. This archaeon has unknown metabolisms as several other microorganisms of the Deep Biosphere.

6.3.3. Response of the microbial community to incubation with acetate as a biodegradation product of drill mud polymers

Due to the high acetate concentration measured in the fluid during the N_2 lift (Morozova et al. 2010), it is likely that microorganisms degraded organic polymers of the residual drill mud and formed acetate. After laboratory incubation of rocks with acetate and synthetic brine, microorganisms like *Variovorax* spp. and *Ralstonia* spp., already detected after the drill mud experiments, were identified. The detection of *Variovorax* after incubation with drill mud was linked to the ability to degrade polymers (Satola et al. 2013). Acetate can also be shunted directly into the central metabolism, as it was shown for *Variovorax paradoxus*-like bacteria (Boersma et al. 2010). In addition, Jin et al. (2012) reported that different strains of *Variovorax* assimilate acetate. Sequences of *Burkholderia*, which were frequently detected after drill mud exposure, were not observed after incubation with acetate; therefore, their higher abundance appears to be related to other degradation products of cellulose polymers than acetate.

After incubation with well fluid and acetate, the microbial community was dominated by two microorganisms that were not identified in the pristine Stuttgart Formation rock or before fluid exchange. One was related to *Pelomonas saccharophila*, a H_2-oxidizing bacterium which uses acetate as carbon source (Doudoroff 1940, Xie and Yokota 2005) and that was already detected in the pristine Exter Formation rock. The other microorganism was related to SRB (*Desulfotomaculum* spp.) that was previously identified in the fresh well fluid. Some species of *Desulfotomaculum* (e.g., *Desulfotomaculum acetoxidans*) can use acetate as their sole energy and carbon source (Widdel and Pfennig 1977, Spring et al. 2009), whereas, others (e.g., *Desulfotomaculum sapomandens*) use acetate only as an electron donor (Vandieken et al. 2006). The detected relative of *Desulfotomaculum* seems to be strongly dependent on acetate because it was not detected when the acetate availability was more restricted (i.e., after drill mud incubation). Some drill mud components may have inhibited the growth of *Desulfotomaculum* spp., or acetate was a limiting growth factor for the microorganisms,

which had to compete with other acetate-metabolizing microorganisms. This may be the reason for the strong increase in SRB cell numbers after the drilling of the injection well, where the same acetate concentration was available. As already mentioned, in the fresh well fluid an archaeal sequence related to *Methanoculleus* spp. was detected. Since several species of the genera *Methanoculleus* are described as hydrogenotrophic organisms, reducing CO_2 to methane (Asakawa and Nagaoka 2003), acetate addition probably did not favor the growth of this archaeon. Moreover, *Methanoculleus* was also not detected after incubation with drill mud. The absence was attributed to the competition with bacteria, which adapted faster to the changed conditions.

6.3.4. Bio-geo interactions

After the incubation experiments with organic carbon as energy source in CO_2 atmosphere, the rock cores showed only minor changes in mineral composition and morphology. Dissolution of feldspar (Appendix II, a) was more advanced in plagioclase than in K-feldspar (Pellizzari et al. 2017). Dissolution of feldspar causes the release of components from which secondary clay minerals are formed. It is likely that clay minerals formed in the pits after feldspar dissolution (Appendix II, b) due to low mobility of certain elements (Pellizzari et al. 2017). The formation of clay minerals seems to be enhanced when bacteria are present (Wagner et al. 2013) or when their activity is enhanced by nutrients, as in the experiments with drill mud or acetate as biodegradation product. Dissolution of K-feldspar in a CO_2-saturated atmosphere and corrosion textures, indicating alteration processes, were previously observed by other authors (Rosenqvist et al. 2014; Huq et al. 2015), also for the Ketzin reservoir rocks (Fischer et al. 2011).

Through SEM observations, indirect demonstrations of microbial activity were present after incubation with organic sources that were not visible in the pristine rocks. The hair-like structures similar to biofilms observed on the surface of Stuttgart Formation rock cores were most likely created as a result of microbial activity (Appendix II, c/e/f) (Pellizzari et al. 2017). Furthermore, the shape and size of small, round iron oxides observed in the Exter Formation rock (Appendix II, d) cores may indicate a microbiological origin (Pellizzari et al. 2017).

The present study enabled the identification of microorganisms that might have been involved in reduction of the permeability of the near-well area and subsequently in the reduced injectivity of the well (Fig.26). The outcomes lead to the assumption that the autochthonous microbial community in the reservoir directly responds to changes in substrate availability. The microbial degradation of drill mud components, and subsequently the supply of low-molecular weight acids such as acetate, are important links in the chain of the bio-geo interactions, allowing a broader community of microorganisms (e.g., SRB) to proliferate. The stimulated SRB reduced sulfate that precipitated together with dissolved iron present in the formation (Zettlitzer et al. 2010; Pellizzari et al. 2017; Kasina et al. 2017), blocking the near-well pores throats and decreasing the permeability and finally the well injectivity.

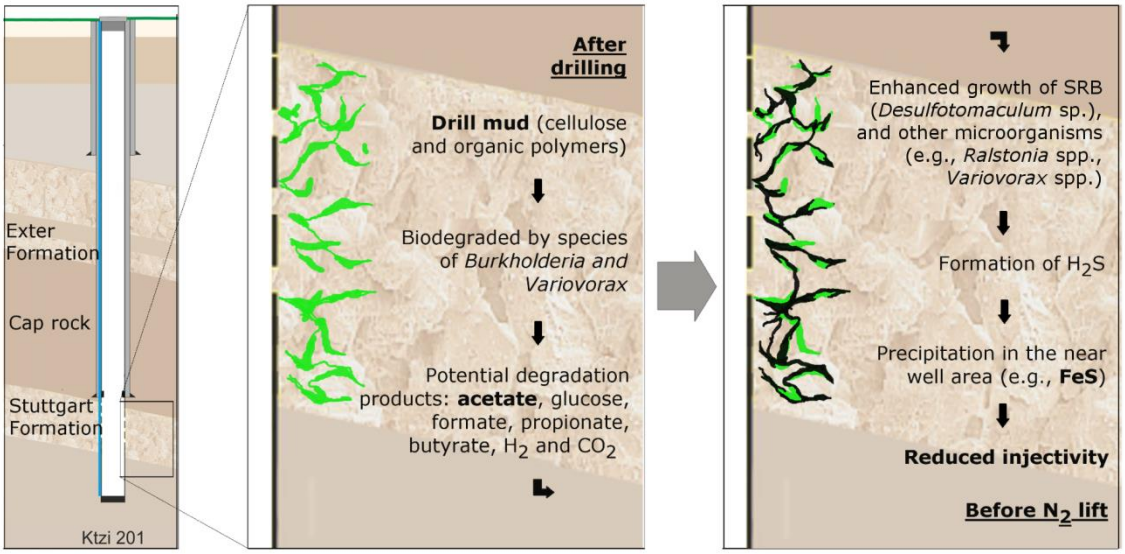

Fig.26: Microbial induced causal chain that provoked the injectivity loss in the well.

7. SUMMARY AND CONCLUSIONS

The present study provides an evaluated sampling procedure to retrieve pristine rock and fluid samples from deep geological formations. The microbial communities, inhabiting rock and fluids of the formations, showed a response to geotechnical operations. The study proved the hypothesis that organic compounds, admixed to many drill mud formulations, are influencing the development of the microbial community. For the fist time, rock cores obtained from the CO_2 injected plume were retrieved and microbiologically analyzed. Differences in the microbial community of the storage formation before and after *in-situ* CO_2 exposure were observed. Furthermore, laboratory experiments indicated that the microbial diversity in rock cores exposed to elevated CO_2 concentrations and nutrients increased. The outcomes demonstrate that microbial processes and bio-geo interactions should be taken into account during drilling operations and long-term use of underground reservoirs. The results indicate that not only one metabolic group of microorganisms but interactions inside the complex autochthonous microbial community are involved in undesired microbial-mediated processes.

This interdisciplinary study provides recommendations that should be considered before the installation and operation of geotechnical facilities in the underground. To prevent the contamination of core samples, wire-line drilling (core retrieval in liners) can help, but the most indispensable approach is to label the drill mud with a tracer. Fluorescein was evaluated as an inexpensive, easy-to-handle and non-hazardous tracer. The new developed procedure for the extraction and detection of fluorescein allows a fast quantification in a high number of rock and fluid samples intended for microbiological and geochemical analyses. Moreover, the long lifetime of fluorescein in the subsurface permitted to detect drill mud contamination also in fluid samples retrieved via pump tests, downhole samplings or U-tube systems after more than three years. As demonstrated in this work, a first microscopic observation of rock cores helps selecting the samples appropriate for inner coring, which is essential to remove the contaminated part of the rock core. Therefore, larger core diameters are more advantageous,

increasing the possibility to gain pristine rock material. For the inner coring, gas core drilling should be preferred to wet drilling, to avoid further contamination.

The use of drill mud is a potential source of contamination with microorganisms and organic substrates not only for the samples retrieved but also for the subsurface. Although cleaning of the well after drilling is a standard procedure, the experiences made in Ketzin indicate that, especially behind filter screens of the well, residual drill mud can be located. As demonstrated through laboratory experiments, autochthonous microorganisms are able to biodegrade organic drill mud and subsequently cell numbers may increase. The degradation products may serve as substrate for a broad microbial population, despite the high salinity of reservoir fluids. In particular, the elevated availability of acetate enhanced the growth of SRB, predominantly present in the well fluid. Hydrogen sulfide produced by SRB precipitates with dissolved iron as iron sulfide and cause plugging of the pore space in the near-well area and may provoke an injectivity loss. The providers of biocides are testing their products under laboratory conditions, but the effectiveness might be reduced under field conditions. Therefore, the well cleaning procedure should be repeated until the tracer concentration of the produced fluid decreases to zero. If subsurface geology and drilling conditions allow (with respect to the core recovery, viscosity requirement and borehole stability), a drill mud without the addition of organic components should be used. Another option to mitigate the risk of disadvantageous consequences of cellulose-based drill mud may be to drill with an “under-balanced” mud system. During this procedure the pressure in the wellbore is lower than the fluid pressure in the formation being drilled (Prevedel et al. 2014). This drilling method, mitigating the loss of drill mud into the formation, protects the formation and allows saving the costs of the mud replacement. When the application of this technique is not possible, a minimum under-balanced drilling should be considered. Further investigations should focus on identifying the long-term effect of allochthonous microorganisms, which are introduced in the geological formations together with the drill mud.

The conclusions of the present study can also be transferred to other geotechnical uses of the underground. Generally, the role of microbial-mediated processes is two-sided. Changes in the environmental conditions can provoke shifts in the microbial community structure or activity. Those alterations may subsequently lead to unintentional changes

in the geochemistry and therefore, undermine the reliability or economy of the geotechnical construction. Nowadays, beside of the underground storage of CO_2, H_2 or CH_4 (Pudlo et al. 2013), the geothermal energy sector is rapidly developing and several studies showed the influence of microbial activity in terms of enhanced corrosion and precipitation (Lerm et al. 2013; Würdemann et al. 2014).

Although the present study highlights detrimental effects of microbial activity, prospective projects will also show up how microbes can be used during underground storage. Recently, Bassil et al. (2014) discussed that microbes can be useful to protect the underground during the storage of nuclear waste, reducing the mobility of radionuclides from an intermediate-level radioactive waste in a multibarrier deep geological disposal facility. Further research is focused on biogeochemical carbon recycling; combining CO_2 sequestration in coal seams to methane production through methanogens or biomimetic approaches (Koide and Yamazaki 2001).

References

Abrams A (1977) Mud design to minimize rock impairment due to particle invasion. J Petrol Technol 29 (5):586-592. SPE-5713-PA. doi:10.2118/5713-PA

Alawi M, Lerm S, Vetter A, Wolfgramm M, Seibt A, Würdemann H (2011) Diversity of sulfate-reducing bacteria in a plant using deep geothermal energy. Grundwasser, 16(2):105-112. doi:10.1007/s00767-011-0164-y

Aldén L, Demoling F, Bååth E (2001) Rapid method of determining factors limiting bacterial growth in soil. Appl Environ Microb 67:1830-1838. doi:10.1128/AEM.67.4.1830–1838.2001

Al Hashem A, Carew JA, Al Borno A (2004) Screening test for six dual biocide regimes against planktonic and sessile populations of Bacteria. NACE International. NACE-04748

Altschul SF, Gish W, Miller W, Myers EW & Lipman DJ (1990) Basic local alignment search tool. J Mol Biol 215(3):403-410. doi:10.1016/S0022-2836(05)80360-2

An D, Caffrey SM, Soh J, Agrawal A, Brown D, Budwill K et al. (2013) Metagenomics of hydrocarbon resource environments indicates aerobic taxa and genes to be unexpectedly common. Environ Sci Technol 47:10708–10717. doi: 10.1021/es4020184

Asakawa S, Nagaoka K. (2003) Methanoculleus bourgensis, Methanoculleus olentangyi and Methanoculleus oldenburgensis are subjective synonyms. Int J Syst Evol Microbiol 53(5):1551-1552. doi:10.1099/ijs.0.02508-0

Baker BJ, Moser DP, MacGregor BJ, Fishbain S, Wagner M, Fry NK, Jackson B et al. (2003) Related assemblages of sulphate-reducing bacteria associated with ultradeep gold mines of south Africa and deep basalt aquifers of Washington state. Environ Microbiol 5:267-277. doi:10.1046/j.1462-2920.2003.00408.x

Balkwill DL, Ghiorse WC (1985) Characterization of subsurface bacteria associated with two shallow aquifers in Oklahoma. Appl Environ Microb 50(3):580-8.

Balkwill DL, Drake GR, Reeves RH, Fredrickson JK, White DC, Ringelberg DB, Chandler DP, Romine MF, Kennedy DW, Spadoni CM (1997) Taxonomic study of

aromatic-degrading Bacteria from deep-terrestrial-subsurface sediments and description of Sphingomonas aromaticivorans sp. nov., Sphingomonas subterranea sp. nov., and Sphingomonas stygia sp. nov. Int J Syst Bacteriol 47(1):191–201. doi:10.1099/00207713-47-1-191

Bassam BJ, Caetano-Anolles G, Gresshoff PM (1991) Fast and sensitive staining of DNA in polyacrylamide gels. Anal Biochem 196:80-83

Bassil NM, Bryan N, Lloyd JR (2014) Microbial degradation of isosaccharinic acid at high pH. ISME J 9:310–320. doi:10.1038/ismej.2014.125

Basso O, Lascourreges JF, Le Borgne F, Le Goff C, Margot M (2009) Characterization by culture and molecular analysis of the microbial diversity of a deep subsurface gas storage aquifer. Res Microbiol 160(2):107-116. doi:10.1016/j.resmic.2008.10.010.

Bauer S, Beyer C, Dethlefsen F, Dietrich P, Duttmann R, Ebert M, Feeser V, Görke U, Köber R, Kolditz O, Rabbel W, Schanz W, Schäfer D, Würdemann H, Dahmke A (2013) Impacts of the use of the geological subsurface for energy storage: an investigation concept. Environ Earth Sci 70:3935-3943. doi:10.1007/s12665-013-2883-0

Beech IB, Sunner JA, Hiraoka K (2005) Microbe-surface interactions in biofouling and biocorrosion processes. Int Microbiol 8(3):157-168.

Bergmann P, Schmidt-Hattenberger C, Kiessling D, Rücker C, Labitzke T, Henninges J, Baumann G, Schütt H (2012) Surface-downhole electrical resistivity tomography applied to monitoring of CO_2 storage at Ketzin, Germany. Geophysics 77:B253-B267, doi:10.1190/geo2011-0515.1.

Berner A (1970) Sedimentary Pyrite formation. Amer J Sci 268:1–23. doi:10.2475/ajs.268.1.1

Bock S, Förster H, Meier A, Pudlo D, Förster A, Gaupp R (2013) Impact of 4-year CO2 injection on reservoir-rock integrity at the CO2 pilot site Ketzin (Germany). Abstracts, AGU 2013 Fall Meeting (San Francisco, USA 2013)

Boersma FGH, Otten R, Warmink JA, Nazir R & van Elsas JD (2010) Selection of Variovorax paradoxus-like bacteria in the mycosphere and the role of fungal-released compounds. Soil Biol Biochem 42(12):2137-2145. doi:10.1016/j.soilbio.2010.08.009

Boivin-Jahns V, Ruimy R, Bianchi A, Daumas S and Christen R (1996) Bacterial diversity in a deep-subsurface clay environment. Appl Environ Microb 62(9):3405–3412

Brockman FJ, Kieft TL, Fredrickson JK, Bjornstad BN, Li SW, Spangenburg W, Long PE (1992) Microbiology of vadose zone paleosols in South-Central Washington State. Microbiol Ecol 23:279-301.

Cadmus MC, Jackson LK, Burton KA, Plattner RD, Slodki ME (1982) Biodegradation of xanthan gum by Bacillus sp. Appl Environ Microb 44(1):5-11.

Cardace D, Hoehler T, Mccollom T, Schrenk M, Carnevale D, Kubo M, Twing K (2013) Establishment of the Coast Range ophiolite microbial observatory (CROMO): drilling objectives and preliminary outcomes. Sci Dril 16:45–55. doi: 10.5194/sd-16-45-2013

Chan CS, Fakra SC, Edwards DC, Emerson D, Banfield JF (2009) Iron oxyhydroxide mineralization on microbial extracellular polysaccharides. Geochim. Cosmochim. Acta 73:3807–3818. doi:10.1016/j.gca.2009.02.036

Czarnetzki AB, Tebbe CC (2004) Diversity of bacteria associated with Collembola – a cultivation-independent survey based on PCR-amplified 16S rRNA genes. FEMS Microbiol Ecol 49:217–227. doi:10.1016/j.femsec.2004.03.007

Darnadatu H (2004) Formation evaluation. – In: Standard Handbook of petroleum and natural gas engineering Eds. Lyons W, Plisga GJ, BS, Michael Lorenz M.

Deng S, Dong H, Lv G, Jiang H, Yu B, Bishop ME (2010) Microbial dolomite precipitation using sulfate reducing and halophilic bacteria: results from Qinghai Lake, Tibetan Plateau, NW China. Chem Geol 278:151–159. doi:10.1016/j.chemgeo.2010.09.008

Detmers J, Strauss H, Schulte U, Bergmann A, Knittel K, Kuever J (2004) FISH shows that Desulfotomaculum spp. are the dominating sulfate-reducing bacteria in a pristine aquifer. Microb Ecol 47(3):236-42. doi: 10.1007/s00248-004-9952-6

Diehl H, Horchak-Morris N (1987) Studies on fluorescein-V The absorbance of fluorescein in the ultraviolet, as a function of pH. Talanta 34(8):739–741. doi:10.1016/0039-9140(87)80232-1

Dohrmann AB, Tebbe CC (2004). Microbial community analysis by PCR-single-strand conformation polymorphism (PCR-SSCP). In Kowalchuk GA, Bruijn FJ de, Head IM, Akkermans AD, van Elsas JD (eds) Molecular Microbial Ecology Manual. 2nd edn. Dodrecht: Kluwer Academic Publisher 3(16):809-838

Douglas S, Beveridge TJ (1998) Mineral formation by bacteria in natural microbial communities. FEMS Microbiol Ecol 26:79-88. doi: 10.1111/j.1574-6941.1998.tb00494.x

Doudoroff M (1940) The oxidative assimilation of sugars and related substances by Pseudomonas saccharophila with a contribution to the problem of the direct respiration of di- and polysaccharides. Enzymologia 9:59–72.

Duan Y, Liu M, Sun W, Wang M, Liu S, Li QX (2009) Recent progress on synthesis of fluorescein probes. Mini-Rev Org Chem 6(1):35–43. doi:10.2174/157019309787316111

Edwards KJ, Becker K & Colwell FS (2012) The deep, dark energy biosphere: intraterrestrial life on Earth. Annu Rev Earth Pl Sc 40:551–568. doi:10.1146/annurev-earth-042711-105500

Ehinger S, Seifert J, Kassahun A, Schmalz L, Hoth N and Schlömann M (2009) Predominance of Methanolobus spp. and Methanoculleus spp. in the Archaeal Communities of Saline Gas Field Formation Fluids. Geomicrobiol J 26(5):326-338. DOI: 10.1080/01490450902754441

Ekperigin MM, (2007) Preliminary studies of cellulase production by Acinetobacter anitratus and Branhamella sp. Afr J of Biotechnol 6(1):028–033

Ezzat EM, Rosser HR, and Al-Hamam AA (1997) Control of microbiological activity in biopolymer - based drilling muds. Soc Petrol Eng J. doi: 10.2118/39285-MS

Falcone-Dias MF, Vaz-Moreira I and Manaia CM (2012) Bottled mineral water as a potential source of antibiotic resistant bacteria. Water Res 46(11):3612-3622. doi: 10.1016/j.watres.2012.04.007

Fendrich C (1988) Halovibrio variabilis gen. nov. sp. nov., Pseudomonas halophila sp. nov. and a new halophilic aerobic coccoid eubacterium from Great Salt Lake, Utah, USA. Syst Appl Microbiol 11(1):36–43. doi:10.1016/S0723-2020(88)80046-8

Fischer S, Liebscher A, Wandrey M and the CO2SINK group (2010) CO2–brine–rockinteraction — First results of long-term exposure experiments at in situ P–T conditions of the Ketzin CO2 reservoir. Chem Erde-Geochem 70(S3):155–164. doi:10.1016/j.chemer.2010.06.001

Fischer S, Zemke K, Liebscher A, Wandrey M, CO2SINK Group (2011) Petrophysical and petrochemical effects of long-term CO2-exposure experiments on brine-saturated reservoir sandstone. En Proc 4:4487-4494. doi:10.1016/j.egypro.2011.02.404

Fisher AT, Cowen J, Wheat CG, Clark JF (2011) Preparation and injection of fluid tracers during IODP expedition 327, eastern flank of Juan de Fuca Ridge. In: Fisher AT, Tsuji T, Petronotis K (eds) The Expedition 327 scientists, proceedings of IODP, 327. Integrated Ocean Drilling Program Management International, Inc., Tokyo. doi:10.2204/iodp.proc.327.108.2011

Fleshman R and Obren-Likic H (1999) Artificial lift for high volume production. Oilfield Review 11(1): 49–63.

Förster A, Norden B, Zinck-Jørgensen K, Frykman P, Kulenkampff J, Spangenberg E, Erzinger J, Zimmer M, Kopp J, Borm G, Juhlin C-G, Cosma C, Hurter S (2006) Baseline characterization of the CO2SINK geological storage site at Ketzin, Germany. Environ Geoscien 13(3):145–161. doi:10.1306/eg.02080605016

Förster A, Giese R, Juhlin C, Norden B, Springer N, CO2SINK Group (2009) The geology of the CO2SINK site: From regional scale to laboratory scale. - En Proc 1(1):2911-2918. doi:10.1016/j.egypro.2009.02.066

Fredrickson JK, Balkwill DL, Romine MF, Shi T (1999) Ecology, physiology, and phylogeny of deep subsurface Sphingomonas sp. J Ind Microbiol Biotechnol. 23(4-5):273-283

Fredrickson JK, Balkwill DL (2006). Geomicrobial processes and biodiversity in the deep terrestrial subsurface. Geomicrobiol J 23(6):345-356. doi:10.1080/01490450600875571

Fry NK, Friedrickson JK, Fishbain S, Wagner M, Stahl D (1997) Population structure of microbial communities associated with two deep, anaerobic, alkaline aquifers. Appl Environ Microb 63(4):1498-1504.

Gantner S, Andersson AF, Alonso-Sáez L, Bertilsson S (2010) Novel primers for 16S rRNA-based archaeal community analysis in environmental samples. J Microbiol Methods 84 (1):12–8. doi: 10.1016/j.mimet.2010.10.001

Geets J, Borremans B, Diels L, Springael D, Vangronsveld J, van der Lelie D, Vanbroekhoven K (2006) DsrB gene-based DGGE for community and diversity surveys of sulfate-reducing bacteria. J Microbiol Methods 66(2):194-205. doi:10.1016/j.mimet.2005.11.002

Ghosh W and Roy P (2006) Mesorhizobium thiogangeticum sp. nov., a novel sulfur-oxidizing chemolithoautotroph from rhizosphere soil of an Indian tropical leguminous plant. Int J Syst Evol Microbiol 56(1):91-7. doi: 10.1099/ijs.0.63967-0

Giangiacorno L, Dennis D (1997) Field testing of the biocompetitive exclusion process for control of iron and hydrogen sulfides. Soc Petrol Eng J SPE 38351:125-135. doi:10.2118/38351-MS

Gieg LM, Jack TR, Foght JM (2011) Biological souring and mitigation in oil reservoirs. Appl Microbiol Biotechnol 92:263. doi:10.1007/s00253-011-3542-6

Glasauer S, Weidler PG, Langley S, Beveridge TJ (2003) Controls on Fe reduction and mineral formation by a subsurface bacterium. Geochim Cosmochim Acta 67:1277-1288. doi:10.1016/S0016-7037(02)01199-7

Gluyas & Mathias (Eds.) (2013) Geological storage of carbon dioxide (CO2), 1st Edition Geoscience, Technologies, Environmental Aspects and Legal Frameworks. Woodhead Publishing

Goldscheider N, Hunkeler D, Rossi P (2006) Review: microbial biocenoses in pristine aquifers and an assessment of investigative methods. Hydrogeol J 14(6):926-941. doi:10.1007/s10040-005-0009-9

Goldscheider N, Meiman J, Pronk M, Smart C (2008) Tracer tests in karst hydrogeology and speleology. Int J Speleol 37(1):27–40

Griebler C and Lueders T (2009) Microbial biodiversity in groundwater ecosystems. Freshwater Biol 54:649–677. doi:10.1111/j.1365-2427.2008.02013.x

Gulliver DM, Lowry GV and Gregory KB (2014*a*) CO2 concentration and pH alters subsurface microbial ecology at reservoir temperature and pressure. RSC Adv 4:17443-17453. doi:10.1039/C4RA02139H.

Gulliver DM, Lowry GV and Gregory KB (2014*b*) Effect of CO2(aq) exposure on a freshwater aquifer microbial community from simulated geologic carbon storage leakage. Environ Sci Technol Lett 1(12):479–483. doi:10.1021/ez500337v

Hazen TC, Jimenez L, Lopez de Victoria G, Fliermans CB (1991) Comparison of bacteria from deep subsurface sediment and adjacent groundwater. Microb Ecol 22(1):293-304. doi:10.1007/BF02540231

Herman D, Frankenberger WT (1998) Microbial-mediated reduction of perchlorate in groundwater. J. Environ. Qual. 27:750–754. doi:10.2134/jeq1998.0047242500270004 0004x

Hill EC, Shennan JL, Watkinson RJ (1987) Microbial problems in the offshore oil industry. The Institute of Petroleum - John Wiley & Sons, Chichester, UK, 257 pp

Hoshino YT, Morimoto S, Hayatsu M, Nagaoka K, Suzuki C, Karasawa T, Takenaka M, Akiyama H (2011). Effect of soil type and fertilizer management on archaeal community in upland field soils. Microbes Environ. 26:307–316. doi:10.1264/jsme2.ME11131

House CH, Cragg BA, Teske A, The Leg 201 Scientific Party (2003) Drilling contamination tests during ODP Leg 201 using chemical and particulate tracers. In: D'Hondt SL, Jørgensen BB, Miller DJ et al (eds) Proceedings of the Ocean drilling program initial reports. 201:1–19

Hötzl H, Käss W, Reichert B (1991) Application of microbial tracers in groundwater studies. Water Sci Tech 24(2):295–300

Huq F, Haderlein SB, Cirpka OA, Nowak M, Blum P, Grathwohl P (2015) Flow-through experiments on water–rock interactions in a sandstone caused by CO2 injection at pressures and temperatures mimicking reservoir conditions. Applied Geochemistry 58: 136–146. doi: 10.1016/j.apgeochem.2015.04.006

IPCC (2005) Metz B, Davidson O,de Coninck H,Loos M, Meyer L (Eds.) Cambridge University Press, UK. 431

IEAGHG (2011*a*) Caprock Systems for CO2 Geological Storage, 2011/01

IEAGHG (2011*b*) Effects of Impurities on Geological Storage of CO2, 2011/04

Ijzerman MM, Hagedorn C, Reneau RB Jr. (1993) Microbial tracers to evaluate an on-site shallow-placed low pressure distribution system. Water Res 27:343–347.

Jaiswal P, Al-Hadrami F, Atekwana EA, and Atekwana EA (2014) Mechanistic models of biofilm growth in porous media, J Geophys Res Biogeosci 119:1418–1431, doi:10.1002/2013JG002440

Jakobsen TF, Kjeldsen KU, Ingvorsen K (2006) Desulfohalobium utahense sp. nov., a moderately halophilic, sulfate-reducing bacterium isolated from Great Salt Lake. Int J Syst Evol Microbiol 56(9):2063-2069. doi:10.1099/ijs.0.64323-0

Jin L, Kim KK, Ahn CY, Oh HM (2012) Variovorax defluvii sp. nov., isolated from sewage. Int J Syst Evol Microbiol 62(8):1779-83. doi: 10.1099/ijs.0.035295-0

Jørgensen BB, Boetius A (2007) Fast and famine-microbial life in the deep-sea bed. Nature 5:770-781. doi:10.1038/nrmicro1745

Kallmeyer J, Mangelsdorf K, Cragg B, Horsfield B (2006) Techniques for contamination assessment during drilling for terrestrial subsurface sediments. Geomicrobiol J 23(3–4):227–239. doi:10.1080/01490450600724258

Kallmeyer J, Pockalny R, Adhikari RR, Smith DC, D'Hondt S (2012) Global distribution of microbial abundance and biomass in subseafloor sediment. - Proceedings of the National Academy of Sciences of the United States of America (PNAS), 109(40):16213-16216. doi: 10.1073/pnas.1203849109

Karkhoff-Schweizer RR, Huber DP, Voordouw G (1995) Conservation of the genes for dissimilatory sulfite reductase from Desulfovibrio vulgaris and Archaeoglobus fulgidus allows their detection by PCR. Appl Environ Microb 61:290–296.

Kasina M, Bock S, Würdemann H, Pudlo D, Picard A, Lichtschlag A, März C, Wagenknecht L, Wehrmann LM, Vogt C, Meister P (2017) Mineralogical and geochemical analysis of Fe-phases in drill-cores from the Triassic Stuttgart

Formation at Ketzin CO2 storage site before CO2 arrival. Environ Earth Sci 76:161. doi: 10.1007/s12665-017-6460-9

Kaszuba JP, Janecky DR (2009) Geochemical impacts of sequestering carbon dioxide in brine formations. In: Sundquist, E, McPherson, B, editors. Carbon sequestration and its role in the global carbon cycle. AGU Monograph;, 183: 239-248.

Kemnitz D, Kolb S, and Conrad R (2007) High abundance of Crenarchaeota in a temperate acidic forest soil. FEMS Microbiol Ecol 60:442–448. doi: 10.1111/j.1574-6941.2007.00310.x

Kempka T, De Lucia M, Kühn M (2014) Geomechanical integrity verification and mineral trapping quantification for the Ketzin CO2 storage pilot site by coupled numerical simulations. En Proc 63:3330-3338. doi:10.1016/j.egypro.2014.11.361

Kieft TL (2010) Sampling the deep sub-surface using drilling and coring techniques. In: Handbook of hydrocarbon and lipid microbiology. Timmis KN (ed.), Springer-Verlag Berlin Heidelberg. doi: 10.1007/978-3-540-77587-4_267

Kim SJ, Moon JY, Weon HY, Hong SB, Seok SJ, Kwon SW (2014) Undibacterium jejuense sp. nov. and Undibacterium seohonense sp. nov., isolated from soil and freshwater, respectively. Int J Syst Evol Microbiol 64(1):236-241 doi: 10.1099/ijs.0.056846-0

Kirk MF, Altman SJ, Santillan EFU, Bennett PC (2016) Interplay between microorganisms and geochemistry in geological carbon storage. Int J Greenh Gas Control 47:386–395. doi:10.1016/j.ijggc.2016.01.041

Koide H Yamazaki K (2001) Subsurface CO2 disposal with enhanced gas recovery and biochemical carbon recycling. Environ Geosci 8(3):218–24.

Kostka JE, Dalton DD, Skelton H, Dollhopf S, Stucki JW (2002) Growth of iron(III)-reducing bacteria on clay minerals as the sole electron acceptor and comparison of growth yields on a variety of oxidized iron forms. Applied and Environmental Microbiology, 68, 6256–6262. doi: 10.1128/AEM.68.12.6256-6262.2002

Kotelnikova S (2002) Microbial production and oxidation of methane in deep subsurface. Earth Sc Reviews 58:367-395. doi:10.1016/S0012-8252(01)00082-4

Kouduka M, Suko T, Morono Y, Inagaki F, Ito K and Suzuki Y (2012) A new DNA extraction method by controlled alkaline treatments from consolidated subsurface sediments. FEMS Microbiol Lett 326:47-54. doi:10.1111/j.1574-6968.2011.02437.x

Ladva HKJ, Tardy P, Howard PR et al. (2000) Multiphase flow and drilling fluid filtrate effects on the onset of production. Presented at the SPE international symposium on formation damage control, Lafayette, Louisiana. SPE-58795-MS. doi:10.2118/58795-MSfckLR

Lane DJ (1991) 16S/23S rRNA sequencing. In: Nucleic acid techniques in bacterial systematics. Stackebrandt E and Goodfellow M eds., John Wiley and Sons, New York, NY. 115-175.

Lappan RE & Fogler HS (1992) The effects of bacterial polysaccharide production on formation damage. Soc Petrol Eng SPE 19418:165-172. doi:10.2118/19418-PA

Lerm S, Westphal A, Miethling-Graff R, Alawi M, Seibt A, Wolfgramm M, Würdemann H (2013) Thermal effects on microbial composition and microbiologically induced corrosion and mineral precipitation affecting operation of a geothermal plant in a deep saline aquifer. Extremophiles 17(2):311-327. doi:10.1007/s00792-013-0518-8

Liang Y-L, Zhang Z, Wu M, Wu Y, Feng J-X (2014) Isolation, screening, and identification of cellulolytic bacteria from natural reserves in the subtropical region of China and optimization of cellulase production by Paenibacillus terrae ME27-1. BioMed Research International doi: 10.1155/2014/512497

Lièvremont D, Bertin PN, Let M-C (2009) Arsenic in contaminated waters: Biogeochemical cycle, microbial metabolism and biotreatment processes. Biochimie 91(2):1229-1237. doi:10.1016/j.biochi.2009.06.016

Lorite MJ, Muñoz S, Olivares J, Soto MJ, Sanjuán J (2010) Characterization of strains unlike Mesorhizobium loti that nodulate Lotus spp. in saline soils of Granada, Spain. Appl Environ Microb 76(12):4019-4026. doi:10.1128/AEM.02555-09.

Lovley DR, Chapelle FH (1995) Deep subsurface microbial processes. Rev Geophys 33:365–381. doi: 10.1029/95RG01305

Martens S, Kempka T, Liebscher A, Lüth S, Möller F, Myrttinen A, Norden B, Schmidt-Hattenberger C, Zimmer M, Kühn M and the Ketzin Group (2012) Europe's longest-operating on-shore CO2 storage site at Ketzin, Germany: a progress report after three years of injection. Environ Earth Sci 67(2):323–334. doi:10.1007/s12665-012-1672-5

Martens S, Liebscher A, Möller F, Henninges J, Kempka T, Lüth S, Norden B, Prevedel B, Szisybalski A, Zimmer M, Kühn M and the Ketzin Group (2013) CO2 Storage at the Ketzin pilot site, Germany: fourth year of injection, monitoring, modelling and verification. En Proc 37:6434-6443. doi.org/10.1016/j.egypro.2013.06.573

Martens S, Möller F, Streibel M, Liebscher A (2014) Completion of five years of safe CO2 injection and transition to the post-closure phase at the Ketzin pilot site. En Proc 59:190-197. doi:10.1016/j.egypro.2014.10.366

Martens S, Conze R, De Lucia M, Henninges J, Kempka T, Liebscher A, Lueth S, Moeller F, Norden B, Prevedel B, Schmidt-Hattenberger C, Szizybalski A, Vieth-Hillebrand A, Würdemann H, Zemke K, Zimmer M (2015) Joint Research Project CO2MAN (CO2MAN Reservoir Management): Continuation of research and development work for CO2 storage at the Ketzin pilot site. - In: Liebscher A, Münch U (Eds.), Geological Storage of CO2 – Long term security aspects, (GEOTECHNOLOGIEN Science Report; 22) (Advanced Technologies in Earth Sciences), Cham : Springer International Publishing 1-32. doi:10.1007/978-3-319-13930-2_1

McKinley JP, Colwell FS (1996) Application of perfluorocarbon tracers to microbial sampling in subsurface environments using mud-rotary and air-rotary drilling techniques. J Microbiol Meth 26:1–9. doi:10.1016/0167-7012(96)00826-3

Ménez B, Dupraz S, Gérard E, Guyot F, Rommevaux–Jestin C, Libert M, Jullien M, Michel C, Delorme F, Battaglia–Brunet F, Ignatiadis I, Garcia B, Blanchet D, Huc AY, Haeseler F, Oger P, Dromart G, Ollivier B, Magot M (2007) Impact of the deep biosphere on CO2 storage performance. Geotechnologien Sci Rep 9:150–163

Mitchell AC, Phillips A, Hiebert R, Gerlach R, Spangler L, Cunningham AB (2009*a*) Biofilm enhanced subsurface sequestration of supercritical CO2. Int J Greenh Gas Control 3:90–99. doi:10.1016/j.ijggc.2008.05.002

Mitchell AC, Phillips AJ, Hamilton MA, Gerlach R, Hollis WK, Kaszuba JP, Cunningham AB (2009*b*) Resilience of planktonic and biofilm cultures to supercritical CO2. J Supercrit Fluids 47:318–325. doi:10.1016/j.supflu.2008.07.005

Mitchell AC, Dideriksen K, Spangler LH, Cunningham AB and Gerlach R (2010) Microbially enhanced carbon capture and storage by mineral-trapping and solubility-trapping. Environ Sci Technol 44, 5270–5276. doi:10.1021/es903270w

Morozova D, Wandrey M, Zimmer M, Pilz P, Zettlitzer M, Würdemann H, the CO2SINK Group (2010) Monitoring of the microbial community composition in saline aquifers during CO2 sequestration by fluorescence in situ hybridisation. Int J Greenh Gas Control 4:981–989. doi:10.1016/j.ijggc.2009.11.014

Morozova D, Alawi M, Shaheed M, Krüger M, Kock D, Würdemann H (2011) The influence of microbial activity on rock fluid interaction: baseline characterization of deep microbial biosphere for Enhanced Gas Recovery in the Altmark natural gas reservoir. En Proc 4:4633-4640. doi:10.1016/j.egypro.2011.02.423

Morozova D, Let D, Würdemann H (2013) Analysis of the microbial community from a saline aquifer prior to CO2 injection in Ketzin using improved Fluorescence in situ Hybridisation method. En Proc 40:276–284. doi:10.1016/j.egypro.2013.08.032

Moser DP, Gihring TM, Brockman FJ, Fredrickson JK, Balkwill DL, Dollhopf ME, Lollar BS, Pratt LM, Boice E, Southam G, Wanger G, Baker BJ, Pfiffner SM, Lin LH, Onstott TC (2005) Desulfotomaculum and Methanobacterium spp. dominate a 4- to 5-kilometer-deep fault. Appl Environ Microb 71(12):8773-83. doi:10.1128/AEM.71.12.8773–8783.2005

Muyzer G, de Waal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microb 59:695–700

Nedelkova M, Radeva G, Selenska-Pobell S (2005) Molecular bacterial diversity in water at the deep-well monitoring site at Tomsk-7. In Underground Injection Science and Technology (eds Tsang CF, Apps J). Elsevier, Amsterdam. 521–536.

Norden B, Förster A, Vu-Hoang D, Marcelis F, Springer N, Le Nir I (2010) Lithological and petrophysical core-log interpretation in CO2SINK, the European CO2 onshore

research storage and verification project. SPE Reserv Eval Eng 13(2):179-192. doi:10.2118/115247-PA

Norden B, Klapperer S (2011) Geologischer Abschlußbericht der Bohrung Hy Ktzi P 300/2011. Deutsches Geoforschungzentrum GFZ, Helmholtz Zentrum Potsdam.

Oelkers EH, Cole DR (2008*a*) Carbon Dioxide Sequestration A Solution to a Global Problem. Elements 4(5):305-310. doi: 10.2113/gselements.4.5.305

Oelkers EH, Gislason SR, Matter J (2008*b*) Mineral Carbonation of CO2. Elements, 4(5): 333-337. doi: 10.2113/gselements.4.5.333

Oren A (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Systems 4:2. doi: 10.1186/1746-1448-4-2

Orphan VJ, Taylor LT, Hafenbradl D, Delong EF (2000) Culture-dependent and culture-independent characterization of microbial assemblages associated with high-temperature petroleum reservoirs. Appl Environ Microb 66(2):700-711. doi: 10.1128/AEM.66.2.700-711.2000

Park JM, Kim TY, Lee SY (2011) Genome-scale reconstruction and in silico analysis of the Ralstonia eutropha H16 for polyhydroxyalkanoate synthesis, lithoautotrophic growth, and 2-methyl citric acid production. BMC Syst Biol 5:101. doi:10.1186/1752-0509-5-101

Paul VG, Wronkiewicz DJ, Mormile MR (2017) Impact of elevated CO2 concentrations on carbonate mineral precipitation ability of sulfate-reducing bacteria and implications for CO2 sequestration. Appl Geochem 78: 250–271. doi: 10.1016/j.apgeochem.2017.01.010

Pchalek, Bergerhoff, Polleschner, Borsdorf, Lange, Oswald (1964) Ergebnisbericht über die geologische Speichererkundung der Struktur Brandenburg – Ketzin, Strukturteil Ost (Knoblauch). VEB UGS Burg

Pedersen K (2014) Microbial life in terrestrial hard rock environments. In Kallmeyer J.and Wagner D. (eds.) Microbial life of the Deep Biosphere. D. De Gruyter, Berlin, 63-81

Pellizzari L, Neumann D, Alawi M, Voigt D, Norden B, Würdemann H (2013) Use of tracers to assess drill mud penetration depth into sandstone rock cores during deep drilling: method development and application. Environ Earth Sci 70:3727–3738. doi:10.1007/s12665-013-2715-2

Pellizzari L, Morozova D, Neumann D, Kasina M, Klapperer S, and Würdemann H (2016). Comparison of the microbial community composition of pristine rock cores and technical influenced well fluids from the Ketzin pilot site for CO_2 storage. Environ Earth Sci 75: 1323. doi:10.1007/s12665-016-6111-6.

Pellizzari L, Lienen T, Kasina M, Würdemann H (2017) Influence of drill mud on the microbial communities of sandstone rocks and well fluids at the Ketzin pilot site for CO_2 storage. Environ Earth Sci 76: 77. doi:10.1007/s12665-016-6381-z;

Pollock TJ (1993) Gellan-related polysaccharides and the genus Sphingomonas. J Gen Microbiol 139:1939–1945.

Prevedel B, Wohlgemuth L, Legarth B, Henninges J, Schütt H, Schmidt-Hattenberger C, Norden B, Förster A, Hurter S (2009) The CO2SINK boreholes for geological CO2 -storage testing. En Proc 1:2087-2094. doi:10.1016/j.egypro.2009.01.272

Prevedel B, Martens S, Norden B, Henninges J, Freifeld BM (2014) Drilling and abandonment preparation of CO2 storage wells – Experience from the Ketzin pilot site. En Proc 63:6067-6078. doi:10.1016/j.egypro.2014.11.639

Pudlo D, Ganzer L, Henkel S, Kühn M, Liebscher A, De Lucia M, Panfilov M, Pilz P, Reitenbach V, Albrecht D, Würdemann H, Gaupp R (2013) The H2STORE Project: Hydrogen underground storage - A feasible way in storing electrical power in geological media? - In: Hou M Z, Xie H, Were P (Eds.), Clean energy systems in the subsurface: production, storage and conversion; Proceedings of the 3rd Sino-German Conference 'Underground storage of CO2 and Energy', Goslar, Germany, 21-23 May 2013, (Springer Series in Geomechanics and Geoengineering), Springer 395-412. doi:10.1007/978-3-642-37849-2_31.

Roscoe Moss (1990) Handbook of Ground Water Development, John Wiley & Sons, New York 276–312

Rosenqvist J, Kilpatrick AD, Yardley BWD, Rochelle CA (2014) Feldspar dissolution at CO2-saturated conditions. Geophysical Research Abstracts Vol. 16, EGU2014-10909, 2014 EGU General Assembly

Rosnes JT, Graue A, & Torleiv L (1991) Activity of Sulfate-Reducing Bacteria under simulated reservoir conditions. Soc Petrol Eng SPE 19429:231-236. doi:10.2118/19429-PA

Russell AD, McDonnell G (2000) Concentration: a major factor in studying biocidal action. J Hosp Infect 44(1):1-3. doi:10.1053/jhin.1999.0654

Sahl JW, Schmidt R, Swanner ED, Mandernack KW, Templeton AS, Kieft TL, Smith RL, Sanford WE, Callighan RL, Mitton JB, Spear JR (2008) Subsurface microbial diversity in deep-granitic-fracture water in Colorado. Appl Environ Microb 74:143-152. doi:10.1128/AEM.01133-07.

Salter SJ, Cox MJ, Turek EJ, Calus SJ, Cookson WO, Moffatt MF, Turner P, Parkhill J, Loman NJ and Walker AW (2014) Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biology 2014, 12:87. doi: 10.1186/s12915-014-0087-z

Sambrook J and Russell DW (2001) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY

Satola B, Wübbeler JH, Steinbüchel A (2013) Metabolic characteristics of the specie Variovorax paradox. Appl Microbiol Biot 97:541–560 doi:10.1007/s00253-012-4585-z

Scherf AK, Zetzl C, Smirnova I, Zettlitzer M, Vieth-Hillebrand A, and CO2SINK-group (2011) Mobilisation of organic compounds from reservoir rocks through the injection of CO2 – Comparison of baseline characterization and laboratory experiments. En Proc 4:4524–4531. doi:10.1016/j.egypro.2011.02.409

Schilling F, Borm G, Würdemann H, Möller F, Kühn M (2009) Status Report on the First European on-shore CO2 Storage Site at Ketzin (Germany). En Proc 1:2029–2035. doi:10.1016/j.egypro.2009.01.264

Schilling F, Bieberstein A, Eckhardt JD, Haist M, Hirsch A, Klumbach S, Kromer M, Mühlbach J, Müller BIR, Müllser HS, Neumann T, Schläger S & Triantafyllidis T

(2015) Long-term safety of well abandonment: First results from large scale laboratory experiments (COBRA). In: A. Liebscher and U. Münch (eds.), Geological storage of CO2 - Long term security aspects. Advanced Technologies in Earth Sciences, Springer International Publishing Switzerland. doi: 10.1007/978-3-319-13930-2_6

Schoonen MAA (2004) Mechanisms of sedimentary pyrite formation. GSA Special Papers 379:117-134. doi:10.1130/0-8137-2379-5.117

Schultze-Lam S, Fortin D, Davis B, Beveridge TJ (1996) Mineralization of bacterial surfaces. Chem Geol 132:171–181. doi:10.1016/S0009-2541(96)00053-8

Shimizu S, Akiyama M, Naganuma T, Fujioka M, Nako M, Ishijima Y (2007) Molecular characterization of microbial communities in deep coal seam groundwater of northern Japan. Geobiology 5(4):423-433. doi:10.1111/j.1472-4669.2007.00123.x

Smith NR, Gordon RE and Clark FE (1952) Aerobic spore-forming bacteria. U.S. Dept Agr Monograph no. 10.

Smith DC, Spivack AJ, Fisk MR, Haveman SA, Staudigel H (2000) Tracer based estimates of drilling-induced microbial contamination of deep-sea crust. Geomicrobiol J 17(3):207–219. doi:10.1080/01490450050121170

Smith SA, Pretorius WA (2002) The conservative behavior of fluorescein. Water SA, Pretoria, 28(4):203-206.

Struchtemeyer CG, Davis JP, Elshahed MS (2011) Influence of the drilling mud formulation process on the bacterial communities in thermogenic natural gas wells of the Barnett Shale. Appl Environ Microb 77(14):4744–4753. doi:10.1128/AEM.00233-11

Stewart EG (2012) Growing Unculturable Bacteria J. Bacteriol. 194(16):4151-4160 doi:10.1128/JB.00345-12

Spark I, Patey I, Duncan B, Hamilton A, Devine C and McGovern-Traa C (2000) The effects of indigenous and introduced microbes on deeply buried hydrocarbon reservoirs, North Sea. Clay Minerals 35:5-12

Spring S, Lapidus A, Schröder M, Gleim D, Sims D, Meincke L, Glavina Del Rio T, Tice H, Copeland A, Cheng JF, Lucas S, Chen F, Nolan M, Bruce D, Goodwin L, Pitluck S, Ivanova N, Mavromatis K, Mikhailova N, Pati A, Chen A, Palaniappan K, Land M, Hauser L, Chang YJ, Jeffries CD, Chain P, Saunders E, Brettin T, Detter JC, Göker M, Bristow J, Eisen JA, Markowitz V, Hugenholtz P, Kyrpides NC, Klenk HP, Han C (2009) Complete genome sequence of Desulfotomaculum acetoxidans type strain (5575). Stand Genomic Sci 1(3):242-53. doi: 10.4056/sigs.39508.

Suihko M-L, Skyttä E (2009) Characterisation of aerobically grown non-spore-forming bacteria from paper mill pulps containing recycled fibres. J Ind Microbiol Biotechnol 36:53–64. doi:10.1007/s10295-008-0472-0

Talia P, Sede SM, Campos E, Rorig M, Principi D, Tosto D, Hopp HE, Grasso D, Cataldi A (2012) Biodiversity characterization of cellulolytic bacteria present on native Chaco soil by comparison of ribosomal RNA genes. Res Microbiol 163:221–232. doi: 10.1016/j.resmic.2011.12.001.

Takai K, Moser DP, DeFlaun M, Onstott TC, Fredrickson JK (2001) Archaeal diversity in waters from deep South African gold mines. Appl Environ Microb 67:5750-5760. doi:10.1128/AEM.67.21.5750-5760.2001

Thordsen JJ, Evans WC, Kharaka YK, Kennedy BM, van Soest M (2005) Chemical and isotopic composition of water and gases from the SAFOD wells: implications to the dynamics of the San Andreas Fault at Parkfield, California, fall meeting 2005. American Geophysical Union, USA

Tian J, Wang Y, Dong X (2010) Methanoculleus hydrogenitrophicus sp. nov., a methanogenic archaeon isolated from wetland soil. Int J Syst Evol Microbiol 60(9):2165-2169. doi: 10.1099/ijs.0.019273-0

Tibbitts GA, Reed MG, McCarter M (1990). New coring system reduces filtrate invasion. EUROCAS London

Vandieken V, Knoblauch C, Jørgensen BB (2006) Desulfotomaculum arcticum sp. nov., a novel spore-forming, moderately thermophilic, sulfate-reducing bacterium isolated from a permanently cold fjord sediment of Svalbard. Int J Syst Evol Microbiol 56(4):687-90. doi:10.1099/ijs.0.64058-0

Verastegui Y, Cheng J, Engel K, Kolczynski D, Mortimer S, Lavigne J, Montalibet J, Romantsov T, Hall M, McConkey BJ, Rose DR, Tomashek JJ, Scott BR, Charles TC, Neufeld JD (2014) Multisubstrate isotope labeling and metagenomic analysis of active soil bacterial communities. mBio 5(4):01157-14. doi:10.1128/mBio.01157-14.

Wagner M, Roger AJ, Flax JL, Brusseau GA and Stahl DA (1998) Phylogeny of Dissimilatory Sulfite Reductases Supports an Early Origin of Sulfate Respiration. J Bacteriol 180(11):2975-2982

Wagner D, Milodowski AE, West JM, Wragg J, Yoshikawa H (2013) Mineralogical comparisons of experimental results investigating the biological impacts on rock transport processes. Environ Sci Process Impacts 15(8):1501-1510. doi: 10.1039/c3em00188a

Wandrey M, Morozova D, Zettlitzer M, Würdemann H, the CO2SINK Group (2010) Assessing drilling mud and technical fluid contamination in rock core and brine samples intended for microbiological monitoring at the CO2 storage site in Ketzin using fluorescent dye tracers. Int J Greenh Gas Control 4(6):972–980. doi:10.1016/j.ijggc.2010.05.012

Wandrey M, Fischer S, Zemke K, Liebscher A, Scherf A-K, Vieth A, Zettlitzer M, Würdemann H (2011a) Monitoring petrophysical, mineralogical, geochemical and microbiological effects of CO2 exposure — results of long-term experiments under in situ condition. En Proc 4: 3644–3650. doi:10.1016/j.egypro.2011.02.295

Wandrey M, Pellizzari L, Zettlitzer M, Würdemann H (2011b) Microbial community and inorganic fluid analysis during CO2 storage within the frame of CO2SINK – Long-term experiments under in situ conditions. En Proc 4:3651–3657. doi:10.1016/j.egypro.2011.02.296

Weaver L, Webber JB, Hickson AC, Abraham PM, Close ME (2015) Biofilm resilience to desiccation in groundwater aquifers: a laboratory and field study. Sci. Total Environ. 514, 281–289. doi:10.1016/j.scitotenv.2014.10.031

Weisburg WG, Barns SM, Pelletier DA and Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. J Bacteriol 173: 697–703

Welch SA, Barker WW, Banfield JF (1999) Microbial extracellular polysaccharides and plagioclase dissolution. Geochim Cosmochim Acta 63(9):1405–1419. doi:10.1016/S0016-7037(99)00031-9

Whitman WB, Coleman DC, Wiebe WJ (1998). Prokaryotes: The unseen majority. Proc. Natl. Acad. Sci. U.S.A. 95:6578–6583. doi:10.1073/pnas.95.12.6578.

Widdel F, Pfennig N (1977) A New Anaerobic, Sporing, Acetate-Oxidizing, Sulfate-Reducing Bacterium, Desulfotomaculum (emend.) acetoxidans. Arch Microbiol 112:119-122. doi: 10.1007/BF00446665

Wiese B, Böhner J, Enachescu C, Würdemann H, Zimmermann G (2010) Hydraulic characterisation of the Stuttgart formation at the pilot test site for CO2 storage, Ketzin, Germany. Int J Greenh Gas Control 4(6):960-971. doi:10.1016/j.ijggc.2010.06.013

Wiese B, Zimmer M, Nowak M, Pellizzari L, Pilz P (2013) Well-based hydraulic and geochemical monitoring of the above zone of the CO2 reservoir at Ketzin, Germany. Environ Earth Sci 70:3709–3726. doi:10.1007/s12665-013-2744-x

Wilkins MJ, Daly R, Mouser PJ, Trexler R, Wrighton KC, Sharma S, Cole DR, Biddle JF, Denis E, Fredrickson JK, Kieft TL, Onstott TC, Petersen L, Pfiffner SM, Phelps TJ, and Schrenk MO (2014) Trends and Future Challenges in Sampling the Deep Terrestrial Biosphere, Frontiers Microbiol 5:481 doi:10.3389/fmicb.2014.00481

Würdemann H, Möller F, Kühn M, Heidung W, Christensen NP, Borm G, Schilling FR (2010) CO2SINK-From site characterisation and risk assessment to monitoring and verification: One year of operational experience with the field laboratory for CO2 storage at Ketzin, Germany. Int J Greenh Gas Control 4(6):938-951. doi:10.1016/j.ijggc.2010.08.010

Würdemann H, Westphal A, Lerm S, Kleyböcker A, Teitz S, Kasina M, Miethling-Graff R, Seibt A, Wolfgramm M (2014) Influence of microbial processes on the operational reliability in a geothermal heat store – Results of long-term monitoring at a full scale plant and first studies in a bypass system. En Proc 59:412-417. doi:10.1016/j.egypro.2014.10.396

Xie CH, Yokota A (2005) Reclassification of Alcaligenes latus strains IAM 12599T and IAM 12664 and Pseudomonas saccharophila as Azohydromonas lata gen. nov., comb. nov., Azohydromonas australica sp. nov. and Pelomonas saccharophila gen. nov., comb. nov., respectively. Int J Syst Evol Microbiol 55(6):2419-25. doi:10.1099/ijs.0.63733-0

Yamazaki M, Thorne L, Mikolajczak M, Armentrout RW and Pollock TJ (1996) Linkage of genes essential for synthesis of a polysaccharide capsule in Sphingomonas strain S88. J Bacteriol 178: 2676–2687.

Yanagawa K, Nunoura T, Mcallister S, Hirai M, Breuker A, Brandt LD, House CH, Moyer CL, Birrien J-L, Aoike K, Sunamura M, Urabe T, Mottl MJ, Takai K (2013) The first microbiological contamination assessment by deep-sea drilling and coring by the D/V Chikyu at the Iheya North hydrothermal field in the Mid-Okinawa Trough (IODPExpedition331). Front Microbiol 4:327. doi:10.3389/fmicb.2013.00327

Youssef N, Elshahed MS, McInerney MJ (2009) Microbial processes in oil fields: culprits, problems and opportunities. Adv Appl Microbiol, 66:141-251.

Zettlitzer M, Möller F, Morozova D, Lokay P, Würdemann H, the CO2SINK Group (2010) Re-establishment of the proper injectivity of the CO2-injection well Ktzi 201 in Ketzin, Germany. Int J Greenh Gas Control 4:952–959. doi:10.1016/j.ijggc.2010.05.006

Verbund-Forschungsvorhaben H2STORE: Untersuchung der geohydraulischen, mineralogischen, geochemischen und biogenen Wechselwirkungen bei der Untertage-Speicherung von H2 in konvertierten Gaslagerstätten. Teilprojekt 4-Mikrobiologie. Abschlussbericht (2016)

www.co2ketzin.de

www.oilfield-biocides.com

www.oecd-ilibrary.org

APPENDIX I

Sequences :

GenBank accession: KR336949

```
        1 agcagtgggg aattttggac aatgggcgaa agcctgatcc agccatgccg cgtgcaggat
       61 gaaggccttc gggttgtaaa ctgcttttgt acggaacgaa acggcctttt ctaataaaga
      121 gggctaatga cggtaccgta agaataagca ccggctaact acgtgccagc agccgcggta
      181 atacgtaggg tgcaagcgtt aatcggaatt actgggcgta aagcgtgcgc aggcggttat
      241 gtaagacagt tgtgaaatcc ccgggctcaa cctgggaact gcatctgtga ctgcatagct
      301 agagtacggt agaggggat ggaattccgc gtgtagcagt gaaatgcgta gatatgcgga
      361 ggaacaccga tggcgaaggc aatcccctgg acctgtactg acgctcatgc acgaaagcgt
      421 ggggagcaaa caggattaga taccctggta gtccacgccc taaacgatgt caactggttg
      481 ttgggtcttc actgactcag taacgaagct aacgcgtgaa gt
```

//

GenBank accession: KR336950

```
        1 gcagcagtag ggaatattgg gcaatggacg agagtctgac ccagccatgc cgcgtgccgg
       61 atgaaggcct tctgggttgt aaacggcttt tctcagggaa gaaaaagacc atgcgtggta
      121 cactgacggt acctgaggaa taagcaccgg ctaactccgt gccagcagcc gcggtaatac
      181 ggagggtgca agcgttgtcc ggatttattg ggtttaaagg gtgcgtaggc ggcttgttaa
      241 gtccggggtg aaagcccaca gctcaactgt ggaactgccc tggatactgg caagcttgag
      301 tccagacgag gttggcggaa tggaaggtgt agcggtgaaa tgcatagata ccttccagaa
      361 ccccgattgc gtaggcagct gactaggctg gtactgacgc tgaggcacga aagcgtgggg
      421 agcgaacagg attagatacc ctggtagtcc acgccgtaaa cgatggatac tcgctggtag
      481 cgatatactg ttactggctt agcgaaagcg gtaagt
```

//

GenBank accession: KR336951

```
        1 gggaggcagc agtggggaat attggacaat gggcgaaagc ctgatccagc catgccgcgt
       61 gtgtgaagaa ggtcttcgga ttgtaaagca ctttaagttg ggaggaaggg cagtaaatta
      121 atactttgct gttttgacgt taccgacaga ataagcaccg gctaactctg tgccagcagc
      181 cgcggtaata cagagggtgc aagcgttaat cggaattact gggcgtaaag cgcgcgtagg
      241 tggttcgtta agttggatgt gaaatccccg ggctcaacct gggaactgca ttcaaaactg
      301 acgagctaga gtatggtaga gggtggtgga atttcctgtg tagcggtgaa atgcgtagat
      361 ataggaagga acaccagtgg cgaaggcgac cacctggact gatactgaca ctgaggtgcg
```

```
      421 aaagcgtggg gagcaaacag gattagatac cctggtagtc cacgccgtaa acgatgtcaa
      481 ctagccgttg ggagccttga gctcttagtg gcgcagctaa cgcattaagt
//
GenBank accession: KR336952
        1 tctttcaccg gagaagataa tgacggtatc cggagaagaa gccccggcta acttcgtgcc
       61 agcagccgcg gtaatacgaa gggggctagc gttgttcgga attactgggc gtaaagcgca
      121 cgtaggcgga tcgatcagtc aggggtgaaa tcccagggct caaccctgga actgcctttg
      181 atactgtcga tctggagtat ggaagaggtg agtggaattc cgagtgtaga ggtgaaattc
      241 gtagatattc ggaggaacac cagtggcgaa ggcggctcac tggtccatta ctgacgctga
      301 ggtgcgaaag cgtggggagc aaacaggatt agataccctg gtagtccacg ccgtaaacga
      361 tgaatgttag ccgtcgggca gtatactgtt cggtggcgca gctaacgcat taaac
//
GenBank accession: KR336953
        1 agcagtgggg aattttggac aatgggcgca agcctgatcc agccatgccg cgtgcgggaa
       61 gaaggccttc gggttgtaaa ccgcttttgt cagggaagaa aaggttctgg ttaatacctg
      121 ggactcatga cggtacctga agaataagca ccggctaact acgtgccagc agccgcggta
      181 atacgtaggg tgcaagcgtt aatcggaatt actgggcgta aagcgtgcgc aggcggttat
      241 gcaagacaga ggtgaaatcc ccgggctcaa cctgggaact gcctttgtga ctgcatagct
      301 agagtacggt agagggggat ggaattccgc gtgtagcagt gaaatgcgta gatatgcgga
      361 ggaacaccga tggcgaaggc aatcccctgg acctgtactg acgctcatgc acgaaagcgt
      421 ggggagcaaa caggattaga taccctggta gtccacgccc taaacgatgt caactggttg
      481 ttgggagggt ttcttctcag taacgtagct aacgcgtgaa gt
//
GenBank accession: KR336954
        1 tggggaatat tggacaatgg gcgcaagcct gatccagcca tgccgcgtga gtgatgaagg
       61 ccctagggtt gtaaagctct ttcaccggtg aagataatga cggtaaccgg agaagaagcc
      121 ccggctaact tcgtgccagc agccgcggta atacgaaggg ggctagcgtt gttcggattt
      181 actgggcgta aagcgcacgt aggcggactt ttaagtcagg ggtgaaatcc cggggctcaa
      241 ccccggaact gcctttgata ctggaagtct tgagtatggt agaggtgagt ggaattccga
      301 gtgtagaggt gaaattcgta gatattcgga ggaacaccag tggcgaaggc ggctcactgg
      361 accattactg acgctgaggt gcgaaagcgt ggggagcaaa caggattaga taccctggta
      421 gtccacgccg taaacgatga atgttagccg ttggggagtt tactcttcgg tggcgcagct
      481 aacgcattaa acatc
```

//

GenBank accession: KR336955

```
        1 agcagtgggg aatattgcgc aatgggcgaa agcctgacgc agcaacgccg cgtgcgggat
       61 gacggccttc gggttgtaaa ccgctttcag cagggacgaa gggtttctga cggtacctgc
      121 agaagaagca ccggctaact acgtgccagc agccgcggtg atacgtaggg tgcgagcgtt
      181 gtccggaatt attgggcgta aagagcttgt aggcggtccg ttgcgtcgga agtgaaaact
      241 cagggcttaa ccctgagcct gctttcgata cgggcggact tgagggatgt aggggagaac
      301 ggaattcctg gtggagcggt ggaatgcgca gatatcagga ggaacaccag tggcgaaggc
      361 ggttctctgg acatttcctg acgctgagaa gcgaaagcgt ggggagcaaa caggcttaga
      421 taccctggta gtccacgccg taaacggtgg gcactaggtg tgggatccat tccacggatt
      481 ccgtgccgaa gctaacgcat taagtgc
```

//

GenBank accession: KR336956

```
        1 tggggtttct tgcgcaatgg gcgaaagcct gacgcagcaa cgccgtgtga atgatgaagg
       61 ccttagggtc gtaaaattct gtcaaggggg aagaagatct gacggtaccc ctggaggaag
      121 ctccggctaa ctacgtgcca gcagccgcgg taatacgtag ggagcgagcg ttgtccggaa
      181 ttactgggcg taaagggcgt gtaggcggcc aatcaagtca ggtgtgaaaa tccaaggctc
      241 aaccttggac gtgcacttga aactggttga cttgagtgca ggagaggaaa gtggaattcc
      301 cagtgtagcg gtgaaatgcg tagatattgg gaggaacacc agtggcgaag gcgactttct
      361 ggcctgtaac tgacgctgag gcgcgaaagc taggggagca aacaggatta gataccctgg
      421 tagtcctagc cgtaaacgat gggtactagg tgtagggggt ttagatgccc tctgtgccgc
      481 agttaacaca ttaagtactc cgc
```

//

GenBank accession: KR336957

```
        1 tggggaatct tgcgcaatgg gcgaaagcct gacgcagcaa cgccgtgtga atgatgaagg
       61 ccttagggtc gtaaaattct gtcaaggggg aagaagatct gacggtaccc ctggaggaag
      121 ctccggctaa ctacgtgcca gcagccgcgg taatacgtag ggagcgagcg ttgtccggaa
      181 ttactgggcg taaagggcgt gtaggcggcc aatcaagtca ggtgtgaaaa tccaaggctc
      241 aaccttggac gtgcacttga aactggttgg cttgagtgca ggagaggaaa gtggaattcc
      301 cagtgtagcg gtgaaatgcg tagatattgg gaggaacacc agtggcgaag gcgactttct
      361 ggcctgtaac tgacgctgag gcgcgaaagc taggggagca aacaggatta gataccctgg
      421 tagtcctagc cgtaaacgat gggtactagg tgtagggggt ttagatgccc tctgtgccgc
      481 agttaacaca
```

//

GenBank accession: KR336958

```
        1 aatgacggta cccttagagg aagccctggc taactacgtg ccagcagccg cggtaatacg
       61 taggggqcta gcgttgtccg gaattactgg gcgtaaaggg cgtgtaggcg gttttttaag
      121 tcagatgtga aagttcgggg ctcaacctcg gaattgcatt tgaaactgga gaacttgagt
      181 actggagagg taagtggaat tcctagtgta gcggtgaaat gcgtagatat taggaggaac
      241 accagtggcg aaggcgactt actggacaga tactgacgct gaggcgcgaa agcgtgggga
      301 gcgaacagga ttagataccc tggtcgtcca cgccgtaaac gatgagtgct aggtgttggg
      361 gcaactcagt gccgtagtta acacaataag cactccg
```

//

GenBank accession: KR336959

```
        1 tgaggaataa gcatcggcta actctgtgcc agcagccgcg gtaagacaga ggatgcaagc
       61 gttatccgga atgattgggc gtaaagcgtc tgtaggtggc ttttcaagtc cgccgtcaaa
      121 tcccagggct caaccctgga caggcggtgg aaactaccaa gctggagtac ggtaggggca
      181 gagggaattt ccggtggagc ggtgaaatgc attgagatcg gaaagaacac caacggcgaa
      241 agcactctgc tgggccgaca ctgacactga gagacgaaag ctaggggagc aaatgggatt
      301 agagacccca gtagtcctag ccgtaaacga tggatactag gtgctgtgcg actcgacccg
      361 tgcagtgctg tagctaacgc gttaag
```

//

GenBank accession: KR336960

```
        1 agcagccgcg gtaatacgta gagtgcaagc gttgtccgga atcattgggc gtaaagggta
       61 cgcaggcgga tatacaagtc aaatgtgaaa ggtgtaggct taacctacag actgcgtttg
      121 aaactgtata tcttgagagc agcagaggaa ggcggaattc ctggtgtagt ggtgaaatac
      181 gtagatatca ggaagaacac cagtggcgaa ggcggtcttc tgggctgcat ctgagcgctc
      241 atgtacgaac gctggggtag ccgaacggga ttrgataccc cggtagtccc aagctgtaaa
      301 cgatggacav tagatgttgg aggttcgaat ccttcagtgc cggag
```

//

GenBank accession: KR336961

```
        1 cagcagccgc ggtaatacgg agggtgcaag cgttattcgg aatcactggg cgtaaagcgc
       61 gcgtaggcgc tgcgcaagtc agatgtgaaa ccctcggcta aaccggggaa ttgcatctga
      121 aactgcgtag ctagagttcg ggagagggcg gcggaactcc cggtgtaggg gtgaaatccg
      181 tagatatcgg gaggaacacc agaggcgaag cggccgcctg gaccgaaact gacgctgagg
      241 cgcgaaagcg tgggtagcaa acaggattag ataccctggt agtccacgcc gtaaacattg
```

```
      301 ggcgctagat gctgggggcg ttagagcttc ggtgtcgt
//
GenBank accession: KR336962
        1 agcagccgcg gtaatacgga ggatccaagc gttatccgga tttattgggt ttaaagggag
       61 cgtaggttga catataagtc agctgtgaaa gtttacggct caaccgtgaa attgcagttg
      121 atactgtatg tcttgagtgt acaagaggtg ggcggaattc gtggtgtagc ggtgaaatgc
      181 ttagatatca cgaagaactc caattgcgaa ggcagctcac tggggtacaa ctgacactga
      241 ggctcgaaag tgtgggtatc aaacaggatt agataccctg gtagtccaca cagtaaacga
      301 tgaatactcg ctgtttgcga tatacagtaa gcggccaagc gaaagcatta agtattccac
      361 ctggggagta cgccggcaa
//
GenBank accession: KR336963
        1 ccgcggtaat acgtaggtgg cgagcgttgt ccggaattac tgggcgtaaa ggatgcgtag
       61 gtggatactt aagtgggatg tgaaattccc gggctcaacc cgggaactgc attccaaact
      121 gggtatctag agtgcaggag aggaaagcgg aattcctagt gtagcggtga aatgcgtaga
      181 tattaggagg aacaccagtg gcgaaggcgg ctttctggac tgtaactgac actgaggcat
      241 gaaagcgtgg gtagcaaaca ggattagata ccctggtagt ccacgccgta aacgatgggt
      301 actaggtgta ggaggtatcg accccttctg tgccgtcgtt aacacaataa gtacccgcct
      361 ggggagtacg gtcgc
//
GenBank accession: KR336964
        1 cagcagccgc ggtaatacgg aggatccaag cgttatccgg aatcattggg tttaaagggt
       61 ccgtaggcgg tttagtaagt cagtggtgaa agcccatcgc tcaacggtgg aacggccatt
      121 gatactgcta aacttgaatt attaggaagt aactagaata tgtagtgtag cggtgaaatg
      181 cttagagatt acatggaata ccaattgcga aggcaggtta ctactaatgg attgacgctg
      241 atggacgaaa gcgtgggtag cgaacaggat tagataccct ggtagtccac gccgtaaacg
      301 atggatacta gctgttgggc gcaatttcag tggctaagcg aaagtgataa gtatccacct
      361 ggggagtacg ttcg
//
GenBank accession: KR336965
        1 cagcagccgc ggtaatacgg aggatccaag cgttatccgg aatcattggg tttaaagggt
       61 ccgtaggcgg tttagtaagt cagtggtgaa agcccatcgc tcaacggtgg aacggccatt
      121 gatactgcta gacttgaatt attaggaagt aactagaata tgtagtgtag cggtgaaatg
```

```
      181 cttagagatt acatggaata ccaattgcga aggcaggtta ctactaatgg attgacgctg
      241 atggacgaaa gcgtgggtag cgaacaggat tagataccct ggtagtccac gccgtaaacg
      301 atggatacta gctgttgggc gcaagttcag tggctaagcg aaagtgataa gtatccacct
      361 ggggagtacg ttcgc
//
GenBank accession: KR336966
        1 cagcagccgc ggtaatacgt agggggcaag cgttatccgg aattactggg cgtaaagggt
       61 gcgtaggcgg tttatcaagt caggggtgaa aggctacggc tcaaccgtag taagcctctg
      121 aaactggtga acttgagtgt aggagaggaa agtggaattc ccagtgtagc ggtgaaatgc
      181 gtagatattg ggaggaacac cagtggcgaa ggcgactttc tggactataa ctgacgctga
      241 ggcacgaaag cgtggggagc aaacaggatt agataccctg gtagtccacg ctgtaaacga
      301 tgagtactag gtgtcggctg tcaaaggtcg gtgccggagt taacacatta agtactccgc
      361 ctgggaagta cgctcgcaa
//
GenBank accession: KR336967
        1 cagcagccgc ggtaatacgt agagtgcaag cgttgtccgg aattattggg cgtaaagggt
       61 acgcaggcgg ataatcaagt caagcgtgaa aggtgtcggc ttaaccgaca gactgcgttt
      121 gaaactggtt atcttgagtg taacagagga gagtggaatt cctagtgtag tggtgaaata
      181 cgtagatatt aggaagaaca ccagtggcga aggcgactct ctgggttaac actgacgctg
      241 aggtacgaaa gctgggggag cgaacgggat tagatacccc ggtagtccca gccgtaaacg
      301 atggatacta ggtgttggag gttcgaatcc ttcagtgccg gagttaacgc attaagtatc
      361 cgcctgggga ttacgatcgc
//
GenBank accession: KU603730
        1 ttggacaatg ggcgcaagcc tgatccagcc atgccgcgtg agtgaagaag gtcttcggat
       61 tgtaaagctc ttttgccagg gacgataatg acggtacctg gagaataagc cccggcaaac
      121 ttcgtgccag cagccgcggt aatacgaagg gggctagcgt tgttcggaat tactgggcgt
      181 aaagcgcacg taggcgggcg attaagtcag gggtgaaatc ccggagctca actccggaac
      241 tgcctttgat actggtcgtc tagagatcgg aagaggtgag tggaattccc agtgtagagg
      301 tgaaattcgt agatattggg aagaacacca gtggcgaagg cggctcactg gtccgatact
      361 gacgctgagg tgcgaaagcg tggggagcaa acaggattag ataccctggt agtccacgcc
      421 gtaaactatg gaagctagcc gtcagtcagc ttgct
//
```

GenBank accession: KU603731

```
        1 cagtggggaa ttttggacaa tgggcgcaag cctgatccag ccatgccgcg tgcgggaaga
       61 aggccttcgg gttgtaaacc gcttttgtca gggaagaaaa ggttctggtt aatacctggg
      121 actcatgacg gtacctgaag aataagcacc ggctaactac gtgccagcag ccgcggtaat
      181 acgtagggtg caagcgttaa tcggaattac tgggcgtaaa gcgtgcgcag gcggttatgc
      241 aagacagagg tgaaatcccc gggctcaacc tgggaactgc ctttgtgact gcatagctag
      301 agtacggtag aggggatgg aattccgcgt gtagcagtga aatgcgtaga tatgcggagg
      361 aacaccgatg gcgaaggcaa tcccctggac ctgtactgac gctcatgcac gaaagcgtgg
      421 ggagcaaaca ggattagata ccctggtagt ccacgcccta aacgatgtca actggttgtt
      481 gggagggttt cttctcagta acgtagctaa cgcg
//
```

GenBank accession: KU603732

```
        1 agtggggaat tttggacaat gggggcaacc ctgatccagc aatgccgcgt gagtgaagaa
       61 ggccctcggg ttgtaaagct cttttgtcag ggaagaaacg gagttctcta atataggatt
      121 ctaatgacgg tacctgaaga ataagcaccg gctaactacg tgccagcagc cgcggtaata
      181 cgtagggtgc aagcgttaat cggaattact gggcgtaaag cgtgcgcagg cggttttgta
      241 attctgatgt gaaatccccg ggctcaacct gggaactgca ttggagactg caaggctaga
      301 gtgtgtcaga ggggggtaga attccacgtg tagcagtgaa atgcgtagag atgtggagga
      361 ataccgatgg cgaaggcagc cccctgggat aacactgacg ctcatgcacg aaagcgtggg
      421 gagcaaacag gattagatac cctggtagtc cacgccctaa acgatgtcta ctagttgtcg
      481 ggacttaatt gtcttggtaa cgcagctaac gcg
//
```

GenBank accession: KU603733

```
        1 cagtggggaa tcttgcgcaa tgggcgaaag cctgacgcag cgacgccgcg tgggggatga
       61 agcttttcgg agtgtaaacc cctttcgact gggaagaatg cccgcaaggg agcgacggta
      121 ccagtagaag aagccccggc taactacgtg ccagcagccg cggtaagacg taggggggca
      181 gcgttgttcg gaattactgg gtgtaaaggg ttcgtaggcg gtgcggcaag ttgggagtga
      241 aatctctggg cttaacccag aggctgcttc caaaactgct gtgcttgagt gtgagagagg
      301 ctcgtggaat tgcaggtgta gcggtgaaat gcgtagatat ctgcaggaac acccgtggcg
      361 aaagcggcga gctggatcac aactgacgct gaggaacgaa agctagggga gcaaacagga
      421 ttagataccc tggtagtcct agccctaaac gatcaggact tggggtgccg cccgttcggg
      481 cgtcgtcccg gagctaacgc g
//
```

GenBank accession: KU603734

```
        1 gggaattttg gacaatgggc gaaagcctga tccagcaatg ccgcgtgtgt gaagaaggcc
       61 ttcgggttgt aaagcacttt tgtccggaaa gaaaacgccg tggttaatac ccgtggcgga
      121 tgacggtacc ggaagaataa gcaccggcta actacgtgcc agcagccgcg gtaatacgta
      181 gggtgcaagc gttaatcgga attactgggc gtaaagcgtg cgcaggcggt ccgctaagac
      241 agatgtgaaa tccccgggct taacctggga actgcatttg tgactggcgg gctagagtat
      301 ggcagagggg ggtagaattc cacgtgtagc agtgaaatgc gtagagatgt ggaggaatac
      361 cgatggcgaa ggcagccccc tgggccaata ctgacgctca tgcacgaaag cgtggggagc
      421 aaacaggatt agataccctg gtagtccacg ccctaaacga tgtcaactag ttgtcgggtc
      481 ttcattgact tggtaacgta gctaacgcg
//
```

GenBank accession: KU603735

```
        1 cagtggggaa tcttgcacaa tgggcgaaag cctgatgcag cgacgccgcg tgggggatga
       61 agcatctcgg tgtgtaaacc cctttcgatt cggacgaatg cccgcaaggg agtgacggta
      121 cggatataag aagccccggc taactacgtg ccagcagccg cggtaagacg taggggggca
      181 gcgttgttcg gaattactgg gtgtaaaggg ttcgtaggcg gtgcggcaag ttgggagtga
      241 aatctctggg cttaacccag aggctgcttc caaaactgct gtgctcgagt gtgggagagg
      301 cgcgtggaat tgcaggtgta gcggtgaaat gcgtagatat ctgcaggaac acccgtggcg
      361 aaagcggcgc gctggaccac tactgacgct gaggaacgaa agctaggggga gcaaacagga
      421 ttagataccc tggtagtcct agccctaaac gatcaggact tggggtgcca cccgttcggg
      481 tgtcgtcccg gagctaacgc g
//
```

GenBank accession: KU603736

```
        1 cagtggggaa tattggacaa tgggcgaaag cctgatccag caatgccgcg tgagtgatga
       61 aggccctagg gttgtaaagc tcttttaccc gggaagataa tgactgtacc gggagaataa
      121 gccccggcta actccgtgcc agcagccgcg gtaatacgga gggggctagc gttgttcgga
      181 attactgggc gtaaagcgca cgtaggcggc tttgtaagtc agaggtgaaa gcctggagct
      241 caactccaga actgcctttg agactgcatc gcttgaatcc aggagaggtc agtggaattc
      301 cgagtgtaga ggtgaaattc gtagatattc ggaagaacac cagtggcgaa ggcggctgac
      361 tggactggta ttgacgctga ggtgcgaaag cgtggggagc aaacaggatt agataccctg
      421 gtagtccacg ccgtaaacga tgataactag ctgtccgggc acttggtgct tgggtggcgc
      481 agctaacgca
//
```

GenBank accession: KU603737

```
        1 ggaattttcc acaatgggcg aaagcctgat ggagcaacgc cgcgtgaacg atgaaggcct
       61 tcgggttgta aagttctgtt gttagggacg aacaagtgcc gttcgaatag ggcggcaccc
      121 tgacggtacc taacgaggaa gccacggcta actacgtgcc agcagccgcg gtaatacgta
      181 ggtggcaagc gttgtccgga attattgggc gtaaagcgcg cgcaggcggt tatgtaagtc
      241 tgatgttaaa gcccggggct caaccccggt tcgcattgga aactgcgtaa cttgagtgca
      301 gaagaggaaa gcggtattcc acgtgtagcg gtgaaatgcg tagagatgtg gaggaacacc
      361 agtggcgaag gcggctttct ggtctgtaac tgacgctgag gcgcgaaagc gtggggagca
      421 aacaggatta gataccctgg tagtccacgc cgtaaacgat gagtgctagg tgttgggggt
      481 ttcaataccc tcagtgccgc agctaacgca
```

//

GenBank accession: KU603738

```
        1 cagtagggaa tattgcgcaa tggacgaaag tctgacgcag ccacgccgcg tgagtgagga
       61 aggccttcgg gttgtaaagc tctgtcgcct gggaaaaacg gctattgggg gaaagttcca
      121 gtagcgtgat ggtaccagga gaggaagcac cggcaaactc tgtgccagca gccgcggtaa
      181 tacagagggt gcaagcgttg ttcggaatta ctgggcgtaa agcgtgcgta gtcggtattg
      241 agagtcgcgg gtgaaatccc agggcttaac cctggaactg cctgcgagac ctcagtacta
      301 gagtgtgaga ggggatagtg gaatacccag tgtagcggtg aaatgcgtag agattgggtg
      361 gaacaccggt ggcgaaggcg gctatctggc tcacaactga cgatcaggca cgaaagcgtg
      421 gggagcaaac aggattagat accctggtag tccacgccgt aaactatgca tacaagctgt
      481 agtgggattt tactcctgct gtggcgaagc taacgca
```

//

GenBank accession: KU603739

```
        1 ggacaatggg cggaagcctg atccagccat gccgcgtgtg tgaagaaggc cttttggttg
       61 taaagcactt taagcgagga ggaggctacc tagattaata ctctgggata gtggacgtta
      121 ctcgcagaat aagcaccggc taactatgtg ccagcagccg cggtaataca gagggtgcga
      181 gcgttaatcg gatttactgg gcgtaaagcg tgcgtaggcg gctgattaag tcggatgtga
      241 aatccctgag cttaacttag gaattgcatt cgatactggt cagctagagt atgggagagg
      301 atggtagaat tccaggtgta gcggtgaaat gcgtagagat ttggaggaat accgatggcg
      361 aaggcagcca tctggcctaa tactgacgct gaggtacgaa agcatgggga gcaaacagga
      421 ttagataccc tggtagtcca tcccgtaaac gatgtttact agccgttggg gcctttgagg
      481 c
```

//

GenBank accession: KU603740

```
        1 cagtggggaa tattggacaa tgggggggaac cctgatccag ccatgccgcg tgtgtgaaga
       61 aggccttatg gttgtaaagc actttaagcg aggaggaggc tactgagatt aatactcttg
      121 gatagtggac gttactcgca gaataagcac cggctaactc tgtgccagca gccgcggtaa
      181 tacagagggt gcgagcgtta atcggattta ctgggcgtaa agcgtgcgta ggcggctttt
      241 taagtcggat gtgaaatccc cgagcttaac ttgggaattg cattcgatac tgggaagcta
      301 gagtatggga gaggatggta gaattccagg tgtagcggtg aaatgcgtag agatctggag
      361 gaataccgat ggcgaaggca gccatctggc ctaatactga cggtgaggta cgaaagcatg
      421 gggagcaaac aggattagat accctggtag tccatgccgt aaacgatgtc tactagccgt
      481 tggggccttt gaggctttag tggcgcagct aacgcg
//
```

GenBank accession: KU603741

```
        1 cagtggggaa tattggacaa tgggggggaac cctgatccag ccatgccgcg tgtgtgaaga
       61 aggccttctg gttgtaaagc actttaagcg aggaggaggc tacttagatt aatactcttg
      121 gatagtggac gttactcgca gaataagcac cggctaactc tgtgccagca gccgcggtaa
      181 tacagagggt gcgagcgtta atcggattta ctgggcgtaa agcgtgcgta ggcggctgat
      241 taagtcggat gtgaaatccc tgagcttaac ttaggaattg cattcgatac tggtcagcta
      301 gagtatggga gaggatggta gaattccagg tgtagcggtg aaatgcgtag agatctggag
      361 gaataccgat ggcgaaggca gccatctggc ctaatactga cgctgaggta cgaaagcatg
      421 gggagcaaac aggattagat accctggtag tccatgccgt aaacgatgtc tactagccgt
      481 tggggccttt gaggctttag tggcgcagct aacgcg
//
```

GenBank accession: KU603742

```
        1 cagtggggaa tattggacaa tgggggcaac cctgatccag ccatgccgcg tgaatgatga
       61 aggccttcgg gttgtaaagt tcttttggcg gggacgatga tgacggtacc cgcagaataa
      121 gctccggcta acttcgtgcc agcagccgcg gtaatacgaa gggagctagc gttgttcgga
      181 tttactgggc gtaaagggcg cgtaggcggt ttagtaagtt ggatgtgaaa gccctgggct
      241 caacccggga attgcattca aaactacttt actcgaattc ggtagaggtt ggtagaattc
      301 ccagtgtaga ggtgaaattc gtagagattg ggaagaatac ccgtggcgaa ggcggccaac
      361 tggaccgaca ttgacgctga ggcgcgaaag cgtgggtagc aaacaggatt agataccctg
      421 gtagtccacg ccgtaaacga tgcatgctag acgttaaaac catcatggtt ttagtgtcgc
      481 agctaacgca
//
```

GenBank accession: KU603743

```
        1 cagtggggaa tcttccgcaa tgggcgaaag cctgacggag caatgccgcg tgagcgaaga
       61 aggccttcgg gtcgtaaagc tatgtccagg gggaagaaca aatgacggta cccctgtacg
      121 agccccggct aactacgtgc cagcagccgc ggtaacacgt agggggcaag cgttgtccgg
      181 aattactggg cgtaaagcgc gcgtaggcgg ccttataagt cagtggtgaa aaccggcggc
      241 tcaaccgcag gcttgcctct gaaactgtaa ggcttgagga caggagagga gagtggaatt
      301 cccagtgtag cggtgaaatg cgtagatatt gggaggaaca ccagtggcga aggcgactct
      361 ctggcctgca cctgacgctg aggcgcgaaa gcgtggggat caaacaggat tagataccct
      421 ggtagtccac gccgtaaacg atgggtgcta ggtgttgggg gtgtaga
//
```

GenBank accession: KU603744

```
        1 cagtggggaa ttttggacaa tgggggcaac cctgatccag caatgccgcg tgtgtgaaga
       61 aggccttcgg gttgtaaagc acttttgtcc ggaaagaaaa cttcggggct aataccccctg
      121 gaggatgacg gtaccggaag aataagcacc ggctaactac gtgccagcag ccgcggtaat
      181 acgtagggtg cgagcgttaa tcggaattac tgggcgtaaa gcgtgcgcag gcggtgctgt
      241 aagaccgatg tgaaatcccc gggcttaacc tgggaactgc attggtgact gcagcgctgg
      301 agtatggcag agggggggtgg aattccacgt gtagcagtga aatgcgtaga gatgtggagg
      361 aacaccgatg gcgaaggcag ccccctgggc caatactgac gctcatgcac gaaagcgtgg
      421 ggagcaaaca ggattagata ccctggtagt ccacgcccta aacgatgtca actggttgtc
      481 gggccttcat tggcttggta acgtagctaa cgcg
//
```

GenBank accession: KU603745

```
        1 cagtggggaa ttttggacaa tgggggcaac cctgatccag caatgccgcg tgtgtgaaga
       61 aggccttcgg gttgtaaagc acttttgtcc gggaagaaat cgcacctgat aataccgggt
      121 gtggatgacg gtaccggaag aataaggacc ggctaactac gtgccagcag ccgcggtaat
      181 acgtagggtc caagcgttaa tcggaattac tgggcgtaaa gcgtgcgcag gcggttgtgc
      241 aagaccgatg tgaaatcccc gggcttaacc tgggaattgc attggtgact gcacggctag
      301 agtgtgtcag agggggggtag aattccacgt gtagcagtga aatgcgtaga gatgtggagg
      361 aataccgatg gcgaaggcag ccccctggga taacactgac gctcatgcac gaaagcgtgg
      421 ggagcaaaca ggattagata ccctggtagt ccacgcccta aacgatgtca actagttgtt
//
```

GenBank accession: KU603746

```
        1 cagtggggaa ttttccgcaa tgggcgcaag cctgacggag caacgccgcg tgggtgacga
```

```
       61 aggatttcgg tctgtaaagc cctttcagtc gggacgagtc gcagacggta ccgacagaag
      121 aagcatcggc taactacgtg ccagcagccg cggtaagacg taggatgcga gcgttgtccg
      181 gaattattgg gcgtaaagcg tacgtaggcg gtcatctaag tctggggtta aagaccaagg
      241 ctcaaccttg ggagtgccct ggatactggg tgactggagg acggcagagg aaagtggaat
      301 tcccagtgta gcggtgaaat gcgtagatat tgggaggaac accagtggcg aaagcgactt
      361 tctgggccgt tcctgacgct gaggtacgaa agccagggga gcaaacggga ttagataccc
      421 cggtagtcct ggccgtaaac gatggatact aggtgtcatc ggtatcaacc ccggtggtgc
      481 cgtagctaac gcg
//
GenBank accession: KU603747
        1 cagtggggaa ttttggacaa tgggcgaaag cctgatccag ccatgccgcg tgcaggatga
       61 aggccttcgg gttgtaaact gcttttgtac ggaacgaaac ggccttttct aataaagagg
      121 gctaatgacg gtaccgtaag aataagcacc ggctaactac gtgccagcag ccgcggtaat
      181 acgtagggtg caagcgttaa tcggaattac tgggcgtaaa gcgtgcgcag gcggttatgt
      241 aagacagttg tgaaatcccc gggctcaacc tgggaactgc atctgtgact gcatagctag
      301 agtacggtag aggggggatgg aattccgcgt gtagcagtga aatgcgtaga tatgcggagg
      361 aacaccgatg gcgaaggcaa tcccctggac ctgtactgac gctcatgcac gaaagcgtgg
      421 ggagcaaaca ggattagata ccctggtagt ccacgcccta aacgatgtca actggttgtt
      481 gggtcttcac tgactcagta acgaagctaa cgcg
//
GenBank accession: KU603748
        1 cagtggggaa ttttacgcaa tgggggaaac cctgacgtag cgacaccgcg tgagcgaaga
       61 agccctttgg ggtgtaaagc tctgtcagca ggaacgaata aaatgacggt acctgcagag
      121 gaagcatcgg ctaactacgt gccagcagcc gcggtaagac gtaggatgca agcgttgtcc
      181 ggatttattg ggcgtaaaga gttcgtaggc ggtttgttaa gtttgatgtt aaagatcggg
      241 gcctaaccct gggagtgcat tgaatactgg cagactggag tgcagtagag gcgagtggaa
      301 ttcccagtgt agcggtgaaa tgcgtagata ttgggaagaa caccggtggc gcaggcgact
      361 cgctgggctg taactgacgc tgaggaacga aagctagggg agcaaatggg attagatacc
      421 ccagtagtcc tagctgtaaa cgatggatac taggcgttgc aggtatcgac ccctgcagtg
      481 ccgcagttaa cgcg
//
GenBank accession: KU603749
        1 cagtggggaa ttttggacaa tgggggcaac cctgatccag caatgccgcg tgtgtgaaga
```

```
       61 aggccttcgg gttgtaaagc acttttgtcc gggaagaaat ccttttcgat aataccgaag
      121 ggggatgacg gtaccggaag aataagcacc ggctaactac gtgccagcag ccgcggtaat
      181 acgtagggtg cgagcgttaa tcggaattac tgggcgtaaa gcgtgcgcag gcggtgatgt
      241 aagaccgatg tgaaatcccc gggcttaacc tgggaactgc attggtgact gcatcgctgg
      301 agtatggcag agggggggtag aattccacgt gtagcagtga aatgcgtaga gatgtggagg
      361 aataccgatg gcgaaggcag ccccctgggc caatactgac gctcatgcac gaaagcgtgg
      421 ggagcaaaca ggattagata ccctggtagt ccacgcccta aacgatgtca actggttgtc
      481 gggccttcat
//
GenBank accession: KU603750
        1 gaagaataag caccggctaa ctacgtgcca gcagccgcgg taatacgtag ggtgcgagcg
       61 ttaatcggaa ttactgggcg taaagcgtgc gcaggcggtt atgcaagaca gatgtgaaat
      121 ccccgggctt aacctgggaa ctgcatttgt gactgcatag ctggagtgcg gcagaggggg
      181 atggaattcc gcgtgtagca gtgaaatgcg tagatatgcg gaggaacacc gatggcgaag
      241 gcaatctcct gggcctgcac tgacgctcat gcacgaaagc gtggggagca aacaggatta
      301 gataccctgg tagtccacgc cctaaacgat gtcaactggt tgt
//
GenBank accession: KU603751
        1 cagtggggaa tattgcacaa tgggcggaag cctgatgcag caacgccgcg tgcgggatga
       61 cggccttcgg gttgtaaacc gctttcgcct gtgacgaagc gtgagtgacg gtaatgggta
      121 aagaagcacc ggctaactac gtgccagcag ccgcggtgat acgtagggtg cgagcgttgt
      181 ccggatttat tgggcgtaaa gggctcgtag gtggttgatc gcgtcggaag tgtaatcttg
      241 gggcttaacc ctgagcgtgc tttcgatacg ggttgacttg aggaaggtag gggagaatgg
      301 aattcctggt ggagcggtgg aatgcgcaga tatcaggagg aacaccagtg gcgaaggcgg
      361 ttctctgggc ctttcctgac gctgaggagc gaaagcgtgg ggagcgaaca ggcttagata
      421 ccctggtagt ccacgctgta aacggtgggt actaggtgtg gggtccattc cacgggttcc
      481 gtgccgtagc taacgc
//
GenBank accession: KU603752
        1 cagtggggaa tattggacaa tgggcgcaag cctgatccag caatgccgcg tgagtgatga
       61 aggccttagg gttgtaaagc tcttttacca gggatgataa tgacagtacc tggagaataa
      121 gctccggcta actccgtgcc agcagccgcg gtaatacgga gggagccagc gttgtttgga
      181 attactgggc gtaaagcgcg cgtaggcggt tactcaagtc agaggtgaaa gcccggggct
```

```
      241 caaccccgga actgcctttg aaactaggtg actagaatct tggagaggtc agtggaattc
      301 cgagtgtaga ggtgaaattc gtagatattc ggaagaacac cagtggcgaa ggcgactgac
      361 tggacaagta ttgacgctga ggtgcgaaag cgtggggagc aaacaggatt agataccctg
      421 gtagtccacg ccgtaaacga tgataactag ctgtc
//
GenBank accession: KU603753
        1 cagtggggaa tattgcacaa tgggcgcaag cctgatgcag ccatgccgcg tgtgtgaaga
       61 aggccttcgg gttgtaaagc actttcagcg gggaggaagg cggtacggtt aataaccgtg
      121 ccgattgacg ttacccgcag aagaagcacc ggctaactcc gtgccagcag ccgcggtaat
      181 acggagggtg caagcgttaa tcggaattac tgggcgtaaa gcgcacgcag gcggtctgtc
      241 aagtcggatg tgaaatcccc gggctcaacc tgggaactgc attcgaaact ggcaggctgg
      301 agtctcgtag agggaggtag aattccaggt gtagcggtga aatgcgtaga gatctggagg
      361 aataccggtg gcgaaggcgg cctcctggac gaagactgac gctcaggtgc gaaagcgtgg
      421 ggagcaaaca ggattagata ccctggtagt ccacgccgta aacgatgtcg atttggaggt
      481 t
//
GenBank accession: KU603754
        1 cagtggggaa tattggacaa tgggcgcaag cctgatccag ccatgccgcg tgagtgatga
       61 aggccctagg gttgtaaagc tctttcaccg gtgaagataa tgacggtaac cggagaagaa
      121 gccccggcta acttcgtgcc agcagccgcg gtaatacgaa gggggctagc gttgttcgga
      181 tttactgggc gtaaagcgca cgtaggcgga ctattaagtc aggggtgaaa tcccggggct
      241 caaccccgga actgcctttg atactggtag tcttgagttc gagagaggtg agtggaattc
      301 cgagtgtaga ggtgaaattc gtagatattc ggaggaacac cagtggcgaa ggcggctcac
      361 tggctcgata ctgacgctga ggtgcgaaag cgtggggagc aaacaggatt agataccctg
      421 gtagtccacg ccgtaaacta tgagagctag ccgtcgggca gtatactgtt cggtggcgca
      481 gcaaacgca
//
GenBank accession: KU603755
        1 gaaagcctga tccagcaatg ccgcgtgtgt gaagaaggcc ttcgggttgt aaagcacttt
       61 tgtccggaaa gaaatggctc tggttaatac ctggggtgga tgacggtacc ggaagaataa
      121 ggaccggcta actacgtgcc agcagccgcg gtaatacgta gggtccaagc gttaatcgga
      181 attactgggc gtaaagcgtg cgcaggcggt tgtgcaagac cgatgtgaaa tccccgagct
      241 taacttggga attgcattgg tgactgcacg tctagagtgt gtcagagggg ggtagaattc
```

```
      301 cacgtgtagc agtgaaatgc gtagagatgt ggaggaatac cgatggcgaa ggcagccccc

      361 tgggataaca ctgacgctca tgcacgaaag cgtggggagc aaacaggatt agataccctg

      421 gtagtccacg ccctaaacga tgtcaactag ttgt

//

GenBank accession: KU603756

        1 cagtggggaa tcttccgcaa tgggcgcaag cctgacggag caacgccgcg tgagcgaaga

       61 aggccttcgg gttgtaaagc tctgtgtagg gggaagaaac aaatgacggt acccctttag

      121 aaagccccgg ctaactacgt gccagcagcc gcggtaaaac gtaggggcga agcgttgtcc

      181 ggaattactg ggcgtaaagg gcgtgtaggc ggcaaagtaa gtccatagtg aaacccggca

      241 gctcaactgc cggctggcta aggaaactgt tttgctctga gtgcaggaga ggggagtgga

      301 attcccagtg tagcggtgaa atgcgtagat attgggagga acaccagtgg cgaaagcggc

      361 tccctggcct gtaactgacg ctgaggcgcg aaagcgtggg tagcaaacag gattagatac

      421 cctggtagtc cacgccgtaa acgatgggtg ctaggtgttg tggg

//

GenBank accession: KU603757

        1 cagtggggaa tattggacaa tgggcgcaag cctgatccag ccatgccgcg tgagtgatga

       61 aggccctagg gttgtaaagc tctttcaacg gtgaagataa tgacggtaac cgtagaagaa

      121 gccccggcta acttcgtgcc agcagccgcg gtaatacgaa gggggctagc gttgttcgga

      181 attactgggc gtaaagcgca cgtaggcgga tacttaagtc aggggtgaaa tcccggggct

      241 caaccccgga actgcctttg atactgggta tctggagtcc ggaagaggtg agtggaattc

      301 cgagtgtaga ggtgaaattc gtagatattc ggaggaacac cagtggcgaa ggcggctcac

      361 tggtccggta ctgacgctga ggtgcgaaag cgtggggagc aaacaggatt agataccctg

      421 gtagtccacg ccgtaaacga tggaagctag ccgttggcaa gtttactgtc ggtggcgcag

      481 ctaacgc

//

GenBank accession: KU603758

        1 cagtggggaa tcttccgcaa tgggcgaaag cctgacggag caacgccgcg tgagcgaaaa

       61 aggccttcgg gttgtaaagc tctgtgatat cagacgaacg gtcttagagt gaataacact

      121 aagacatgac ggtatggtaa gaggaagccc cggctaacta cgtgccagca gccgcggtaa

      181 tacgtagggg gcaaacgttg tccggaatga ctgggcgtaa agggcgagta ggtggtttgc

      241 caagtcagga gtgaaaactc cgggctcaac ctggagattg ctactgaaac tggcagactt

      301 gagtgcagga gagggaagcg gaattcctag tgtagcggtg aaatgcgtag atattaggag

      361 gaacaccagt ggcgaaggcg gcttcctggc ctgtaactga cactgaggcg cgaaagcgtg
```

```
      421 gggagcgaac aggattagat accctggtag tccacgccgt aaacgatgag tgctaggtgt
      481 tgggggtatc gacccccctca gtgccgcagt aaacgca
//
GenBank accession: KU603759
        1 cagtggggaa ttttggacaa tgggcgaaag cctgatccag ccatgccgcg tgcaggatga
       61 aggccttcgg gttgtaaact gcttttgtac ggaacgaaaa ggtttggcct aataagctga
      121 gctcatgacg gtaccgtaag aataagcacc ggctaactac gtgccagcag ccgcggtaat
      181 acgtagggtg cgagcgttaa tcggaattac tgggcgtaaa gcgtgcgcag gcggttatgt
      241 aagacagatg tgaaatcccc gggctcaacc tgggaactgc attagtgact gcatagctgg
      301 agtacggcag agggggatgg aattccgcgt gtagcagtga aatgcgtaga tatgcggagg
      361 aacaccgatg gcgaaggcaa tcccctgggc ctgtactgac gctcatgcac gaaagcgtgg
      421 ggagcaaaca ggattagata ccctggtagt ccacgcccta aacgatgtca actggttgtt
      481 gggtcttcac tgactcagta acgaagctaa cgcg
//
GenBank accession: KU603760
        1 cagtggggaa tattgcacaa tgggcgcaag cctgatgcag cgacgccgcg tgggggatga
       61 cggccttcgg gttgtaaacc tctttcgaca gggacgaagc tttggtgacg gtacctgtat
      121 aagaagcacc ggctaactac gtgccagcag ccgcggtaat acgtagggtg cgagcgttgt
      181 ccggaattac tgggcgtaaa gagctcgtag gtggtttgtc gcgtcgtctg tgaaattccg
      241 gggcttaact tcgggcgtgc aggcgatacg ggcataactt gagtgctgta ggggagactg
      301 gaattcctgg tgtagcggtg gaatgcgcag atatcaggag gaacaccgat ggcgaaggca
      361 ggtctctggg cagtaactga cgctgaggag cgaaagcatg gggagcgaac aggattagat
      421 accctggtag tccatgccgt aaacggtggg cgctaggtgt aggggtcttc cacgacttct
      481 gtgccgtagc taac
//
GenBank accession: KU603761
        1 cagtggggaa tattggacaa tgggcgcaag cctgatccag caatgccgcg tgtgtgaaga
       61 aggccttcgg gttgtaaagc acttttatca ggagcgaaat accatcggct aatacccggt
      121 ggggctgacg gtacctgagg aataagcacc ggctaacttc gtgccagcag ccgcggtaat
      181 acgaagggtg caagcgttaa tcggaattac tgggcgtaaa gcgtgcgtag gcggtttttt
      241 aagtctgctg tgaaatcccc gggctcaacc tgggaatggc agtggatact ggaaagctag
      301 agtgtgatag aggatggtgg aattcccggt gtagcggtga aatgcgtaga gatcgggagg
      361 aacatcagtg gcgaaggcgg ccatctggat caacactgac gctgaggcac gaaagcgtgg
```

421 ggagcaaaca ggattagata ccctggtagt ccacgcccta aacgatgcga actggatgtt

481 ggtctcaact cggagatcag tgtcgaagct aacgcg

//

GenBank accession: KU603762

1 agtggggaat attggacaat gggggcaacc ctgatccagc aatgccgcgt gtgtgaagaa

61 ggccttcggg ttgtaaagca ctttcagtag ggaggaaaag cttgatgcta ataccattga

121 gtcttgacgt tacttacaga agaagcaccg gctaactctg tgccagcagc cgcggtaata

181 cagagggtgc aagcgttaat cggaattact gggcgtaaag ggcgcgtagg tggtttggta

241 agttggatgt gaaatccccg ggctcaacct gggaatggca ttcaatactg cctcactaga

301 gtatggtaga gggaagcgga attccacatg tagcggtgaa atgcgtagat atgtggagga

361 acatcaatgg cgaaggcagc ttcctggacc aatactgaca ctgaggcgcg aaagcgtggg

421 tagcaaacag gattagatac cctggtagtc cacgccctaa acgatgagaa ctagatgttg

481 ggaagtatat cttcttagtg tcgcagctaa cgcg

//

GenBank accession: KU603763

1 cagtggggaa tattgcacaa tgggcgaaag cctgatgcag cgacgccgcg tgagggatgg

61 aggccttcgg gttgtaaacc tctttcggca gggaagaagc gttaagtgac ggtacctgca

121 gaagaagcgc cggctaacta cgtgccagca gccgcggtaa tacgtagggc gcaagcgttg

181 tccggaatta ttgggcgtaa agagctcgta ggtggtttgt cgcgtctggt gtgaaatccc

241 ggggcttaac ttcgggcgtg catcgggtac gggcaggcta gagtgcggta ggggagactg

301 gaattcctgg tgtagcggtg gaatgcgcag atatcaggag gaacaccgat ggcgaaggca

361 ggtctctggg ccgcaactga cgctgaggag cgaaagcatg gggagcgaac aggattagat

421 accctggtag tccatgccgt aaacgttggg cactaggtgt ggggcctatt ccacgggttc

481 cgtgccgtag ctaacgca

//

GenBank accession: KU603764

1 cagtggggaa ttttggacaa tgggcgcaag cctgatccag ccatgccgcg tgcaggatga

61 aggccttcgg gttgtaaact gcttttgtac ggaacgaaag gttctgccct aatacggtgg

121 gaatatgacg gtaccgtaag aataagcacc ggctaactac gtgccagcag ccgcggtaat

181 acgtagggtg cgagcgttaa tcggaattac tgggcgtaaa gcgtgcgcag gcggttatgt

241 aagacagatg tgaaatcccc gggctcaacc tgggaactgc attagtgact gcatagctgg

301 agtacggcag aggggatgg aattccgcgt gtagcagtga aatgcgtaga tatgcggagg

361 aacaccgatg gcgaaggcaa tcccctgggc ctgtactgac gctcatgcac gaaagcgtgg

```
      421 ggagcaaaca ggattagata ccctggtagt ccacgcccta aacgatgtca actggttgtt

      481 gggtcttcac tgactcagta acgaagctaa cgcg

/
```

APPENDIX II

SEM images of rock samples after long-term experiments. Analyses performed by Dr. M. Kasina (in Pellizzari et al. 2017). a. Partially dissolved feldspar grain (StF 4-W); b. Clay minerals formed on the surface of a partially dissolved feldspar grain (StF 4-W); c. Biofilm-like structures produced on the partially dissolved feldspar grain (StF 4-W); d. Rounded precipitates composed of Fe-ox (ExF 6-W); e. and f. Hair-like structures similar to biofilms covering the mineral surface (StF 1-W).