

Influence of drill mud on the microbial communities of sandstone rocks and well fluids at the Ketzin pilot site for CO₂ storage

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Received: 27 May 2016 / Accepted: 26 December 2016 / Published online: 11 January 2017
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Abstract At a pilot site for CO₂ storage in Ketzin (Germany), a drastic decrease in injectivity occurred in a well intended for injection. This was attributed to an obstruction of the pore throats due to microbial degradation of the organic drill mud and subsequent iron sulfide (FeS) precipitation in the highly saline brine (240 g L⁻¹). To better understand the biogeochemical processes, the response of the autochthonous microbial community to drill mud exposure was investigated. Pristine cores of two aquifers with different salinity were incubated under simulated in situ conditions (50 bar, 40 °C and 45 bar, 25 °C, respectively) and CO₂ atmosphere. For the first time, rock cores obtained from the CO₂ plume of the storage formation were investigated. The influence of acetate as a biodegradation product of drill mud polymers and the effectiveness of a biocide were additionally tested. Increased microbial diversities were observed in all long-term (8–20 weeks) incubations, even including biocide. Biofilm-like structures and small round-shaped minerals of probable microbiological origin were found. The results indicate that the microbial community remains viable after long-term CO₂ exposure. Microorganisms hydrolyzing

cellulose polymers (e.g., *Burkholderia* spp., *Variovorax* spp.) biodegraded organic components of the drill mud and most likely produced low molecular weight acids. Although the effects of drill mud were less strong as observed in situ, it was demonstrated that acetate supports the growth of sulfate-reducing bacteria (i.e., *Desulfotomaculum* spp.). The microbial-induced precipitation of amorphous FeS reduced the injectivity in the near-well area. Therefore, when using organic drill mud, the well must be cleaned intensively to minimize the hazards of bacterial stimulation.

Keywords Drill mud · CO₂ storage · Reduced injectivity · Sulfate-reducing bacteria

Introduction

Planning of subsurface use requires consideration of thermal, hydraulic, mechanical and chemical effects for efficient geotechnical use of geological reservoirs (Bauer et al. 2013). During drilling and coring of deep boreholes, the use of drill mud is necessary to lubricate the drill bit, transport cuttings to the surface and stabilize and maintain bottom-hole pressure. To preserve the environment, drill muds are composed of biodegradable ingredients. Drill mud can influence natural conditions and the geochemical equilibrium of rock formation (Zettlitzer et al. 2010). Organic constituents of the drill fluids may promote bacterial growth by acting as energy and carbon sources (Zettlitzer et al. 2010; Struchtemeyer et al. 2011). Consequently, proliferation of microorganisms can damage the rock formation or the filter screens of the well and lower injection rates by blocking the pores through production of biofilms or promoting mineral precipitation

This article is part of a Topical Collection in Environmental Earth Sciences on 'Subsurface Energy storage.' Guest edited by Sebastian Bauer, Andreas Dahmke and Olaf Kolditz.

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(Rosnes et al. 1991; Lappan and Fogler 1992; Spark et al. 2000; Schwartz et al. 2009; Zettlitzer et al. 2010; Jaiswal et al. 2014). Bacteria can provoke precipitation of clay minerals, silicates and carbonates (Douglas and Beveridge 1998; Deng et al. 2010), but currently the most discussed effect on oil- and gas-producing formations is the precipitation of metal sulfides (e.g., pyrite) induced by sulfate-reducing bacteria (SRB) (Giangiaco and Dennis 1997; Spark et al. 2000).

In 2007, three deep wells (between 700 and 800 m) were drilled into the Stuttgart Formation (here named StF) (Prevedel et al. 2009), the saline aquifer target for CO₂ injection at Europe's longest-operating onshore pilot site for CO₂ storage, located in the North German Basin near the city of Ketzin (Würdemann et al. 2010). A water-based CaCO₃/bentonite/organic polymer drill mud containing carboxymethylcellulose (CMC) and the tracer fluorescein was used for drilling (Wandrey et al. 2010). For the completion phase, a swelling rubber packer technology requiring the use of fresh water was used to facilitate stage cementing. Hydraulic tests performed 12 weeks after well completion revealed that the injectivity was much lower than expected (approximately 0.07 mD) (Wiese et al. 2010). The low well injectivity was attributed to amorphous iron sulfide, which blocked the pore throats (Zettlitzer et al. 2010). The iron sulfide was generated by the reaction of dissolved iron with hydrogen sulfide, a product of the metabolism of SRB (Morozova et al. 2010). Despite the use of biocide in the drill mud formulation, the remaining organic polymers acted as organic substrates or nutrients for the bacterial community, whose activity was favored by a reduced salinity due to fresh water injection during the completion phase. A gas lift was performed to back-produce the well and to remove the blocking material. The injection of gas reduces the density of the fluids in the tubing, lowering the pressure at the bottom of the well (Fleshman and Obren-Likic 1999). For the lift, nitrogen (N₂) was chosen over air to avoid corrosion and oxidation of dissolved ferrous ions (Zettlitzer et al. 2010). Analyses of the fluid revealed that the content of suspended solids (mainly iron sulfide), total organic carbon (TOC) and fluorescein drastically decreased from the beginning to the end of the lift, indicating the gradual removal of the blocking material. High TOC content has been linked to the organic components of drill mud. The highest TOC measured was 380 mg L⁻¹ (Zettlitzer et al. 2010), of which 50% was acetate. The high amount of acetate suggested that the organic drill mud was microbiologically degraded. High cell numbers and the presence of SRB, as well as a reduced sulfate content in the samples with a high content of iron sulfide (Zettlitzer et al. 2010; Morozova et al. 2010), supported this assumption. Downhole sampling performed before and after the N₂ lift showed

drastically decreased cell numbers of SRB due to the cleaning procedure (Morozova et al. 2010; Pellizzari et al. 2016).

Struchtemeyer et al. (2011) observed that the microbial community in drill water almost completely changed after incubation with autoclaved drill mud. This demonstrated that the drill mud had a significant impact on the microbial populations that are introduced into natural gas wells during drilling. In the present study, laboratory experiments were performed to better understand the influence of CO₂ exposure and drill mud on the autochthonous microbial communities of sandstone rocks and well fluids at the Ketzin pilot site. It should be figured out whether the drill mud components act as an energy source for the autochthonous microbial community supporting microbial growth and sulfate reduction. The goal of this investigation was to get insights into the causal chain which provoked the injectivity loss. Shifts in the autochthonous microbial community of pristine rock cores, as a consequence of incubation with organic drill mud under simulated in situ conditions, were investigated by means of genetic fingerprinting (denaturing gradient gel electrophoresis, DGGE). Mineralogical analyses (field emission scanning electron microscope, FE-SEM) were applied to investigate the changes due to CO₂ exposure and the microbial interaction with the rock and fluid components.

Materials and methods

Sampling of rock cores and well fluid

Rock cores used in the laboratory experiments were acquired during two different coring campaigns at the CO₂ storage site in Ketzin. Pristine StF rock cores were collected in 2012, during the coring of the deep (~700 m) well CO₂ Ktzi 203/2012 (short name: Ktzi 203) across the storage formation (Martens et al. 2014). Pristine Exter Formation (here named ExF) rocks (first aquifer above the StF caprock) were acquired in 2011, during the drilling of the shallow (~450 m) hydraulic and geochemical monitoring well Hy Ktzi P300/2011 (short name: Ktzi P300) (Pellizzari et al. 2013).

The core sections selected for microbiological analyses were collected directly after recovery and stored in aluminum bags under N₂ atmosphere. To obtain pristine rock cores, the drill mud was labeled with fluorescein, and the influenced outer part of the core was removed through inner coring. Only uncontaminated rock core samples were used in the laboratory experiments. The drill mud contamination control using fluorescein and the preparation of pristine inner core plugs are explained in detail in Pellizzari et al. (2013). The microbial baseline characterization of the

ExF rock cores (ExF 4-BL, ExF 6-BL and ExF 9-BL) is included in Pellizzari et al. (2016) and is compared to the results of this work in the discussion part.

Fresh well fluid was obtained via downhole sampling from the Ktzi 201 injection well after the injection operations were completed. The fluid was collected in October 2013, at a depth of 675 m using a cleaned flow through sampler and double ball liner sampler. The fluid was then aseptically transferred to sterilized, cooled glass vessels and transported to the laboratory.

Experimental setup

Immediately after inner coring, each pristine rock core (ExF 4-BL; ExF 6-BL; ExF 9-BL; StF 1-BL; StF 3-BL; StF 4-BL) was placed in a high-pressure vessel. Because no fresh well fluid was available directly after the coring campaign, two synthetic brines (one for ExF and one for StF, Table 1) were sterilized by autoclaving and incubated with the rock cores. The ExF samples, which were stored under in situ pressure and temperature conditions (45 bar, 25 °C), were initially maintained under a N₂ atmosphere. After an equilibrium phase of six months, the gas was changed to CO₂. The in situ pressure for StF, 70–75 bar at a depth of 700 m, could not be reproduced due to technical reasons; therefore, the StF setups were incubated at 50 bar at the in situ temperature (40 °C). The StF samples were incubated in a pure CO₂ atmosphere, without the intermediate N₂ incubation, because at the time of coring, after four years of storage operations, the plume of injected CO₂ likely already reached the rock formation in the near-well area (Bergmann et al. 2012). After one year of incubation

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

KCl/CaCO₃/CMC-based drill mud was synthesized according to the composition of the drill mud used in Ketzin during the 2007 coring campaign (Table 2). The biocide used (M-I Cide, ingredient name: 1,3,5-triazine-1,3,5-(2H,4H,6H)-triethanol) is non-bioaccumulating and completely biodegradable, according to the OECD 306 test (biodegradability in seawater) (www.oilfield-biocides.com). Before being used for the experiments, the drill mud (and the acetate solution) was autoclaved.

Six high-pressure vessels (three for StF and three for ExF) were employed for the incubation experiments. A total of ten different setups were established (Table 3). The first six experiments involved the incorporation of laboratory-made drill mud using a standard formulation (StF 4-S; ExF 4-S; ExF 6-S) or a formulation that excluded the biocide component (StF 3-S; ExF 9-S) or simply acetate (StF 1-S) mixed with synthetic brine. The acetate concentration (190 mg L⁻¹) was chosen based on the in situ concentration measured at the beginning of the N₂ lift. After 8–16 weeks of incubation, the vessels were opened, 10–20 g of rock was sampled, and the brine and mud (or acetate) mixture (75–170 mL) was removed and analyzed. Four additional experiments with fresh well fluid were performed with the rock samples that had been previously incubated. 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. 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 the well-fluid chemical composition on the microbial community inhabiting the rock. After 15–20 weeks, rock and fluid samples were collected and analyzed.

Table 1 Composition of the synthetic brines

Formation	Compound	Conc. (g L ⁻¹)
Exter	NaCl	53.15
	K ₂ SO ₄	0.22
	CaCl ₂	2.81
	MgSO ₄	1.53
	KCl	0.18
	K ₂ HPO ₄	0.01
Stuttgart	NaCl	216.56
	KCl	0.66
	MgCl ₂ , 6H ₂ O	6.19
	CaCl ₂ , 2H ₂ O	7.27
	SrCl ₂ , 6H ₂ O	0.13
	Na ₂ SO ₄ , 10H ₂ O	14.41
	NaBr	0.05

The composition of the ExF brine was chosen according to the chemical measurements of the Exter fluid retrieved from the Ktzi 117-63 observation well in the Ketzin area (Pchalek et al. 1964). StF brine was synthesized as described in Pellizzari et al. (2013)

Table 2 Composition of the synthetic drill mud used for the long-term experiments

Component	Ingredient	Amount
Water	Water	920 mL
Sodium carboxymethyl cellulose	CMC-LV	25 g
Potassium chloride	KCl	150 g
Calcium carbonate	M-I Cal SL	60 g
Biocide	M-I Cide	1 mL
Xanthan gum	XC polymer	2 g

Ingredients and a simplified formulation were provided by Mi SWACO Deutschland GmbH (Celle, Germany), the company responsible for the drill mud in the field during the 2007 coring campaign

Table 3 Experimental setup for the long-term experiments of pristine rock core incubation with synthetic brine (blue) and fresh collected well fluid (yellow)

Formation	Incubation with synthetic brine					Incubation with well fluid				
	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 (190 mg L ⁻¹)					Addition of acetate (190 mg L ⁻¹)				
Stuttgart	StF 1-S	280	-	170	16	StF 1-W	260	-	160	20

* Fluid was filtrated

DNA extraction

Rock samples were crushed into small pieces using sterilized hammer and chisel and subjected to cryogenic grinding using a laboratory mill (Mixer Mill MM 400 from Retsch GmbH, Germany). The complete fluid (75–170 mL) of each setup was progressively centrifuged in 50-mL sterile falcon tubes at 14,000×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. For each fluid sample, two pellets resulting from two centrifugation steps were produced and separately analyzed by genetic fingerprinting analyses. Well fluid (250 mL) obtained during the downhole sampling was filtered (50-mm, 0.2-µm PC filter, WhatmanTM, GE Healthcare Europe GmbH, Freiburg, Germany) using a Stainless Steel Filter Holder (Sartorius AG, Göttingen, Germany).

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. With respect to the high contamination risk, blank controls were always included during DNA extraction and were analyzed in parallel with the environmental samples. After extraction, the DNA concentration was fluorometrically measured (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).

PCR and DGGE analyses

Partial bacterial 16S rRNA genes were amplified by polymerase chain reaction (PCR). Due to low DNA concentrations even after long-term experiments with an organic carbon source, nested PCR was required to acquire enough products for the DGGE analyses. The universal 16S rRNA primer pairs 27F (5'-AGAGTTTGATCMTGGCTCAG-3'; Lane 1991) and 1492R (5'-TACGGYTACCTTGTACACTT-3'; Weisburg et al. 1991) were used to amplify a long DNA fragment, followed by 341F-GC (5'-CGCCCGCCGC GCCCGCGCCCGTCCCGCCGCCCGCCCGCCCGCCTAC GGGAGGCAGCAG-3'; Muyzer et al. 1993) and 907R (5'-CCGTCAATTCMTTTRAGTTT-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. For all of the described PCRs, the reaction tubes contained 2.5 µL of 10x PCR buffer COMPLETE II (Bioron GmbH, Ludwigshafen, Germany), 2.5 µL of dNTPs (2.5 mM each, Thermo Scientific), 0.5 µL of MgCl₂ (50 mM, Bioron), 1 µL of each 10 mM primer stock, 0.25 µL of DFS-Taq DNA Polymerase (Bioron), 1 µL

Table 4 Phylogenetic affiliation of partial bacterial 16S ribosomal RNA gene sequences from DGGE profiles of fluid and rock samples

Sample	Band	GenBank accession number	Phylum	Closest relative	Similarity (%)
ExF 4-S	19	KU603752	Proteobacteria	<i>Novosphingobium</i> spp.	99
ExF 6-W	50	KU603736	Proteobacteria	<i>Sphingomonas</i> spp.	100
StF 4-BL	3				
StF 4-W	32				
StF 4-BL	2	KU603742	Proteobacteria	Uncultured Alphaproteobacterium	98
ExF 6-W	48	KU603757	Proteobacteria	<i>Mesorhizobium</i> spp.	100
StF 4-S	16	KU603754	Proteobacteria	<i>Phyllobacterium myrsinacearum</i> (T)	100
ExF 6-W	46	KU603759	Proteobacteria	<i>Curvibacter lanceolatus</i>	100
ExF 4-W	36	KU603764	Proteobacteria	<i>Curvibacter</i> spp.	100
ExF 9-S	7	KU603747	Proteobacteria	<i>Variovorax</i> spp.	100
ExF 4-S	20				
ExF 6-S	24				
StF 4-S	14				
StF 1-S	58				
	57				
StF 1-W	59	KU603731	Proteobacteria	<i>Pelomonas</i> sp	100
ExF 9-S	10	KU603745	Proteobacteria	<i>Ralstonia</i> spp.seq. 1	100
	9				
	6				
	11				
ExF 4-W	43				
StF 3-S	4				
StF 1-S	53				
StF 4-S	15	KU603755	Proteobacteria	<i>Ralstonia</i> spp.seq. 2	99
StF 1-BL	1	KU603732	Proteobacteria	<i>Undibacterium</i> spp.	100
ExF 4-W	44	KU603749	Proteobacteria	<i>Burkholderia heleaia</i>	100
ExF 4-W	39	KU603744	Proteobacteria	<i>Burkholderia tuberum</i> (T)	100
ExF 9-S	12				
ExF 6-S	26				
StF 3-S	5				
StF 4-W	33	KU603734	Proteobacteria	<i>Burkholderia</i> spp.	100
ExF 4-W	38	KU603750	Proteobacteria	Unclassified Burkholderiales	99
ExF 6-S	23	KU603762	Proteobacteria	Uncultured Gammaproteobacterium	99
StF 4-S	18	KU603753	Proteobacteria	<i>Kosakonia</i> spp.	100
StF 1-S	56				
ExF 6-S	25	KU603761	Proteobacteria	<i>Dyella</i> spp.	99
ExF 4-W	42	KU603740	Proteobacteria	<i>Acinetobacter junii</i> (T)	100
ExF 4-W	41	KU603741	Proteobacteria	<i>Acinetobacter</i> spp.seq. 1	100
StF 4-W	29	KU603739	Proteobacteria	<i>Acinetobacter</i> spp.seq. 2	99
StF 1-W	60	KU603730	Proteobacteria	Unclassified Rhizobiales	98
ExF 9-S	13	KU603735	Acidobacteria	Uncultured bacterium seq. 1	100
ExF 4-W	40				
ExF 6-W	49				
StF 4-W	34	KU603733	Acidobacteria	Uncultured bacterium seq. 2	100
StF 1-S	55				
ExF 6-S	27	KU603760	Actinobacteria	<i>Corynebacterium vitaeruminis</i>	100
ExF 6-S	22	KU603763	Actinobacteria	<i>Isoptericola variabilis</i>	98
ExF 4-S	21	KU603751	Actinobacteria	<i>Propionibacterium acnes</i>	100

Table 4 continued

Sample	Band	GenBank accession number	Phylum	Closest relative	Similarity (%)
StF 4-S	17				
StF 4-W	31	KU603737	Firmicutes	<i>Brevibacillus</i> spp.	100
Ktzi fluid	28	KU603743	Firmicutes	Uncultured <i>Desulfotomaculum</i> spp.seq. 1	100
StF 1-W	61				
ExF 6-W	52	KU603756	Firmicutes	Uncultured <i>Desulfotomaculum</i> spp.seq. 2	100
ExF 6-W	47	KU603758	Firmicutes	<i>Desulfotomaculum intricatum</i>	99
ExF 4-W	45	KU603748	n.d.	Uncultured bacterium seq. 3	100
ExF 9-S	8	KU603746	n.d.	Uncultured bacterium seq. 4	100
ExF 4-W	37				
ExF 6-W	51				
StF 1-S	54				
ExF 4-W	35	KU603738	n.d.	Uncultured bacterium seq. 5	100
StF 4-W	30				

of DNA template and DNase/RNase-free water (Thermo Scientific) for a final volume of 25 μ L.

Denaturing gradient gel electrophoresis (DGGE) was performed using the DCode system (Bio-Rad Laboratories, Hercules, CA, USA). The denaturing gels included a 35–70% urea gradient to separate the bacterial amplification products. After the electrophoresis, which was conducted at 60 °C with 100 V for 17 h, the gel was silver stained. The DNA was extracted from the excised bands using the ‘crush and soak’ method (Sambrook and Russell 2001; Czarnetzki and Tebbe 2004), re-amplified using the primer set 341F and 907R (without GC clamp), purified using the GeneJet PCR purification kit (Thermo Scientific) and subjected to sequencing (GATC Biotech AG, Konstanz, Germany). Sequence homologies were analyzed using BLAST (Basic Local Alignment Search Tool) (Altschul et al. 1990). The sequences are deposited at GenBank under the accession numbers KU603730–KU603764 (Table 4). The similarities of the communities in the ExF 4-BL, ExF 4-S, ExF 4-W, ExF 6-BL, ExF 6-S and ExF 6-W setups were calculated according to the Sørensen coefficient (Sørensen 1948). DGGE gel analyses and band matching with a distance height of 0.5% were conducted using the PyElph software (Pavel and Vasile 2012). The similarities were calculated using the formula $CC = 2C / (S1 + S2)$ [C: number of bands the two communities have in common; S1: number of bands in community 1; S2: number of bands in community 2]. Furthermore, the Shannon diversity index and the Simpson index of dominance were calculated according to Wakil et al. (2008).

Mineralogical analyses of rock cores

After incubation in synthetic brine and drill mud without biocide (ExF 9-S and StF 3-S), in well fluid with drill mud

containing biocide (ExF 4-W, ExF 6-W and StF 4-W) or in well fluid and acetate (StF 1-W), small pieces of the rock cores were collected for mineralogical analyses. A field emission scanning electron microscope (FE-SEM; Hitachi S-4700 microscope) was used to determine the morphology and relationship between the rock components and cements. Sandstone fragments ca. 1 cm large were placed on the sample holder and coated with carbon. SEM observations were performed at an accelerating voltage of 20 kV. Chemical composition was quantified using an energy-dispersive spectrometer (EDS). The elements in the spectra were identified by spot analysis using the analytical Thermo Scientific NSS software.

Results

Microbial community composition of rocks and fluids

Pristine rock cores of Stuttgart and Exter Formations from the CO₂ storage site in Ketzin were exposed to different combinations of drill mud, synthetic brine, fresh well fluid and acetate. The alterations in relative abundance and diversity compared to the autochthonous microbial community of the rocks were investigated by genetic fingerprinting (Fig. 1).

Autochthonous microbial community of pristine rock cores

The microorganisms detected in the StF pristine rock cores were affiliated to the genera *Undibacterium* and *Sphingomonas* and to an uncultured Alphaproteobacterium (Fig. 2; Table 5: StF 3-BL; StF 4-BL; StF 1-BL). A previous investigation revealed that the pristine ExF rock cores were inhabited by microorganisms related to

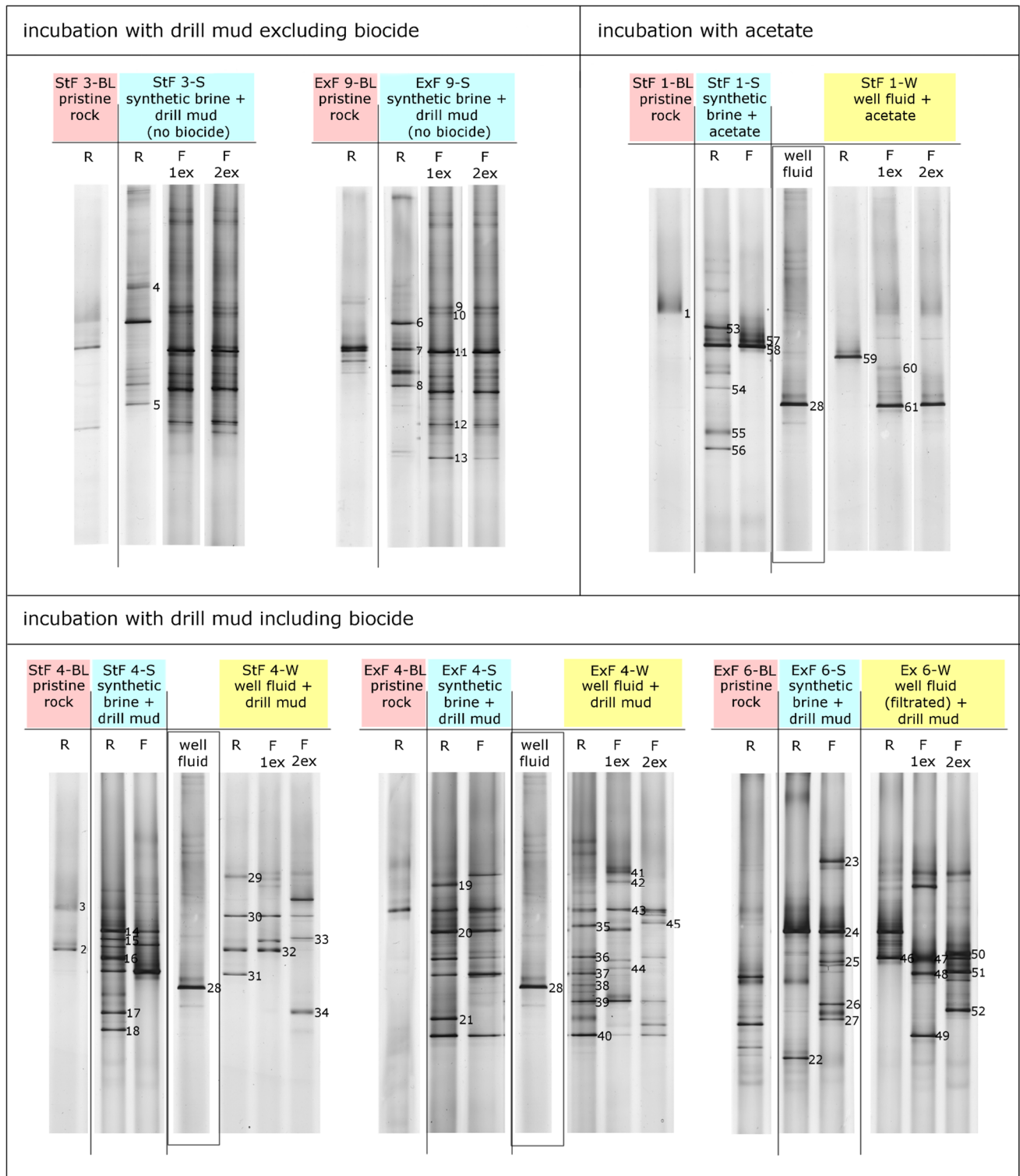


Fig. 1 Comparative DGGE analysis of bacterial 16S rRNA gene fragments in the pristine rock cores (-BL, red) and in samples incubated with drill mud, or acetate and synthetic brine (-S, blue) or

well fluid (-W, yellow). Sequenced bands are marked with numbers. R rock, F fluid, 1ex extraction after first centrifugation cycle, 2ex extraction after second centrifugation cycle

Rhizobium spp., *Pelomonas* spp., *Pseudomonas* spp., *Ochrobactrum* spp. and *Propionicimonas paludicola* (Pelizzari et al. 2016). The genetic fingerprinting analyses of

ExF rock cores showed up to 14 bands, while in the fingerprinting of the StF rock cores a maximum of 5 bands were counted (Fig. 1; Table 6).

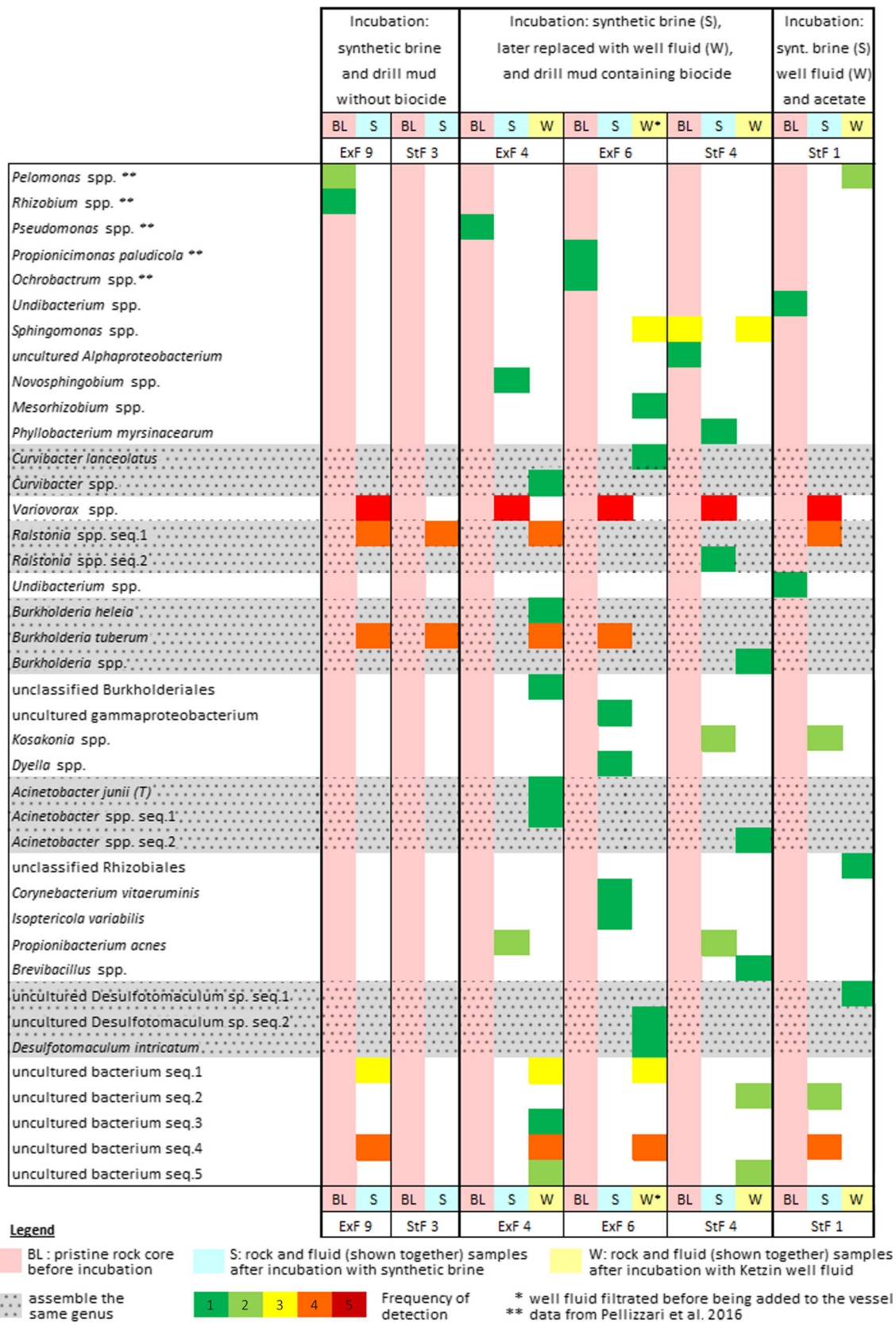


Fig. 2 Identified bacterial species in the pristine rocks and incubation experiments. The *different colors indicate* the relative abundance of detection

Table 5 Overview of the microorganisms identified through genetic fingerprinting analyses in pristine rock cores (red), in rock and fluid after incubation with drill mud or acetate and synthetic brine (blue) or well fluid (yellow)

Formation	Lithology	Before incubation		After incubation with synthetic brine		After incubation with well fluid	
		Sample	Microorganisms	Sample (incubation time)	Microorganisms	Sample (incubation time)	Microorganisms
Addition of drill mud without biocide							
Stuttgart	ss, ms-si	StF 3-BL	N.s.	StF 3-S (8 weeks)	<i>Ralstonia</i> spp. seq.1; <i>Burkholderia tuberum</i>		
Exter	ss, ms-si	ExF 9-BL**	<i>Rhizobium</i> spp.; <i>Pelomonas</i> spp.	ExF 9-S (8 weeks)	<i>Ralstonia</i> spp. seq.1; <i>Burkholderia tuberum</i> ; <i>Variovorax</i> spp.; uncultured bacterium (seq.1; seq.4)		
Addition of drill mud							
Stuttgart	fg, ss, si	StF 4-BL	<i>Sphingomonas</i> spp.; uncultured Alphaproteobacterium	StF 4-S (16 weeks)	<i>Ralstonia</i> spp. seq.2; <i>Variovorax</i> spp.; <i>Phyllobacterium myrsinacearum</i> ; <i>Kosakonia</i> spp.; <i>Propionibacterium acnes</i>	StF 4-W (20 weeks)	<i>Sphingomonas</i> spp.; <i>Burkholderia</i> spp.; <i>Acinetobacter</i> spp. seq.2; <i>Brevibacillus</i> spp.; uncultured bacterium (seq.2; seq.5)
Exter	fg-mg, ss	ExF 4-BL**	<i>Pseudomonas</i> spp.	ExF 4-S (16 weeks)	<i>Variovorax</i> spp.; <i>Novosphingobium</i> spp.; <i>Propionibacterium acnes</i>	ExF 4-W (15 weeks)	<i>Ralstonia</i> spp. seq.1; <i>Curvibacter</i> spp.; <i>Burkholderia heleaia</i> ; <i>Burkholderia tuberum</i> ; <i>Acinetobacter junii</i> ; <i>Acinetobacter</i> spp. seq.1; unclassified Burkholderiales; uncultured bacterium (seq.1; seq.3; seq.4; seq.5)
Exter	ss	ExF 6-BL**	<i>Ochrobactrum</i> spp.; <i>Propionimonas paludicola</i>	ExF 6-S (16 weeks)	<i>Variovorax</i> spp.; <i>Burkholderia tuberum</i> ; <i>Corynebacterium vitaeruminis</i> ; <i>IsotERICOLA variabilis</i> ; <i>Dyella</i> spp.; uncultured Gammaproteobacterium;	ExF 6-W* (20 weeks)	<i>Sphingomonas</i> spp.; <i>Mesorhizobium</i> spp.; <i>Curvibacter lanceolatus</i> ; <i>Desulfotomaculum intricatum</i> ; uncultured <i>Desulfotomaculum</i> spp.seq.2; uncultured bacterium (seq.1; seq.4)
Addition of acetate (190mg/L)							
Stuttgart	ss	StF 1-BL	<i>Undibacterium</i> spp.	StF 1-S (16 weeks)	<i>Ralstonia</i> spp. seq.1; <i>Variovorax</i> spp.; <i>Kosakonia</i> spp.; uncultured bacterium (seq.2; seq.4)	StF 1-W (20 weeks)	<i>Pelomonas</i> spp.; unclassified Rhizobiales; uncultured <i>Desulfotomaculum</i> 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 well fluid was filtrated before being added to the vessel, ** data from Pellizzari et al. 2016

Incubation of rock cores with drill mud without biocide and synthetic brine

The incubation with synthetic brine and drill mud without biocide caused an increase in the number of bands (Table 6: StF 4-S and ExF 4-S). Similar genetic fingerprints were observed between the fluids of ExF and StF (Fig. 1). The dominant microorganisms of rocks and fluids of both setups were affiliated to *Ralstonia* spp. and *Burkholderia tuberum* (Fig. 2; Table 5). Moreover, in the ExF setup, DNA sequences related to *Variovorax* spp. and an uncultured Acidobacteria were identified (Fig. 2; Table 5) in the rock and fluid, respectively.

Incubation of rock cores with drill mud containing biocide and synthetic brine

An increase in the bacterial diversity was also observed after incubation with synthetic brine and drill mud containing biocide (Fig. 1; Table 6: StF 4-S and ExF 4-S). The total number of bands decreased only in the ExF 6-S setup; however, the number of bands with higher intensities

increased in this setup. The changes in the community structures of ExF 4-S and ExF 6-S setups were also indicated by differing Sørensen coefficients, Shannon and Simpson indices (Table 7). Microorganisms related to *Variovorax* spp. were detected in all setups (Fig. 2; Table 5). In ExF 6-S, the bacterial community was additionally dominated by bacteria related to *Burkholderia tuberum*, *Dyella* spp., *Corynebacterium vitaeruminis*, *IsotERICOLA variabilis* and an uncultured Gammaproteobacteria (Fig. 2; Table 5). Besides *Variovorax* spp., in ExF 4-S, microorganisms affiliated to *Novosphingobium* spp. and *Propionibacterium acnes* were identified, whereas in StF 4-S, bacteria related to *Propionibacterium acnes*, *Ralstonia* spp., *Kosakonia* spp. and *Phyllobacterium myrsinacearum* were detected (Fig. 2; Table 5).

Incubation of rock cores with drill mud containing biocide and well fluid

The dominant microorganism detected in the freshly collected well fluid was affiliated to an uncultured *Desulfotomaculum* spp.. A decrease in bacterial diversity in rocks

Table 6 Number of DGGE bands (16S rRNA gene) in each sample

Before incubation		After incubation with synthetic brine			Well fluid	After incubation with well fluid			
Sample	Pristine rock	Sample (incubation time)	Rock	Fluid		Sample (incubation time)	Rock	Fluid 1ex	Fluid 2ex
		Addition of drill mud without biocide							
StF 3-BL	5	StF 3-S (8 weeks)	17	20					
ExF 9-BL	8	ExF 9-S (8 weeks)	14	22					
		Addition of drill mud including biocide							
StF 4-BL	4	StF 4-S (16 weeks)	16	9	17	StF 4-W (20 weeks)	6	7	10
ExF 4-BL	3	ExF 4-S (16 weeks)	18	15	17	ExF 4-W (15 weeks)	20	13	10
ExF 6-BL	14	ExF 6-S (16 weeks)	11	13	17*	ExF 6-W* (20 weeks)	15	11	17
		Addition of acetate (190 mg L ⁻¹)							
StF 1-BL	2	StF 1-S (16 weeks)	12	5	17	StF 1-W (20 weeks)	2	10	10

The pristine rock samples before incubation are indicated in red, the rock and fluid samples after incubation with synthetic brine and organics (drill mud or acetate) are indicated in blue, and the rock and fluid samples after incubation with fresh collected well fluid and organics (drill mud or acetate) are indicated in yellow

* The Ketzin well fluid was sterile filtrated before being added to the vessel

Table 7 Calculations of the Shannon diversity index, Simpson index of dominance and Sørensen coefficient of community similarity in the ExF 4 and ExF 6 setups

	Shannon index	Simpson index	Sørensen coefficient	
ExF 4-BL	1.02	0.46	ExF 4-BL/ExF 4-S Rock	0.10
ExF 4-S Rock	2.13	0.15	ExF 4-S/ExF 4-W Rock	0.32
ExF 4-S Fluid	2.15	0.15	ExF 4-S/ExF 4-W Fluid	0.21
ExF 4-W Rock	2.21	0.13		
ExF 4-W Fluid	2.10	0.15		
ExF 6-BL	2.02	0.20	ExF 6-BL/ExF 6-S Rock	0.48
ExF 6-S Rock	1.37	0.33	ExF 6-S/ExF 6-W Rock	0.23
ExF 6-S Fluid	1.79	0.19	ExF 6-S/ExF 6-W Fluid	0.25
ExF 6-W Rock	1.14	0.39		
ExF 6-W Fluid	1.63	0.22		

The Sørensen coefficient gives a value between 0 and 1. The closer the value is to 1, the more the communities have in common. Higher Shannon values give increased diversities. Simpson's index gives more weight to the more abundant species in a sample. The band intensities were detected with Gel-Quant.NET software, provided by biochemlabsolutions.com

was identified when comparing StF 4-S, pre-fluid exchange, to StF 4-W, after incubation with well fluid and drill mud containing biocide (Fig. 1; Table 6). For the ExF setup (ExF 4-W), after fluid replacement the bacterial diversity increased in the rock but decreased in the fluid (Fig. 1; Tables 6, 7). In both StF and ExF setups, sequences related to uncultured Acidobacteria, *Acinetobacter* and

Burkholderia were detected (Table 5). In ExF 4-W, microorganisms related to *Ralstonia* spp., *Curvibacter* spp. and *Cyanobacterium vitaeruminis* were also identified. Bacteria ascribed to *Sphingomonas* spp. and *Brevibacillus* spp. were observed in StF 4-W (Fig. 2; Table 5).

In the setup with filtered well fluid (ExF 6-W), the bacterial diversity increased (Fig. 1; Tables 6, 7).

Microorganisms related to the genera *Mesorhizobium*, *Sphingomonas*, to the organism *Curvibacter lanceolatus* and to an uncultured Acidobacteria were detected (Fig. 2; Table 5). Additionally, two sequences related to two different species of *Desulfotomaculum* were identified (Fig. 2; Table 5).

Incubation with acetate and synthetic brine or well fluid

After incubation with acetate and synthetic brine (StF 1-S), the diversity increased in the rock and fluid (Fig. 1; Table 6). Microorganisms related to *Variovorax* spp., *Ralstonia* spp., *Kosakonia* spp. and an uncultured Acidobacteria were observed (Fig. 2; Table 5). After incubation with unfiltered well fluid and acetate (StF 1-W), the number of dominant bands decreased in both rock and fluid (Fig. 1; Table 6). A DNA sequence affiliated to the genus *Pelomonas* was observed, as well as the same sequence assigned to the uncultured *Desulfotomaculum* spp. that was previously detected in the well fluid, as described in the above paragraph (Fig. 2; Table 5).

Mineral composition and morphology of rock cores after incubation

Besides the identification of the microbial community structure determined by genetic fingerprinting, FE-SEM analyses were conducted to investigate the impact of the different additives on the rock cores and to illustrate possible microbe–mineral interactions. The main minerals detected in the rock cores are indicated in Table 8.

Incubation of rock cores with drill mud without biocide and synthetic brine

After incubation in synthetic brine and drill mud, in the StF rock (StF 3-S), quartz was usually covered with clay minerals, whereas feldspar was partially dissolved (Table 8). Small anhydrite crystals (a few micrometers) were precipitated as a secondary mineral among clay minerals. The biotite was compressed between the rock-forming minerals. In the ExF rock core (ExF 9-S), in addition to the main mineral phases (Table 8), spot analyses revealed relatively high concentrations of potassium,

Table 8 Overview of the mineral composition of rock cores determined through FE-SEM analyses

Formation	Lithology	Sample (incubation time)	Main minerals
After incubation with synthetic brine and drill mud without biocide			
Stuttgart	ss, ms–si	StF 3-S (8 weeks)	Quartz, feldspar, clay minerals, biotite and anhydrite.
Exter	ss, ms–si	ExF 9-S (8 weeks)	Quartz, feldspar, clay minerals (overgrown with ankerite), calcite, barite and halite.
After incubation with well fluid and drill mud			
Stuttgart	fg, ss, si	StF 4-W (20 weeks)	Quartz, feldspar (plagioclase and K-feldspar) and clay minerals (mostly chlorite and illite)
Exter	fg–mg, ss	ExF 4-W (15 weeks)	Quartz, feldspar (plagioclase and K-feldspar) mica, clay minerals, calcite, ankerite and iron sulfides
Exter	ss	ExF 6-W* (20 weeks)	Quartz, feldspar (plagioclase and K-feldspar) carbonates (ankerite and in minor amount calcite), mica and iron oxide
After incubation with well fluid and acetate (190mg/L)			
Stuttgart	ss	StF 1-W (20 weeks)	Quartz, feldspar (plagioclase and K-feldspar), biotite, clay minerals and halite.

Results obtained after incubating the rock cores with synthetic brine and drill mud without biocide (blue) and well fluid and drill mud or acetate (yellow)

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

* The well fluid was filtrated before being added to the vessel

calcium and chlorine commonly identified in salts. Indications of microbial activity were not observed.

Incubation of rock cores with drill mud containing biocide and well fluid

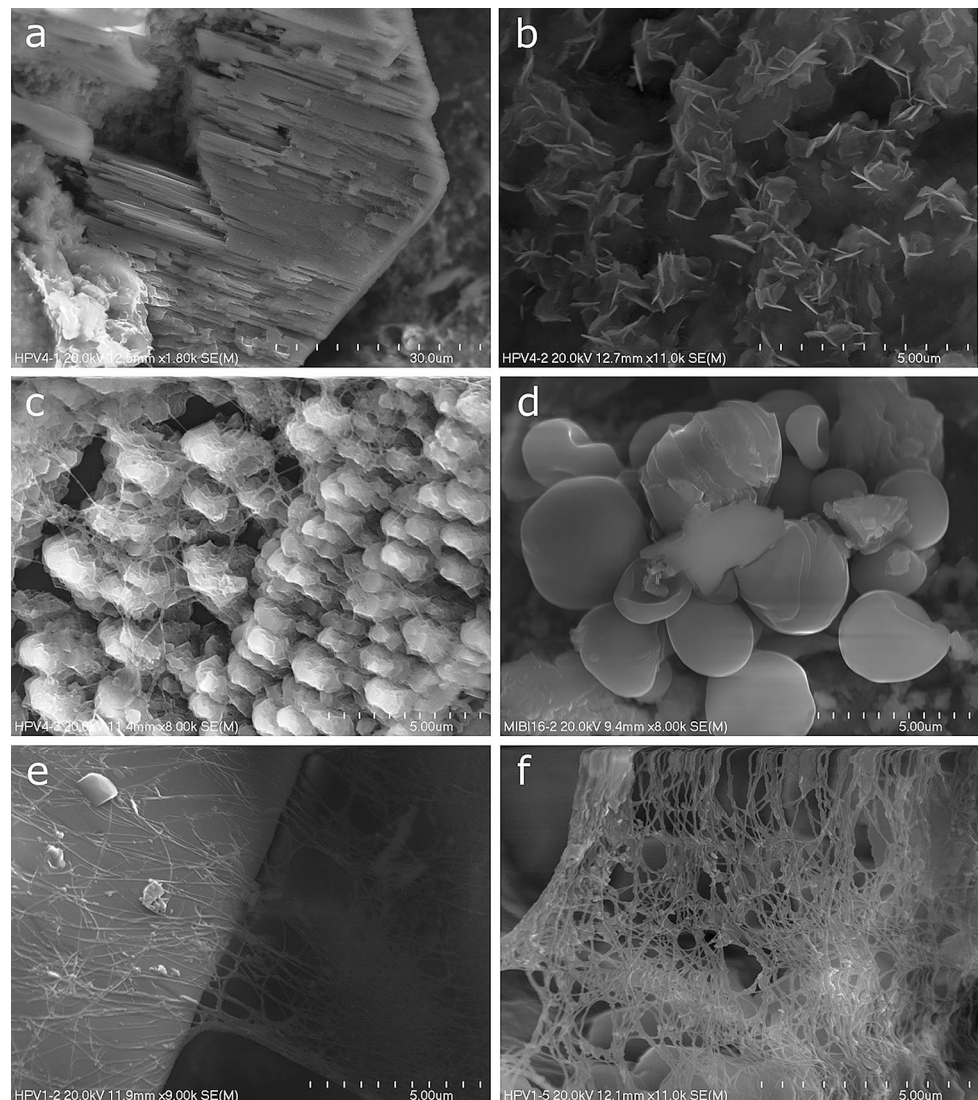
After incubation with unfiltered well fluid and drill mud containing biocide, in the StF rock (StF 4-W), feldspar was subjected to dissolution. Hair-like structures similar to biofilms were frequently produced on partly dissolved mineral surfaces (Fig. 3a/b/c). However, single microorganisms were not observed, likely due to the low cell numbers. In the ExF rock (ExF 4-W), besides the main minerals observed (Table 8), few EDS spot analyses detected iron sulfide in the anhedral form, usually in a close relationship with clay minerals. After incubation with filtered well fluid and drill mud (ExF 6-W), the quartz surface was affected by the dissolution process,

whereas K-feldspar and plagioclase were characterized by smooth surfaces. The surface of ankerite was subjected to partial dissolution, with visible, characteristic etch pits. Rounded precipitates ca. 2 μm in diameter were observed (Fig. 3d). Spot analyses indicated that they were composed of iron oxide. Single examples of metallic iron were detected.

Incubation of rock cores with acetate and well fluid

After incubation with well fluid and acetate, the quartz surface was usually rugged and covered with clay minerals, whereas K-feldspar and plagioclase showed signs of dissolution (Table 8). Biotite was usually creased between grains of quartz and feldspar and slightly folded, likely as a result of compaction. Precipitation of large amounts of halite was observed in the form of euhedral crystals (ca. 1 μm in size) or as coatings on the surfaces and edges of

Fig. 3 SEM images of rock samples after long-term experiments. **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**, **f** hair-like structures similar to biofilms covering the mineral surface (StF 1-W)



the rock-forming minerals. Complex structures similar to biofilms were observed (Fig. 3e/f).

Discussion

In this study, the influence of long-term CO₂ exposure and drill mud on the autochthonous microbial community compositions of rock cores and fluids under simulated in situ conditions was investigated. The microbial response to acetate, as a biodegradation product of drill mud, was also analyzed. 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.

Differences in microbial community composition of StF and ExF pristine rocks

Pristine StF rock cores, which were collected after approximately four years of CO₂ storage operation, showed a low microbial diversity. In addition to *Sphingomonas* spp., a commonly detected genus in the deep subsurface (Balkwill et al. 1997; Fredrickson et al. 1999), another Alphaproteobacterium was detected. The sequence showed high similarity (98%) to an uncultured representative, which had previously been identified in oligotrophic groundwater from a deep (290–324 m) monitoring well of a radioactive waste depository in Siberia, Russia (Nedelkova et al. 2005). Furthermore, a sequence affiliated to the genus *Undibacterium* (Betaproteobacteria) was detected. Species of this genus are commonly identified in soil and freshwater (Kim et al. 2014).

The microbial community of the pristine ExF rock cores was composed of microorganisms related to *Rhizobium* spp., *Pelomonas* spp., *Pseudomonas* spp., *Ochrobactrum* spp. and *Propionicimonas paludicola* (Pellizzari et al. 2016). Both, the ExF and the StF, are inhabited by microorganisms typically found in soil and freshwater habitats. However, the detected DNA sequences might also represent uncultured species of these genera, adapted to high salinity and rather oligotrophic environments (Pellizzari et al. 2016). Differences in the community structures between the two formations are generally explained by the different depths, ages and salinity (four times higher salinity in StF than in ExF) (Pellizzari et al. 2016). In addition, the high lithological heterogeneity of the two formations entails differences in the mineralogical composition, influencing microbial diversity and causing dissimilar communities not only between two different formations but also between rocks of the same formation.

Changes in microbial diversity and relative abundance in rocks and fluids due to drill mud and acetate exposure

Altered microbial community composition after exposure to substrate and synthetic brine

Due to incubation with synthetic brine and drill mud, the microbial diversity and relative quantities increased considerably in all five setups. This was indicated by a higher intensity of bands in the genetic fingerprints. Dissimilarities in the community structures, which were present after the incubations without biocide, were mainly observed in the rocks, whereas only minor differences were observed in the fluids of all setups. A similar experiment incubating pristine ExF rock under the same conditions but in N₂ atmosphere was performed (data not shown). The genetic fingerprinting revealed a microbial composition similar to the outcomes of the experiments in CO₂ atmosphere. This demonstrated that the microbial community remains viable after long-term CO₂ exposure and that only minor changes in the composition of the microbial community occurred after incubation in CO₂ atmosphere. Also Wandrey et al. (2011) observed only minor changes in the microbial community structure of StF rock cores after incubation in CO₂ atmosphere (without the addition of organic sources).

The StF exhibited higher salinity than the ExF (Pellizzari et al. 2016), and different microbial community inhabited the two rock formations before incubation. Nevertheless, similar genera dominated in both StF and ExF approaches, indicating a stronger effect of drill mud components than of rock material on the microbial community composition. The common microorganisms detected after incubation with drill mud in StF and ExF approaches were ascribed to three genera: *Ralstonia*, *Burkholderia* and *Variovorax*. These microorganisms are described as typical for the environment and demonstrated to be well adaptable to the in situ conditions as well as to the incubation in CO₂ atmosphere and organic drill mud. For instance, several species of *Ralstonia* can utilize CO₂ and H₂ as carbon and energy sources but switch over to organotrophic metabolism in the presence of substrates such as acetate (Park et al. 2011). The cellulose-based drill mud enhanced the relative abundance of bacteria from the genera *Burkholderia*, *Variovorax*, *Corynebacterium* and *Dyella*, species of which are known to be 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 that cellulose-based drill mud is a valuable substrate for many rock-inhabiting microorganisms and can cause remarkable growth acceleration, as previously shown in

Ketzin (Zettlitzer et al. 2010). Microorganisms (e.g., members of the genera *Variovorax* and *Ralstonia*) that had been already detected after incubation with drill mud were identified after acetate and synthetic brine exposure. This indicated that the drill mud was probably degraded to acetate. Acetate can be shunted into the central metabolism of *Variovorax*, as it was shown for *Variovorax paradoxus*-like bacteria (Boersma et al. 2010). 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. Their abundance appears to be related to the degradation of cellulose polymers of the drill mud. Species of the genus *Burkholderia* could be relevant for CO₂ storage because they may play an important role in altering the minerals of the formation. As discussed by Ménez et al. (2007), these bacteria need Ca²⁺ and Mg²⁺ ions and acidify their medium during growth on a carbon source, likely by releasing organic acids. This acidification enhances the mineral dissolution/alteration and facilitates carbonate precipitation in the presence of CO₂ (Ménez et al. 2007), which is also relevant for the Ketzin storage site (Fischer et al. 2010).

Altered microbial community composition after exposure to substrate and well fluid

The bacterial population of ExF and StF responded differently to the incubation with fresh well fluid. The community structure of the rock and fluid of the ExF setups slightly varied after the fluid was replaced with unfiltered well fluid, as indicated by the Sørensen coefficient. Many microorganisms that were detected in the synthetic brine and drill mud setup were also identified after fluid replacement. The fresh well fluid had a four times higher salinity than the ExF brine. It was obtained from a depth corresponding to the StF and was therefore similar in composition (especially in salinity) to the StF synthetic brine. The community structure and diversity in the StF setup changed when the fluid was replaced. In contrast, the population of ExF had probably more difficulties to evolve in consequence of the increased salinity, and it varied only slightly. In addition, the five-week-longer incubation time of the StF setup likely influenced the results as well. In the StF setup, microorganisms related to *Variovorax*, *Phyllobacterium* and *Propionibacterium* disappeared and relatives of the genera *Sphingomonas*, *Burkholderia* and *Acinetobacter* became dominant. It was shown that species of *Acinetobacter*, which are commonly detected in deep biosphere, use CMC as a carbon source (Ekperigin 2007). 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 in the ExF and StF setups only after the fluid was replaced. Accordingly, it may have been present in concentrations below detection limit in the fresh collected fluid and after incubation with drill mud its activity increased, resulting in higher DNA concentrations.

The depletion of important growth factors such as trace elements or low molecular weight acids before the fluid exchange may have influenced the microbial response to the increased substrate availability. It is assumed that the simplified synthetic brine could not support the growth of several species inherent in the StF rock. The well fluid may have had a strong impact on bacterial growth through introducing a complex mixture of essential substances. The five-week-longer incubation time of the StF approach likely enhanced the depletion process through microbial activity. This assumption is strengthened by the results of the setup with the ExF rock and filtered well fluid, which was incubated for the same duration as the StF approach. In this case, the bacterial community structure changed as well after the fluid was replaced. However, the most abundant bacterial genus found before the fluid exchange, namely *Variovorax*, was detected after the fluid replacement at the same intensity. The other microorganisms that appeared after the fluid exchange (i.e., next relatives of *Curvibacter lanceolatus* and the genus *Mesorhizobium*) were previously observed in mineral water and soils (Ghosh and Roy 2006; Lorite et al. 2010; Falcone-Dias et al. 2012) and can be part of the autochthonous community of the rock, thus enhancing their growth. In addition, two SRB affiliated to the genus *Desulfotomaculum* were detected. *Desulfotomaculum* species have previously been identified in the deep biosphere (Detmers et al. 2004; Moser et al. 2005; Ehinger et al. 2009) and in the Ketzin storage reservoir (Morozova et al. 2013; Pellizzari et al. 2016). Although a SRB that was likely related to a novel species of *Desulfotomaculum* was already observed in the well fluid obtained from the injection well (Ktzi 201), both sequences after incubation differed from the sequence detected in the well fluid. It is assumed that only a low cell number of these SRB resided as dormant cells or spores in the ExF rock and then proliferated after replacement of synthetic brine with well fluid and drill mud.

Acetate addition increased the relative abundance of the *Desulfotomaculum*-like organism previously detected in the well fluid. Some species of *Desulfotomaculum* (e.g., *Desulfotomaculum acetoxidans*) 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 relative of *Desulfotomaculum* likely has analogous metabolism because it was not

observed after incubation with the drill mud, where the availability of acetate was more restricted. Acetate was likely a limiting growth factor for the *Desulfotomaculum* species, which had to compete with other acetate-metabolizing microorganisms, or some drill mud components may have inhibited the growth of *Desulfotomaculum*. This may be the reason for the strong increase in the cell numbers of SRB after the drilling event in Ketzin, where acetate was available at the same concentration as was applied in this experiment. Incubation with well fluid and acetate also resulted in a higher relative abundance of an organism related to *Pelomonas saccharophila*, a H₂-oxidizing bacterium that was already detected in the pristine rock of the ExF and utilizes acetate as a carbon source (Doudoroff 1940; Xie and Yokota 2005). Therefore, the acetate addition likely triggered growth of the *Pelomonas*-like organism.

Response of the autochthonous microbial community to biocide included in the drill mud formulation

A conspicuous inhibitory effect on bacterial growth was expected when biocide was used. In contrast, bacterial diversity increased compared with the pristine rock and the setups without biocide, as shown by the calculated Shannon and Simpson indices. This assumption is supported by the low Sørensen coefficient, which indicates a significant change in bacterial community composition. One plausible explanation for the higher diversity may be the eight-week-longer incubation time of the setups including biocide, which may have allowed the bacterial community to further evolve. Another explanation may be that the biocide decreased the dominant species to an extent that less abundant microorganisms became visible. Furthermore, the manufacturers showed that each bacterial species is affected by a specific concentration of biocide in laboratory experiments (www.oilfield-biocides.com). The use of biocide may have mitigated bacterial growth but did not completely inhibit it. An aspect that has to be considered is whether biodegradable biocide can become a carbon and nitrogen source for microorganisms after a certain incubation time and in specific environmental conditions. The applied biocide is completely biodegradable under aerobic conditions (www.oecd-ilibrary.org). The storage formation is considered to be an anaerobic environment, but the presence of oxygen cannot be excluded because aerobic or facultative anaerobic microorganisms and oxidized iron were detected (Wandrey et al. 2011; Pellizzari et al. 2016; Kasina et al. 2017, this issue). Likely oxygen was introduced into the deep biosphere during drilling and injection of fresh water; therefore, contact between biocide and oxygen in the near-well area and subsequent biocide degradation cannot be ruled out. Reduced effectiveness of

the biocide may occur due to dilution effects in the near-wellbore area, and already low concentrations of organics can stimulate microbial growth. The biocide concentration is regarded as the most important factor that affects its efficacy (Russell and McDonnell 2000), and for this reason, it should be always optimized during treatments (Al Hashem et al. 2004).

Microbiological–mineralogical interaction

After incubation in CO₂ atmosphere, dissolution was more advanced in the plagioclase than in K-feldspar. Albite seemed to undergo dissolution faster and more easily. The dissolution of K-feldspar in CO₂-saturated atmosphere was previously observed by Rosenqvist et al. (2014). Corrosion textures, indicating alteration processes, were also detected on feldspars in the experiments where StF cores were incubated in a CO₂ atmosphere (Fischer et al. 2011). Dissolution of feldspar causes a release of components from which secondary clay minerals are formed. It is likely that clay minerals formed in the pits after feldspar dissolution, due to low mobility of certain elements. The formation of clay minerals appears to be enhanced when bacteria are present (Wagner et al. 2013). After incubation, indirect evidence of microbial interaction with the rock components was observed. The large biofilm-like structures that were draped over the surfaces of minerals in the StF rock cores were most likely created as a result of microbial metabolic activity. However, embedded microorganisms were not observed. The shape and size of the small, round iron oxides observed in the ExF rock cores may indicate microbiological origin. As for the pristine StF (Kasina et al. 2017, this issue), the presence of oxygen in the ExF approach is demonstrated by the observation of rounded iron oxides. Nevertheless, the spread of oxygen may be restricted to some areas as not all of the iron was oxidized. In some cases, EDS spot analyses detected relatively high concentrations of K, Ca and Cl, which were likely derived from the drill mud components (e.g., KCl and M-I Cal SL: calcium carbonate) or from the brine. EDS spot analyses indicated the presence of iron sulfide in anhedral form in one ExF rock core. Even though the presence of iron sulfide can be linked to biologically induced mineralization, its presence as a consequence of drill mud exposure cannot be proven, because pyrite was observed in the rock before the incubation as well (Pellizzari et al. 2016).

The present outcomes lead to the assumption that the autochthonous microbial community in the reservoir directly responds to drill mud exposure and changes in substrate availability. The investigation shows that 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

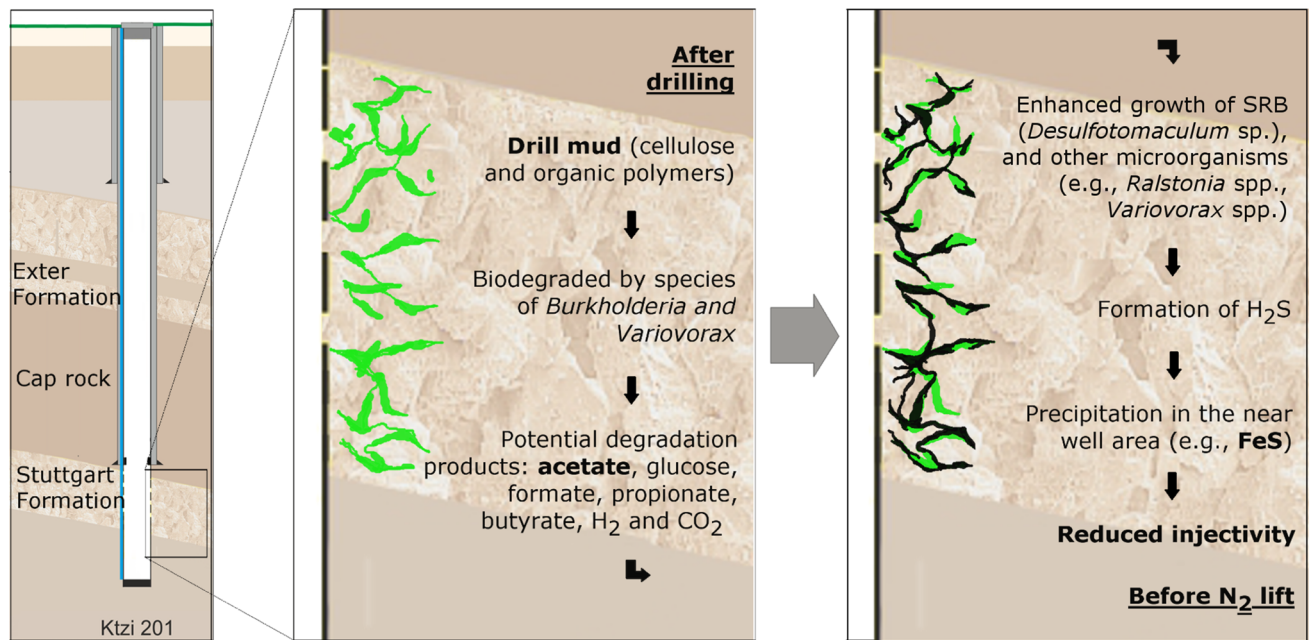


Fig. 4 Microbial-induced chain of events that caused injectivity loss in the Ketzin well

interactions that occurred during the reduced well injectivity, allowing a broader community of microorganisms (e.g., SRB) to proliferate (Fig. 4). As a consequence, hydrogen sulfide can be generated by SRB, and it reacts with dissolved iron and precipitates as amorphous FeS (van Beek and Kooper 1980; Zettlitzer et al. 2010; Morozova et al. 2010; Würdemann et al. 2010).

Conclusions

This study shows that after long-term CO₂ exposure the autochthonous microbial community of the rock formations remains viable and responds to the addition of organic drill mud as energy and carbon source. The present outcomes help on describing the causal chain which caused the injectivity loss in Ketzin. Cellulose-based organic components of the drill mud were likely degraded by bacteria of the genera *Burkholderia* and *Variovorax*. Acetate, a typical degradation product, subsequently triggered microbial sulfate reduction (*Desulfotomaculum* spp.). Previous in situ observations had shown that microbial sulfate reduction leads to an enhanced precipitation of iron sulfides that decreased the well injectivity. More efforts should be taken to clean the wells from technical fluids right after drilling in order to avoid unintentional microbial processes that may negatively influence the performance and reliability of geotechnical installations. Although the efficiency of the biocides used during drilling is tested by the manufacturers under specific laboratory conditions, the results of the present study revealed that several SRBs were

not inhibited in the long term, most likely due to dilution effects. The study shows that complex processes occurring in the deep subsurface can be, to a certain degree, simulated through laboratory experiments. To improve the economy and reliability of the geotechnical use of the subsurface such as gas storage or geothermal energy, further investigations should focus on long-term effects of drilling operations on the inhabiting microbial community.

Acknowledgements We would like to thank all partners in the Ketzin projects and CO₂ storage center for their continued support and contributions. Dr. Stephanie Lerm and Dr. Hannah Halm are acknowledged for critical reading of the manuscript. This research was funded by the Federal Ministry for Education and Research within the Geotechnologien program in the framework of CO₂MAN (CO₂ Reservoir Management 03G0760A-F) and within the H₂STORE (hydrogen to store 03SF0434B) projects.

References

- 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
- 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
- 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
- Bergmann P, Schmidt-Hattenberger C, Kiessling D, Rücker C, Labitzke T, Hennings J, Baumann G, Schütt H (2012) Surface-downhole electrical resistivity tomography applied to monitoring of CO₂ storage at Ketzin, Germany. *Geophysics* 77:B253–B267. doi:10.1190/geo2011-0515.1
- 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
- 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
- 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):236–242. doi:10.1007/s00248-004-9952-6
- 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
- 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
- Ehinger S, Seifert J, Kassahun A, Schmalz L, Hoth N, Schlömann M (2009) Predominance of *Methanobolus* 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 Biotechnol* 6(1):028–033
- Falcone-Dias MF, Vaz-Moreira I, 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
- Fischer S, Liebscher A, Wandrey M, The CO₂SINK group (2010) CO₂–brine–rock interaction—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, The CO₂SINK Group (2011) Petrophysical and petrochemical effects of long-term CO₂-exposure experiments on brine-saturated reservoir sandstone. *Energy Proc* 4:4487–4494. doi:10.1016/j.egypro.2011.02.404
- Fleshman R, Obren-Likic H (1999) Artificial lift for high volume production. *Oilfield Rev* 11(1):49–63
- 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
- Ghosh W, Roy P (2006) *Mesorhizobium thioanganeticum* sp. nov., a novel sulfur-oxidizing chemolithoautotroph from rhizosphere soil of an Indian tropical leguminous plant. *Int J Syst Evol Microbiol* 56(1):91–97. doi:10.1099/ijs.0.63967-0
- Giangiacomo LA, Dennis DM (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
- Jaiswal P, Al-Hadrami F, Atekwana EA, Atekwana EA (2014) Mechanistic models of biofilm growth in porous media. *J Geophys Res Biogeosci* 119:1418–1431. doi:10.1002/2013JG002440
- Jin L, Kim KK, Ahn CY, Oh HM (2012) *Variovorax defluvi* sp. nov., isolated from sewage. *Int J Syst Evol Microbiol* 62(8):1779–1783. doi:10.1099/ijs.0.035295-0
- 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 (submitted in this issue)
- 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
- Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. Wiley, New York, pp 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
- 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 Res Int*. doi:10.1155/2014/512497
- 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 Microbiol* 76(12):4019–4026. doi:10.1128/AEM.02555-09
- 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. *Energy Proc* 59:190–197. doi:10.1016/j.egypro.2014.10.366
- 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. *Geotechnol Sci Rep* 9:150–163
- 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, 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. *Energy 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, Piffner SM, Lin LH, Onstott TC (2005) *Desulfotomaculum* and *Methanobacterium* spp. dominate a 4- to 5-kilometer-deep fault. *Appl Environ Microbiol* 71(12):8773–8783. 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 Microbiol* 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: Tsang CF, Apps J (eds) *Underground injection science and technology*. Elsevier, Amsterdam, pp 521–536
- 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
- Pavel AB, Vasile CI (2012) PyElph—a software tool for gel images analysis and phylogenetics. *BMC Bioinform* 13:9
- Pchalek J, Bergerhoff H, Polleschner G, Borsdorf K, Lange M, Oswald B (1964) Ergebnisbericht über die geologische Speicherkundung der Struktur Brandenburg, Ketzin, Strukturteil Ost (Knoblauch). VEB UGS Burg
- 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 Morozova D, Neumann D, Kasina M, Klapperer S, Zettlitzer 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 CO₂ storage. *Environ Earth Sci* 75:1323. doi:10.1007/s12665-016-6111-6
- Prevedel B, Wohlgemuth L, Legarth B, Henniges J, Schütt H, Schmidt-Hattenberger C, Norden B, Förster A, Hurter S (2009) The CO₂SINK boreholes for geological CO₂-storage testing. *Energy Proc* 1:2087–2094. doi:10.1016/j.egypro.2009.01.272
- 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
- Sambrook J, 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 Biotechnol* 97:541–560. doi:10.1007/s00253-012-4585-z
- Schwartz T, Jungfer C, Heissler S, Friedrich F, Faubel W, Obst U (2009) Combined use of molecular biology taxonomy, Raman spectrometry, and ESEM imaging to study natural biofilms grown on filter materials at waterworks. *Chemosphere* 77(2):249–257. doi:10.1016/j.chemosphere.2009.07.002
- Sørensen T (1948) A method of establishing groups of equal amplitude in plant sociology based on similarity of species and its application to analyses of the vegetation on Danish commons. *Kongelige Danske Videnskaberne Selskab* 5(4):1–34
- Spark I, Patey I, Duncan B, Hamilton A, Devine C, McGovern-Traa C (2000) The effects of indigenous and introduced microbes on deeply buried hydrocarbon reservoirs, North Sea. *Clay Miner* 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 Genom Sci* 1(3):242–253. doi:10.4056/sigs.39508
- 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 Microbiol* 77(14):4744–4753. doi:10.1128/AEM.00233-11
- 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
- Van Beek CGDW, Kooper WF (1980) The clogging of shallow discharge wells in the Netherlands' river region. *Ground Water* 18(6):578–586
- Vandiekén 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):687–690. 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 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
- Wakil SM, Onilude AA, Ball AS (2008) Dynamics and diversity of bacterial communities of fermented weaning foods via denaturing gradient gel electrophoresis PCR-DGGE. *Res J Microbiol* 3(11):630–640
- 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, Pellizzari L, Zettlitzer M, Würdemann H (2011) Microbial community and inorganic fluid analysis during CO₂ storage within the frame of CO₂SINK—long-term experiments under in situ conditions. *Energy Proc* 4:3651–3657. doi:10.1016/j.egypro.2011.02.296
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ (1991) 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173:697–703
- 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
- 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
- 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):2419–2425. doi:[10.1099/ijms.0.63733-0](https://doi.org/10.1099/ijms.0.63733-0)

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](https://doi.org/10.1016/j.ijggc.2010.05.006)