



Potential impacts of oxygen impurities in carbon capture and storage on microbial community composition and activity

Hayden Morgan^{a,b}, David J. Large^b, Keith Bateman^a, David Hanstock^c, Simon P. Gregory^{a,*}

^a British Geological Survey, Nicker Hill, Keyworth, Nottingham NG12 5GG, UK

^b University of Nottingham, University Park, Nottingham NG7 2QL, UK

^c Progressive Energy, Bonds Mill, Stonehouse, Gloucestershire GL10 3RF, UK

ARTICLE INFO

Keywords:

Carbon dioxide storage
Oxygen impurities
Microbial communities
Hydrogen sulphide
Saline aquifer

ABSTRACT

Gaseous impurities, such as O₂, are expected to be present within CO₂ captured for storage. This could stimulate microbial activity in a geological CO₂ storage site which has the potential to lead to operational issues such as injection well blockages, corrosion and oil souring. A series of experiments were carried out to examine the effect of 10 ppm and 100 ppm O₂ in an anoxic (CO₂ or N₂) atmosphere on microbial communities and microbial gas production in laboratory scale experiments. Experiments inoculated with sulphate reducing bacteria enrichments were compared to uninoculated controls. The results show that H₂S production is delayed in a CO₂ atmosphere compared to the N₂ atmosphere. 100 ppm O₂ in CO₂ resulted in a spike of H₂S production as well as greater bacterial biomass when compared to the 10 ppm O₂ in CO₂ atmosphere. The inoculated N₂ experiments showed similar patterns in H₂S production and biomass regardless of O₂ concentration. These results suggest that a concentration of O₂ lower than 100 ppm in CO₂ could reduce the potential for microbial growth and H₂S production in CO₂ storage sites. CH₄ production was observed in some microcosms subsequent to H₂S production, highlighting the potential for microbial methanogenesis in the in CCS reservoirs.

1. Introduction

The capture and storage of CO₂ (CCS) in geological formations, such as deep saline aquifers, has the potential to reduce greenhouse gas emissions, which is needed to meet national and international targets (Committee on Climate Change, 2019; Parliament of the United Kingdom, 2008; UNFCCC Conference of the Parties, 1998). The captured CO₂ will contain a range of impurities depending upon the source of the gas, the combustion process and the capture process. O₂ is expected to be one of those impurities when CO₂ is captured after the combustion of fuels for energy generation and it is estimated that its concentration could be as high as 6% depending on the combustion and capture technology used (Porter et al., 2015). Current recommendations from the European 6th framework programme DYNAMIS are that O₂ concentrations within injected gas should be limited to between 10 ppm and 100 ppm (de Visser et al., 2008; Jensen, 2016). The current specifications for O₂ limits appear to be based on transport issues and do not take into consideration any potential effects on microbial activity. The introduction of small concentrations of O₂ into anoxic environments will change the redox conditions as O₂ has a greater redox potential

compared to other common electron acceptors found in anaerobic environments. Low concentrations of O₂ have been shown to increase microbial breakdown of complex carbon sources and encourage the growth of anaerobic microbes (Chayabutra and Ju, 2000; Jordan and Walsh, 2004). The concentration of O₂ recommended for CCS (i.e. 10 - 100 ppm) is high enough for microaerobic bacteria to respire and still allow some level of anaerobic metabolism (Marschall et al., 1993; Stolper et al., 2010). Although CO₂ will be injected in its supercritical form, which is generally regarded as a sterilising agent, certain Bacteria have been shown to survive and grow in its presence (Li et al., 2017; Mitchell et al., 2009; Peet et al., 2015). Spore forming bacteria, in particular, have shown resilience to supercritical CO₂ (Peet et al., 2015). Many sulphate reducing bacteria (SRB), such as *Desulfotomaculum*, form spores (O'Sullivan et al., 2014). This is of particular concern as SRB in subsurface engineered environments have been connected to issues such as blockage of injection wells (Pellizzari et al., 2016; Zettlitzer et al., 2010), corrosion of the injection well pump in geothermal energy (Lerm et al., 2013), oil souring (Jordan and Walsh, 2004) and oil degradation during enhanced oil recovery (Jobson et al., 1979; Sherry et al., 2013). It is now recognised that both archaeal and bacterial groups are capable of

* Corresponding author.

E-mail address: simongr@bgs.ac.uk (S.P. Gregory).

<https://doi.org/10.1016/j.ijggc.2021.103479>

Received 27 January 2021; Received in revised form 14 September 2021; Accepted 27 September 2021

Available online 7 October 2021

1750-5836/© 2021 British Geological Survey (c) UKRI 2021. All rights reserved. Published by Elsevier Ltd. This is an open access article under the CC BY license

(<http://creativecommons.org/licenses/by/4.0/>).

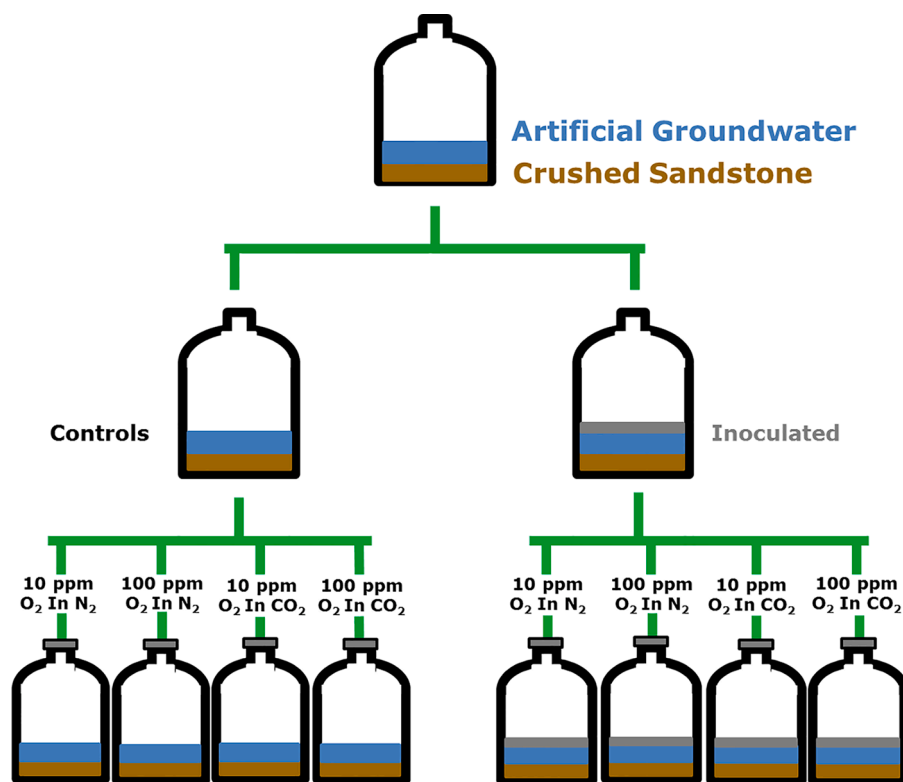


Fig. 1. Diagram showing the different conditions used in the experiments. Triplicate microcosms were set up for each of the conditions.

sulphate reduction, and could be implicated in these operational issues. In this study the term sulphate reducing bacteria (SRB) is used in this paper to describe microbes capable of performing sulphate reduction to maintain consistency with literature, but it is recognised that archaeal sulphate reducers could also be present.

The CO₂SINK project serves as an example of the response of microbial communities in subsurface environments used for CCS. This pilot scale project started in 2004 and involved the storage and monitoring of CO₂ in a sandstone formation at Ketzin, Germany (Martens et al., 2012). Prior to CO₂ injection, well blockages were encountered and were revealed to be caused by iron sulphide precipitation. SRB were detected within the downhole water samples and were determined to be the cause of the blockage due to the production of H₂S which reacted with iron within the storage site forming these deposits (Morozova et al., 2010; Zettlitzer et al., 2010). In addition to SRB, a range of microbial groups were shown to respond to CO₂ injection, and after injection, methanogenic archaea outcompeted SRB for a short period before SRB became dominant again (Morozova et al., 2010). As well as production of CH₄, methanogens have been implicated in microbially influenced corrosion (Lahme et al., 2020), so their presence in CCS reservoirs may also be of concern.

To better understand the potential for microbial activity in CCS reservoirs the experiments described in this paper examined microbial community composition and activity in N₂ and CO₂ when saturated with a gas containing 10 ppm and 100 ppm of O₂. The inclusion of a N₂ atmosphere allowed investigation of the specific effect of CO₂ to be distinguished from the response to microaerobic N₂ atmosphere. In addition to resembling the deep saline aquifer environment, the inclusion of the sandstone will provide a surface for microbial biofilm growth and the groundwater will provide additional nutrients required for microbial proliferation. In a CCS reservoir, the amount of dissolved O₂ available to microbes will vary depending on the groundwater composition, temperature and pressure, but this study will focus on creating an environment by saturating with gas mixtures containing 10 ppm and 100 ppm O₂ as this is the recommended range for use in the CO₂ storage

industry. The aim of this work was to observe changes in the microbial communities and related gas production in relation to the different O₂ concentrations, to inform operators of storage sites the recommendations for O₂ limits in the injected CO₂.

2. Material and methods

2.1. Material selection

The sandstone used in experiments was obtained from core at the British Geological Survey core store from the Sherwood Sandstone Group (formerly Bunter Sandstone) in the southern North Sea (map reference: 54.20125, 2.464). The sandstone was from well number 44/23-3 (SSK61176-81) and subsamples were taken from the 1380 – 1399 m depth range where a saline aquifer is situated. These samples are from a location and depth considered to be appropriate for CO₂ storage (Chadwick et al., 2008). Sandstone was crushed and sieved to <500 µm in size for use in experiments. Crushing the sandstone allowed for greater consistency between replicate experiments but also created a greater surface area for potential reactions with atmosphere, groundwater and microbial communities. By having a greater surface area, the reactions could occur more quickly and will allow the observation of interactions within the experimental run that might be occurring more slowly in the deep surface environment.

2.2. Microbial inoculum

Inoculum for the experiments were prepared from sandstone using a specific medium for enriching and indicating sulphate reducing bacteria, Postgate's medium B (Postgate, 1984), and incubated at 30C for 14 days under an anoxic atmosphere. Attempts to revive suitable organisms from the Sherwood Sandstone Group core failed, which may have been related to the age of the core (this material had been stored without any measures to preserve microorganisms for a minimum of 25 years). Therefore, to prepare the inoculum, fresh samples from the Godstone

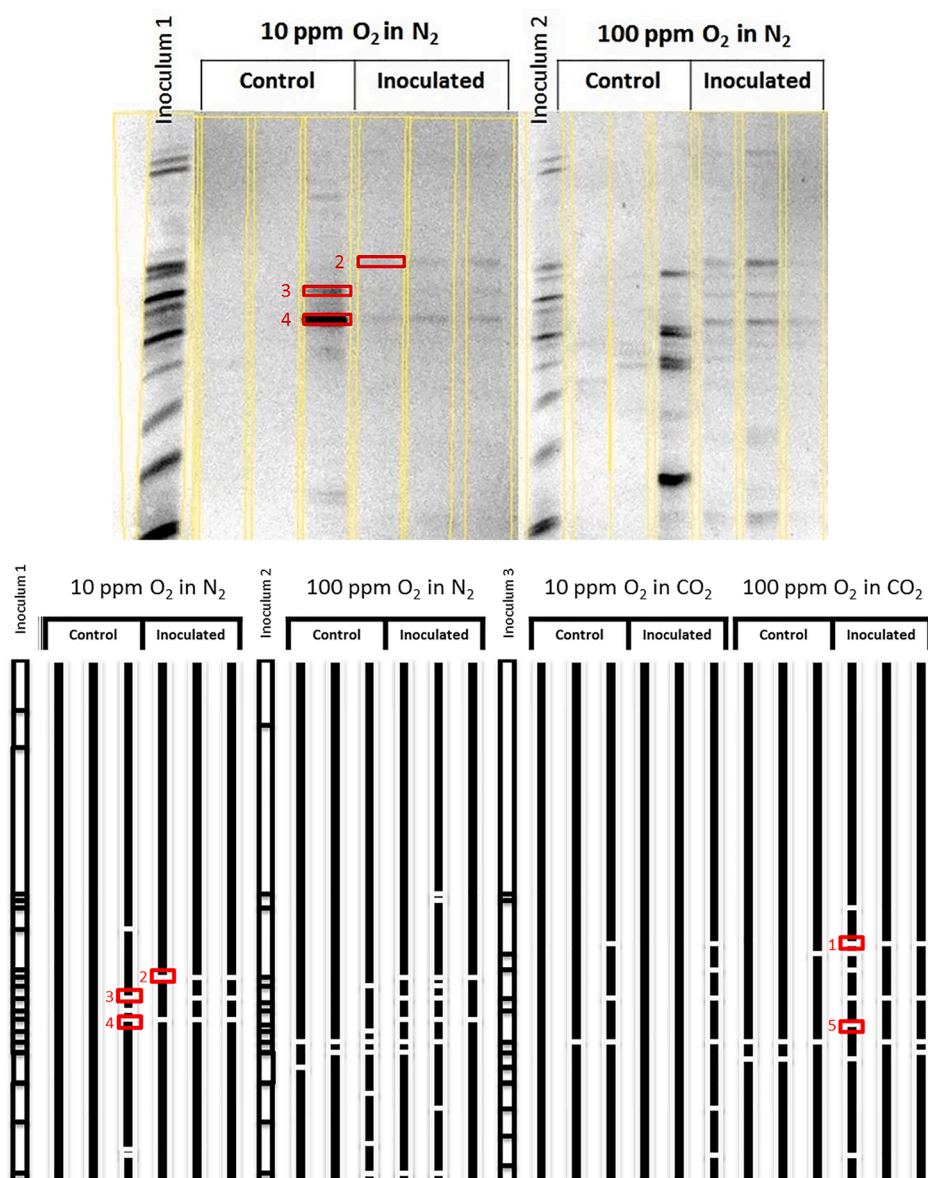


Fig. 2. Denaturing gradient gel electrophoresis (DGGE) profile of bacterial gene 16S rRNA for each experiment. Top shows the actual gel from the N_2 and the bottom image shows all three gels used in the experiment which were normalised using inoculum samples. Bands were aligned to 0.1 confidence level using T-align (Smith et al., 2005). Identities of excised and sequenced bands labelled (red outline) and numbered can be found in Table 4. Bacteria sequences identified by DNA sequencing of DGGE bands from the material sampled at the end of experiments.

and Marden sandstone were collected from a depth of 100–200 m in an underground stone quarry in Godstone, Surrey (UK). The quarry is in the Upper Greensand bed and this site was chosen as it has similar depositional environments to the middle Cretaceous of the central North Sea (Johnson et al., 2005) which is being investigated for potential CO_2 storage (Heinemann et al., 2012). Cultures for inoculum from these samples were set up within 1 week of collection of the sandstone. Prior to sampling, the sandstone was stored in a cool dry storage room, covered by a plastic tarp to reduce exposure to contamination and sunlight. Subsamples for enrichment were taken from the inside of the sandstone, to avoid the exposed exterior and reduce possible contamination. Positive SRB cultures in Postgate's B medium, determined by the formation of iron sulphide visible as a black precipitate, were selected for use. To obtain information on the dominant bacteria in the enrichments, DNA sequencing of selected cloned 16S rRNA gene PCR products was carried out (detailed in section DNA techniques).

To increase the consistency of inoculum between experiments, 15% glycerol stocks from the enrichment culture were prepared and stored at $-80^\circ C$. Prior to use in experiments, 100 μl of the glycerol stocks were cultured under optimal conditions for SRB growth which were in 15 ml Postgate's medium B at $31^\circ C$ under an atmosphere of 5% H_2 and 95% N_2

for 14 days. After growth, the sample was centrifuged and washed in sterile water three times to remove spent media before being used as inoculum.

2.3. Artificial groundwater

An artificial groundwater was prepared based on water chemistry data from groundwater obtained from the Sherwood Sandstone Group in Lincoln, UK below the saline interface at approximately 800 m in depth (Smedley and Edmunds, 2002). The artificial groundwater was prepared using deionised water and contained the following components Na_2SO_4 2.22 g l $^{-1}$; Calcium chloride dihydrate 4.40 g l $^{-1}$; Sodium chloride 4.18 g l $^{-1}$; Magnesium chloride hexahydrate 2.43 g l $^{-1}$; Potassium chloride 0.07 g l $^{-1}$; Sodium carbonate 0.10 g l $^{-1}$; Yeast extract 0.05 g l $^{-1}$. It was then autoclaved and adjusted to pH 7.77 using sterile 1 M hydrochloric acid. Yeast extract (0.005% w/v) was added to represent the presence of natural organic compounds within the aquifer and any that may be introduced during operations e.g. in drilling fluids.

Table 1
PCR and primer details used in this study.

Gene target	Primer pair	Start (°C/ min)	Denaturing (°C/ s)	Annealing(°C/ s)	Extension (°C/ s)	Cycles	Final extension (°C/ min)	Reference
16S rRNA (Bacteria)	BAC341F/ BAC534R	95/5	95/30	55/30	72/30	34x	72/30	(Muyzer et al., 1993)
16S rRNA (Archaea)	Arc344F / Arc915R	95/3	95/30	66/30	72/120	34x	72/30	(Raskin et al., 1994)
<i>mcrA</i> (methanogens)	mlas / <i>mcrA</i> -rev	95/3	95/30	55/45	72/30	40x	72/5	(Luton et al., 2002)
<i>dsrB</i> (sulphate reducing gene)	<i>dsr2060F</i> / <i>dsr4R</i>	94/4	94/60	55/60	72/60	34x	72/15	(Geets et al., 2006)

Table 2
Analysis performed on the batch experiments with timings.

Timing	Method
Day 0	Assembly of batch experiments
Day 7	Gas analysis
Day 15	Gas analysis
Day 22/23	Gas analysis (N ₂ /CO ₂)
Day 30	Gas analysis
Day 37	Gas analysis
Day 43 (end point)	Gas analysis Disassembly of batch experiments pH Preservation of samples
Post incubation	DNA extraction and analysis Microbial enumeration

2.4. Experimental setup

The experiments were set up in sterile 125 ml Wheaton™ serum bottles containing unsterilised crushed sandstone, artificial groundwater and a microbial inoculum (except in controls). Gas headspace mixtures of 10 ppm O₂ in N₂, 100 ppm O₂ in N₂, 10 ppm O₂ in CO₂ and 100 ppm O₂ in CO₂ (gas mixes were supplied by Calgaz™ Ltd, Newcastle-Under-Lyme, UK) were used. Gas mixtures other than 10 ppm O₂ in CO₂ were certified as accurate to 1 ppm O₂, where accuracy was ± 5 ppm. For each gas mixture triplicate serum bottles were set up for both the inoculated experiments and uninoculated controls (Fig. 1). All experiments were set up inside an anaerobic chamber with a nominal atmosphere of 5% H₂ and 95% N₂. 5 g of sandstone and 30 ml of artificial groundwater was added to each bottle, leaving approximately 90 ml of headspace. 1 ml of the prepared inoculum, resuspended in sterile water, was added to each of the inoculated experiments. The inoculum was from the same source for all inoculated experiments and was prepared in the same way; however, the inoculum for the N₂ experiments was prepared separately to the inoculum for the CO₂ experiments due to available equipment and space. Gas mixtures were bubbled through artificial groundwater for 5 minutes to displace the anaerobic chamber atmosphere and then the serum bottle was crimp sealed with an autoclaved butyl rubber stopper. Experiments were incubated in the anaerobic chamber at 31°C for 43 days. pH measurements of the starting groundwater and from each serum bottle at the end of the experiment. Timings of analysis performed can be found in Table 2.

2.5. DNA techniques

Samples for DNA extraction were taken from the inoculum and from the serum bottles at the end of each experiment. Serum bottles were mixed well prior to taking a sample to obtain representation of planktonic and sessile organisms and frozen at -80°C until analysed. DNA extraction was performed using FastDNA™ SPIN kit for soil (MP Bio Science Ltd, Derby, UK) according to the manufacturer's instructions. PCR was conducted to detect presence of selected microbial groups in the microcosms. PCR conditions for the amplification of Bacteria and Archaea (16S rRNA), sulphate reducers (*dsrB*) and methanogens (*mcrA*) are given in Table 1. PCR reactions were set up with 3.6 µl nuclease free water, 0.2 µl of 10 µM forward primer, 0.2 µl of 10 µM reverse primer,

5.0 µl of DreamTaq™ polymerase master mix (ThermoFisher Scientific®, Loughborough, UK) and 1 µl of template DNA. 3 µl of PCR products were run on 1% agarose gel and visualised on Gel Doc™ XR+ and Image Lab™ software (Bio-Rad®, CA, USA) to confirm amplification.

Denaturing gradient gel electrophoresis (DGGE) was performed on PCR products from the bacterial 16S rRNA gene extractions. The DCode™ system (Bio-Rad®) was used for DGGE casting and electrophoresis, according to manufacturer's instructions. PCR amplification was carried out using BAC341F (with GC clamp) and BAC534R primers using the same program as 16S rRNA bacteria (Table 1). For DGGE, 10 µl PCR reactions were set up in triplicate and pooled to reduce PCR bias (Wagner et al., 1994). 25 µl samples of PCR product was run on an 8% polyacrylamide gel with a denaturing gradient of 40–70% urea and formamide at 80 V at 60°C for 16 hours. After staining with 1X SYBR™ Gold (ThermoFisher Scientific®) in 1X TAE buffer, the gel was visualised using the Gel Doc™ XR+ with the XcitaBlue™ conversion screen and Image Lab™ software (Bio-Rad®). Bands of interest were excised from the DGGE and cloned into pCR2.1 following the Invitrogen TA Cloning kit manufacturer's instructions (Invitrogen, 2013) in preparation for DNA sequencing. Sequencing of PCR inserts was conducted at Eurofins Genomic (Wolverhampton, UK) and sequences were checked for nearest relatives using BLAST search in the GenBank Data Library (<https://blast.ncbi.nlm.nih.gov/Blast.cgi> [accessed 31/07/2021]). DNA sequences generated in this project were deposited in NCBI GenBank (Accession numbers MH513315 -MH513318)

The starting inoculum was run on each gel, for direct comparison with samples and to allow gels to be compared more easily. The migration distance of bands and relative intensity were used to create a multivariate cluster with the unweighted pair group method using arithmetic averages (UPGMA) using the Bray-Curtis dissimilarity index in the PAST software package (Hammer et al., 2001). UPGMA clusters samples based on the highest similarities and then by the arithmetic average of the similarities. Comparison of DGGE profiles from different gels was performed by consolidating bands using T-align with a confidence level of 0.1 (Smith et al., 2005).

2.6. Gas measurement

Approximately 4 ml gas sample headspace gas was collected on days 7, 15, 22 (N₂ only) 23 (CO₂ only), 30, 37 and 43 of incubation. For H₂S gas analysis a Dräger Xam-2000 gas detector was used. For analysis of CH₄ an Agilent Technologies gas chromatography (GC) 7820A with a Restek® 80468-810 packed column with a He carrier gas was used. 4 ml of the headspace gas samples were injected into the GC for analysis. The heating profile for analysis was 3 minutes hold at 23°C, 3°C/minute ramp to 40°C, 5°C/minute to 90°C, 2 minutes hold at 90°C, having a total run time of 20.7 minutes. Gas standards of air and 100 ppm CH₄ in N₂ were used to calculate CH₄ gas concentration. CH₄ measurements were compared against standards assuming a linear relationship and linearly extrapolated when outside this range.

Table 3

Gene detection for each experiment. Experiments in which the target gene could be detected by PCR are shown with a tick.

Experiments	Target Gene			
	16S rRNA (Bacteria)	16S rRNA (Archaea)	<i>dsrB</i> (Sulphate reducers)	<i>mcrA</i> (Methanogens)
10 ppm O ₂ in N ₂ control 1				
10 ppm O ₂ in N ₂ control 2		✓		
10 ppm O ₂ in N ₂ control 3	✓	✓	✓	
10 ppm O ₂ in N ₂ inoculated 1	✓		✓	
10 ppm O ₂ in N ₂ inoculated 2	✓		✓	
10 ppm O ₂ in N ₂ inoculated 3	✓	✓	✓	
100 ppm O ₂ in N ₂ control 1	✓	✓	✓	✓
100 ppm O ₂ in N ₂ control 2	✓	✓		
100 ppm O ₂ in N ₂ control 3	✓	✓	✓	
100 ppm O ₂ in N ₂ inoculated 1	✓	✓	✓	
100 ppm O ₂ in N ₂ inoculated 2	✓			
100 ppm O ₂ in N ₂ inoculated 3	✓	✓	✓	
10 ppm O ₂ in CO ₂ Control 1		✓		
10 ppm O ₂ in CO ₂ Control 2				
10 ppm O ₂ in CO ₂ Control 3		✓		
10 ppm O ₂ in CO ₂ Inoculated 1			✓	
10 ppm O ₂ in CO ₂ Inoculated 2	✓		✓	
10 ppm O ₂ in CO ₂ Inoculated 3	✓			✓
100 ppm O ₂ in CO ₂ Control 1				
100 ppm O ₂ in CO ₂ Control 2				
100 ppm O ₂ in CO ₂ Control 3				
100 ppm O ₂ in CO ₂ Inoculated 1	✓	✓	✓	
100 ppm O ₂ in CO ₂ Inoculated 2		✓	✓	
100 ppm O ₂ in CO ₂ Inoculated 3	✓	✓	✓	✓

2.7. Microbial enumeration

At the end of the experiments, pH was measured and 1 ml subsamples (mixed sandstone and groundwater) were preserved in 1% glutaraldehyde and stored at 4°C before staining with 0.01% acridine orange and preparation for epifluorescence microscopy (Darzynkiewicz and Kapuscinski, 1990). Microscopy was carried out using the Zeiss microscope with an epifluorescence head, containing filter set 09 (40 - 490 nm). 20 fields of view were counted and the mean cells per ml were calculated (Hobbie et al., 1977). All samples included particulates in the

Table 4

Bacteria sequences identified by DNA sequencing of DGGE bands from the material sampled at the end of experiments. Bands as in Fig. 2.

	DGGE band (GenBank accession number)	Experiment Condition	Closest matching sequence in NCBI nucleotide database (% match)
1	MH513315	100 ppm O ₂ in CO ₂ inoculated 1	AB245379.1 <i>Bacillus ginsengihumi</i> (99%)
2	sequence too short to be submitted to NCBI	10 ppm O ₂ in N ₂ inoculated 1	JQ897424.1 <i>Desulfotomaculum halophilum</i> (97%)
3	MH513316	10 ppm O ₂ in N ₂ control 3	AB245379.1 <i>Bacillus ginsengihumi</i> (99%)
4	MH513317	10 ppm O ₂ in N ₂ control 3	KY753308.1 <i>Bacillus niacini</i> (98%)
5	MH513318	100 ppm O ₂ in CO ₂ inoculated 1	AJ630291.1 <i>Alkaliphilus transvaalensis</i> (100%)

16S rRNA gene DGGE results from multiple gels were combined and aligned using the inoculum sample which was run on all gels. This data was then compiled into the chart shown in Fig. 2 which shows the bands present in each sample. Error! Reference source not found. With the exception of the 10 ppm O₂ in N₂ experiment, the controls showed fewer unique bands compared to the inoculated experiments.

form of sandstone from the experiments. Microbial cells are known to attach to particulates causing greater cell counts in presence of particulates. However cells can also be masked by the particulate, making them impossible to count. Therefore, it was assumed that the loss in cell numbers caused by particulate masking was offset by the greater number of cells being attached to the particulate (Schallenberg et al., 1989).

3. Results

3.1. Detection of key microbial groups

Bacterial 16S rRNA and *dsrB* genes were detected in most of the inoculated experiments (Table 3). Bacterial 16S rRNA and *dsrB* genes were also detected in the uninoculated control experiments in a N₂ atmosphere. Archaeal 16S rRNA genes were detected across all experiments in a N₂ atmosphere. The *mcrA* gene was only detected in 3 individual replicates (each of which also showed CH₄ production). However, the *mcrA* gene could not be detected in two of the microcosms that produced lower CH₄ concentrations (10 ppm O₂ in N₂ inoculated 2 and 100 ppm O₂ in CO₂ inoculated 2), or in the starting material.

3.2. Bacterial community analysis

Abundant members of the bacterial community in initial SRB enrichment inoculum and at the end of experiments were identified by sequencing key DGGE gel bands (Table). The closest matching sequences to bands from the inoculum in NCBI's genbank database were a facultatively anaerobic *Pseudomonas* species (KT991031), a spore forming SRB *Desulfotomaculum* species (KM494501), the iron reducing spore former *Pelosinus fermentans* (JBW45) and a facultatively anaerobic saline tolerant *Halomonas* species (KP241932). *Desulfotomaculum* spp. was also identified from bands at the end of experiments, with other bands most closely related to spore-forming *Alkaliphilus transvaalensis* and *Bacillus* species.

To compare the communities in different samples, multivariate clustering using the unweighted pair group method with arithmetic mean method and Bray-Curtis dissimilarity index was performed (Fig. 3). The inoculated N₂ experiments (along with one of the 100 ppm O₂ in N₂ control samples) clustered separately from CO₂ atmospheres, with the 10 ppm O₂ in N₂ samples showing more similarity to each other than the 100 ppm in N₂ samples. This cluster of samples was most closely related in its composition to two of the inoculum samples. The third inoculum sample was more similar to the communities that developed in

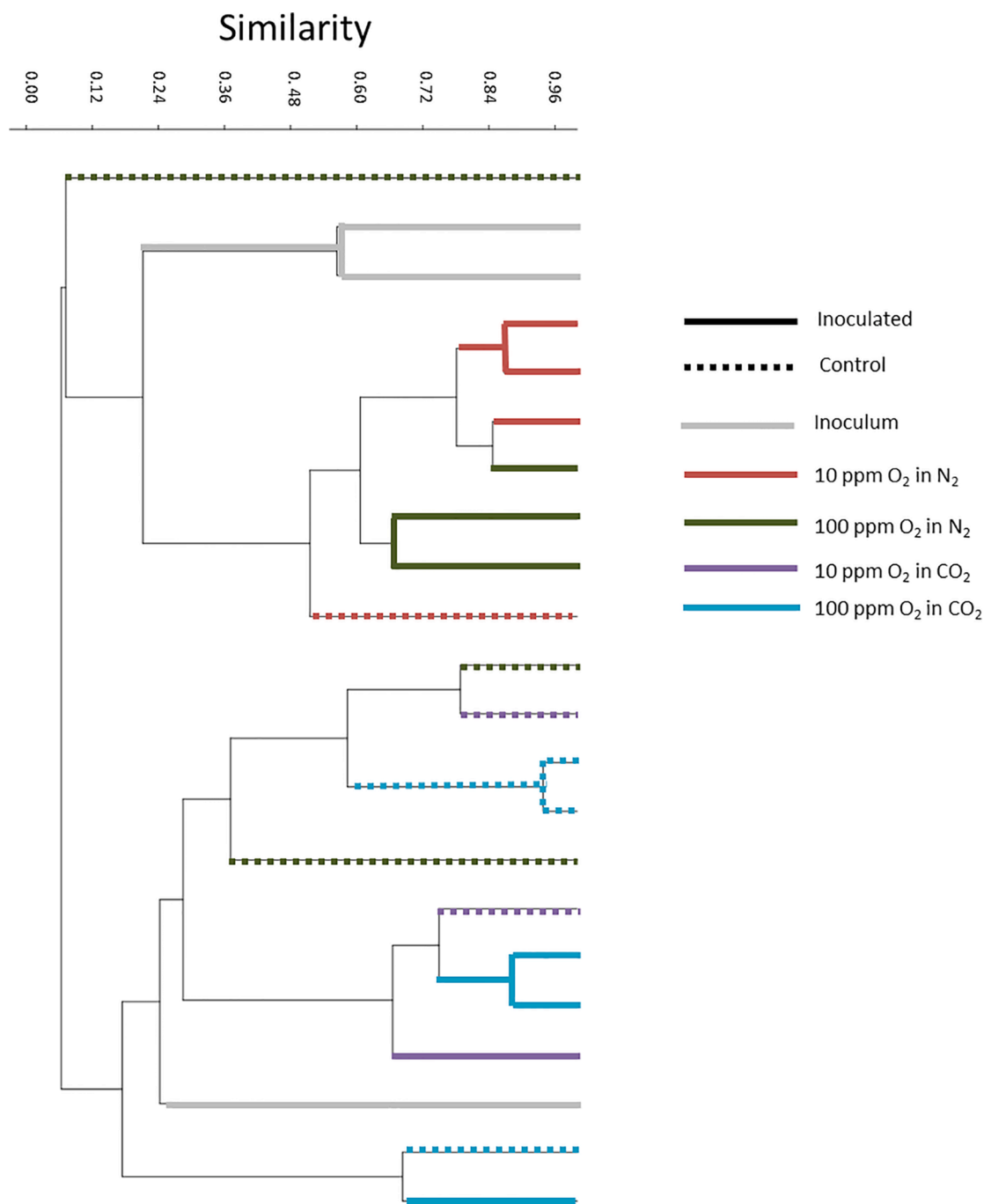


Fig. 3. Dendrogram showing dissimilarity between microbial communities in samples taken from all atmospheres. Dendrogram was constructed using unweighted pair group method with arithmetic mean (UPGMA) method and using Bray-Curtis dissimilarity index, based on DGGE profiles of bacterial 16S rRNA genes. The solid lines are inoculated experiments and the inoculum and the dotted lines are uninoculated controls.

the CO₂ headspace. Despite attempts to standardise the inoculum by preparing enrichment cultures from a stock culture stored in glycerol, these differences likely reflect differences in community dynamics during this enrichment stage. Except for one of the 100 ppm O₂ control, the remaining samples formed a looser cluster. Within this cluster, as might be expected, similarities between at least two replicates (e.g. the 100 ppm O₂ in CO₂ samples) were observed.

3.3. Epifluorescence cell counts

The starting inoculum had an average cell count of 5.1×10^7 . The experimental cell counts were all within an order of magnitude of each

other, between 0.9 And 3.3×10^6 cells per ml (Fig. 4). The mean cell counts in the inoculated experiments were higher than the relevant control, with the exception of the 100 ppm O₂ in N₂ controls (where control and inoculated samples were within mean standard error of each other). The mean cells counts in both the control and inoculated experiments were lower with the CO₂ headspace gas than the equivalent O₂ concentration in N₂.

3.4. Gas analysis and pH measurements

H₂S and CH₄ were detected in at least one microcosm under all headspace gas compositions. H₂S was produced in all inoculated

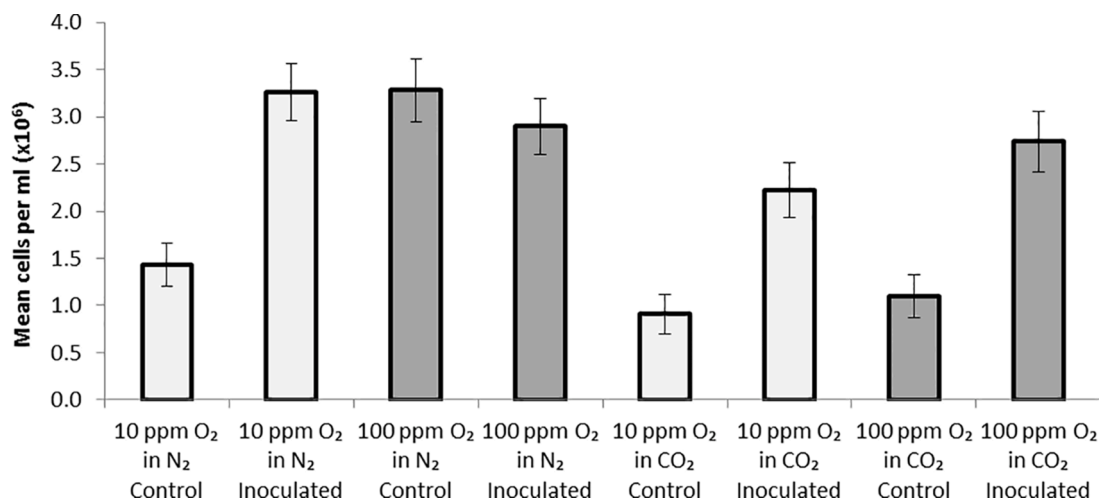


Fig. 4. Mean cell counts ($\times 10^6$) per ml at the end of the experiment. Error bars show the mean standard error for the cell count calculations.

microcosms, but only detected in one of the control experiments (100 ppm O₂ in N₂). Production of H₂S was delayed in the CO₂ headspace compared to N₂ headspace (Fig. 5). CH₄ was only detected in five out of the 24 microcosms. When detected, CH₄ production typically followed H₂S production.

At the first sampling point (day 7) H₂S was only detected in inoculated N₂ headspace microcosms. The inoculated 10 ppm and 100 ppm O₂ in N₂ experiments showed similar patterns in H₂S production to each other. Means of 0.30 ppm H₂S (10 ppm O₂ in N₂) and 0.17 ppm H₂S (100 ppm O₂ in N₂) were recorded on day 7. On day 15, 100 ppm H₂S was detected in the O₂ in N₂ microcosms (0.47 ppm), other than this, no H₂S was detected after day 7 until day 30 when H₂S was detected in both sets of inoculated microcosms and in the 100 ppm O₂ in N₂ control microcosms. Thereafter, there was a general increase H₂S in these three microcosms until the end of the experiment on day 43, when the two inoculated microcosms showed the higher H₂S concentrations (1.20 ppm for 10 ppm O₂ and 0.83 ppm in the 100 ppm O₂ microcosms) than the 100 ppm O₂ control microcosms (0.60 ppm) on day 43. No H₂S was detected in the 10 ppm O₂ in N₂ microcosms. Mean concentrations in the 100 ppm control experiments were always lower than the comparable inoculated experiment.

In comparison, under the CO₂ headspace, H₂S production was delayed and was detected only in the inoculated microcosms and only from day 23 onwards. The highest mean H₂S concentration in both 10 ppm and 100 ppm O₂ in CO₂ inoculated experiments was on day 23 (2.03 ppm for 10 ppm and 0.83 for 100 ppm). Afterwards the mean concentration of H₂S fell to 0.13–0.20 ppm for the remaining readings in the 10 ppm microcosms and fluctuated between 0.40 ppm (day 37) and 1.13 ppm (day 30) for the remainder of the experiment. High standard deviations seen in the 100 ppm O₂ in CO₂ inoculated experiments reflect the fact that one replicate showed considerably higher H₂S concentrations than the others.

CH₄ production was only observed in five of the 24 replicate microcosms (Fig. 5). One of the 10 ppm O₂ in N₂ inoculated replicates had CH₄ present (458 ppm) on day 5 only. Other than this no CH₄ was detected until day 30 when CH₄ was detected in two of the microcosms with a CO₂ headspace (one 10 ppm O₂, and one 100 ppm O₂ microcosms). In both, mean CH₄ concentration reached maximum on day 37 (4882 ppm in the 10 ppm O₂ and 5011 ppm in the 100 ppm O₂) microcosm. On day 37 another of the 100 ppm O₂ in CO₂ microcosms showed CH₄ production, along with the only control microcosm where CH₄ was detected (100 ppm O₂ in N₂). In those inoculated microcosms where CH₄ was detected (all of which had a CO₂ headspace), this was preceded by the maximum concentration of H₂S observed.

Results from the end point pH measurements can be found in Table 5.

The CO₂ experiments showed a lower final pH (5.77 - 5.91) compared to the N₂ experiments (pH 6.89 - 7.15). This was the expected observation as CO₂ dissolves in water to form carbonic acid. All experiments decreased in pH from the starting pH 7.77. This suggests that the sandstone and microbial activity from native and inoculated communities may also contribute to the decrease in pH. This might be from the degradation of organic compounds to form volatile organic acids.

4. Discussion

The communities that developed in a CO₂ atmosphere were different to those developed under an N₂ atmosphere, as revealed by the DGGE analysis, PCR and cell counts, which indicate that microbial abundance and activity was reduced or delayed in the presence of CO₂. Evidence of sulphate reduction was observed in both N₂ and CO₂ atmospheres, but the *dsrA* gene was less commonly detected in the CO₂ atmosphere which could be because of lower microbial activity. Targeted sequencing was used to identify OTUs represented by key DGGE bands, which provided some insight into dominant Bacteria in the microcosms. The SRB *Desulfotomaculum halophilum* was detected in the inoculum, the inoculated 10 ppm and 100 ppm O₂ in N₂ experiments. The band relating to *D. halophilum* was not detected in the CO₂ experiments, but the gene for sulphate reduction (*dsrA*) and H₂S production was detected, this indicates that other SRB must be present, either represented by bands that were not selected for sequencing or not detected using this primer set.

The 100 ppm O₂ in CO₂ showed a greater initial H₂S production compared to 10 ppm O₂. After this initial H₂S production, concentration decreased in the headspace. This contrasts with the generally increasing H₂S levels seen in the both the N₂ experiments and the 10 ppm O₂ in CO₂. The fact that H₂S could only be detected in CO₂ microcosms after day 23, despite being inoculated with SRB from an enrichment culture, may reflect the initially inhibitive conditions for SRB produced upon initial injection of CO₂ as observed in the CO₂SINK project. The decrease in SRB was thought to be related to the decrease in pH of the environment (Morozova et al., 2011). Decreases seen in H₂S concentrations observed in the CO₂ experiments could be because of the H₂S being oxidised, either chemically or biologically by microbes, such as autotrophic sulphide-oxidizing *Thiobacillus* species, which can be found in subsurface environments (Hose et al., 2000). In both the inoculated and control CO₂ microcosms, cell counts were higher in the 100 ppm O₂ condition compared to the 10 ppm O₂ conditions. This result supports the links seen between O₂ increase and increase in activity of microbes that are able to use O₂ and the microbes that are able to use the products of aerobic reactions such as aerobic digestion (Chayabutra and Ju, 2000; Jobson et al., 1979; Jordan and Walsh, 2004).

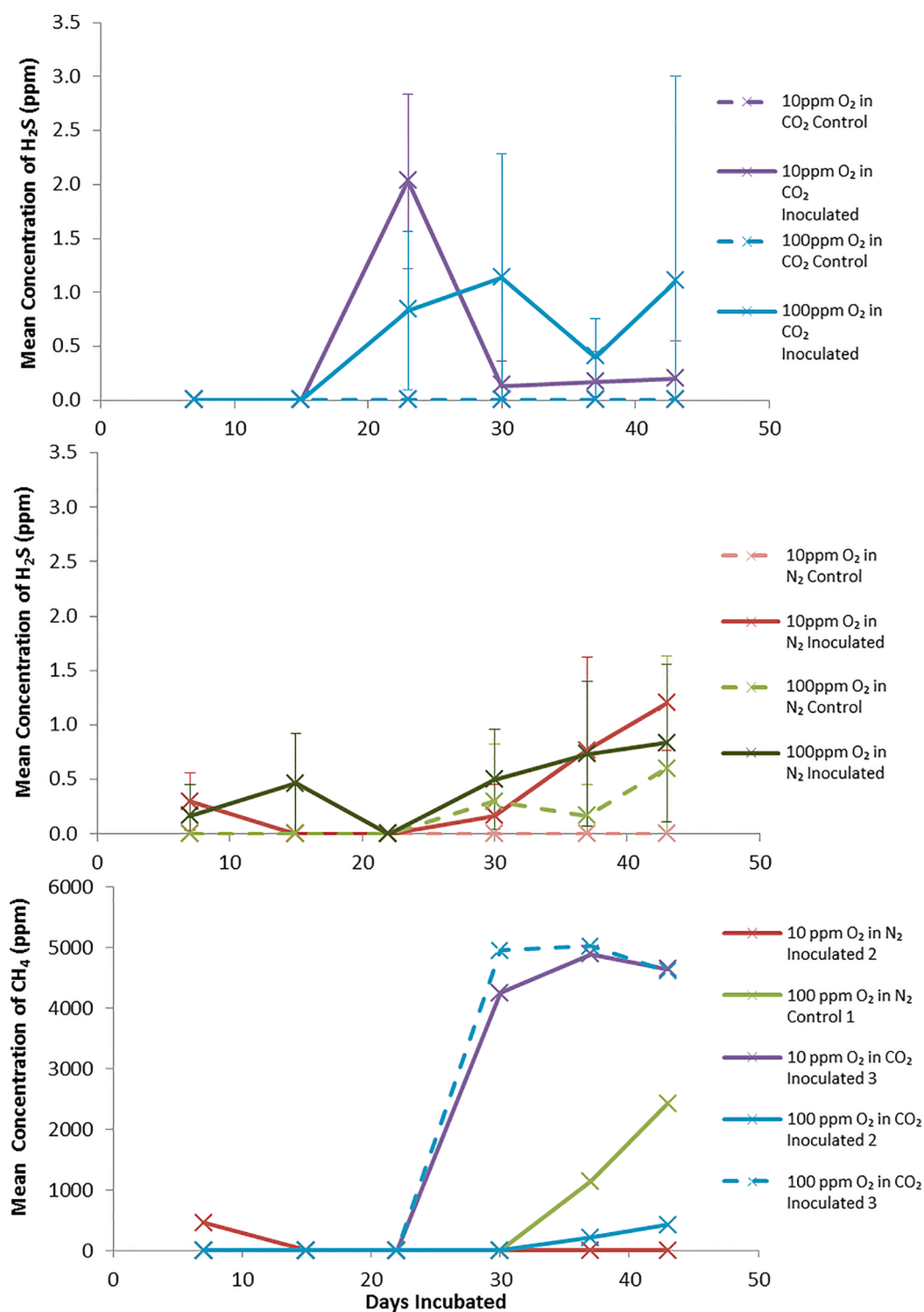


Fig. 5. H₂S and CH₄ concentrations in ppm. Average H₂S in the O₂ in CO₂ (top) and in N₂ experiments (middle), and individual replicates showing CH₄ concentrations in both experiments (bottom). Error bars show standard deviation from triplicates.

Methanogenesis was less commonly detected than sulphate reduction (in three CO₂ microcosms and two N₂ microcosms – one of which had CH₄ only at the start of the experiment). The *mcrA* gene was detected in the experiments which produced the three highest concentrations of CH₄. An increase in CH₄ was seen after a drop in H₂S concentration in a single replicate with a 100 ppm O₂ in CO₂ atmosphere. Methanogenesis was not expected as the inoculum used was from an enrichment culture designed to select SRB not methanogens. Methanogens are sensitive to oxygen (at least in the laboratory) (Wolfe, 2011),

but they have previously been shown to be cultivable from a number of desert and other oxic-soils under appropriate conditions (Peters and Conrad, 1995, Angel et al., 2011) suggesting they possess mechanisms for surviving dry, oxic conditions as would be encountered in a core store. The presence of methanogens/methanogenesis was more common in the CO₂ experiments and is consistent with observations that methanogenesis is increased in high CO₂ atmospheres mimicking reservoir conditions (Mayumi et al., 2013). If we assume that some loss in viability occurs during storage of core material, stimulation of methanogenesis in

Table 5
pH for each condition taken at the end of the experiment.

Sample	pH (+/- SD)
Starting artificial groundwater	7.77
10 ppm O ₂ in N ₂ control	6.99 (± 0.37)
10 ppm O ₂ in N ₂ inoculated	6.89 (± 0.07)
100 ppm O ₂ in N ₂ control	7.14 (± 0.04)
100 ppm O ₂ in N ₂ inoculated	7.15 (± 0.03)
10 ppm O ₂ in CO ₂ Control	5.89 (± 0.05)
10 ppm O ₂ in CO ₂ Inoculated	5.77 (± 0.35)
100 ppm O ₂ in CO ₂ Control	5.91 (± 0.05)
100 ppm O ₂ in CO ₂ Inoculated	5.81 (± 0.06)

pH of N₂ experiments were taken at 19.5°C and CO₂ experiments at 19°C. Standard deviations were calculated for 3 replicates of each sample.

subsurface environments such as hydrocarbon reservoirs and saline aquifers in the presence of CO₂ could be more common than observed in these experiments. CH₄ was detected after H₂S in these experiments but in a CCS reservoir a redox gradient may form from the O₂ introduction near the injection well. This could cause sulphate reduction to be more prominent near the injection well and methanogenesis further was from the injection well. Because of the low numbers of microcosms showing CH₄ production, it is difficult to make any definitive statements about the effect of O₂ concentration, the number of microcosms producing CH₄, the earlier production and maximum production in these microcosms does hint that methanogenesis could be more likely with the higher concentration of O₂. The presence of CH₄ within a storage site could reduce the storage capacity, by making CO₂ solubility and residual trapping less efficient and reduce injectivity (Blanco et al., 2012).

Spore forming organisms are considered important in many subsurface environments (Muyzer and Stams 2008, Nilsen et al., 1996) including CCS reservoirs (Peet et al., 2015) because this lifestyle allows them to survive harsh conditions. Although, a complete community analysis was not done in this study, it is interesting to note that while two out of five of the sequences obtained from the inoculum belonged to non-spore forming bacteria, all of the sequences retrieved post experimentally belonged to spore forming bacteria. Bacteria capable of forming spores may do so when challenged with inhospitable conditions in order to survive. Being able to form spores would also aid survival when coming into contact with supercritical CO₂ (Peet et al., 2015). *D. halophilum* is a strict anaerobe (Tardy-Jacquenod et al., 1998) and a related sequence detected in the within 10 ppm and 100 ppm O₂ in N₂ indicate its tolerance of the levels of O₂ used in CO₂ storage. A sequence relating to *Bacillus ginsengihumi* was detected in four out of six inoculated CO₂ replicates, and one control CO₂ experiment. This organism has been shown to grow at pH 5 (Ten et al., 2006) and could have had a competitive advantage in the weakly acidic conditions observed within the CO₂ experiments.

The DGGE analysis showed the bacterial communities in samples from N₂ inoculated atmospheres were different to those from the CO₂ atmospheres. Samples from experiments containing the same O₂ concentrations in N₂ cluster together, whereas the CO₂ samples are more heterogeneous with controls and inoculated experiments of different O₂ concentration appearing on the same clusters. Similarly, bacterial cell counts showed some response to the concentration of O₂ and in all cases, except for inoculated N₂ microcosms, biomass was higher in the 100 ppm condition. In all cases microcosms appeared to be dominated by a relatively small number of taxa. This study did not aim to fully characterise the microbial communities, rather to use DGGE, targeted sequencing and the other methods to identify whether there were differences in the communities and what some of the dominant organisms were. Given that the DGGE data combined with all the other data presented indicate there are differences in the composition and activity of the microbial communities when exposed to CO₂ and to a lesser extent to different levels of O₂ impurities in CO₂, applying -omics technologies in

future studies will help to understand how the communities differ and predict what impacts this could have on the operation of CCS.

5. Conclusions

The different O₂ concentrations investigated show differences in microbial communities and in H₂S production. A CO₂ atmosphere retards or reduces the amount of sulphate reducing activity. In CO₂ with 100 ppm O₂ there was an initial peak of H₂S production followed by reduced concentrations, which contrasted with gradually increasing H₂S production under all other atmospheres. Biomass was also higher in the presence of 100 ppm O₂. The observation of CH₄ showed that there is the potential for CH₄ production under CO₂ storage conditions, particularly as methanogens are more likely to be found in anoxic subsurface environments. This highlights the need for further studies to investigate methanogens in future research. However, as CH₄ production was only observed in a few microcosms, it is not possible to determine the effect of O₂ on CH₄ production. These results suggest that a level of 10 ppm O₂ in the injected CO₂ would result in a lower H₂S production compared to 100 ppm O₂. A reduction in H₂S production would reduce CO₂ storage issues from microbial sources and should be taken into consideration when setting limits for acceptable amounts of O₂ in injected CO₂. These results support previous links between O₂ being used within microbial communities and increasing anaerobic activity. To confirmation and expand on these findings, experiments could be conducted in a continuous flow system and/or under elevated pressures relevant to CO₂ storage sites.

CRedit authorship contribution statement

Hayden Morgan: Conceptualization, Methodology, Investigation, Writing – original draft. **David J. Large:** Conceptualization, Writing – review & editing, Supervision. **Keith Bateman:** Conceptualization, Writing – review & editing, Supervision. **David Hanstock:** Conceptualization, Writing – review & editing, Supervision. **Simon P. Gregory:** Conceptualization, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

This work was funded by the EPSRC as part of the centre for doctoral training in carbon capture and storage and cleaner fossil energy at the University of Nottingham. The authors would like to acknowledge the Inorganic Geochemistry Facility at the British Geological Survey (Keyworth, England) for sample preparation work. Hayden Morgan, Keith Bateman and Simon Gregory publish with the permission of the Director, British Geological Survey.

References

- Angel, R., Matthies, D., Conrad, R., 2011. Activation of methanogenesis in arid biological soil crusts despite the presence of oxygen. *PLoS ONE* 6, e20453, 5.
- Blanco, T., Rivas, C., Ferna, J., Artal, M., Velasco, I., 2012. Influence of methane in CO₂ transport and storage for CCS technology. *Environ. Sci. Technol.* 46, 13016–13023. <https://doi.org/10.1021/es3037737>.
- Chadwick, A., Arts, R., Bernstone, C., May, F., Thibeau, S., Zweigel, P., 2008. *Best Practice for the Storage of CO₂ in Saline Aquifers - Observations and Guidelines from the SACS and CO₂STORE Projects*. Keyworth, Nottingham: ISBN: 978-0-85272-610-5.
- Chayabutra, C., Ju, L., 2000. Degradation of n-hexadecane and its metabolites by *Pseudomonas aeruginosa* under microaerobic and anaerobic denitrifying conditions. *Appl. Environ. Microbiol.* 66, 493–498, 0099-2240/00/\$04.00+0.

- Committee on Climate Change, 2019. Reducing UK emissions - 2019 progress report to parliament - committee on climate change. *Comm. Clim. Change*.
- Darzynkiewicz, Z., Kapuscinski, J., 1990. Acridine orange: a versatile probe of nucleic acids and other cell constituents. *Flow Cytometry and Sorting*, second ed. Wiley-Liss Inc, New York.
- de Visser, E., Hendriks, C., Barrio, M., Mølnvik, M.J., de Koeijer, G., Liljemark, S., Le Gallo, Y., 2008. Dynamics CO₂ quality recommendations. *Int. J. Greenh. Gas Control* 2, 478–484. <https://doi.org/10.1016/j.ijggc.2008.04.006>.
- 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*. <https://doi.org/10.1016/j.mimet.2005.11.002>.
- Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: paleontological statistics software package for education and data analysis. *Palaentol. Electron.* 4, 9.
- Heinemann, N., Wilkinson, M., Pickup, G., Haszeldine, S., Cutler, N., 2012. CO₂ storage in the offshore UK Bunter sandstone formation. *Int. J. Greenh. Gas Control* 6, 210–219. <https://doi.org/10.1016/j.ijggc.2011.11.002>.
- Hobbie, J.E., Daley, R.J., Jasper, S., 1977. Use of nucleopore filter counting bacteria by fluorescence microscopy. *Appl. Environ. Microbiol.* 33, 1225–1228 doi:citeulike-article-id:4408959.
- Hose, L., Palmer, A., Palmer, M., Northup, D., Boston, P., DuChene, H., 2000. Microbiology and geochemistry in a hydrogen-sulphide-rich karst environment. *Chem. Geol.* 169, 399–423.
- Invitrogen, 2013. Invitrogen TA Cloning Kit. Available at: <https://www.thermofisher.com/order/catalog/product/K204001#/K204001> (accessed 31/07/2021).
- Jensen, M., 2016. CO₂ transport: IEAGHG 10th international CCS summer school, Saskatchewan, Canada, July 21, 2016. Available at: <http://documents.ieaghg.org/index.php/s/71ht8eZsocEaes8?path=%2FSummer%20School%202016%2FPresentations> (accessed 31/07/2021).
- Jobson, A.M., Cook, F.D., Westlake, D.W.S., 1979. Interaction of aerobic and anaerobic bacteria in petroleum biodegradation. *Chem. Geol.* 24, 355–365. [https://doi.org/10.1016/0009-2541\(79\)90133-5](https://doi.org/10.1016/0009-2541(79)90133-5).
- Johnson, H.A.B., Leslie, A.B., Wilson, C.K., Andrews, L.J., Cooper, R.M., 2005. Middle Jurassic, upper Jurassic and lower Cretaceous of the UK central and northern North Sea, British Geological Survey. Keyworth, Nottingham. doi:ISBN - 0852725094.
- Jordan, L.C., Walsh, J.M., 2004. Selection of an active souring management solution for a Gulf of Mexico waterflood. *CORROSION* 2004, 1–11 doi:NACE-04759.
- Lahme, S., Mand, J., Longwell, J., Smith, R., Enning, D., 2020. Severe corrosion of carbon steel in oil field produced water can be linked to methanogenic archaea containing a special type of [NiFe] hydrogenase. *Appl. Environ. Microbiol.* 87, e01819–e01820.
- Lerm, S., Westphal, A., Miethling-Graff, R., Alawi, M., Seibt, A., Wolfram, 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, 311–327. <https://doi.org/10.1007/s00792-013-0518-8>.
- Li, C., Zhong, S., Zhang, F., Wang, Z., Jiang, F., Wan, Y., 2017. Response of microbial communities to supercritical CO₂ and biogeochemical influences on microbially mediated CO₂-saline-sandstone interactions. *Chem. Geol.* 473, 1–9. <https://doi.org/10.1016/j.chemgeo.2017.09.026>.
- Luton, P.E., Wayne, J.M., Sharp, R.J., Riley, P.W., 2002. The *mcrA* gene as an alternative to 16S rRNA in the phylogenetic analysis of methanogen populations in landfill b. *Microbiology* 148, 3521–3530. <https://doi.org/10.1099/00221287-148-11-3521>.
- Marschall, C., Frenzl, P., Cypionka, H., 1993. Influence of oxygen on sulfate reduction and growth of sulfate-reducing bacteria. *Arch. Microbiol.* 159, 168–173. <https://doi.org/10.1007/BF00250278>.
- Martens, S., Kempka, T., Liebscher, A., Lüth, S., Möller, F., Myrntinen, A., Norden, B., Schmidt-Hattenberger, C., Zimmer, M., Kühn, M., 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, 323–334. <https://doi.org/10.1007/s12665-012-1672-5>.
- Mayumi, D., Dolfing, J., Sakata, S., Maeda, H., Miyagawa, Y., Ikarashi, M., Tamaki, H., Takeuchi, M., Nakatsu, C., Kamagata, Y., 2013. Carbon dioxide concentration dictates alternative methanogenic pathways in oil reservoirs. *Nat. Commun.* 4 (1), 1–6. <https://doi.org/10.1038/ncomms2998>, 1998.
- Mitchell, A.C., Phillips, A.J., Hiebert, R., Gerlach, R., Spangler, L.H., Cunningham, A.B., 2009. Biofilm enhanced geologic sequestration of supercritical CO₂. *Int. J. Greenh. Gas Control* 3, 90–99. <https://doi.org/10.1016/j.ijggc.2008.05.002>.
- Morozova, D., Wandrey, M., Alawi, M., Zimmer, M., Vieth, A., Zettlitzer, M., Würdemann, H., 2010. Monitoring of the microbial community composition in saline aquifers during CO₂ storage by fluorescence in situ hybridisation. *Int. J. Greenh. Gas Control* 4, 981–989. <https://doi.org/10.1016/j.ijggc.2009.11.014>.
- Morozova, D., Zettlitzer, M., Let, D., Würdemann, H., 2011. Monitoring of the microbial community composition in deep subsurface saline aquifers during CO₂ storage in Ketzin, Germany. *Energy Proc.* 4, 4362–4370. <https://doi.org/10.1016/j.egypro.2011.02.388>.
- Muyzer, G., Dewaal, E.C., Uitterlinden, A.G., 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, 0099-2240/93/030695-06\$02.00/0.
- Nilsen, R., Torsvik, T., Lien, T., 1996. *Desulfotomaculum thermocisternum* sp. Nov., a sulfate reducer isolated from hot North Sea oil reservoir. *Int. J. Syst. Bacteriol.* 46 (2), 397–402. <https://doi.org/10.1099/00207713-46-2-397>.
- Parliament of the United Kingdom, 2008. Climate change act 2008. HM Gov. 1–103. doi:10.1136/bmj.39469.569815.47.
- O'Sullivan, L.A., Roussel, E.G., Weightman, A.J., Webster, G., Hubert, C.R., Bell, E., Head, I., Sass, H., Parkes, R.J., 2014. Survival of *Desulfotomaculum* spores from estuarine sediments after serial autoclaving and high-temperature exposure. *ISME J.* 9, 922–933. <https://doi.org/10.1038/ismej.2014.190>.
- Peet, K.C., Freedman, A.J.E., Hernandez, H.H., Britto, V., Boreham, C., Ajo-Franklin, J.B., Thompson, J.R., 2015. Microbial growth under supercritical CO₂. *Appl. Environ. Microbiol.* 81, 2881–2892. <https://doi.org/10.1128/aem.03162-14>.
- Pellizzari, L., 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. <https://doi.org/10.1007/s12665-016-6111-6>.
- Peters, V., Conrad, R., 1995. Methanogenic and other strictly anaerobic bacteria in desert soil and other oxic soils. *Appl. Environ. Microbiol.* 61 (4), 1673–1676.
- Porter, R.T.J., Fairweather, M., Pourkashanian, M., Woolley, R.M., 2015. The range and level of impurities in CO₂ streams from different carbon capture sources. *Int. J. Greenh. Gas Control* 36, 161–174. <https://doi.org/10.1016/j.ijggc.2015.02.016>.
- Postgate, J.R., 1984. *The Sulphate-Reducing Bacteria*, second ed. Cambridge University Press, Cambridge.
- Raskin, L., Stromley, J., Rittmann, B.E., Stahl, D.A., 1994. Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens. *Appl. Environ. Microbiol.* 60, 1232–1240 doi:0099-2240/94/\$04.00 +0.
- Schallenberg, M., Kalf, J., Rasmussen, J.B., 1989. Solutions to problems in enumerating sediment bacteria by direct counts. *Appl. Environ. Microbiol.* 55, 1214–1219.
- Sherry, A., Gray, N.D., Ditchfield, A.K., Aitken, C.M., Jones, D.M., Röling, W.F.M., Hallmann, C., Larter, S.R., Bowler, B.F.J., Head, I.M., 2013. Anaerobic biodegradation of crude oil under sulphate-reducing conditions leads to only modest enrichment of recognized sulphate-reducing taxa. *Int. Biodeterior. Biodegrad.* 81, 105–113. <https://doi.org/10.1016/j.ibiod.2012.04.009>.
- Smedley, P.L., Edmunds, M., 2002. Redox patterns and trace-elements behaviour in the East Midlands triassic sandstone aquifer, U.K. *Ground Water* 40, 44–58. <https://doi.org/10.1111/j.1745-6584.2002.tb02490.x>.
- Smith, C.J., Danilowicz, B.S., Clear, A.K., Costello, F.J., Wilson, B., Meijer, W.G., 2005. T-Align, a web-based tool for comparison of multiple terminal restriction fragment length polymorphism profiles. *FEMS Microbiol. Ecol.* 54, 375–380. <https://doi.org/10.1016/j.femsec.2005.05.002>.
- Stolper, D.A., Revsbech, N.P., Canfield, D.E., 2010. Aerobic growth at nanomolar oxygen concentrations. *Proc. Natl. Acad. Sci. U. S. A.* 107, 18755–18760. <https://doi.org/10.1073/pnas.1013435107>.
- Tardy-Jacquod, C., Magot, M., Patel, B.K., Matheron, R., Caumete, P., 1998. *Desulfotomaculum halophilum* sp. nov., a halophilic sulfate-reducing bacterium isolated from oil production facilities. *Int. J. Syst. Bacteriol.* 48, 333–338. <https://doi.org/10.1099/00207713-48-2-333>.
- Ten, L., Im, W., Baek, S., Lee, J., 2006. *Bacillus ginsengihumi* sp. nov., a novel species isolated from soil of a ginseng field in Pocheon province, South Korea. *J. Microbiol. Biotechnol.* 16, 1554–1560 doi:8242869561.
- UNFCCC Conference of the Parties, 1998. Kyoto protocol to the United Nations framework, Review of European Community and International Environmental Law. doi:10.1111/1467-9388.00150.
- Wagner, A., Blackstone, N., Cartwright, P., Dick, M., Misof, B., Snow, P., Wagner, G., Bartels, J., Murtha, M., Pendleton, J., 1994. Surveys of gene families using polymerase chain reaction: PCR selection and PCR drift. *Syst. Biol.* 43 (2), 250–261 doi:10.2307.2413465.
- Wolfe, R.S., 2011. *Techniques for cultivating methanogens. Methods Enzymol.* 494, 1–22.
- Zettlitzer, M., Moeller, F., Morozova, D., Lokay, P., Würdemann, H., 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. <https://doi.org/10.1016/j.ijggc.2010.05.006>.