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The effect of variable oxygen impurities on microbial activity in conditions resembling geological storage sites

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Abstract

Current specifications on carbon dioxide (CO_2) storage do not take into account the effect of oxygen (O_2) present as an impurity, on storage site microbiology. Some microbiology related impacts related to the CCS process include the potential blockage of injection well, corrosion of pipes, oil souring and oil degradation. To investigate this, microcosm experiments were set up using the O_2 concentrations of 0 ppm, 10 ppm, 100 ppm and atmospheric. Artificial groundwater and sandstone microcosms were inoculated with a mixed microbial community, incubated for 29 days and regularly sampled for gases produced and sampled at the end of the experiment to analyse the microbiology. Gas chromatography analysis of these microcosms showed no hydrogen sulphide (H_2S) production and a variable amount of CO_2 production. Microbial analysis of the microcosms show that the microbial inoculum (including sulphate reducing bacteria) was able to survive/grow better in the microcosms with 10 ppm and below compared to the higher levels of O_2. The levels of CO_2 for 100 ppm and atmospheric levels of O_2 were similar indicating the introduction of 100 ppm of O_2 could promote aerobic processes. This experiment has shown that small differences in O_2 concentrations affects microbial communities relevant to geological storage sites which could cause operational issues. Further investigation is required to properly assess the effect of small O_2 changes on H_2S production.

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Keywords: CCS; Sulphate Reducing Bacteria; Saline Aquifers; Oxygen; Hydrogen Sulphide; Impurities.

1. Introduction

The storage of carbon dioxide (CO_2) in geological storage sites introduces a new range of gases to native microbial communities. In addition to CO_2, impurities are present within the injected gas such as oxygen (O_2). The presence and concentration of these impurities depends on the source of CO_2 and the type of capture used. It is predicted that the amount of O_2 could be as high as 6% [1]. Recommendations on the amount of O_2 impurities allowed within gas streams are mainly focused on the transport of the gas and not on the impacts upon storage.

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Transport specifications of O_2 vary on the operator, some of which are 10 - 100 ppm [2]. Microbes can still survive in deep geological storage sites for CO_2, at depths 800 - 2000 m beneath the surface [3]. The introduction of low levels of O_2 is likely to affect microbial communities within the geological storage sites, which could lead to operational issues. Undesirable microbial impacts have been linked to such carbon capture and storage (CCS) operational issues such as injection blockages [4], pipeline corrosion [5], oil souring [6] and oil degradation [7]. These issues have been connected to hydrogen sulphide (H_2S) production by sulphate reducing bacteria (SRB). Sulphate reduction to H_2S is a process which occurs when there is no O_2 and the presence of O_2 should reduce the production of H_2S. However, the presence of O_2 encourages the activity of microorganisms capable of using O_2. Some microbes can survive without O_2 but can preferentially use O_2 when it is available. These O_2 utilising microorganisms can interact with SRB potentially stimulating the production of H_2S [8]. An experimental programme was conducted in order to investigate the effects of 0 ppm, 10 ppm, 100 ppm and atmospheric levels of O_2 to observe any changes in microbial communities and gases produced. The production of different gases, related to microbial activity, such as increase CO_2 could be because of increased respiration. Increases in H_2S from increased SRB activity could cause CCS operational issues. The analysis of microbial communities would indicate which conditions are more favourable for microbial growth and survival. This paper looks at the preliminary data from the variable O_2 microcosms.

2. Methods

2.1 Rock sample and preparation

The sandstone used in the experiments was a section of a core from the Cleethorpes borehole in North East Lincolnshire, England. The samples used were from approximately 900 m below the surface. The sandstone was crushed to particle sizes smaller than 500 µm. Sterilisation of the sandstone was performed by gamma irradiation using Gammacell® 1000 Elite with a total dose of 30 kGy from Casesium^{137}.

2.2 Artificial groundwater (AGW)

Using water chemistry data from Sherwood sandstone saline aquifer [9], the Welton borehole in the Sherwood sandstone saline aquifer, artificial ground water (AGW) was formulated as follows: $MgCl_2.6H_2O$ (0.24 g), Na_2SO_4 (2.22 g), $CaCl_2.2H_2O$ (4.40 g), NaCl (3.25 g), KCl (0.007 g), $NaHCO_3$ (1.00 g) and yeast extract (0.05 g), made up to a litre with deionised water and autoclaved to sterilise. Yeast extract was added to the AGW to represent the introduction of potential nutrients associated from drilling fluids aquifer. Prior to addition to the microcosms AGW measured pH 8.18.

2.3 Microbial inoculum

The inoculum added to the experiments was an enrichment culture of sandstone samples in Postgate's Medium B [10]. Attempts to obtain a sulphate reducing enrichment culture from sandstone obtained from Cleethorpes borehole were used in the microcosms, but they did not show positive growth for SRB. Instead, the sandstones samples from Godstone and Marden from the Upper Cretaceous Greensands formation in Kent were used for enrichment cultures. These were obtained at an approximate depth of 100-200 m below the surface in an underground cave network. Positive identification of the growth of SRB was indicated by a black precipitate of iron sulphate. The enrichment community was pelleted and washed three times with the AGW to remove the enrichment media. Some of the dominant bacteria within the enrichment culture were identified using Sanger DNA sequencing [11]. These were identified as being most closely related to a *Pseudomonas* species (KT991031), *Desulfotomaculum* species (KM494501), uncultured Firmicute (AB874517) and *Halomonas* species (KP241932). This demonstrates that the inoculum contained not only the key organisms of interest (SRB), but also a range of other bacteria likely to be encountered within a CO_2 storage site and which may have ecological interactions with the SRB in a storage site [12,13,14].

2.4 Experimental set up

Microcosms were set up containing 5 g crushed sandstone, 30 ml AGW and 1 ml microbial inoculum in 250 ml glass bottles. A control was set up without microbial inoculum. Both the experimental and the control microcosms were set up in triplicate. The microcosms were set up under 4 conditions to investigate the role of O_2 contaminants with CO_2 storage sites (atmospheric concentrations - approximately 209,500 ppm O_2), 100 ppm, 10 ppm and 0 ppm O_2. The atmospheric concentration microcosm experiments were set up in a laminar flow hood. The 0 ppm O_2 was achieved by putting the equipment into an anaerobic chamber, with an atmosphere of 95% nitrogen (N_2) and 5% hydrogen (H_2). The 10 ppm and 100 ppm levels of O_2 were achieved by bubbling gas of appropriate composition for 5 minutes to remove excess O_2 from the microcosms (10 ppm O_2 with N_2 balance and 100 ppm O_2 with N_2 balance, Calgaz Ltd) and sealing immediately. These were set up in an anaerobic chamber of N_2 and 200 ppm O_2 to reduce any contamination with atmospheric O_2 and to reduce starting levels of H_2 in the experiments. The microcosms were then sealed with a butyl rubber stopper and aluminium seal crimp tops. The microcosms were incubated at 37°C for 29 days.

2.5 Gas, pH and Eh measurements

Gas samples were taken from each microcosm every 5 days using a gas syringe. 2 ml samples were taken and analysed using Agilent Technologies 7820A GC for gas chromatography (GC). The carrier gas used for GC was helium. The program for GC analysis was held at 23°C for 3 minutes followed by a 3°C/min increase to 40°C and finally a 5°C/min increase to 90°C with a hold at 90°C for 2 minutes. Gases detected by GC analysis were H_2, N_2 and CO_2. Further gas analysis was performed using a Dräger gas monitor X-am 2000 used for the detection of H_2S and H_2/methane (CH_4). 2 ml gas samples were taken for Dräger gas analysis.

Groundwater samples were taken from the microcosms at the end of the experiment. pH and redox potential (Eh) were measured immediately after the experiment.

2.6 Microbial analysis

After 29 days, the microcosms were disassembled and samples were taken for microbial analysis. 1 ml mix of sandstone and groundwater from each microcosm was placed in 9 ml 1% glutaraldehyde fixative for epifluorescence cell counts. Epifluorescence cell counts will be completed later using an Acridine orange stain, which fluorescens between 500-526 for DNA, and visualised with a Zeiss III RS epifluorescence head filter. 1 ml mix of sandstone and groundwater was also taken for DNA extraction using a FastDNA™ SPIN kit for soil (MP Bio) according to the manufacturer's instructions. The extracted DNA was tested for the presence of bacterial and SRB DNA sequences by performing polymerase chain reaction (PCR) with the primers BAC341F-GC and BAC534R [15], which are specific for the bacterial 16S rRNA gene, and DSRp2060F and DSR4R, which are specific of the gene *dsrB* which is found in SRB[16][17]. This technique amplifies the targeted gene, if it is present within the extracted DNA, to detectable levels. The DNA was used to create community profiles to observe differences between the microcosms through denaturing gradient gel electrophoresis (DGGE). DGGE separates DNA by a denaturing gradient which creates a fingerprint of a microbial community [18]. Gene fingerprints for the gene 16S rRNA were obtained using a DGGE of 40-70% denaturing gradient. Range-weighted richness is one way to interpret data from a DGGE to give a value to the number and range of bands in a given sample. It is calculated by squaring the number of bands and multiplying it by the percentage of denaturing gradient between the first and last band. A range-weighted richness value above 30 is considered a highly habitable environment, 10-30 is considered moderately habitable and below 10 is considered show restricted colonisation [19].

3. Results

3.1 Gas analysis

Table 1. Mean Dräger gas readings for 0 ppm O_2 microcosms with standard deviation.

Days Incubated	Mean H_2/CH_4 (LEL%)			
	Control	Standard Deviation	Inoculated	Standard Deviation
5	41	5.69	49	4.93
8	53	2.08	48	10.41
12	31	1.53	25	2.52
20	43	2.08	33	5.13
25	36	2.08	29	6.66

No H_2S was detected during the experiments in any microcosm. H_2/CH_4 was only detected in the 0 ppm O_2 microcosms (Table 1). The H_2/CH_4 levels detected using the Dräger were measured in terms of the lower explosive limit (%). The uninoculated control showed higher levels of H_2/CH_4 compared to the inoculated microcosm.

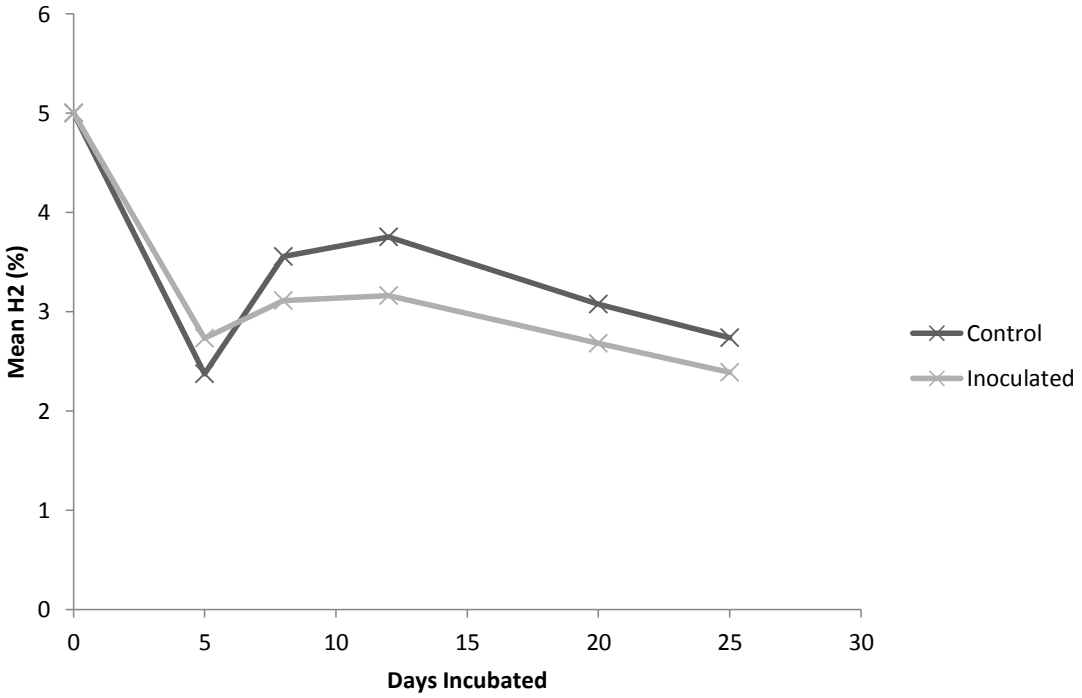

Figure 1. Mean H_2 (%) in 0 ppm O_2 microcosms.

Table 2. Standard deviations for mean H_2 (%) in 0 ppm O_2 microcosms.

Days Incubated	Standard deviations for mean H_2 (%) in 0 ppm O_2 microcosm	
	Control	Inoculated
5	0.04	0.08
8	0.02	0.02
12	0.08	0.06
20	0.27	0.43
25	0.52	0.29

Figure 1 shows hydrogen concentration in the microcosms throughout the experiment measured by GC. A decrease from the starting 5% H_2 after 5 days was observed for both the uninoculated control and inoculated microcosm. Readings peaked at 12 days with 3.2% and 3.8% for inoculated and uninoculated microcosms. There was then a drop on 20 and 25 days. The control microcosms showed a higher mean level of H_2 than the inoculated microcosm.

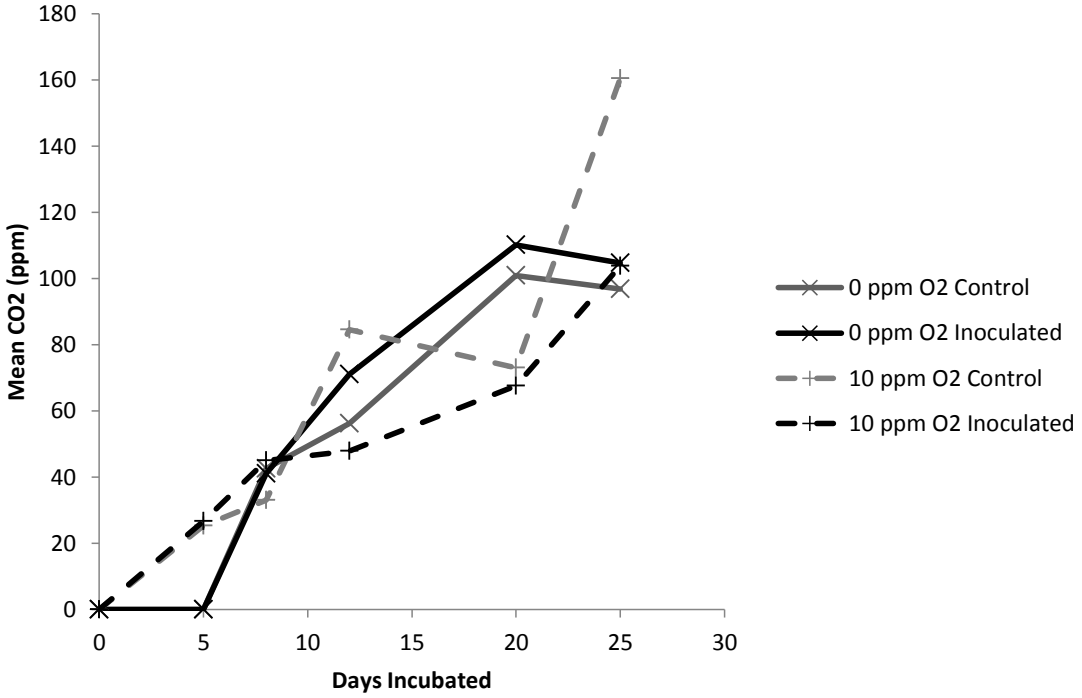

Figure 2. Mean CO_2 (ppm) in 0 ppm and 10 ppm O_2 microcosms.

Table 3. Standard deviations for mean CO_2 (ppm) in 0 ppm and 10 ppm O_2 microcosms.

Days Incubated	Standard deviations for mean CO_2 (ppm) in 0 ppm O_2 microcosms		Standard deviations for mean CO_2 (ppm) in 10 ppm O_2 microcosms	
	Control	Inoculated	Control	Inoculated
5	-	-	11.08	18.55
8	4.88	6.60	9.35	12.24
12	3.83	6.57	5.36	9.59
20	8.01	10.54	3.39	2.84
25	16.14	8.67	84.25	34.43

Figure 2 shows the average CO_2 production from the 0 ppm and 10 ppm O_2 microcosms from GC measurement. CO_2 was not detected after 5 days in the either 0 ppm O_2 microcosm. The readings at 8 and 12 days showed an increase in CO_2 levels in the 0 ppm O_2 microcosms and the CO_2 levels showed a small drop after 20 and

25 days. The inoculated microcosm at 0 ppm O_2 had higher levels of CO_2 production than the control. Highest CO_2 readings of around 100 ppm were seen at the final reading.

In the 10 ppm O_2 microcosms the CO_2 was detected in all GC readings. The levels of CO_2 in the 10 ppm O_2 microcosm were generally higher in the control than the inoculated microcosm. The inoculated microcosm reached a CO_2 peak of 104 ppm (day 25) with the control microcosm producing levels greater than 160 ppm CO_2. The results for day 25 of 10 ppm O_2 microcosms had high standard deviations values (Table 3).

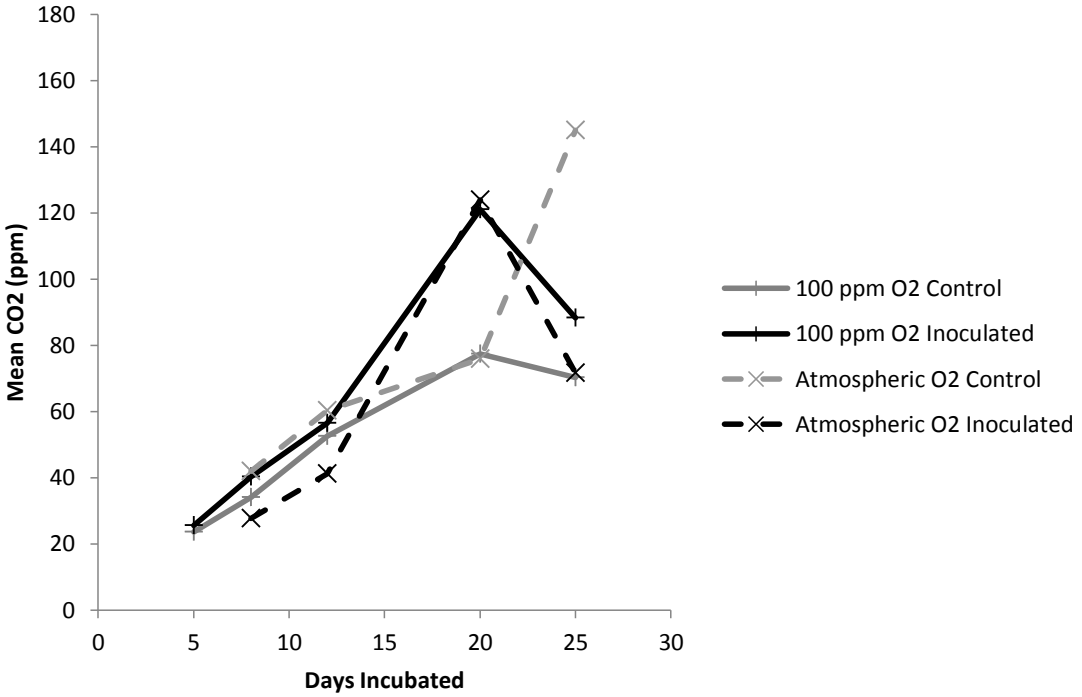

Figure 3. Mean CO_2 (ppm) in 100 ppm O_2 and atmospheric microcosms.

Table 4. Standard deviations for mean CO_2 (ppm) in 100 ppm and atmospheric O_2 microcosms.

Days Incubated	Standard deviations for mean CO_2 (ppm) in 100 ppm O_2 microcosms		Standard deviations for mean CO_2 (ppm) in atmospheric O_2 microcosms	
	Control	Inoculated	Control	Inoculated
5	7.52	6.76	-	-
8	7.52	7.91	27.13	8.80
12	2.71	10.86	24.94	0.45
20	11.46	66.91	13.56	58.47
25	4.65	5.22	118.66	12.22

CO_2 was detected in all readings for the 100 ppm O_2 microcosm (Figure 3). An increase in CO_2 was detected in both microcosms peaking on day 20 before dropping in the final reading. The inoculated microcosm showed higher levels of CO_2 than the uninoculated control. A high standard deviation (Table 4) for the 100 ppm microcosm can be seen for the inoculated microcosms on day 20.

In the microcosms with atmospheric conditions (Figure 3), CO_2 was not detected in the first reading. The control microcosm for atmospheric conditions showed higher levels of CO_2 in the experiment apart from the reading on day 20 where the inoculated microcosm produced higher levels of CO_2. Standard deviations are highest for atmospheric microcosms on day 25 for the control and day 20 for the inoculated microcosms.

3.2 Microbial analysis

PCR using the primers DSRp2060F and DSR4R were able to detect the presence of *dsrB* gene in one out of three of the atmospheric, one out of three of the 100 ppm O_2, two out of three of the 10 ppm O_2 and two out of three of the 0 ppm O_2 microcosms. No positive results for the *dsrB* gene were seen in the control microcosm.

Bands for the 16S rRNA gene were seen in all microcosms. The control microcosms showed 1-3 bands per lane and the inoculated microcosms showed 1-7 bands per lane. The inoculated microcosms had more bands of a greater intensity compared to the control microcosms which all had weak band intensity. The highest number of bands per group is 10 ppm O_2 with 19 bands in total. The lowest number of bands for an inoculated microcosm group is 100 ppm with 7 bands. The atmospheric inoculated microcosms had 13 bands and the 0 ppm O_2 had 16. All of the range-weighted richness values were below 10 which means that all of the microcosms would be considered to only allow restricted colonisation.

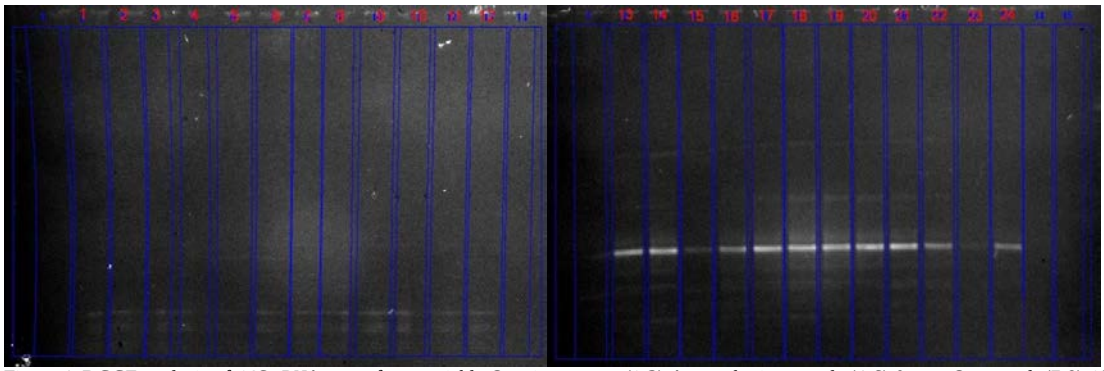

Figure 4. DGGE analysis of *16S rRNA* genes from variable O_2 microcosms. (1-3) Atmospheric controls,(4-6) 0 ppm O_2 control, (7-9) 10 ppm O_2 control, (10-12) 100 ppm O_2 control, (13-15) atmospheric inoculated, (16-18) 0 ppm O_2 inoculated, (19-21) 10 ppm O_2 inoculated and (22-24) 100 ppm O_2 inoculated.

Table 5. Range-weighted richness of each lane of DGGE.

Microcosm	Range-Weighted Richness
Atmospheric Control 1	0
Atmospheric Control 2	0.115
Atmospheric Control 3	0.044
0 ppm Control 1	0.044
0 ppm Control 2	0.425
0 ppm Control 3	0.425
10 ppm Control 1	0.044
10 ppm Control 2	0.051
10 ppm Control 3	0.115
100 ppm Control 1	0.115
100 ppm Control 2	0.044
100 ppm Control 3	0.044
Atmospheric Inoculated 1	2.716
Atmospheric Inoculated 2	2.716
Atmospheric Inoculated 3	0.256
0 ppm Inoculated 1	0.256
0 ppm Inoculated 2	5.323
0 ppm Inoculated 3	5.323
10 ppm Inoculated 1	5.323
10 ppm Inoculated 2	5.323
10 ppm Inoculated 3	2.183
100 ppm Inoculated 1	1.397
100 ppm Inoculated 2	0
100 ppm Inoculated 3	0.256

3.3 pH and Eh measurements

Table 6. pH and Eh measurements for the microcosms at the end of the experiment.

Microcosm	pH	Eh (mV)
Artificial Groundwater	10.50	162.6
0 ppm Control 1	10.33	38.5
0 ppm Control 2	10.17	-32.0
0 ppm Control 3	9.94	-0.3
0 ppm Inoculated 1	10.07	-29.4
0 ppm Inoculated 2	9.97	-6.1
0 ppm Inoculated 3	10.02	-23.5
10 ppm Control 1	10.22	164.0
10 ppm Control 2	10.16	132.6
10 ppm Control 3	10.43	141.4
10 ppm Inoculated 1	9.72	170.5
10 ppm Inoculated 2	10.12	204.1
10 ppm Inoculated 3	10.30	149.2
100 ppm Control 1	10.36	155.2
100 ppm Control 2	10.20	164.9
100 ppm Control 3	10.38	171.0
100 ppm Inoculated 1	10.25	189.1
100 ppm Inoculated 2	10.16	184.6
100 ppm Inoculated 3	10.18	189.9
Atmospheric Control 1	9.65	205.1
Atmospheric Control 2	10.07	78.0
Atmospheric Control 3	9.95	133.0
Atmospheric Inoculated 1	9.91	122.9
Atmospheric Inoculated 2	9.94	140.0
Atmospheric Inoculated 3	9.89	134.4

ZoBell's solution standard value for atmospheric and 0 ppm 0 ppm was 261.0 mV and for 10 ppm and 100 ppm O_2 was 262.8 mV.

pH and Eh values can be found in Table 6. They can influence microbial and chemical reactions within an environment. The pH of the ground water had evolved over the course of the experiment from 8.18 to 10.5 making the environment more alkaline than was originally planned. This can be seen in all the microcosms, with pH ranging from 9.65 to 10.43. The lowest average pH readings are seen in the atmospheric microcosms. Microcosms set up with 0 ppm O_2 have the lowest average Eh.

4. Discussion

H_2S was not detected in any microcosm which was unexpected since because of the identification of SRB in the enrichment cultures used as inoculum (as detected by the presence of a black precipitate in the culture media and the retrieval of SRB DNA sequence from the same) and the amplification of the *dsrB* gene in inoculated, but not control microcosms. The *dsrB* gene was detected in the microcosms with lower O_2 (0 ppm and 10 ppm O_2) than the microcosms with higher levels of O_2 (100 ppm and atmospheric). This is consistent with literature on O_2 conditions tolerances of SRB as they have been observed to have reduced activity in increasing O_2 concentrations but can still grow in O_2 concentrations up to 144ppm [20]. If a balance was achieved where the O_2 could suppress microorganisms which need O_2 and those that have reduced activity in its presence this could be a way to prevent any undesirable microbial impacts. Quantitative PCR (a method for quantifying DNA) has not been carried out but the low intensity of positive PCR bands suggests that only low levels of DNA were present. With more sulphate

reducing genes being seen in 0 ppm and 10 ppm O_2 this could suggest that H_2S is more likely at these O_2 concentrations.

The absence of detectable SRB activity could be because the pH measured at the end of the experiments was much higher than expected and high pH can inhibit microbial sulphate reduction [21]. For sulphate reduction to take place the Eh also needs to be below -100 mV [22]. All of the microcosms had an Eh value above this and could be why no H_2S was detected. For CH_4 to take place the Eh needs to be below -150 mV [23] and so this indicates that the H_2/CH_4 detected by the Dräger metre is most likely H_2. As SRB were detected in the inoculated microcosms it is possible that with favourable Eh, H_2S production would still be possible.

Faint bands were seen on the DGGE for control microcosms, which indicates that there were microbes present even within the control microcosms. The inoculated microcosms that showed the least number of bands was 100 ppm O_2 with a higher numbers being seen in the O_2 concentrations that were higher and lower. This could be because 100 ppm O_2 is too low for good growth with O_2 but it also has enough O_2 reduce other processes such as H_2S production. Despite the lowest number of bands, 100 ppm O_2 still had similar levels of CO_2 to those seen in the atmospheric microcosms. This shows that the bands that are missing in the 100 ppm O_2 microcosms are not contributing greatly to CO_2 production.

The range-weighted richness was calculated for each sample. All microcosms had a range-weighted richness value of less than 10 meaning that all microcosms could allow restricted colonisation. This can be expected as the microcosms were inoculated with an enrichment culture that grew under conditions without O_2 within SRB enrichment media. The greatest range-weighted richness values were seen in the 100 ppm microcosms followed by 0 ppm microcosm. This indicates that these conditions have the biggest diversity within the microcosms. Epifluorescence microscopy will be performed on these samples to identify difference in the cell numbers under different conditions. To further help identify which microbes were present in the microcosms DNA sequencing of DGGE bands will be performed in the future. This will allow a better understanding of the potential microbial reactions occurring different conditions in this experiment.

The two main sources of CO_2 production within the microcosms would be microbiological and geochemical. The microbiological CO_2 is most likely from respiration by the O_2 utilising microbes. The formation of CO_2 through geochemical processes would be from carbonates in the sandstone. Another source for CO_2 in the atmospheric O_2 microcosms would be from the atmosphere. The inoculated microcosms for the atmospheric and 100 ppm O_2 show similar CO_2 production, both reaching a peak above 100 ppm CO_2 before dropping at day 25. These peaks at day 20 have high standard deviation values because of high variability in the microcosms. The CO_2 seen in the inoculated microcosms is likely a combination of both microbial and geochemical sources. The control microcosms for atmospheric conditions and 100 ppm O_2 have similar levels of CO_2 production until day 25 where the atmospheric control has a higher average of 145 ppm CO_2. This peak has a high standard deviation value. Although the controls should have been sterile, DNA results show that they were not and therefore the variability within the control microcosm could be because one control microcosm had a higher initial biomass than the others. The results suggest that 100 ppm O_2 could promote aerobic processes during the injection of CO_2. This could lead to some problems when using it for enhanced oil recovery as it could promote the degradation of oil. The initial degradation of oil by O_2 utilising organisms has also been linked to increased SRB activity [8] which could further lead to H_2S production and H_2S related issues.

The 10 ppm and 100 ppm O_2 inoculated microcosms show different trends in CO_2 levels. The 10 ppm O_2 inoculated microcosm shows a more gradual increase whereas the 100 ppm O_2 microcosm showed a peak in day 20 and then a drop in day 25. The peaks seen in both 10 ppm and 100 ppm O_2 microcosms have high standard deviation values because of high variability between the replicates. .

Although the different O_2 concentrations produced different graph shapes, the final reading for all of the inoculated microcosms was around 100 ppm CO_2. In comparison the un-inoculated control microcosms had greater differences in their CO_2 production. This could be because further respiration and growth was limited by the availability of nutrients. Extending the experiment to observe any further increase in CO_2 levels would be one option to investigate if this is the case.

5. Conclusion

- The 100 ppm O_2 microcosms showed the fewest number of bands on the DGGE which indicates a restriction in what can grow under these conditions. If an O_2 concentration was able to restrict growth further it could be a way to prevent undesirable microbial impacts.
- The detection of SRB and bacterial genes showed that there was a greatest diversity seen in microcosms 10 ppm O_2 and below. This suggests that the SRB activity is more likely at these O_2 levels, which could lead to CCS related issues.
- Under these experimental conditions, 100 ppm O_2 inoculated microcosms show similar patterns of CO_2 production to the atmospheric inoculated microcosm. This indicates that the introduction of 100 ppm O_2 could promote the aerobic processes, such as the degradation of oil.
- Although no H_2S was detected in this experiment, there is evidence for greater microbial and SRB growth at 10 ppm O_2 and below, when compared to 100 ppm O_2 and above. Further investigation will be conducted, at more suitable pH and Eh conditions for H_2S production, to further examine the influence of O_2 concentration on H_2S production.

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