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# MICROBIAL EFFECTS ON CO<sub>2</sub> STORAGE

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## Executive Summary

Microorganisms are thought to be responsible for over half the biomass on the planet, with a substantial fraction of them in the subsurface. Some microorganisms are known to be able to exist in extreme conditions and have been found in the subsurface at depths greater than 3km and can therefore potentially exist at some locations considered for geological storage of CO<sub>2</sub>. In general the chemoautotrophic nature of subsurface ecosystems increases with depth, i.e. microbes in the deeper subsurface are more likely to be using CO<sub>2</sub> to synthesise necessary organic compounds. Therefore these are what you may expect to find at the depth of a typical CO<sub>2</sub> storage reservoir.

If CO<sub>2</sub> is injected into the subsurface where microorganisms reside it is likely to cause an initial decrease in numbers, followed by a gradual increase as some microorganisms will be able to utilise the CO<sub>2</sub> to produce necessary organic compounds. CO<sub>2</sub> can also affect the pH, which will affect bacterial communities, but the extent to which this will occur is dependent on the microorganisms present as well as the lithology and mineralogy of the storage formation and the chemistry of the formation waters. Overall, studies show that microbial redox reactions are favourable improved with greater CO<sub>2</sub> pressure.

Microorganisms can potentially have a positive or negative effect on the storage formation. Negative effects include corrosion or biofouling of wellbore materials as well as potentially affecting injectivity by causing pore blocking. Positive effects include increasing storage security by the use of biofilms or enhanced solubility and mineral trapping through ureolysis. The use of biofilms as a barrier has also been considered as a potential mitigation method to stop the flow of the CO<sub>2</sub> plume if this becomes necessary.

This topic may become increasingly significant as more demonstration projects start to take place. There are several research projects happening, mostly at the stage of laboratory testing as well as monitoring for microbial activity at storage demonstration projects, such as the Ketzin and Otway sites. As knowledge and understanding of potential effects and uses of microbial activity increases, this may need to be taken account of at the site characterisation phase of a project as well as the risk assessment.

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# Microbial Effects on CO<sub>2</sub> Geological Storage

## 1. Introduction

Microorganisms are thought to be responsible for over half the biomass on planet earth with a substantial fraction of this in the subsurface. Microorganisms can exist in extreme environments from the ice sheets of Antarctica to submarine hydrothermal vents. Microbial activity in the subsurface may exist as deep as 3.5km, assuming a 110°C temperature limit for microbial activity and a geothermal gradient of 25°C/ km (Krumholz, 1998) and microbial communities have been found as deep as 3.2 km in goldmines in South Africa (Takai et al, 2001). They are therefore likely to exist in formations considered for geological storage of CO<sub>2</sub>. Additionally, microbes are likely to be introduced into these geological formations during the construction and operation of a storage site, mainly through drilling fluids.

In general the chemoautotrophic nature of subsurface ecosystems increases with depth, i.e. microbes in the deeper subsurface are more likely to be using CO<sub>2</sub> to synthesise necessary organic compounds. Therefore these are what you may expect to find at the depth of a typical CO<sub>2</sub> storage reservoir. Deep subsurface microbial communities are dominated by four anaerobic, physiological types, methanogens, sulphate or sulphur reducing bacteria, fermentative anaerobes and Fe(III) reducing bacteria (Onstott, 2005).

Microbial activity in deep subsurface environments is controlled by nutrient and energy source availability and their activity in subsurface environments is generally slow due to limited availability and supply rates of energy sources. Their activity will cause both direct effects (mineral formation/degradation, porosity change through biofilm formation, corrosion) and indirect effects (changes in pH, redox). Biofilms are complex aggregations of microorganisms growing on a solid substrate and are characterised by structural heterogeneity, genetic diversity, complex community interactions, and an extracellular matrix of polymeric substances.

Introduction of injected CO<sub>2</sub> and associated impurities into a geological environment, either dissolved in groundwater or in the gaseous phase, will stimulate these effects as they can be utilised by microbes in energy generating redox reactions or as a nutrient source. Many microbes may not survive the initial stages due to the presence of supercritical CO<sub>2</sub>; however once injection has stopped, microbes that use CO<sub>2</sub> as an energy source are likely to propagate more rapidly and may end up in greater quantities than before injection.

If, as a result of CO<sub>2</sub> injection the level of microbial activity is increased or decreased, this can affect the porosity and permeability and hence the storage capacity and injectivity of the formation. Geochemical parameters are affected and a variety of geochemical reactions may occur, affecting migration of potentially hazardous elements. These effects will likely be site specific.

## 2. Effect of CO<sub>2</sub> on Microbial Activity

Injection of supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) into the subsurface has been found to have a negative effect on the majority of bacteria and other microorganisms. SC-CO<sub>2</sub> is particularly damaging to cells because of its low viscosity and low surface tension and can therefore quickly penetrate cellular material. This is enhanced at higher temperatures, which will increase the fluidity of cell membranes, and higher pressures, which increases CO<sub>2</sub> solubility in water and therefore penetration through cell walls. Experiments on the comparison of CO<sub>2</sub> stress response on three model organisms, showed that biofilm formation and cell wall thickness may be two very important factors in resisting CO<sub>2</sub> toxicity as they create a reactive barrier that slows the diffusion of CO<sub>2</sub> into cytoplasmic membranes (Santillan et al, 2011).

As the CO<sub>2</sub> plume moves through the storage formation, microbial communities may reside in residual brine left behind in cracks, dead flow zones and upstream of the front; this brine will have a gradient of dissolved CO<sub>2</sub> in which microbial interaction may behave differently. Experiments looking at formation waters from the Arbuckle formation, USA (Gulliver and Gregory, 2011) show different families of bacteria reside when varying the CO<sub>2</sub> partial pressure. Knowledge of surviving and thriving microbial populations may enable improved models for predicting the fate of CO<sub>2</sub> following injection and lead to better strategies for ensuring the security of CO<sub>2</sub> in the subsurface. Other experiments (Peet et al, 2010) identified a strain of bacteria resistant to SC-CO<sub>2</sub> and tolerant of conditions expected at geological storage sites. The bacteria were termed MIT0214, are similar to the Bacillus strain of bacteria and were taken from formation waters of the Frio storage test site. The findings of the study suggest adaptation to a supercritical CO<sub>2</sub> environment may simply reflect thermodynamic adaptations to growth under high pressure and differential regulation of genomic content. Colwell et al (2011) also show that native microorganisms at the proposed injection site into the Wallula Basalts, Columbia River are able to survive in water incubated with SC-CO<sub>2</sub>. Planned future work will compare communities at different depths, accurately determine microbial concentrations in samples and characterise microbial diversity using pyrosequencing.

Monitoring studies in Ketzin (Morozova et al, 2010) show that initial CO<sub>2</sub> injection initially causes a decrease in total microbial activity, which after injection starts to increase again. However, the diversity of microorganisms is reduced. The study revealed temporal out competition of sulphate-reducing bacteria by methanogenic bacteria (methane producing microorganisms). In addition, enhanced activity of the microbial population after five months of CO<sub>2</sub> storage indicate that the bacterial community was able to adapt to the extreme conditions of the deep biosphere and to the extreme changes of these atypical conditions.

Onstott (2005) carried out a modelling exercise considering the main redox reactions associated with microbial activity and relevant to H, C, N, O and S. The free energy in an aquifer prior to CO<sub>2</sub> injection was calculated during injection and post injection looking at different groundwater types. The results show that the most significant impact of CO<sub>2</sub> injection is the reduction of pH.

The pH affects which reactions are more likely to take place, which may affect the energy available for microbial activity as lithotrophic microorganisms utilise the energy of redox reactions for their life processes.

For the groundwater hosted in the siliciclastic reservoir, the pH is reduced by one unit. This makes microbial Fe(III) reduction reactions more significant. If sufficient electron donors are available for both biotic and abiotic Fe(III) reducing reactions and sufficient Fe(III) bearing oxides are present in the aquifer then these reactions will restore the aquifer's pH to its initial, pre-injection value. CO<sub>2</sub> injection should cause a short term stimulation of Fe(III) reducing communities.

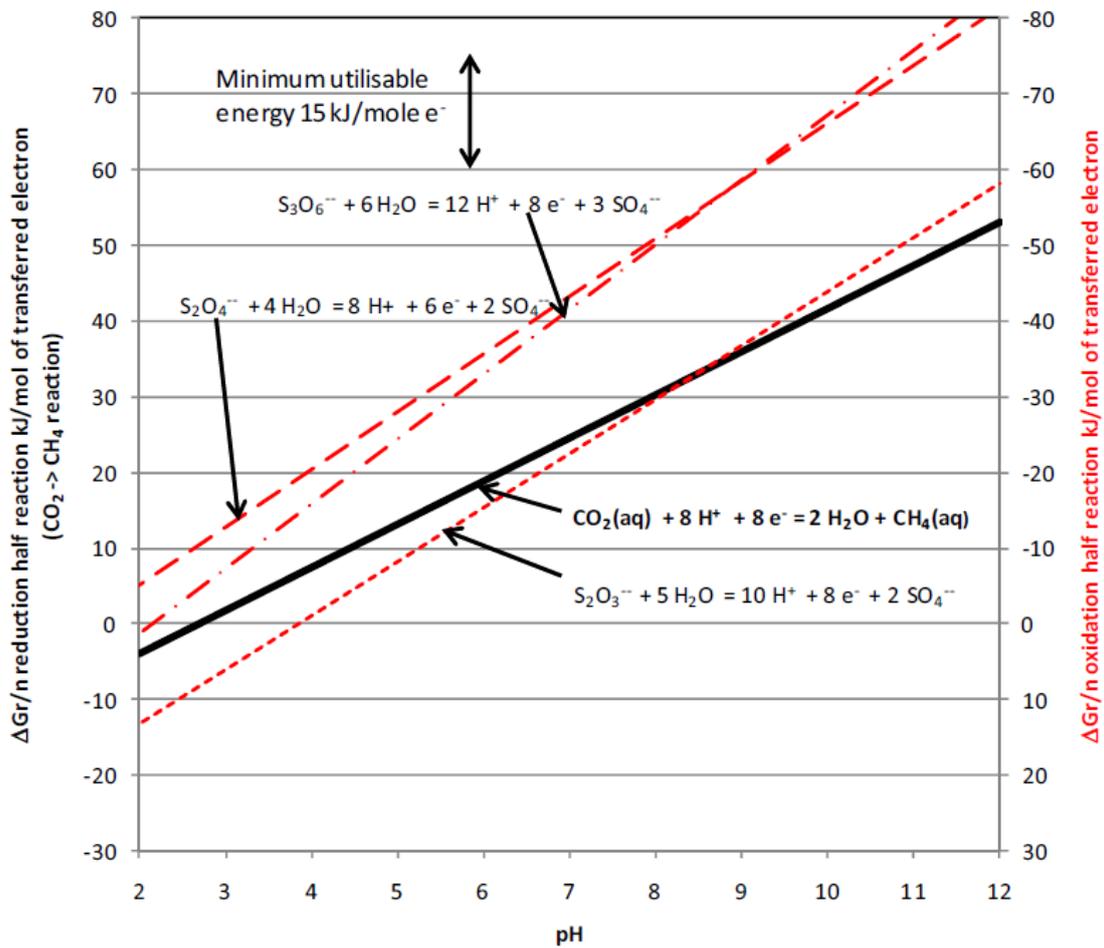
Dolomitic or carbonate aquifers may be more severely impacted by CO<sub>2</sub> injection; the modelling study showed that the dissolution of carbonate failed to restore the pH to a range that is conducive to metabolism of some microorganisms.

Another factor associated with lower pH caused by CO<sub>2</sub> injection is that it facilitates proton pumping reactions across the cell membrane. Microorganisms need to maintain an internal pH that is 1–2 units less than the external pH in order for the proton pumps to generate ATP (Adenosine-5'-triphosphate; used to transport chemical energy within cells for metabolism). For pH values approaching 8.5–9, the high internal pH values affect the aqueous species of phosphate making it more difficult to synthesis ATP. The microorganism is then required to expend energy in ion transport across the membrane to correct for this problem. A more neutral pH of 6–7 alleviates this energy drain. The greater availability of energy will also facilitate the fixation of N<sub>2</sub> which would help support growth of the microbial population. The lower pH values should also help solubilise phosphate for growth. Overall CO<sub>2</sub> injection should increase the availability of N and P to microbial communities.

These findings are confirmed and added to in later research (Onstott, 2011), which states that many microbial redox reactions are favourably improved with greater CO<sub>2</sub> pressure. If there are sufficient electron donors and Fe(III)/ sulphate electron acceptors CO<sub>2</sub> injection should lead to short term stimulation of anaerobic activity. For long term storage in siliciclastic reservoirs this should lead to carbonate precipitation. Injection into carbonate/ dolomite may produce a greater impact on subsurface microbial ecosystems with dissolution of carbonate depending on pH range of indigenous microorganisms.

It is important to understand which reactions in the storage reservoir are likely to enhance microbial activity. This can be analysed with an evaluation tool using a microbial energetics approach (West et al, 2011). As lithotrophic microorganisms utilise the energy of redox reactions for their life processes, it is necessary to consider what reactions may be coupled with the reduction of CO<sub>2</sub>. The energy difference will show whether a particular reaction will provide enough energy to be utilised by the microbes. This is demonstrated for sulphur oxidation in Figure 1, which shows that some intermediate sulphur oxidation above approximately pH 4, when coupled with CO<sub>2</sub> reduction, could potentially provide enough energy for microbial usage.

**Figure 1: Diagrammatic illustration variety of free energy of some potential intermediate S oxidation half reactions coupled to CO<sub>2</sub> reduction half reaction as a function of pH. CO<sub>2</sub> reduction (solid line) refers to the left hand scale while oxidations (dashed lines) refer to the right hand scale. From West et al, 2011**



If a reservoir is geochemically characterised before CO<sub>2</sub> injection it can be determined beforehand if there are any potential reactions that could cause an increase in microbial activity. Modelling studies as those described above can be used to predict what is likely to occur

### 3. Effect of Microbial Activity on CO<sub>2</sub> Storage

The reactions between microorganisms and the minerals of the reservoir and caprock may cause changes in the structure and chemical composition and corrosion of the casing and cement around the well. Biofilms have been known to cause corrosive effects on materials used in oil and gas drilling operations and is a fairly common occurrence, known as biofouling. The usual solution in most cases is the addition of chemicals into the wellbore to reduce the buildup.

Buildup of biofilm in the aquifer itself could cause pore blocking, which could potentially affect injectivity of CO<sub>2</sub> into the reservoir. However, there is not expected to be much microbial activity in the area around the wellbore in the initial stages of injection as most microorganisms will be negatively affected by SC-CO<sub>2</sub>.

Experiments show the ability of microbial biofilms to decrease permeability of natural and artificial porous media, survive exposure to scCO<sub>2</sub> and facilitate conversion of CO<sub>2</sub> into long-term stable carbonate phases as well as increase solubility of CO<sub>2</sub> in brines (Gerlach et al, 2010). Reactive transport models describing the influence of biological processes on CO<sub>2</sub> storage security have been developed and are continuously being modified to include relevant processes. Kirk et al (2010) show the effect of pH on hydraulic conductivity (K) of biologically clogged media. A reduction in pH showed an increase in K, but that clogging persisted. The results suggest that biomass in porous media will remain largely in place following exposure to acidic water in a CO<sub>2</sub> storage reservoir, particularly where buffering is able to limit to extent of acidification.

There have been studies considering the potential positive effects of microbial activity on storage security. Mitchell et al (2010) consider the potential of microorganisms for enhancing mineral and solubility trapping by utilising the bacterial hydrolysis of urea (ureolysis). This has the effect of increasing the pH, which causes increased solubility of CO<sub>2</sub>. Any carbon from the urea undergoes mineralisation to CaCO<sub>3</sub>, which in turn may increase storage security by creating an impermeable barrier. It is also suggested that waste water containing urea could be utilised, which will also reduce the amount of labile surface carbon. Figure 2 shows a schematic representation of what such microbially enhanced storage would look like.

Numerical models are also being developed that can simulate the development of a biofilm, barrier near the injection well (Ebigbo et al, 2010). This will account for the transport of bacteria, biofilm accumulation as well as the role of increased ureolysis, which leads to the precipitation of carbonate minerals. Influence of precipitates and biofilm of the two fluid phases (water and CO<sub>2</sub>) are accounted for and the model may be a useful tool for optimisation of strategies for the proposed technology involving the use of such microbially induced barriers to increase storage security near wellbores.

Cappacio et al (2010) show that even metabolically inactive microorganisms can have a positive effect on mineral trapping. Experiments using mutated bacteria showed that crystalline surface layer proteins can selectively attract Ca<sup>2+</sup> ions, serving as nucleation sites for CaCO<sub>3</sub>, thereby accelerating crystal formation.

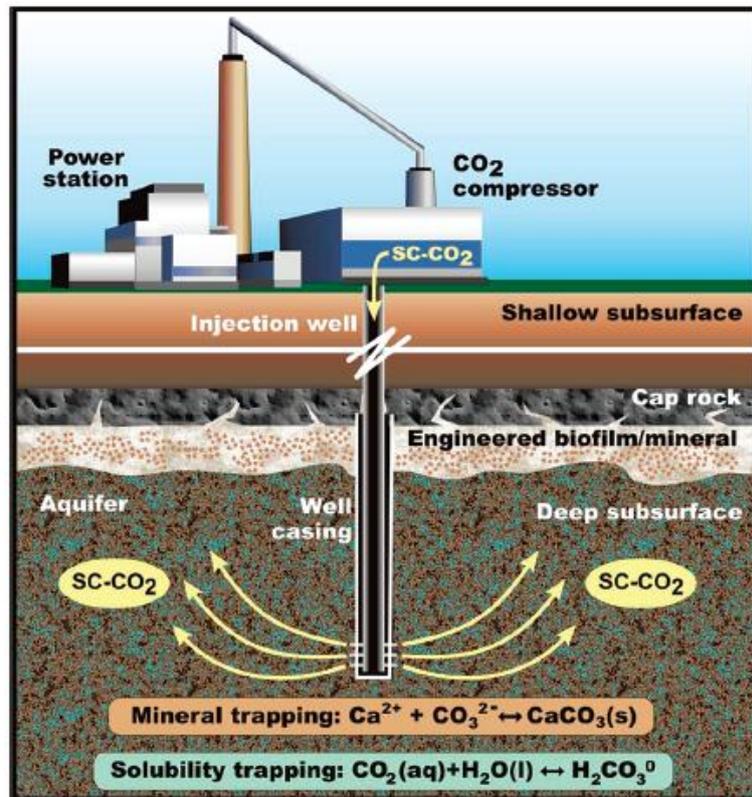
Model systems have been developed (Freedman et al, 2010) to look at the effects of SC-CO<sub>2</sub> on the SC-CO<sub>2</sub> tolerant strain of bacteria (MIT0214), discovered in the experiment mentioned in the previous section, with the intent to understand how to optimise mineral trapping techniques. At Otway, after initial identification of a CO<sub>2</sub> resistance strain of microorganism, further study is underway with the possible aim of engineering biofilms to enhance trapping of CO<sub>2</sub> in saline aquifers (Mu et al, 2011).

Mitchell et al (2009) considered the use of biofilms as a mitigation method for the leakage of CO<sub>2</sub> out of the storage reservoir. Experiments were carried out to investigate the growth of biofilm under high pressure and salinity conditions and its utility for reducing sandstone permeability, and how flowing SC-CO<sub>2</sub> and biofilm starvation affect viability and permeability of the biofilm barrier, and its structural resilience to mechanical stress.

The results show that permeability of the sandstone decreased in both high salinity and high pressure conditions. The effect of starvation of the microorganisms showed negligible changes in permeability. The effect of SC-CO<sub>2</sub> showed an increase in permeability, but by less than 5% after 71 hrs. This suggests that subsurface biofilm barriers do not require continued nutrient feeds in order to sustain the long-term integrity of the barrier. However, continued feeding of starved biofilms may promote further biofilm growth and permeability reduction.

It has also been suggested that stimulating methanogenic bacteria in coal samples can enhance the production of methane (Jones et al 2010). Tang et al (2012) carried out a study analysing indigenous microorganisms in coalbeds to see if this is feasible. The study, while focused on the Ordos basin, China, compared other sites worldwide. The bacterial community was more diverse than those in coals reported so far and the existence of an intact methanogenic microbial community was revealed in low-ranking coal. In contrast, the bacterial diversity decreased remarkably in the presence of sulphate reducing bacteria with no methanogens detected in coal with high coalification levels. An analysis of the microbial community showed it to be very site specific and more diverse in some areas and less so in others.

Figure 2: Schematic representation of microbially enhanced storage (Mitchell et al, 2010)



#### 4. Monitoring of Microbial Activity

Monitoring of CO<sub>2</sub> storage has tended not to focus highly on microorganisms, though there have been several studies related to various sites. At Ketzin, however a particular effort was

made to conduct an analysis on the subsurface microbial community from pre-injection to post injection.

One of the major challenges was to account for any microbial activity that would have been introduced externally through the drilling fluids. A tracer-based method for determining the infiltration of drilling mud and technical fluids into rock cores and fluid samples was applied (Wandrey et al, 2010), using a fluorescent dye, Na-fluorescein to be added to the drilling fluids. It was found that outer core regions of mildly permeable sandstone sections were significantly infiltrated with drilling mud, though the tracer concentration in the inner core was below the visual detection limit. To make sure that inner core samples are not affected by drilling mud in the future, the fluorescein concentration of any samples will need to be quantified.

Microbial monitoring was then able to take place using fluorescence in situ hybridisation (FISH), (Morozova et al, 2010). This is one of the most used nucleic acid techniques to study microorganisms in their natural environments. FISH coupled with rRNA-targeted oligonucleotide probes allows direct visualisation, identification and localisation of bacterial cells from selected phylogenetic groups in environmental samples. This showed the microbial community to be strongly influenced by CO<sub>2</sub> injection. Before CO<sub>2</sub> arrival, up to  $6 \times 10^6$  cells/ ml were detected by DAPI staining (epifluorescent microscopy) at a depth of 647 m below the surface. The microbial community was dominated by the domain Bacteria that represented approximately 60% to 90% of the total cell number, with Proteobacteria and Firmicutes as the most abundant phyla comprising up to 47% and 45% of the entire population, respectively. Both the total cell counts as well as the counts of the specific physiological groups revealed quantitative and qualitative changes after CO<sub>2</sub> arrival. The study revealed out competition of sulphate-reducing bacteria by methanogenic Archaea. In addition, an enhanced activity of the microbial population after five months of CO<sub>2</sub> storage indicated that the bacterial community was able to adapt to the extreme conditions of the deep biosphere and to the extreme changes of these atypical conditions.

All current methods of microbial monitoring and observation are from samples taken from observation wells. In Otway a U-tube was used which isolated formation water from sources of contamination, while maintaining the formation pressure (Mu et al, 2011). From this, DAPI staining highlighted abundance of filamentous cells ranging from 5 to 45µm. The microorganisms found to be resistance to CO<sub>2</sub> are currently being investigated.

## **5. Conclusions**

There has been much work carried out on microbial activity in the subsurface, though there is limited information regarding the effect on CO<sub>2</sub> storage. There are however, several research projects and studies underway looking into this topic.

CO<sub>2</sub> can have a large effect on microbial activity, with the majority of microorganisms having a negative reaction to supercritical CO<sub>2</sub>. Microorganisms likely to thrive in a CO<sub>2</sub> environment are those with thicker cell walls and those that are able to produce a biofilm. Microorganisms that can survive CO<sub>2</sub> injection are being identified and studied through

laboratory experimentation and observation at current CO<sub>2</sub> storage sites. Models are also being developed that will be able to take account of reactions and changes caused by microbial activity.

Microbial activity can potentially have negative effects, such as bio-corrosion in the wellbore and potential pore blocking, which may affect injectivity, though there was not much information found related specifically to this area.

There has been and is current research on the potential utilisation of microorganisms to enhance storage security by enhanced mineral and solubility trapping, such as through microbially enhanced ureolysis or just by their presence of crystalline surface layers that can act as nucleation sites. Biofilms can also act as a hydraulic barrier to prevent flow of the CO<sub>2</sub> plume, which may be particularly useful as a mitigation method. Laboratory experiments show high resistance of biofilms to starvation of the microorganisms as well as supercritical CO<sub>2</sub>, making it a potentially viable solution.

## **6. Recommendations**

This topic is becoming increasingly significant as more demonstration projects start to take place. Microbial activity may need to be part of any site characterisation and the effects may need to be taken into account in the risk assessment phase. Knowledge regarding bioengineering is also increasing.

It is therefore recommended that IEAGHG consider a full study at the appropriate time documenting the effects of and on microbial activity on geological storage of CO<sub>2</sub> and highlighting the research gaps.

## List of Figures

Figure 1: Diagrammatic illustration variety of free energy from S redox half reactions (from West et al, 2011)

Figure 2: Schematic representation of microbially enhanced storage (From Mitchell et al, 2010)

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