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Biofilm enhanced geologic sequestration of supercritical CO_2

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ABSTRACT

In order to develop subsurface CO_2 storage as a viable engineered mechanism to reduce the emission of CO_2 into the atmosphere, any potential leakage of injected supercritical CO_2 (SC-CO_2) from the deep subsurface to the atmosphere must be reduced. Here, we investigate the utility of biofilms, which are microorganism assemblages firmly attached to a surface, as a means of reducing the permeability of deep subsurface porous geological matrices under high pressure and in the presence of SC-CO_2, using a unique high pressure (8.9 MPa), moderate temperature (32 °C) flow reactor containing 40 millidarcy Berea sandstone cores. The flow reactor containing the sandstone core was inoculated with the biofilm forming organism *Shewanella fridgidimarina*. Electron microscopy of the rock core revealed substantial biofilm growth and accumulation under high-pressure conditions in the rock pore space which caused >95% reduction in core permeability. Permeability increased only slightly in response to SC-CO_2 challenges of up to 71 h and starvation for up to 363 h in length. Viable population assays of microorganisms in the effluent indicated survival of the cells following SC-CO_2 challenges and starvation, although *S. fridgidimarina* was succeeded by *Bacillus mojavensis* and *Citrobacter* sp. which were native in the core. These observations suggest that engineered biofilm barriers may be used to enhance the geologic sequestration of atmospheric CO_2.

1. Introduction

Geologic sequestration of carbon dioxide is one strategy to reduce the emission of greenhouse gases generated through the combustion of fossil fuels. Geologic sequestration of CO_2 involves the injection of CO_2 into underground formations such as oil-bearing formations, deep un-mineable coal seams, and deep saline aquifers (White et al., 2003; Zakkour and Haines, 2007). During operation of geologic CO_2 sequestration sites, supercritical CO_2 (SC-CO_2; critical point = 31.1 °C and 7.4 MPa) would be injected into the receiving formation resulting in elevated pressure in the region surrounding the point of injection. As a result, an upward hydrodynamic pressure gradient may develop across the trapping cap-rock. Upward "leakage" of CO_2 could occur due to the primary permeability of the cap-rock, through fractures, or near injection wells (White et al., 2003). It is therefore imperative to develop methods that reduce CO_2 leakage and develop subsurface CO_2 storage as a viable engineered mechanism to reduce concentrations of atmospheric CO_2 (UNEP, 2006).

In order to reduce SC-CO_2 leakage, we are investigating the utility of biofilms as a means of reducing the porosity and permeability of deep subsurface geological matrices (Fig. 1). Biofilms are microorganism assemblages firmly attached to a surface, which form and are encased within self produced extracellular polymeric substances (EPS), a hydrated matrix

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of mostly polysaccharides and proteins (Costerton and Stewart, 2001; Lewandowski and Beyenal, 2007). Biofilms are the predominant way of life for most microorganisms in the environment because matrix enclosed multi-cellular communities offer structural support, protection from physical, chemical and biological stresses, optimal location relative to substrates required for metabolic function, and symbiotic benefits in multi-species communities (Watnick and Kolter, 2000; Costerton and Stewart, 2001; Stoodley et al., 2002; Lewandowski and Beyenal, 2007). The concept of using biofilms to enhance CO_2 sequestration as proposed herein is to contain CO_2 in the subsurface and reduce upward leakage through the cap-rock, by biofilm plugging of pore space and fractures present in the cap-rock (Fig. 1). This may be achieved by the injection of biofilm forming organisms and growth nutrients into the CO_2 injection well before, during, or after CO_2 injection. Subsequent control over the spatial extent and mass of the biofilm will be achieved by the flux and composition of subsequent nutrient feeds. CO_2 sequestration could be enhanced if biofilm growth can be fueled by autotrophic fixation of the injected CO_2, and if biofilm can induce the mineralization of carbonate minerals (Mitchell and Ferris, 2005; Mitchell et al., in press-a).

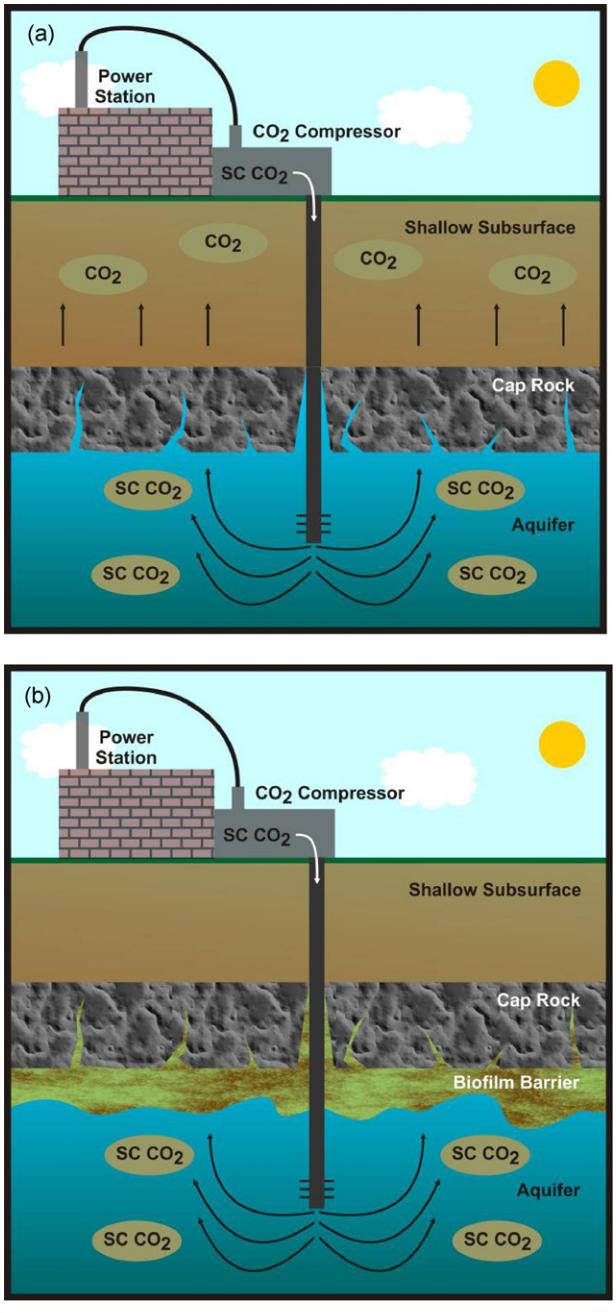

Fig. 1 – Conceptual illustration of supercritical CO_2 leakage mitigation using deep subsurface biofilm barriers. (a) Potential upward leakage of CO_2 from deep subsurface. (b) Reduced upward leakage of CO_2 due to engineered biofilm barrier.

Biofilms have many undesirable properties, including human infection, souring of oil formations, and microbially influenced corrosion (Lewandowski and Beyenal, 2007). However, over the last two decades, understanding biofilm formation has developed the discipline of biofilm engineering, allowing control over mechanisms of biofilm formation for desirable human purposes, including the bioremediation of toxic compounds (Bouwer et al., 2000), wastewater treatment (Lazarova and Manem, 2000), and enhanced oil recovery (Shaw et al., 1985; Macleod et al., 1988). In the subsurface, biofilm-enhanced technologies have evolved which focus upon the utility of biofilm communities to form barriers to flow and mass transport. Such engineered biofilm barriers often involve the injection and transport of starved bacterial cultures followed by resuscitation with injected growth substrates (Cunningham et al., 2007). This process can produce a cohesive mass of cells and the production of copious amounts of EPS, which plug the free pore space of the aquifer, reducing permeability by as much as four orders of magnitude (Cunningham et al., 1997). Biofilm barrier technology has been successfully demonstrated at the field scale for containment and remediation of nitrate-contaminated groundwater (Cunningham et al., 2003) and has been researched as strategy for enhancing secondary oil recovery (Shaw et al., 1985; Macleod et al., 1988). This provides the incentive for undertaking a unique research and development effort aimed at examining the problems and opportunities of biofilms in CO_2 sequestration.

Underground formations in which SC-CO_2 will be stored will be subject to pressures >7.4 MPa from the injected SC-CO_2. Therefore biofilm barriers must be able to be resilient to the effect of high pressure and SC-CO_2. While pressure is a thermodynamically well-known physical parameter, the effects of high pressure on microorganisms remain poorly characterized, unlike those of heat (Aertsen et al., 2004). Mechanisms by which high pressure can degrade microorganisms include protein denaturation, enzyme inhibition (Simpson and Gilmour, 1997; Erijman and Clegg, 1998; Niven et al., 1999), cellular disruption, and phase transition in membranes (Kobori et al., 1995; Pagan and Mackey, 2000; Ganzle et al., 2001). However, organisms that are adapted to normal atmospheric pressure (0.1 MPa) (mesophilic), including *Escherichia coli*, can often grow in planktonic cultures at pressures up to a few tens of megapascals, albeit at much lower rates (Aertsen et al., 2004). This contrasts to high-pressure-adapted organisms (piezophilic), which are often found in high-pressure deep-sea environments (30–100 MPa)

and require higher pressure to grow. For a range of microorganism species grown planktonically, only pressures of 100–1000 MPa will generate a complete kill of the culture (Aertsen et al., 2004).

High-pressure CO_2 has been shown to reduce the number of living or 'viable' cells for a range of microorganisms grown in planktonic cultures (Zhang et al., 2006). The viability of 20 out of 22 tested vegetative species of microorganisms reported in the literature was completely deactivated at some combination of pressure and temperature in the presence of SC-CO_2 (Zhang et al., 2006). SC-CO_2 is particularly damaging to cells because it has a low viscosity [3–7 $\times$ 10^{-5} N s m^{-2}, between 7.4 and 15 MPa, at 37 °C; McHugh and Krukonis, 1994] and low surface tension, so it can quickly penetrate complex cellular material. This is enhanced at higher temperatures, which increase the fluidity of the cell membrane (Hong et al., 1997), and higher pressures, which facilities CO_2 solubilization in water and penetration through cell walls (Lin et al., 1994). Gram-positive cells are more resilient to high-pressure CO_2 than Gram-negative cells, owing to their thick cell walls which have high peptidoglycan content, making them less permeable and increasing their resilience to mechanical stress (Zhang et al., 2006). Both mechanical cell rupture during CO_2 pressurization and depressurization and physiological deactivation (from low intracellular pH, enzyme denaturation, and liquid extraction by SC-CO_2) have been proposed as potential mechanisms for cell deactivation in a range of microorganisms (Zhang et al., 2006). Conversely, bacterial endospores (a dormant, tough, and non-reproductive structure that is adapted for dispersion and surviving for extended periods of time in unfavorable conditions) are highly resistant to high-pressure CO_2 treatment (Zhang et al., 2006). However, the resilience of biofilms to SC-CO_2, in terms of the mechanical structure of the biofilm and its continued viability, has never been investigated. Mechanical erosion on the biofilm by the fluid shear stress of SC-CO_2 may degrade the biofilm, as has been shown with shear stress applied by non-pressurized aqueous media (Rittmann, 1982; Peyton and Characklis, 1993; Picioreanu et al., 2001). EPS may also provide a protective barrier for the microorganisms, as has been demonstrated for the resistance of biofilms to antimicrobial agents (Costerton and Stewart, 2001; Stewart, 2003; Lewandowski and Beyenal, 2007).

Nutrient starvation has been shown to lead to the rapid detachment of cells from biofilms for a range of organisms including *Pseudomonas aeruginosa* and *Shewanella oneidensis* (Hunt et al., 2004; Thormann et al., 2005, 2006). When the vitamin, nutrient, and energy needs of the organism are no longer satisfied, cells will detach in order to find microenvironments that will satisfy these requirements. Therefore, subsurface biofilm barriers may require continued feeding with media, which contains sufficient vitamin, nutrient, and energy sources, in order to sustain the integrity of the barrier over the long term.

In this study we describe and use a unique high pressure (<8.9 MPa), moderate temperature (32 °C) flow reactor containing a 40 millidarcy (md) Berea sandstone core. We provide the first data pertinent to the application of biofilms for the subsurface sequestration of CO_2, investigating (i) the growth of biofilm under high pressure and salinity conditions and its utility for reducing sandstone permeability, and (ii) how flowing SC-CO_2 and biofilm starvation affect the viability and permeability of the biofilm barrier, and its structural resilience to mechanical stress.

2. Methodology

2.1. High-pressure equipment setup

A high-pressure biofilm and SC-CO_2 test system was built to form a biofilm barrier in sandstone cores and measure *in situ* changes in permeability over time. The system allowed a differential pressure to be established between two high-pressure accumulators causing media to flow through a core housed in a Hassler-type core holder (TEMKO, Tulsa, OK). Differential pressure and flow rate measurements were used to calculate changes in permeability as the biofilm developed. Two experiments were performed with separate 2.54 cm diameter Berea sandstone cores (97% quartz, 3% accessory clay and opaque minerals) (Dunning and Miller, 1984). Each had an approximate porosity of 22% and permeability of 40 millidarcy. The core length was 5.08 cm in experiment 1 and 11.85 cm in experiment 2.

The high-pressure system was constructed of 1/4 in. stainless steel tubing and Swagelok fittings housed in an incubator to control temperature at 32 °C. The media and SC-CO_2 reservoirs were water service piston-type accumulators (Parker, Inc., Cleveland, OH) designed to withstand pressures of 21 MPa (Figs. 2 and 3). Prior to loading the sandstone core, the media accumulator and influent tubing were sterilized by pumping through 10% bleach in 6 g L^{-1} TWEEN 80 solution, followed by 2.25 g L^{-1} sodium thiosulphate solution, followed by a 70% ethanol solution (Barkley and Richardson, 1994). Next, the sandstone core was loaded into a Buna-N (TEMCO Inc., Tulsa, OK) sleeve and the core holder was reassembled. The core and core sleeve were not sterilized by autoclaving nor chemical treatment to avoid inducing mineral transformation and changing the pore structure. The annulus of the core holder was filled with water and an overburden pressure of 10.5 MPa was provided by a hydraulic jack pump. The core holder and media accumulator were connected to the high-pressure system. Nitrogen gas was used to pressurize the system. The nitrogen pressure regulator was slowly opened to pressurize the system in 0.7 MPa increments until it reached 8.9 MPa.

2.2. System inoculation

Shewanella frigidimarina was used as an inoculum for the experiments. This organism was isolated from oil field brine and therefore had adapted to high salt environments and high pressure. It was also selected because it produces copious amounts of EPS which are likely to clog pore spaces in the core (Garth James, personal communication). *S. frigidimarina* was grown to the exponential growth phase prior to inoculating the high-pressure system in autoclaved saline nutrient media containing 18 g L^{-1} brain heart infusion (BHI), 0.75 g L^{-1} NH_4Cl, 40 g L^{-1} NaCl, and 3 g L^{-1} $NaNO_3$. 40 mL of the exponential growth phase culture of *S. frigidimarina* were injected into the

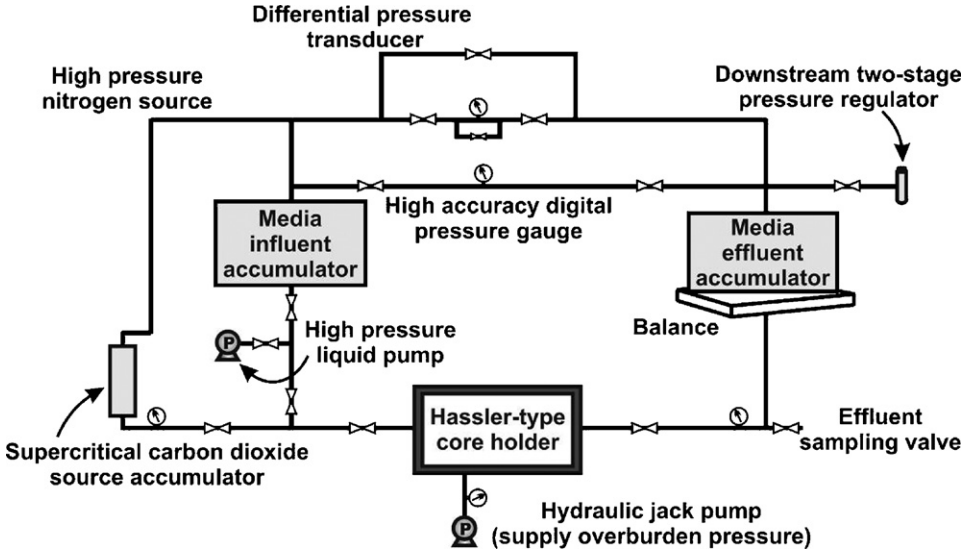

Fig. 2 – Schematic of high-pressure biofilm and supercritical CO_2 flow system.

core using a 60 mL sterile syringe attached to a Swagelok fitting located at the influent side of the core holder. The inoculum remained in the core for 16 h to allow ample attachment time to the Berea sandstone. Previous studies have shown significant attachment of microorganisms such as *P. aeruginosa* to glass in a matter of 10's of minutes to hours (Sauer et al., 2002). Next, the media accumulator was removed from the system and sterilized using the sterilization procedure described in Section 2.1. It was then filled with 1100 mL of sterile saline nutrient media and placed back on line. After being allowed to warm up to the system temperature, saline nutrient media was pulsed through the core using a constant differential pressure across the core, which was maintained at values ranging from 0.29 to 0.39 MPa. The duration of each pulse was set so as to pass at least one pore volume of fluid through the core. The initial pore volumes were 5.66 and 13.2 mL for the 5.08 and 11.85 cm cores, respectively.

Initially the saline nutrient media pulses were about 20 min in length. These gradually increased to almost 200 min because flow rates decreased with decreasing core permeability. The liquid that passed through the core into the effluent accumulator was transferred into a flask. The resulting volume of the effluent was divided by the pulse time to determine a flow rate and subsequently the permeability of the core, according to:

$$K = \frac{Q \times \mu \times L}{A \times \Delta P}$$

where K is the permeability in Darcy, Q is the flow rate in mL s^{-1}, μ is the viscosity in cp, L is the length of core in cm, A is the cross sectional area in cm^2, ΔP is the differential pressure in atm.

This was repeated once or twice a day for 20 days in experiment 1, and 13 days in experiment 2. After each pulse, the core holder was isolated from the rest of the system by closing the valves on either side to maintain pressure in the system. The overburden pressure on the core sleeve remained constant at 10.5 MPa and the system pressure at 8.9 Mpa throughout the experiments.

2.3. *Starvation challenge*

Cores were subjected to vitamin, nutrient, and energy limited conditions by switching the saline nutrient media to a brine solution with the same salt (NaCl) concentration as the saline nutrient medium (40 g L^{-1}) in order to determine if the biofilm barrier became less resilient to fluid flow due to cell starvation and associated detachment and degradation. Brine pulses were performed analogously to the previous nutrient pulses. Permeability was calculated over time as described previously. Liquid viscosity was assumed to be 1.0 cp (for H_2O) for permeability calculations using brine and the saline nutrient media since the same pressure and temperature conditions apply, and the NaCl concentrations are the same. The saline nutrient medium has slightly higher salinity than the brine from the inclusion of BHI, but the effect on viscosity was considered negligible.

2.4. *Supercritical carbon dioxide challenge*

Cores were subjected to several SC-CO_2 challenges. SC-CO_2 was prepared by pumping liquid CO_2 from a pressure tank into the SC-CO_2 accumulator using an HPLC pump (Fig. 2). The pump and accumulator were located inside the incubator to maintain the CO_2 at a temperature of 32 °C. Supercritical conditions were verified by measuring the pressure of the SC-CO_2 accumulator and the temperature of the incubator. After preparing about 950 mL of SC-CO_2 the valves were opened and a pulse of SC-CO_2 was allowed to flow through the core. Following the SC-CO_2 challenge, the core was re-saturated with brine solution and the permeability of the core was determined as described previously. The number of viable cells in the reactor effluent was determined for each of the brine and nutrient pulses using serial dilution, plating and counting on BHI + Salt agar plates. Field Emission Scanning

Fig. 3 – Pictures of high-pressure biofilm and supercritical CO_2 flow system: top—series of valves and gauges that allow differential pressure to be controlled. Bottom left: Nutrient media accumulator, supercritical CO_2 storage accumulator, Hassler-type core holder containing Berea sandstone core, and hydraulic jack pump used to apply overburden pressure. All housed inside an incubator for temperature control. Bottom right: Accumulator and sample port used to collect effluent samples.

Electron Microscopy (FESEM) was performed on a Zeiss® Supra 55VP on core sections prior to inoculation and at the termination of the experiments.

3. Results

3.1. *Biofilm growth and permeability change under high pressure*

A permeability decrease was observed in both experiments (Fig. 4). Initial core permeability was 39 and 47 md in experiments 1 and 2, respectively. Permeability decreased rapidly by between 82 and 85% during the first 50–80 h to ~7 md. During the period over which the decrease in permeability was approximately linear with time, the rate of permeability reduction was 0.97 md h^{-1} from 0 to 23 h during experiment 1, and 1.2 md h^{-1} from 0 to 15 h during experiment 2. Subsequent permeability exhibited a slight increase up to 120 h in experiment 1, and 140 h in experiment 2, and then decreased to below 2.5 md by 200 h. The maximum reduction in permeability relative to the initial permeability of the core was 95 and 99% in experiments 1 and 2, respectively.

FESEM images of the Berea core prior to inoculation of the experiments indicate the microorganism-free mineral surfaces of the mostly quartz grains which make up the sandstone. By comparison, FESEM images of the core at the termination of the experiment indicate an assemblage of microorganisms covering most of the mineral surfaces in the rock core (Fig. 5). These data demonstrate that a biofilm was able to grow in the sandstone core under pressures of 8.9 MPa and significantly decrease its permeability.

3.2. *Effect of biofilm starvation on core permeability*

During experiment 2, the saline nutrient media was switched to a 4% brine solution between 300 and 663 h in order to starve the biofilm. This 363 hour starvation period resulted in negligible changes in the permeability of the sandstone core,

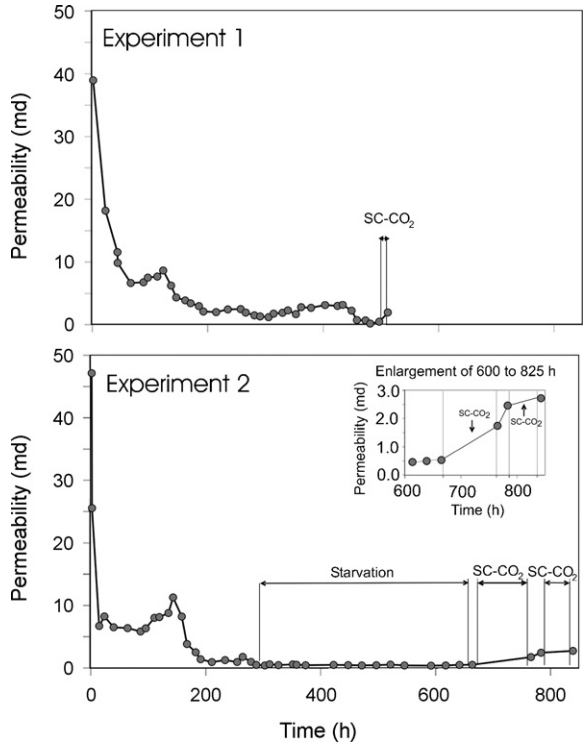

Fig. 4 – Changes in permeability over time during experiments 1 and 2. During experiment 1 the core was challenged with supercritical CO_2 for 16 h at 494 h. During experiment 2, the core biofilm was (i) starved with a 4% brine solution at 300 h, (ii) challenged with supercritical CO_2 for 71 h at 694 h, and (iii) again for 48 h at 783 h.

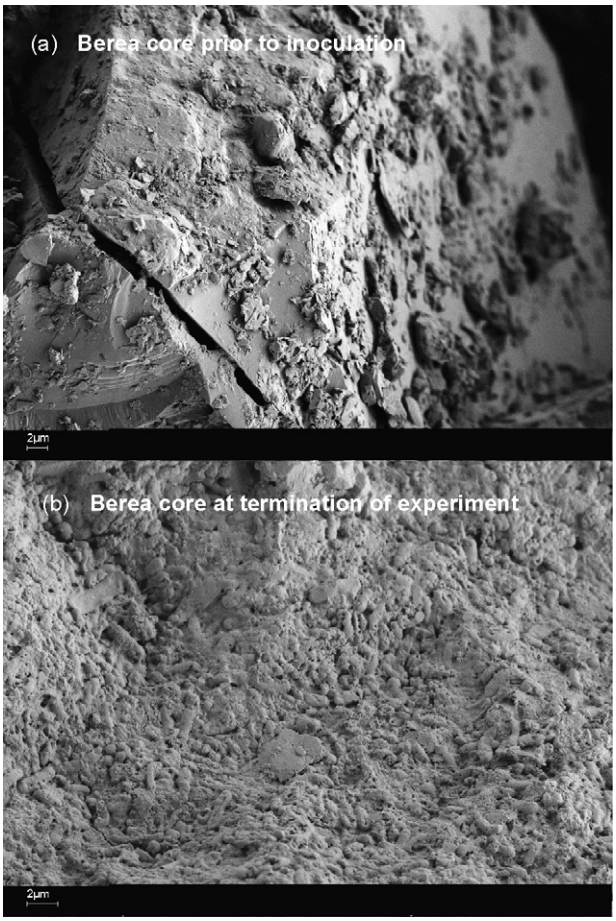

Fig. 5 – FESEM images of (a) Berea sandstone core prior to inoculation showing the microorganism-free mineral surfaces of the quartz grains which make up the sandstone, and (b) at termination of the experiment showing an assemblage of microorganisms attached to the mineral surfaces.

which remained at ~0.5 md throughout the starvation period (Fig. 4). These data demonstrate that starving the biofilm did not result in a significant increase in permeability.

3.3. *Effect of supercritical CO_2 on biofilm-affected core permeability*

$SC\text{-}CO_2$ challenges of different durations were performed. During experiment 1, a 16 h $SC\text{-}CO_2$ challenge was performed between 494 and 510 h (Fig. 4). Permeability before the challenge was 0.46 md and increased to 1.93 md after 16 h, which corresponds to a 3.8% increase relative to the initial permeability of the core. During experiment 2, two $SC\text{-}CO_2$ challenges were performed. The first $SC\text{-}CO_2$ challenge was 71 h long which increased the permeability from 0.51 to 1.74 md, or by 2.6% compared to the initial permeability of the core (Fig. 4). During the second challenge, a 48 h challenge increased the permeability from 2.45 md before the challenge, to 2.75 md after. This was only a 0.64% increase relative to the initial permeability of the core. These data clearly demonstrate that although $SC\text{-}CO_2$ challenges increased biofilm-affected core permeability slightly, the resulting permeability was still less than 5% of the initial permeability of the cores, and that there was no correlation between the duration of the $SC\text{-}CO_2$ challenge and the increase in permeability, even when the challenge lasted for prolonged periods.

3.4. *Microbial populations in reactor effluent*

Viable microbial populations in the core effluent were measured throughout experiments 1 and 2 (Fig. 6). During experiment 1, plate counts for the inoculated organism *S. frigidimarina* decreased during the duration of the experiment from an initial high of 2.73×10^6 colony forming units (cfu) mL^{-1} to 7.7×10^3 cfu mL^{-1} by the end of the experiment. A white mucoid organism was detected 43 h after the beginning of the experiment, at a concentration of 5.6×10^6 cfu mL^{-1}, greater than the number of *S. frigidimarina* colony forming units. The colony numbers of the white mucoid organism decreased to 3.0×10^5 cfu mL^{-1} by 88 h, but remained two orders of magnitude greater than the number of *S. frigidimarina* colony forming units. Identification of the white mucoid colony unit by small subunit ribosomal DNA (16S rDNA) sequencing (Midi Labs, Newark, DE), identified the organism as most similar to the species *Bacillus mojavensis* (0.09% base pair difference). The $SC\text{-}CO_2$ challenge at the end of experiment 1 did not have an appreciable effect on the number and assemblage of colony forming units measured in the reactor effluent.

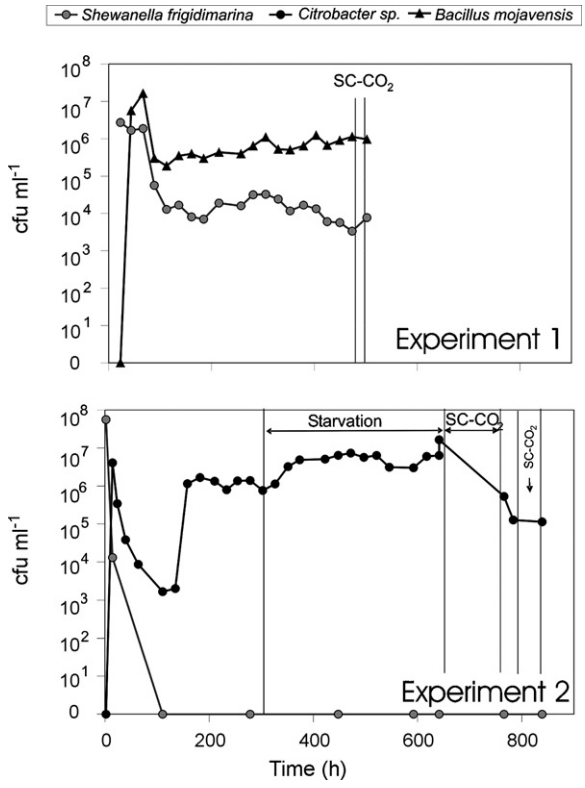

Fig. 6 – Effluent population counts (colony forming units (cfu) mL^{-1}) from experiments 1 and 2, determined from drop plating on BHI agar plates.

Plate counts of *S. frigidimarina* also decreased during the course of the second experiment, from an initial high of 5.57×10^7 cfu mL^{-1} to less than 10 cfu mL^{-1} 5 days later (Fig. 6). A white mucoid organism dominated the plate counts shortly after the beginning of the experiment. 16S rDNA sequencing of this colony unit identified it as a *Citrobacter* sp. (0.38% base pair difference). As the experiment progressed their numbers remained relatively constant between 10^6 and 10^7 cfu ml^{-1}. The first SC-CO_2 challenge between 694 and 765 h generated an approximately 1 order of magnitude decrease in colony forming units of *Citrobacter* sp. However, the following challenge between 783 and 838 h had a negligible effect on the number of colony forming units. In contrast, the starvation challenge increased the number of colony forming units in the reactor effluent by around 1 order of magnitude between 300 and 663 h. These data demonstrate that SC-CO_2 challenges and starvation do not lead to complete sterilization of the biofilm or a complete loss of permeability reduction by the biofilm in the sandstone core.

4. Discussion

4.1. *Biofilm formation under high-pressure saline conditions*

The development of a unique high-pressure moderate temperature flow system has provided the means to investigate biofilm growth under high pressure and in the presence of SC-CO_2. Flow experiments revealed that a biofilm was able to form under high-pressure pulsed-flow conditions which caused appreciable plugging of the Berea sandstone core. The magnitude of permeability reduction (95 and 99% in experiments 1 and 2, respectively) was as great or greater than reported by a range of biofilm plugging experiments at atmospheric conditions (Shaw et al., 1985; Macleod et al., 1988; Hill and Sleep, 2002; Kim, 2004) demonstrating high-pressure conditions do not reduce the structural resilience of the biofilm to fluid flow.

Investigation into the effects of high pressure on the growth of planktonic cells reveals that the metabolic functions of mesophilic microorganisms are affected by pressure (Abe et al., 1999). Growth rate is significantly reduced at pressures of 30–50 MPa, due to the sensitivity of DNA replication at elevated pressures (Yayanos and Pollard, 1969). Protein synthesis is also affected, due to the destabilization of uncharged ribosomes (Gross et al., 1993). However, the rapid formation of a biofilm suggests that it is possible to establish a biofilm at high pressures. Biofilms are often more resilient to physical, chemical, and biological stresses than the same organisms in the planktonic form (Lewandowski and Beyenal, 2007). This is often attributed to the EPS biofilm matrix which provides a protective barrier against such stresses (Costerton and Stewart, 2001; Stewart, 2003; Lewandowski and Beyenal, 2007). Indeed, the measurement of viable cells between 10^4 and 10^8 cfu mL^{-1} in the reactor effluent throughout the entire experiment suggests continuous biofilm viability in the pressurized sandstone core.

The shift in microorganism assemblages measured in the reactor effluent from the inoculated organism *S. frigidimarina* to *B. mojavensis* and *Citrobacter* sp. in experiments 1 and 2, respectively, indicates that native microorganisms present in the sandstone core were able to out-compete the inoculum, or that there is a fractionation of species types between the solid sandstone phase, and liquid nutrient phase. The saline nutrient medium has previously been shown to support *S. frigidimarina* growth under low pressure batch experiment conditions (Garth James, personal communication). Therefore, the dominance of *B. mojavensis* and *Citrobacter* like species suggests that these native organisms were more tolerant to the high-pressure conditions or that they had an affinity for the liquid phase and were not able to remain attached to the mineral surface as effectively as *S. frigidimarina*. Such species fractionation has been observed previously in porous media flow experiments (Lehman et al., 2001). However, the complete absence of *S. frigidimarina* in the reactor effluent in experiment 2, suggests that this species was almost entirely absent from the biofilm, and that the native organism had out-competed *S. frigidimarina*. This is somewhat surprising since *S. frigidimarina* was isolated from oil field brine, had survived in high pressure and high salt environments, and would therefore be expected to be well adapted to the high pressure of the flow system. However, *B. mojavensis* also appears well adapted to high-pressure environments due to its spore forming capabilities (Roberts et al., 1994). Spores have a thick outer coat and a dehydrated state and are therefore resilient to high-pressure conditions (Kamihira et al., 1987; Enomoto et al., 1997; Zhang et al., 2006). This suggests the continued viability of *B.*

mojavensis in the high-pressure sandstone core may result in part from enhanced spore formation. *Citrobacter* is a common bacterium found in soils and water, and thus its presence in Berea sandstone is not unsurprising, although it has no specific properties to suggest tolerance to high-pressure saline environments.

4.2. Permeability changes

The reduction in permeability with time (95 and 99% in experiments 1 and 2, respectively) was quite similar between experiments 1 and 2. Any differences are likely to be attributable to the fact that different species, *B. mojavensis* and *Citrobacter* sp., became dominant in experiments 1 and 2, respectively, which are likely to exhibit different organism morphologies and biofilm structures. The increase in permeability at 120 and 140 h in experiments 1 and 2 correlates with a decrease in the number of viable cells in the reactor effluent (Fig. 6). This suggests the viable biomass of the biofilm decreased in this period allowing a decrease in pore space clogging and an increase in permeability, although it remains unclear why this occurred at approximately the same time in both the experiments.

The starvation challenge during experiment 2 was associated with an order of magnitude increase in the number of colony forming units in the reactor effluent from $\sim 10^6$ to 10^7 cfu, likely reflecting starvation-induced detachment of viable biofilm cells. Such starvation-induced detachment has been previously reported (Marshall, 1988; Delaquis et al., 1989; Sawyer and Hermanowicz, 1998; Hunt et al., 2004), although mechanisms by which nutrient limitation affects the physiology of the cell to induce detachment are not well understood. However, even though starvation-induced detachment appeared to have occurred, it had only a negligible effect on the permeability of the sandstone core under high-pressure conditions. This indicates the proportion of detaching cells was negligible relative to the biomass of the biofilm, as observed in flow reactor studies at atmospheric pressure (Lehman et al., 2001), or that the EPS produced by the biofilm is mostly responsible for the permeability reduction. Previous experiments investigating the utility of biofilm barriers for reducing aquifer hydraulic conductivity in the near subsurface under near atmospheric pressure conditions have also shown starved biofilm barriers to be stable for weeks to months without a significant increase in permeability (Cunningham et al., 2003). Significantly, these data suggest 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, as has been in observed in biofilm barriers in the near subsurface under near atmospheric pressures (Cunningham et al., 2003, 2007).

SC-CO_2 challenges in our study did not lead to a significant increase in core permeability, and while challenges were associated with a subsequent decrease in the number of colony forming units in the reactor effluent, significant viable cell numbers (between 10^4 and 10^7 cfu mL^{-1}) were still measured in the effluent. Therefore, SC-CO_2 challenges for periods of up to 71 h at pressures of approximately 8.9 MPa and a temperature of 32 °C did not lead to any significant destruction of the biofilm structure or killing of the biofilm organisms. Biofilm structure undergoes some desiccation in the presence of SC-CO_2 (Mitchell et al., in press-b), and while the nature of the rock core does not allow us to observe this *in situ* in the current experiment, it is likely to have occurred. This suggests desiccation does not severely destroy the structure of the biofilm to a point that it develops a significant increase in core permeability.

A number of previous studies investigating the effect of SC-CO_2 upon the viability of planktonic cells and spores of both Gram-negative and Gram-positive bacteria demonstrates that there is a large range in the temperature and pressure conditions in the presence of SC-CO_2 that is required to completely kill microorganisms (Zhang et al., 2006). For example, at 7.4 MPa CO_2 and 38 °C, *B. subtilis* required an exposure of 2.5 min for complete deactivation, whereas *S. marcescens* was deactivated immediately after the initial temperature and pressure had been attained (Debs-Louka et al., 1999; Dillow et al., 1999; Elvassore et al., 2000). However, generally, the pressures and durations that microorganisms have been exposed to SC-CO_2 to allow complete deactivation are lower than we applied in our experiments (Zhang et al., 2006). This suggests the biofilm that formed within the sandstone core in our current experiments was extremely resilient to SC-CO_2 challenges. This is likely a reflection of the EPS which form the biofilm matrix and which provide a protective barrier, likely reducing the ability of the SC-CO_2 to penetrate deep into the biofilm (Mitchell et al., in press-b), as has been exhibited for the inhibition of antimicrobial agent penetration into biofilms (Costerton and Stewart, 2001; Stewart, 2003; Lewandowski and Beyenal, 2007). The stability of the biofilm matrix is also likely to provide resistance to the mechanical shear stress applied by the SC-CO_2, compared to planktonic cell cultures.

5. Conclusions and recommendations

The development of a unique high pressure (8.9 MPa), moderate temperature (32 °C) flow reactor has allowed the interaction of hydrodynamics, microbiology, and geology in the presence of SC-CO_2 to be investigated. This reactor system has facilitated the investigation into the growth of biofilm under high pressure, the utility for reducing sandstone core permeability, and how repeated SC-CO_2 challenges and biofilm starvation affect the stability of a biofilm barrier. The preliminary data presented demonstrate that biofilm communities can grow under high-pressure conditions which can lead to a 95–99% reduction in core permeability. Starvation led to an order of magnitude increase in the detachment of cells from the core biofilm, but had a negligible effect on core permeability. Repeated SC-CO_2 challenges also had little effect on core permeability, and viable cells were collected in the effluent of the flow system demonstrating that SC-CO_2 challenges lasting up to 71 h did not lead to a significant destruction of the biofilm structure or killing of the biofilm organisms. The continued resilience and viability of the biofilm which maintained the reduction in core permeability to >95% demonstrates that biofilm barriers may serve as an

effective means to enhance SC-CO_2 sequestration by reducing leakage during engineered subsurface carbon sequestration. If biofilms can be developed in the deep subsurface which use the injected CO_2 as their carbon source, and potentially induce the mineralization of carbonate minerals (Mitchell and Ferris, 2005; Mitchell et al., in press-a), the permanent sequestration of CO_2 could be enhanced. Further research is required into biofilm formation in the presence of and interactions with SC-CO_2, particularly the direct measurement of CO_2 transport through biofilms and CO_2 fixing (autotrophic) biofilms.

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