MONTANA STATE UNIVERSITY
Center for Biofilm Engineering

ZERT

Microbially Enhanced Geologic Sequestration of Supercritical CO_2

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Abstract

Geologic sequestration of CO_2 involves injection into underground formations. According to White et. al.[1] multiple geologic formations are possible for storage including oil beds (CO_2 is currently used in enhanced oil recovery (EOR) projects), deep un-minable coal seams, and deep saline aquifers. There are several conditions which must be met for successful sequestration in these formations including: 1) temperature and pressure conditions must be such that CO_2 will be supercritical, 2) the aquifer must have a suitable aquitard trap, and 3) the receiving aquifer should have appropriate porosity and permeability. Supercritical CO_2 will 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 aquitard. Upward "leakage" of CO_2 can subsequently occur due to the primary permeability of the aquitard, through fractures or near injection wells.

This poster focuses on microbially-based strategies and technologies for controlling leakage of supercritical CO_2 ($scCO_2$) during geologic sequestration. We will examine the concept of using engineered microbial biofilm barriers as a method for 1) reducing permeability of confining aquitard layers in the vicinity of CO_2 injection, 2) providing a zone of reduced permeability to mitigate further migration of CO_2 leaks which have penetrated the aquitard and reached overlying strata, and 3) stimulating irreversible precipitation of mineral phases for long-term permeability reduction.

Biofilm grown in a 2.54 cm diameter, 5 cm long, 40 millidarcy Berea sandstone core resulted in a two order-of-magnitude reduction in permeability over the 500 hour experiment. Permeability did not increase significantly in response to a 12 hour long $scCO_2$ challenge. The *Shewanella fridgidimarina* inoculum was replaced by another species (possibly a *Vibrio* species) in the effluent samples from the daily pulses. SEM analysis of rock core revealed substantial biofilm accumulation in rock pore channels. Application of a DNA stain SYTO 9® (Molecular Probes, Inc.) demonstrated that biofilm remained viable after a $scCO_2$ challenge. These observations are encouraging to the use of engineered biofilm barriers for controlling leakage of geologically sequestered CO_2.

Rationale

Engineered microbial biofilm barrier technology involves the injection and subsurface transport of starved bacterial cultures followed by resuscitation with injected growth substrates. This process results in the production of copious amounts of extracellular polymer (EPS), which plugs the free pore space of the aquifer thereby reducing porosity and hydraulic conductivity. This zone of reduced hydraulic conductivity forms a barrier which serves as a means for controlling migration of contaminants as wells as providing the potential for contaminant biotransformation. Biofilm barrier technology has been successfully demonstrated at the field scale (Cunningham et al., 2003)[2] thereby providing the incentive for undertaking a research and development effort aimed at examining the problems and opportunities associated with the application to geologic CO_2 sequestration.

Goals and Objectives

The ***GOAL*** of this project is *to address critical research questions related to developing engineered microbial processes for the geologic containment of supercritical CO_2*. The CBE work plan contains the following objectives:

CURRENT PROJECT OBJECTIVES

1) Identification of microorganisms which can serve as inoculum (pressure tolerant, mesophillic, salt tolerant, mucoid) for biofilm formation. Assessment of different combinations of inoculum, growth substrate, and electron acceptor to produce biofilms which effectively plug porous media.

2) Conduct biofilm plugging experiments in both packed columns and core rock columns to determine the individual response to challenges from high pressure, salinity, and supercritical CO_2. A high pressure, temperature controlled core testing system will be built for this purpose. We will also explore the use of Magnetic Resonance Imaging as a tool to image biofilm growth and its effect on flow through porous media.

3) CBE will develop methods to quantify the effects of biofilm growth on the multi-phase fluid properties of core rock samples. This will involve direct measurement of the capillary pressure-saturation curves and the relative permeability-saturation relationships for CO_2 and brine, with and without the presence of biofilm growth in the core samples. CBE will work with other ZERT partners who have experience in making these types of measurements. This information is important for calibrating computational simulation models for analyzing CO_2 injection schemes and performing risk assessments.

Experimental Approach

In accordance with objective #2, a high pressure system was built to measure in situ hydraulic conductivity of the core sections and biofilm. The system allows a differential pressure to be established between two high pressure accumulators causing media to flow through a core housed in the Hassler-type core holder. Differential pressure and flow rate measurements were used to calculate changes in hydraulic conductivity as the biofilm develops.

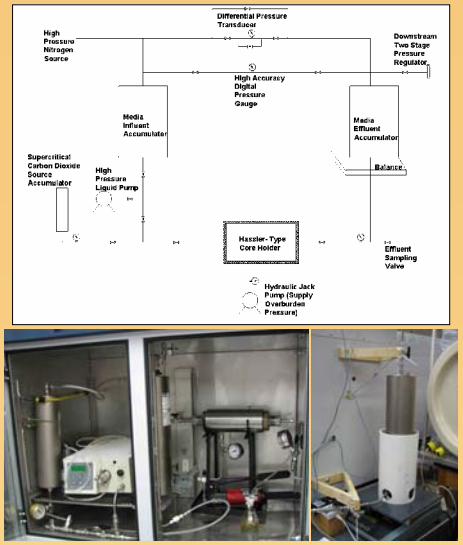


Figure 1. High Pressure Core and Biofilm Testing Apparatus

TOP - Generalized schematic showing the testing apparatus design.
BOTTOM LEFT - Influent accumulator for the storage of Modified BHI + Salt Media until pulses, Supercritical CO_2 accumulator (far left) for the production and storage of $scCO_2$ and the Hassler-type core holder with Berea sandstone core. All items are inside an incubator for temperature control.
BOTTOM RIGHT - Effluent accumulator, balance and sample port used to collect the effluent after each pulse.

Experimental Protocol

- **Incubator temperature: 32° C**
- **Overall System Pressure: > 1228 PSI (1356 Max)**
- **Nutrient: Modified BHI + Salt Media (per liter H_2O)**
  - **18.0 g Brain Heart Infusion Broth, 40.0 g NaCl, 3.0 g $NaNO_3$, 0.75 g NH_4Cl**
- **Organism Inoculated: *Shewanella frigidimarina* (oilfield isolate)**
- **Organism Incubation: ~22 hours of incubation before 1st pulse**
- **Hassler-type core holder with overburden pressure ~1500psi**
- **Berea sandstone core 5 cm long, 2.54 cm diameter, 40 md (Temco)**
- **Pulsed flow: ~one original pore volume per pulse**
- **Pulsed flow: 2X daily during the week, once daily on the weekend**
- **Pulsed flow differential pressure: ~40psi**
- **Pulsed flow: effluent collected for each pulse**
- **Supercritical CO_2 flood: ~12 hours flood @ 1320psi, 32.5° C**
- **Effluent sampling occurred after each pulse**
- **Volume of collected effluent measured after bubbles dissipated**
- **Effluent sample population assay (drop plate) (10^{-3} to 10^{-6})**
- **Plates counted after 3+ days of growth**

Results

1) PERMEABILITY REDUCTION DUE TO BIOFILM GROWTH
Biofilm growing in a 5 cm long, 2.54 cm diameter, 40 millidarcy rock core resulted in a two-order-of-magnitude reduction in permeability over the 500 hour experiment. Permeability did not increase significantly in response to the 12 hour supercritical CO_2 flood challenge.

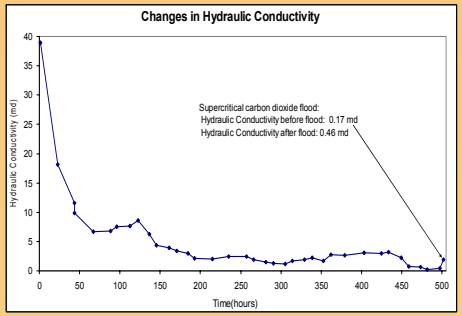


Figure 2. Changes in hydraulic conductivity (md). Hydraulic conductivity decreased 2 orders of magnitude over 500 hours of the experiment in Berea sandstone core due to biofilm growth (initial hydraulic conductivity 40 md).

2) BACTERIAL SUCCESSION DURING EXPERIMENT
Population assays were performed on each effluent sample and the colony types were observed over the course of the experiment. Bacterial succession was observed as the characteristic pinkish *S. frigidimarina* colony type is replaced by a mucoid white colony tentatively identified by API 20 NE® (BioMerieux, Inc.) test strips as *Vibrio fluvialis*. 16s rDNA phylogenetic analysis is pending.

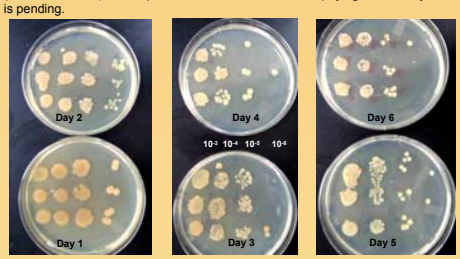


Figure 3. Drop plate method population assay plates (10^{-3} to 10^{-6}). The plates show the pinkish *S. frigidimarina* colony morphology and the white mucoid colony later identified as *V. fluvialis*. Media is modified BHI+Salt Agar.

The number of cells/ml from the collected effluent of both colony types were calculated from the drop plate method counts. *S. frigidimarina* was the initial organism inoculated but the dominant population shifted from *S. frigidimarina* to the mucoid white colony morphology (*V. fluvialis*) within the first 2 days.

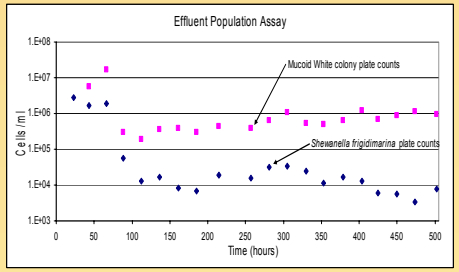
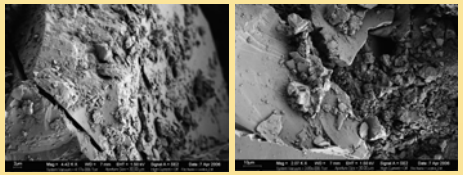


Figure 4. Effluent Population (cells/ml effluent collected)

3) BIOFILM ACCUMULATION IN CORE SECTIONS (SEM)
Destructive sampling occurred after 502 hours. The core was removed from the Hassler-type core holder. Thin sections of the core were cut and images were taken with the Field Emission SEM Zeiss® Supra 55VP (Montana State University Image and Chemical Analysis Lab). SEM analysis of rock core revealed substantial biofilm accumulation in rock pore channels. Application of a DNA stain Syto 9® (Molecular Probes, Inc.) demonstrated biofilm remained viable after $scCO_2$ challenge.



Figures 4 and 5. Scanning Electron Microscopy Images of Berea Sandstone Core Thin Sections - Control (no biofilm, no supercritical CO_2)

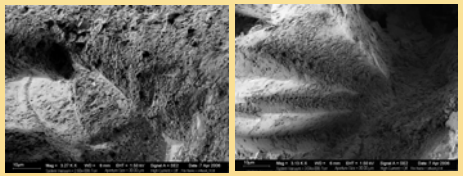


Figure 6 and 7. Scanning Electron Microscopy Images of Berea Sandstone Core Thin Sections after 500 hours of Biofilm Growth and Supercritical CO_2 (12 hour flood) challenge.

Future Work

RELATIVE PERMEABILITY OF CO_2 AND BRINE. CBE will develop methods to quantify the effects of biofilm growth on the multi-phase fluid properties of core rock samples. This will involve direct measurement of the capillary pressure-saturation curves and the relative permeability-saturation relationships for CO_2 and brine, with and without the presence of biofilm growth in the core samples. This information is important for calibrating computational simulation models for analyzing CO_2 injection schemes and performing risk assessments.

BIOMINERALIZATION. Various soil bacteria including *Bacillus pasteurii* have been shown to precipitate $CaCO_3$ thereby reducing the porosity and permeability of porous media. CBE will investigate incorporating this biomineralization process into the biofilm barrier concept in an attempt to provide a technology for permanently sealing cap rock and well bore leakage pathways. We will also explore the potential benefits associated with biofilm accumulation and biomineralization directly on the surface of well-bore concrete. It is currently hypothesized that, in the absence of buffering capacity, CO_2 may lower the pH near the well bore resulting in acidic attack on the surrounding concrete. We will examine the concept of using microbial biofilms containing calcite-precipitating species to form a "sacrificial" calcite layer on concrete which will buffer pH thereby reducing the susceptibility to acidic attack.

CO_2 CHALLENGES. CBE, in collaboration with Dr. John Kaszuba of Los Alamos National Laboratory, will develop methods to examine the survival of various microbial species when challenged by supercritical CO_2 under representative pressure and temperature conditions. We will begin by growing cultures of *Cellulomonas* sp. strain *ES6*, an organism adept at forming biofilms, and various *Clostridia*, which are spore forming species. These species will be investigated in both vegetative and starved condition as well as grown on crushed core-rock samples and subjected to supercritical CO_2 challenge. The challenge experiments will be done at Los Alamos using an existing supercritical fluid extractor system. Once developed, this method will provide a rapid tool for assessing microbial survival when subjected to supercritical CO_2.

Acknowledgements

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References

[1] Curt M. White; Brian R. Strazisar; Evan J. Granite; James S. Hoffman; Henry W. Pennline. Separation and Capture of CO2 from large stationary sources and sequestration in geological formations--coal beds and deep saline aquifers. *Journal of the Air & Waste Management Association*, June 2003 v53 i6 p645

[2] Cunningham, A.B, R. R. Sharp, R. Hiebert and G. James. Subsurface Biofilm Barriers for the Containment and Remediation of Contaminated Groundwater. *Bioremediation Journal.*7(3/4) 151-164, 2003.