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ABSTRACT

This research project addresses one of the goals of the U.S. Department of Energy (DOE) Carbon Storage Program (CSP) aimed at developing Advanced Wellbore Integrity Technologies to Ensure Permanent Geologic Carbon Storage. The technology field-tested in this research project is referred to as microbially induced calcite precipitation (MICP), which utilizes a biologically-based process to precipitate calcium carbonate. If properly controlled MICP can successfully seal fractures, high permeability zones, and compromised wellbore cement in the vicinity of wellbores and in nearby caprock, thereby improving the storage security of geologically-stored carbon dioxide. This report describes an MICP sealing field test performed on a 24.4 cm (9.625 inch) diameter well located on the Gorgas Steam Generation facility near Jasper, Alabama. The research was aimed at (1) developing methods for delivering MICP promoting fluids downhole using conventional oil field technologies and (2) assessing the ability of MICP to seal cement and formation fractures in the near wellbore region in a sandstone formation. Both objectives were accomplished successfully during a field test performed during the period April 1-11, 2014. The test resulted in complete biomineralization sealing of a horizontal fracture located 340.7 m (1118 feet) below ground surface. A total of 24 calcium injections and six microbial inoculation injections were required over a three day period in order to achieve complete sealing. The fractured region was considered completely sealed when it was no longer possible to inject fluids into the formation without exceeding the initial formation fracture pressure. The test was accomplished using conventional oil field technology including an 11.4 L (3.0 gallon) wireline dump bailer for injecting the biomineralization materials downhole. Metrics indicating successful MICP sealing included reduced injectivity during seal formation, reduction in pressure falloff, and demonstration of MICP by-products including calcium carbonate (CaCO3) in treated regions of side wall cores. This project successfully integrated mesoscale laboratory experiments at the Center for Biofilm Engineering (CBE) together with simulation modeling conducted at the University of Stuttgart to develop the protocol for conducting the biomineralization sealing test in the field well.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

A significant challenge for advancing subsurface carbon capture and storage (CCS) is developing ways to control unwanted upward migration of CO_2 which may occur through the confining layers (i.e. cap rock) or near wellbores in the receiving reservoir. This research project was conducted in response to National Energy Technology Laboratory Funding Opportunity Number DE-FOA-0000652 entitled Technologies to Ensure Permanent Geologic Carbon Storage, CFDA Number: 81.089 Fossil Energy Research and Development, *Area 2: Advanced Wellbore Integrity Technologies.* The research project investigates the potential of microbially induced calcium carbonate precipitation (MICP) as a technology for mitigating subsurface leakage potential in the near-wellbore environment through field testing at an existing 1498 M (4915-foot) deep, 24.4 cm (9.625 inch) diameter Alabama Power Company well located at the Gorgas Power plant near Jasper, Alabama. The goal of this project was to develop a biomineralization-based technology for sealing preferential flow pathways in the vicinity of wellbores. The project goal was accomplished by way of the following four objectives: (1) Characterize the Alabama well test site, (2) Design protocol for field test, (3) Perform field tests, and (4) Evaluate results of the field test.

The field test was carried out during the period April 1-11, 2014. The MICP sealing protocol test resulted in complete biomineralization sealing of a horizontal fracture located 340.7 m (1118 feet) below ground surface (bgs). The test was accomplished using conventional oil field technology including an 11.4 L (3.0 gallon) wireline dump bailer for injecting the biomineralization materials downhole. A total of 24 calcium injections and six microbial inoculation injections were required over a three day period in order to achieve complete sealing. The fractured region was considered completely sealed when it was no longer possible to inject fluids into the formation without exceeding the initial formation fracture pressure. Field plugging results compared favorably with model predictions (25 Ca, six inoculation injections) made using an MICP simulation model developed at the University of Stuttgart. Model predictions were made prior to the field test based on planned injection strategy. This project has successfully integrated mesoscale laboratory experiments at CBE together with simulation modeling conducted at the University of Stuttgart to develop the protocol for conducting the biomineralization sealing test in the field well.

At the termination of the experiment the fracture extension pressure (i.e. the pressure necessary to re-fracture the formation) was significantly higher 111.6 bar (1640 psi) compared to 96.6 bar (1420 psi) compared with pre-experiment. This suggests the MICP seal was stronger than the formation prior to treatment following the field test the effectiveness of the biomineralization seal was evaluated. Metrics indicating successful MICP sealing included reduced injectivity during seal formation, reduction in pressure falloff, and demonstration of MICP by products (CaCO3) in treated regions of side wall cores.

The inoculum microorganism, *Sporosarcina pasteurii,* was not present in formation water and sediment samples taken prior to inoculation and biomineralization at the Gorgas site. However *S. pasteurii* was detected in formation fluids three days later at the conclusion of the

biomineralization experiment. This outcome indicates the presence of an active culture of the organism responsible for ureolytic biomineralization as the field test progressed.

The development of biomineralization sealing technology to seal preferential flow pathways near wellbores provides the following benefits to CO_2 storage operations: (1) an alternative technology to cement for plugging preferential CO_2 leakage pathways in the vicinity of wellbores; and, (2) a low viscosity technology which can penetrate significant distances away from the well bore and reach small aperture pathways not easily reachable by traditional cement-based sealing technologies. This technology can be applied before and after closure to reduce risk of release of CO_2 around the well casing and cement. The technology developed in this project contributes to the DOE program's effort of ensuring 99% CO_2 storage performance in the injection zone(s) for 100 years or more.

Investigations are on-going (beyond the scope of this project report) which are focused on using MICP sealing to mitigate leakage pathways through compromised wellbore cement, thereby improving wellbore integrity. Also both enzymatic and thermal-based processes are being examined as alternatives for the microbially-based mineral precipitation process currently in use. Successful enzymatic and thermal-based mineral precipitation will result in more robust biomineralization technologies capable of being applied at much greater depths than are currently achievable.

This research was conducted at the Center for Biofilm Engineering (CBE) at Montana State University (MSU) in collaboration with MSU's Energy Research Institute (ERI) in Bozeman, Montana; Southern Company (SC) in Birmingham, Alabama; the University of Alabama at Birmingham (UAB) in Birmingham, Alabama; The University of Stuttgart in Stuttgart, Germany; Schlumberger (SLB) (formerly Schlumberger Carbon Services) in Houston, Texas; Shell Exploration and Production B.V. (Shell) in Rijswijk, The Netherlands.

INTRODUCTION AND REPORT ORGANIZATION

Project Concept

An advanced method for sealing wells (e.g. engineered biomineralization) has been evaluated in the field using an existing 1498 m (4915-foot) deep Alabama Power Company well located at the Gorgas Power plant near Jasper, Alabama. Access to the well was granted through a site access agreement obtained through Southern Company Services which authorized MSU and SLB to conduct field testing at the Gorgas well. The sealing method tested is based on microbial biofilm formation, together with precipitation of calcium carbonate minerals, to plug preferential flow paths outside the well. As referred to in the Executive Summary, this particular biomineralization technology is referred to hereafter as microbially induced calcite precipitation (MICP).

Biomineralization Overview

The basic principle of MICP sealing technology can be described as follows: Carbonate mineral formation can be induced by bacterial hydrolysis of urea (also known as ureolysis). Ureolysis can occur under dark subsurface conditions and results in the production of ammonium (NH_4^+), an increase in pH (OH^--ion production), an increase in alkalinity (Eqs. 1-5), and ultimately oversaturation of the aqueous phase with respect to carbonate minerals, such as $CaCO_3$ (Eq. 6, Ferris et al., 1996; Ferris et al., 2003; Mitchell and Ferris, 2005, Mitchell et al., 2010). Carbonate mineral formation can be controlled by altering the concentration and activity of the microorganism, the supply of Ca^{2+}, the pH, carbonate speciation, carbon source, and urea availability. Urease (the enzyme responsible for urea hydrolysis) is common in a wide variety of microorganisms, including in subsurface groundwater communities, (Swensen and Bakken, 1998) and can be readily induced by adding inexpensive urea. Microorganisms are thought to act as nucleation points for mineral formation, but the mineral formation may also lead to further reduced ureolysis and carbonate precipitation due to diffusion limitation or cell inactivation (Stocks-Fisher, 1999; DeMuynck, 2010).

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad \text{(Eq. 1)}$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad \text{(Eq. 2)}$$

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \quad \text{(Eq. 3)}$$

$$H_2CO_3 + OH^- \leftrightarrow HCO_3^- + H_2O \quad \text{(Eq. 4)}$$

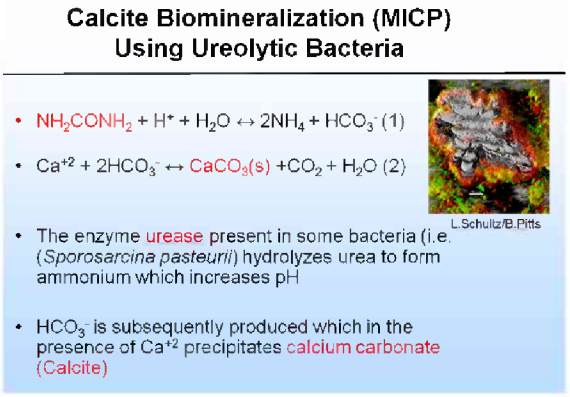
$$HCO_3^- + OH^- \leftrightarrow CO_3^{2-} + H_2O \quad \text{(Eq. 5)}$$

$$CO_3^{2-} + Ca^{2+} \leftrightarrow CaCO_3 \ (K_{SO} = 3.8 \times 10^{-9}) \quad \text{(Eq. 6)}$$

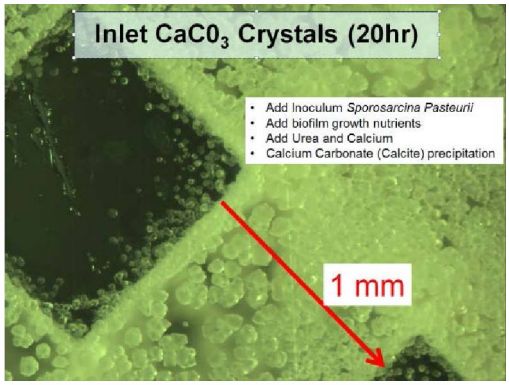
Previous MICP research

Mineral precipitation purposely induced by microbial activity in the subsurface (particularly through the urea-hydrolysis pathway) can be exploited for a variety of applications including the immobilization of calcium and contaminants (Fujita et al., 2008; Curti, 1999; Hammes et al., 2003; Mitchell and Ferris, 2006), mineral plugging of underground formations (Ferris and

Stehmeier, 1992; Ferris et al., 1996), ground reinforcement (Harkes et al., 2010; Whiffin et al., 2007; van Paassen et al., 2010), the improvement of construction materials and the creation reactive barriers in the subsurface (Cunningham et al., 2009). This project focuses on the use of MICP to deposit calcite in preferential flow paths in porous media and in the near-well bore environment to mitigate leakage potential. The primary advantage of the MICP technology is that aqueous solutions with low viscosity are used to promote precipitation that can reduce permeability and seal unwanted flow paths in the near wellbore environment. Success of the MICP treatment depends on the manipulation of the microbial activity, the reaction and transport of the substrates, and the saturation conditions of the minerals. The MICP process is summarized below in Figure 1.



(a)



(b)

Figure 1. *a) Stoichiometry governing urea hydrolysis by the urease enzyme (in this study the species Sporosarcina pasteurii was used as the microbial catalyst along with the subsequent precipitation of calcium carbonate (calcite). (b)Photograph of calcite deposition in a 1 mm (0.039 inches) flow channel in a flat-plate laboratory reactor. The steps involved in calcite precipitation include 1) add inoculum (Sporosarcina pasteurii) to reactor under flowing conditions, 2) add biofilm growth nutrients to form biofilm colonies within the reactor, 3) add urea and calcium resulting in calcium carbonate precipitation. These steps comprise the MICP sealing process.*

Bench and mesoscale MICP research has been carried out at CBE/MSU under a previous DOE-funded project entitled "*Advanced CO_2 Leakage Mitigation using Engineered Biomineralization Sealing Technologies"* (DOE Award #: DE-FE0004478). Progress under this project advanced the MICP technology to the point where a field pilot test was feasible.

Project Collaborators

Collaborators on this project include MSU, Southern Company (SC), the University of Alabama at Birmingham (UAB), Schlumberger (SLB), Shell International Exploration and Production B.V. (Shell), and the University of Stuttgart (Stuttgart). Drs Spangler, Cunningham, Phillips, Gerlach (CBE/MSU) have managed the project, designed the field test protocol, oversaw testing and analyzed results. Dr Rainer Helmig (Stuttgart) supervised simulation modeling in

collaboration with CBE researchers. Dr. Richard Esposito (SC) conducted geologic site characterization and obtained rock core samples from the field for laboratory analysis. Dr. Esposito also helped coordinate field operations with Schlumberger. Dr. Peter Walsh (UAB) conducted multiple core tests on field and laboratory sandstone rock core samples. Dr. Claus Otto, Joe Westrich, and Bart Lomans (Shell) assisted in designing the field test and analyzing results. All collaborators actively participated in decision making and evaluation for each stage of the project. This project integrated expertise from practitioners (SC, SLB, and Shell) with experimental research (MSU/CBE) and simulation modeling (Stuttgart) to achieve a successful project and a thorough evaluation of the field injection protocol, field delivery system, and effectiveness of the biomineralization sealing process.

Report Organization

The goal of this project was to develop a biomineralization-based technology for sealing preferential flow pathways in the vicinity of injection wells. The project goal has been accomplished with the following four objectives:

Objective 1 Characterize the Alabama well test site
Objective 2 Design protocol for field test
Objective 3 Perform field test
Objective 4 Evaluate results of the field test

This final report is organized by addressing EXPERIMENTAL METHODS, RESULTS AND DISCUSSION, as requested. Contents of subheadings under each major heading address each of the four project objectives. A comprehensive CONCLUSION section follows.

EXPERIMENTAL METHODS

Field site characterization

Well information. In April 2014, collaborators from Southern Company, Schlumberger, Shell and Montana State University mobilized to the William Crawford Gorgas Power Plant (Alabama Power, Southern Company) in Walker County, Alabama (hereafter referred to as the plant) to initiate an ureolysis-driven MICP field experiment. The Gorgas # 1 well, located at the plant site had been a stratigraphic test well drilled in the fall of 2011. The drilling effort was performed as part of the DOE's efforts to characterize the geologic formations to look for opportunities to safely sequester carbon dioxide in deep saline reservoirs. The Gorgas #1 well, drilled to 1498.1 m (4915 feet) below top of the 24.4 cm (9 5/8 inch) casing, resides at 114.6 m (376.1 feet) above sea level (Permanent Datum) in Walker County Alabama, as shown in Figure 2.

Based on a review of the petrophysical ELAN (EUDICO Linguistic Annotator) analysis (well log) prepared by Schlumberger, multiple layers of shale, sandstone and coals were assessed as possible field test formation candidates. The Fayette sandstone group at a depth of 338.3 to 341.4

m (1110 to 1120 feet) was determined to be the best candidate for performing the field test (Figure 2). The Fayette sand in this zone was a medium to fine-grained sandstone with a porosity of approximately 10% and a permeability of approximately 3 millidarcies (mD). The cement bond log (not shown) indicated good cement across the zone so good hydraulic isolation was expected. Prior to the actual biomineralization sealing test a bridge plug was installed in the 24.4 cm (9.625 inch) well at elevation 343.5 m (1127 feet) bgs. This plug established the lower boundary of the injection zone for injection of test fluids.

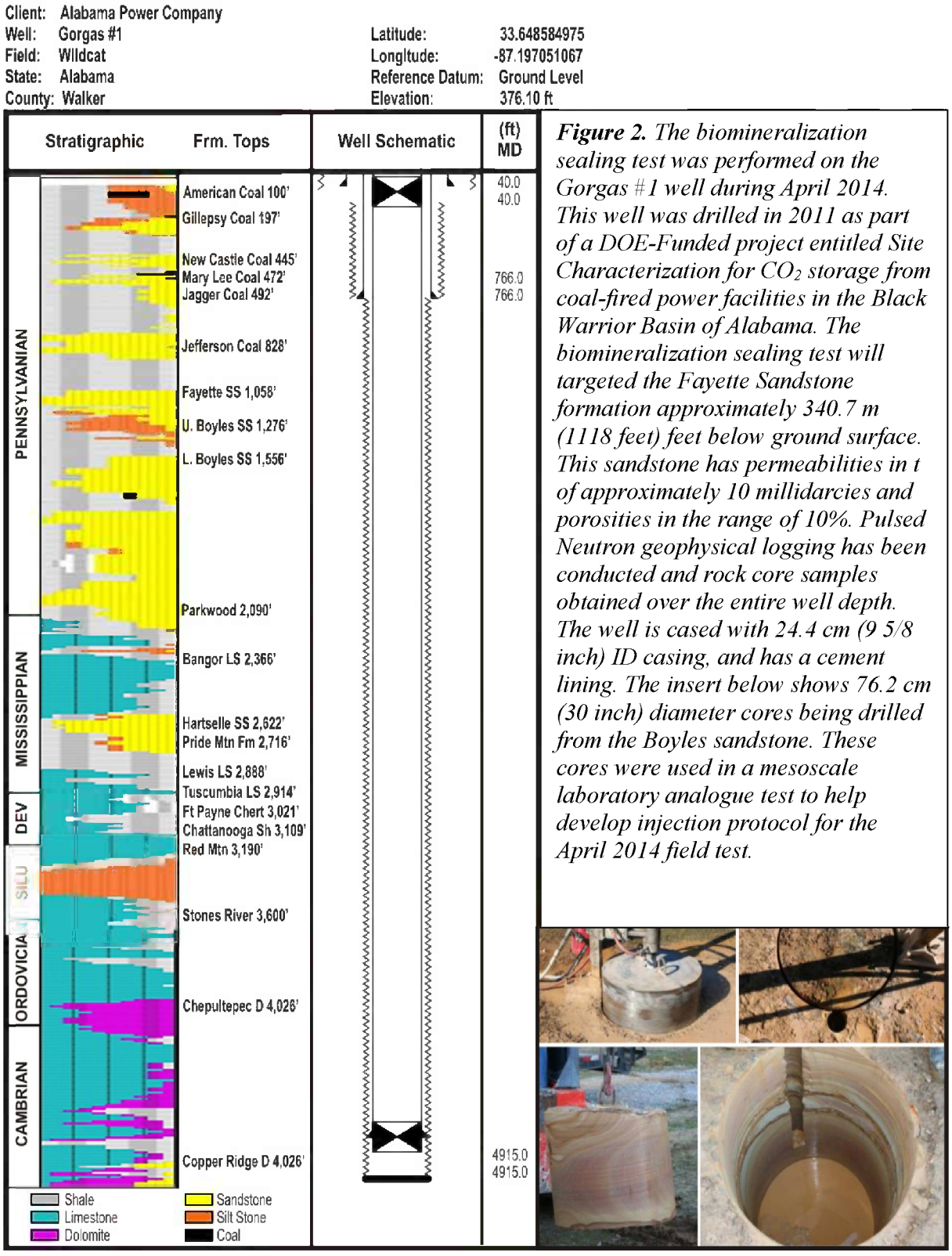


Figure 2. *The biomineralization sealing test was performed on the Gorgas #1 well during April 2014. This well was drilled in 2011 as part of a DOE-Funded project entitled Site Characterization for CO_2 storage from coal-fired power facilities in the Black Warrior Basin of Alabama. The biomineralization sealing test will targeted the Fayette Sandstone formation approximately 340.7 m (1118 feet) feet below ground surface. This sandstone has permeabilities in t of approximately 10 millidarcies and porosities in the range of 10%. Pulsed Neutron geophysical logging has been conducted and rock core samples obtained over the entire well depth. The well is cased with 24.4 cm (9 5/8 inch) ID casing, and has a cement lining. The insert below shows 76.2 cm (30 inch) diameter cores being drilled from the Boyles sandstone. These cores were used in a mesoscale laboratory analogue test to help develop injection protocol for the April 2014 field test.*

Mobilization and injection zone perforation. On Monday, March 31, 2014, SLB moved on-site and began equipment mobilization. SLB began by running 7.30 cm (2.875 inch) tubing (referred to here after as "tubing") with the packer attached to tubing but not engaged at this point. The well was cased completely to the bottom, so the target test region was perforated in order to access the formation. Perforation of the casing and the formation occurred on Tuesday, April 1, 2014, with six shots at 60 degree phasing within the zone from 340.7 to 341.1 m (1118-1119 feet) below ground surface. The perforations were estimated to have a 0.0089 m (0.35 inch entry hole) and extend approximately 0.51m (20 inches) into the formation.

Injection zone detail. The details of the zone of injection are shown below in Figure 3.

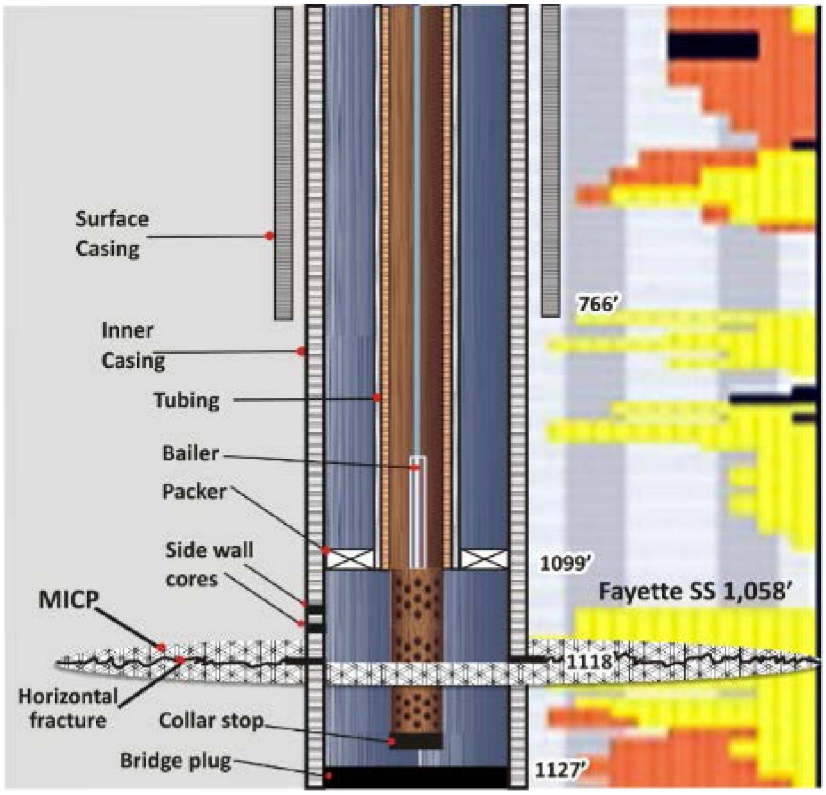


Figure 3. *Configuration of subsurface injection system. A bailer on a wireline was used to deliver concentrated MICP promoting solutions to the region of the fracture that was formed at 340.7m (1118 feet) bgs in the Fayette sandstone formation. An existing bridge plug was set at 343.5m (1127 feet) bgs and the packer with 7.3 cm (2.875 inch) tubing string was set at 335m (1099 feet) bgs. The tubing string ran to surface and was used to deliver brine which served to push and dilute concentrated bailer contents into the fracture. MICP is shown in black and white hatch pattern in the horizontal fracture.*

Injection System. The tubing string was equipped with downhole pressure memory gauges (Omega Engineering, Connecticut, USA) attached to the outside of the pipe below the packer. A collar stop was placed between two 1.2 m (4 foot) perforated pipes (pup joints) on which to land the bailer. The tubing was set in the well with the end of the tubing at 341.7 m (1121 feet). Black Warrior River water was trucked from the plant to two 1893 L (550 gallon) holding tanks where it was amended with NaCl (Mix-N-Fine) to 2.4% (hereafter referred to as the brine). The flow rate from the Cat Model 310 (Cat Pumps, Minneapolis, MN) injection pump powered by a 5 HP 230V motor with a variable speed drive was monitored by a Hoffer flow meter (Hoffer Inc., North Carolina, USA) with an Omega (Omega Engineering Inc., Connecticut, USA) pressure

data logger to record surface pressure. The injection pump was connected to the tubing to pump brine into the subsurface.

Sump amendment. The 3.05 m (10 foot) zone between the bridge plug (located at 343.5 m (1127 feet bgs) and the perforation zone in the 24.4 cm (9.625 inch) well (referred to herein as the "rat hole") was filled with sodium chloride-river water solution of approximately 1.1 specific gravity (see Appendix A, Supporting Information, for details). By filling the rat hole with fluid of higher density than the other injection fluids, injection fluids were encouraged to flow into the formation instead of being diluted in the rat hole. Once the high density fluid had been placed in the rat hole, the packer was engaged and set at elevation 334.98 m (1099 feet) bgs to isolate the Fayette formation.

Formation Breakdown and target injection flow rate. To create injectivity into the formation, the formation was stimulated by increasing the brine injection flow rate until formation breakdown (fracturing) occurred. The break-down flow rate was found to be 13.3 L/min (3.5 gpm) and the corresponding downhole pressure was 105.9 atmospheres (atm) (1557 psi). Immediately after fracturing the formation, the injection flow was stopped in order to avoid further fracture propagation. In order to determine a "safe" injection flow rate for the biomineralization sealing test, that is an injection flow rate which would avoid any further fracture propagation, a second pressure-flow injection test was performed by pumping brine at 1.9 L/min (0.5 gpm) for six hours. This test resulted in a steady downhole pressure of 68 atm (1000 psi), which indicated that the 1.9 L/min flow rate was entering the formation without extending the fracture. The target flow rate for the biomineralization sealing test was set at 1.9 L/min (0.5 gpm) as a result. These results were obtained on Wednesday April 1, 2014.

Pressure fall off test. On Thursday April 3, 2014, after the six hour injection test, the well was shut in for an 88 hour pressure fall off test. This test was completed Sunday, April 6, 2014. Results from this test were used by SLB to obtain additional formation properties.

Field test protocol

Pre-injection sampling. After the 88 hour pressure fall-off test, injectivity was re-established to confirm the pressure flow relationship of 1.9L/min and 68 atm downhole pressure. Fluids in the well casing from the region of the fracture were sampled using the Wireline Kuster Sampling tool (Schlumberger, Florida, USA). The Kuster sampler was set on a timer to open after delivery at the region of the fracture in the mixing zone and close after 20 minutes. When closed, the tool was retrieved and the sample collected in an autoclaved bottle (Nalgene, Thermo Fisher Scientific, New York, USA). Additional samples from the region of the fracture were collected on the morning of the second day and the evening of the third day. The sample collected on the evening of the third day was done after injection of the inoculum and a pull-back of fluids from the fracture into the mixing zone of the packered off well casing. Portions of the sample were used to assess the microbial activity, concentration of urea, calcium and pH. Concentration of urea was assessed by modified method of the Jung Assay (Jung et al., 1075, Phillips et al., 2013b). The pH was assessed with a two-point calibrated meter (Accumet AP71, Thermo Fisher Scientific, New York, USA). Calcium concentration was assessed via IC as previously described

after the sample was filtered, placed on ice, and shipped back to MSU (Phillips et al. 2013a, Ebigbo et al., 2012). (Additional details found in Appendix A, Tables A1, A2).

Delivery of MICP components: field test design. The MICP field test occurred over four days (April 7-11, 2014) using a pulsed injection strategy with delivery of concentrated solutions via slickline dump bailer**.** There were two types of brine fluid mixtures delivered with the bailer: (1) inoculum (culture prepared as described in the supporting information) amended with 24 g/L urea fertilizer (Potash Corporation, Illinois, USA) and 3 g/L Nutrient Broth (Research Products International, Illinois, USA), and (2) calcium containing growth solution (90.5 g/L $CaCl_2$ Oxy Chem Ice Melt (Michigan, USA), 23.3 g/L NH_4Cl BASF (New Jersey, USA), 56 g/L Urea, and 7.0 g/L Becton Dickinson Nutrient Broth (New Jersey, USA) mixed with brine. These concentrations were chosen such that the dilution with brine would yield substrate concentrations in the fracture supportive of MICP conditions. The inoculum and calcium containing growth solution were injected separately with brine rinse in between in order to avoid mixing and unwanted MICP reaction within the 0.0073 m diameter (2 7/8 inch) tubing or mixing zone in the packer region.

Microbial Inoculum Preparation. The microbial inoculum *Sporosarcina pasteurii,* ATCC 11859, formally *Bacillus pasteurii,* (Yoon et al., 2001) was prepared for injection as detailed in Appendix A (Table A3, A4).

Bailer Delivery and Injection Strategy. The use of the 9.1m (30 feet) tall 0.051m (2 inch) diameter 11.4L (3.0 gallon) slickline dump bailer delivery technique was the most economical conventional oil and gas field methodology to deliver the substrates to the subsurface. Substrates were mixed and then pumped to the top of the bailer to fill. During inoculation stages, the 24 hour microbial inoculum was amended with 24 g/L urea fertilizer and 3 g/L nutrient broth into the mixing tank. After filling, the bailer was lowered into the well into the region of the fracture where it opened to release the inoculum. The inoculum was pushed into the fracture and formation by pumping brine through the tubing string. The injection of the brine also served to dilute the growth solution (and subsequently also the microbial suspension) to the desired concentration to promote ureolysis in the fracture. A one hour attachment period was allowed prior to three bailer deliveries of concentrated calcium growth solutions each followed by brine dilution. Prior to an overnight shut in period, a second microbial inoculum was delivered on day one. Pressure and flow rate during injection and pressure falloff in between flowing periods were monitored and recorded by the surface and subsurface pressure gauges. At the end of each day the bailer was rinsed with brine, the well was shut in overnight, and the site secured. Injection occurred for four days with the pulsed bailer delivery injection strategy (Table A5). Pictures of the overall field deployment system are shown below in Figure 4.

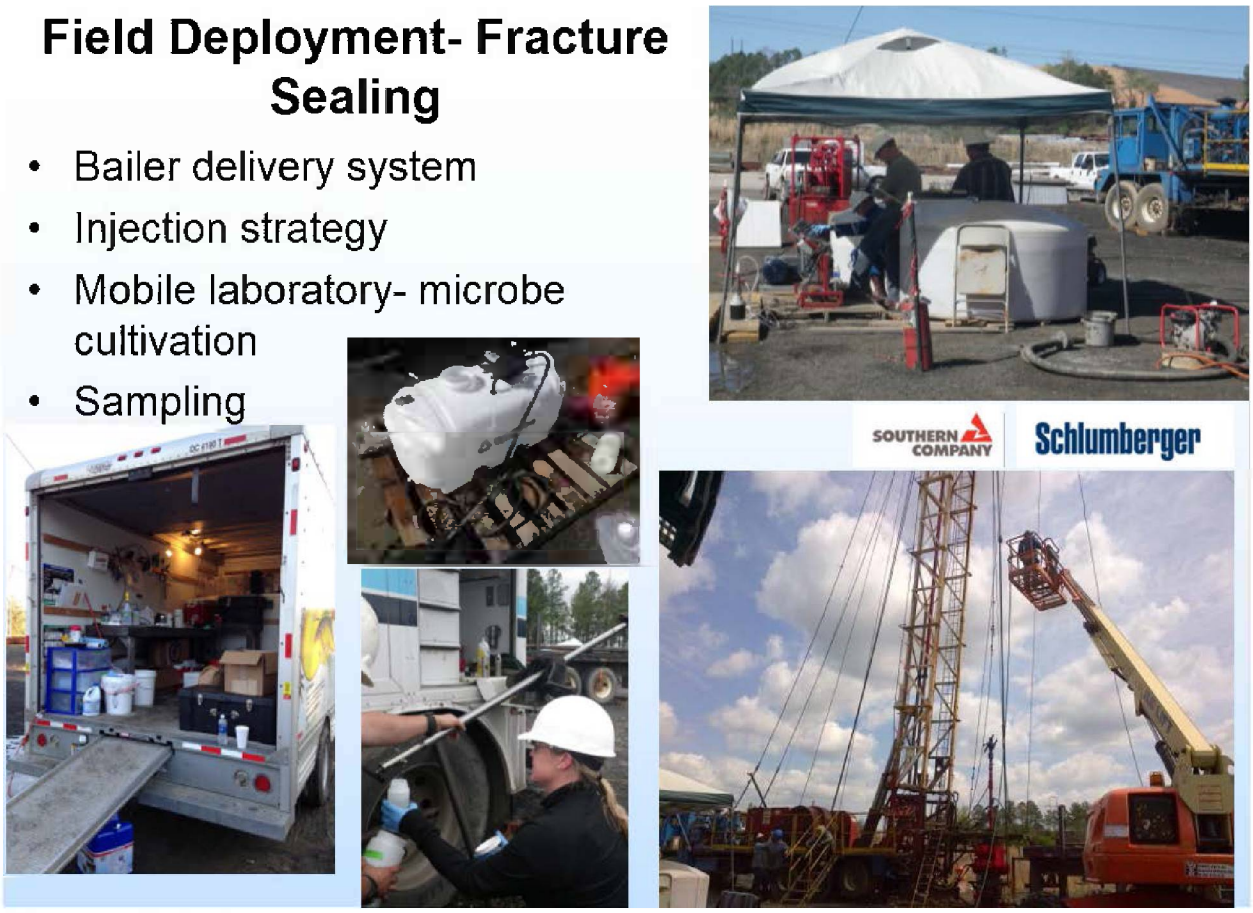


Figure 4. *Field deployment system used to conduct the April 2014 MICP sealing test at the Gorgas well.*

Formation threshold Pressure and Flow Rate. Determination to terminate the experiment was based on the inability to inject fluids through the tubing without exceeding the formation threshold pressure (TP) which was set at 81.6 atm (1200 psi, down hole pressure) to remain below the fracture extension pressure. As formation pressure increased, the injection flow rate was adjusted (reduced) from the original 1.9 L/min (0.5 gpm) in order to prevent formation threshold pressure from being exceeded. Pressure decay was monitored over the course of five minutes after the pumping was terminated (well shut in) during no flow periods after injection. At the end of the experiment, a step rate test was performed where pumping pressures were raised until fracture breakdown occurred.

Microbial analysis. Analysis of microbial samples obtained before, during, and after the biomineralization sealing test were analyzed using the following protocol. Unfiltered samples from each mixing zone sample measuring at 1 ml (0.034 ounces) were plated onto Brain Heart Infusion (BHI) + Urea agar 37g/L BHI, (Becton Dickinson, New Jersey, USA), 20 g/L Urea, (Fisher Scientific, New Jersey, USA), and 15 g/L Difco Agar (Becton Dickinson, New Jersey, USA) to assess culturability of microorganisms. Unique colonies, which grew up on the BHI+Urea agar plates, were streaked for isolation on fresh agar plates after which they were

inoculated into autoclaved BHI+urea liquid medium and cultured at room temperature. Identification of the isolates via 16s RNA analysis was also performed. The remaining 350 ml (11.83 ounces) of the samples were filtered through a 0.2µm bottle top filter (Thermo Fisher Scientific, New York, USA). The filter portion out of the plastic case was aseptically cut from the filter top and transferred to a sterile 50 ml (1.69 ounces) centrifuge tube which was placed on dry ice and shipped to MSU. The filtered samples were received at MSU with ice intact and placed into a -80 °C freezer for storage until microbial community analysis commenced with methods detailed in Appendix A.

Model predictions for MICP field test. Predictions for the April 2014 MICP field test were made using the Stuttgart MICP model. These model predictions were made in March 2014 using the planned injection scheme discussed above and in Appendix A along with formation permeability and fracture data representative of the Gorgas site. The model predicted that 25 Calcium and six inoculation injections would be required to form a substantial MICP seal in the fracture near the well bore as well as in the surrounding formation. These predictions were used to guide decision making during the April 2014 field test.

Post-field test analysis

Post- experiment side wall coring. In May 2015, Schlumberger retrieved samples from the region of the fracture by drilling side wall cores at 337.7 m and 338.9 m (1108 and 1112 feet) below ground surface. A cement plug and piece of the casing was retrieved from the 338.9 m location and the cement portion was scanned at 100kV and no filter with X-ray Micro Computed Tomography (Micro-CT) (Sky Scan 1173, Bruker USA, Wisconsin). The cement core was also analyzed with a Leica M205FA stereomicroscope using reflected white light and for fluorescence, a DAPI cube (ex 350/50, em 460/50) (Leica Microsystems, Illinois). Mud type material was retrieved from the 337.7 m (1108 foot) location which in the laboratory was dried in a sterile petri dish on the benchtop prior to the collected particles analyzed by X-Ray Powder Diffraction Spectrometer (XRD) (Scintag X-GEN 4000 XRD) at Montana State University's Image and Chemical Analysis Laboratory.

Microbial transport through sandstone cores. This activity was motivated by discussions with Shell following the April 2014 Gorgas field test in which questions arose regarding the degree of microbial penetration into the sandstone likely achieved during biomineralization sealing. A permeability of around 10 mD was determined for the Gorgas formation in the vicinity of the fracture targeted for sealing. The question to be addressed was: is it possible that the injected microbial inoculum (*Sporosarcina pasteurii*) could have penetrated a significant distance into the sandstone formation surrounding the Gorgas well? (This question has been answered affirmatively as discussed below in the Results and Discussion section).

A laboratory experimental system was developed to study the transport of ureolytic bacteria (*Sporosarcina pasteurii*) through rock cores of similar permeability to the target formation we biomineralized at Gorgas to address this question.

The aim of this laboratory effort was to investigate the lower permeability limit for the transport of bacterial cells through the pore spaces of intact sandstone cores. To this end, 2.54 cm (1 inch)

diameter sandstone cores representing a range of permeability values were tested in a core system. The pore size distribution of a porous medium can typically be positively correlated with measured permeability. Thus, we hypothesized that the injection efficiency of bacterial cells into the cores would decrease with decreasing permeability due to the inability of cells to pass through smaller pore throats. In addition to investigating cell transport through cores with different permeability values and pore throat sizes, the viability and activity of transported cells was also investigated.

A stand-alone report was prepared for this activity and appears in Appendix B, Microbial Transport through Rock Cores Report.

RESULTS AND DISCUSSION

Summary of April 2014 field test

The field test at the Gorgas well in Alabama, which was performed during the period April 1-11, 2014, resulted in complete biomineralization sealing of a horizontal fracture located 340.7 m (1118 feet) below ground surface, as shown in Figure 5. A total of 24 calcium injections and six inoculation injections were required over a three day period in order to achieve complete biomineralization sealing. The fractured region was considered completely sealed when it was no longer possible to inject fluids into the formation without exceeding the initial formation fracture pressure of 740 psi, measured at the ground surface.

Comparison with MICP model predictions

As previously discussed *a priori* predictions for the April 2014 MICP field test were made using the Stuttgart MICP model. These model predictions were made in March 2014 using the planned injection scheme discussed above and in Appendix A along with formation permeability and fracture data representative of the Gorgas site. Field plugging results (24 calcium and six inoculation pulses) compared favorably with model predictions (25 calcium and six inoculation injections) made using the Stuttgart MICP model. The similarity of model predictions with actual field experiment observations provides validation that this model provides a valuable tool for future MICP field projects.

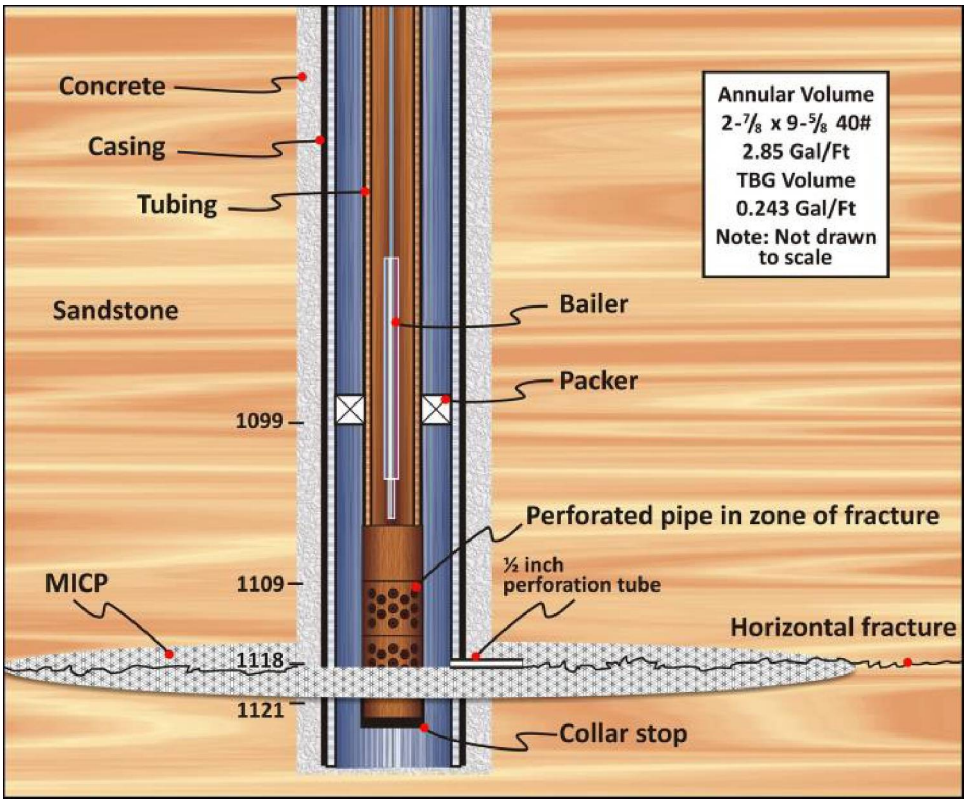


Figure 5. *Graphic showing the configuration of the down-hole tubing and fluid injection components. The field protocol involved directly injecting biomineralization fluids including microbial inoculum, growth nutrient, urea, and calcium. An 11.4 L (3.0 gallon) capacity injection (dump) bailer was used to transport these fluids to the point of application where they were released directly into the region surrounding the horizontal fracture.*

Injection protocol

Conventional oil field technology was used to successfully deliver MICP promoting materials to the subsurface fracture region. A pulsed strategy (Table A5) was used to inject microbes and separately inject calcium growth solutions with the bailer followed by a brine rinse to push microbes or substrates into the formation. This strategy was deployed to minimize instantaneous precipitation which was hypothesized to reduce near injection point plugging. Assessment of the microbial community in the water sampled from the mixing zone in the region of the fracture revealed *S. pasteurii* was recovered and made up the predominant species after MICP treatment.

Analysis of MICP fracture seal

The MICP treatment altered the flow characteristics into the formation. After MICP treatment, it was observed that there was reduced injectivity, a reduction in pressure decay after pumping ceased, and an increase in the fracture extension pressure.

Reduced Injectivity

Over the course of four days, 24 calcium pulses and six microbial inoculations were delivered into the re-perforated zone. By calcium pulse #22, fluids could not be pumped at the original flow rate without exceeding the threshold pressure (81.6 atm, 1200 psi) (Figure 6).

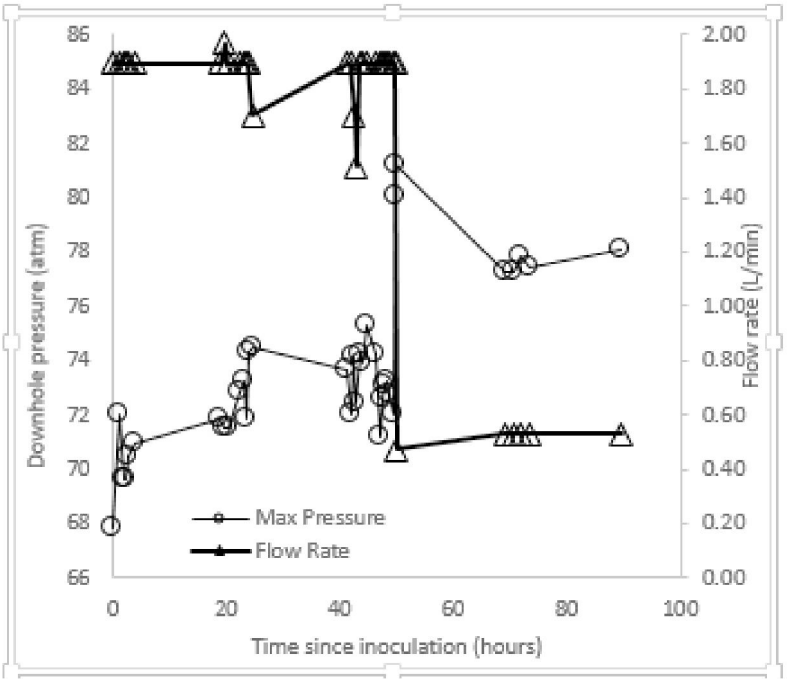


Figure 6. *The flow rate (Δ) was decreased over time to remain below a threshold pressure (○) of 1200 psi to avoid re-fracturing during the treatment period. Pressure increased and the flow rate was decreased over the course of the experiment.*

The initial flow rate was 1.9 L/min (0.5 gpm) which was decreased to as low as 0.47 L/min (0.125 gpm) to remain below the TP as MICP treatment progressed. The original pressure increased from approximately 1020 to as high as 81.6 atm (1200 psi) over the course of the treatment. The reduction of injectivity was attributed to the MICP filling void spaces and blocking flow pathways of the fracture as the mineral precipitation occurred over time.

Pressure Falloff

For injections wells, Title 40 of the Code of Federal Regulations Section 146.8 defines mechanical integrity (MI) as there having no significant leak in the casing, tubing, or packer (Koplos et al., 2006). One method to measure that leakage is an annulus pressure test. In this experiment, the well was pressured with liquid during every injection and pressure decay was monitored to assess the efficacy of MICP treatment toward improving mechanical integrity of the well. The pressure decay continued to improve (less pressure decay over time) over the

course of the experiment and ended with a promising 7% pressure falloff in five minutes down from greater than 30% over five minutes prior to biomineralization treatment (Figure 7). The positive result observed in the reduction of pressure decay suggested that wellbore integrity might be improved by treatment with MICP.

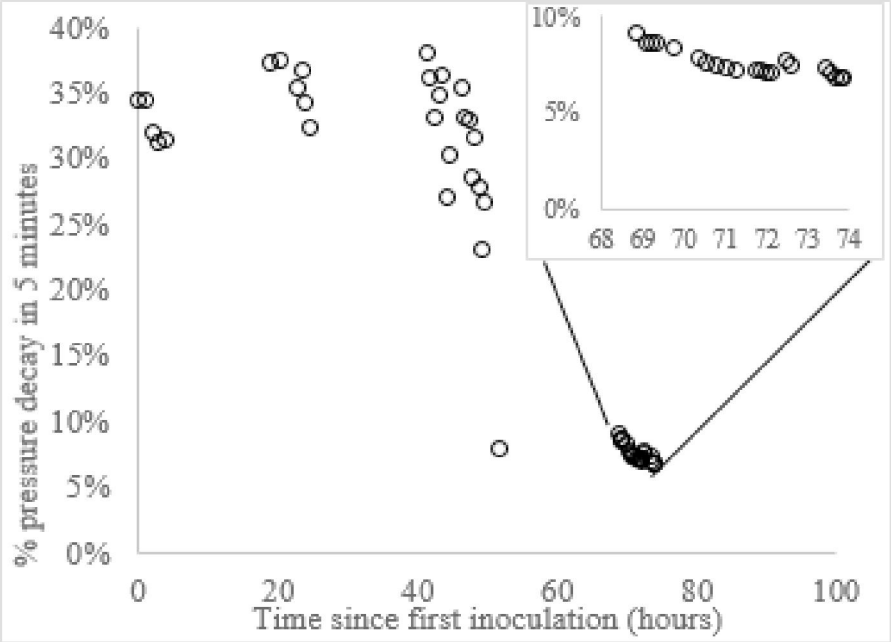


Figure 7. *The pressure decay was monitored for five minutes after pumping ceased and was observed to decrease over the course of the experiment from greater than 30% to 7%.*

Fracture Extension Pressure. At the termination of the experiment, the formation was again broken down to assess the post experiment fracturing pressure. While re-fracturing, it was determined that the fracture extension pressure downhole was 111.6 atm (1640 psi) compared to 96.6 atm (1420 psi) pre-experiment (Figure 8). This suggests the MICP seal was stronger than prior to treatment as was also observed in prior laboratory studies (Phillips et al. 2013a, Phillips et al., 2015). While the fracture extension pressure was greater than the original fracturing pressure prior to biomineralization treatment, it cannot be determined whether the original fracture re-opened or the formation fractured in a new location. However, because the fracture extension pressure was significantly higher in the post experiment formation, it was speculated that a new fracture was likely opened. The increase of fracture extension pressure indicated that the pressure required to flow fluid through the fracture and the pressure required to create new fracture had both increased with biomineralization treatment (Fjar et al., 2008). Additionally, after re-fracturing a downhole pressure of 109.3 bar (1607 psi) at 12.5 L/min (3.3 gpm) was necessary to inject brine after biomineralization treatment compared to 95.9 bar (1410 psi) at the same flow rate before treatment. The increased pressure needed after re-fracturing indicated reduced injectivity into the formation.

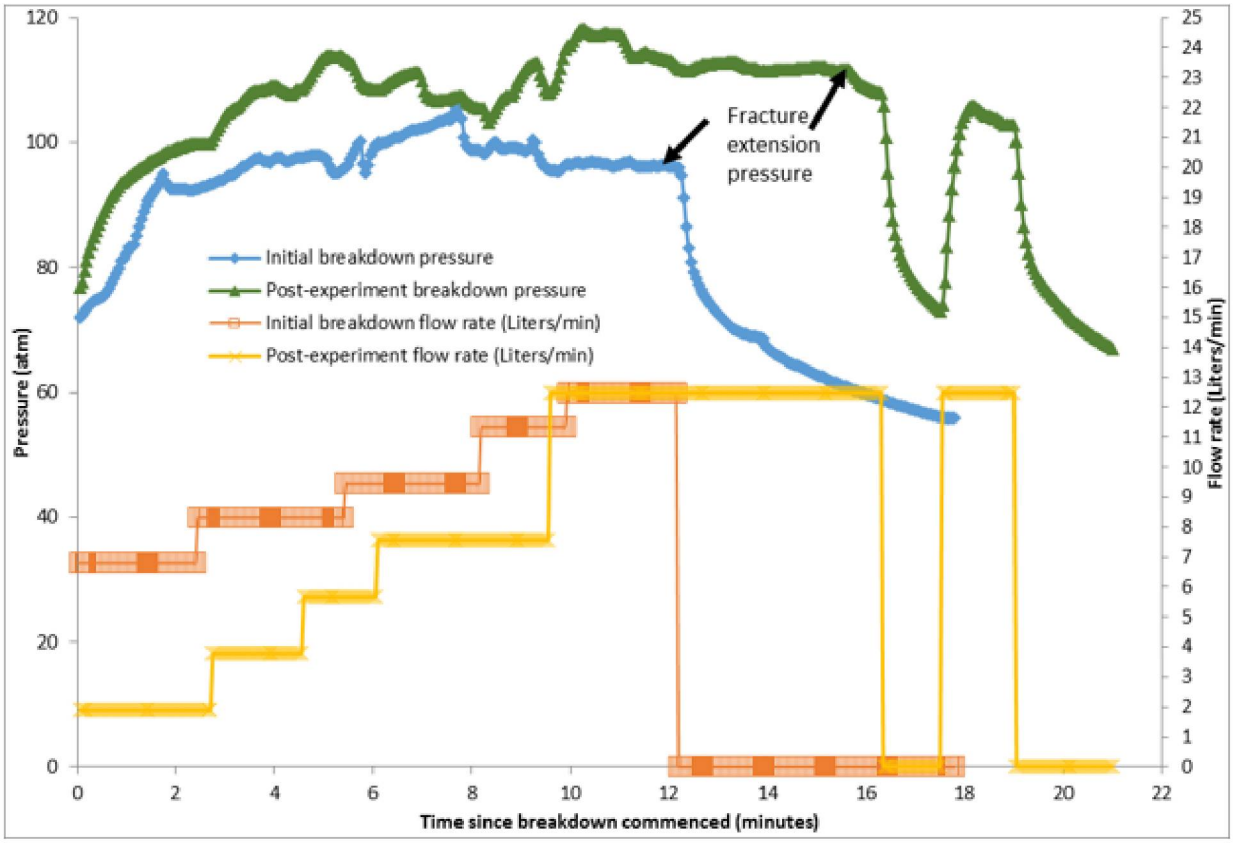


Figure 8. *The formation was stimulated before and after the experiment to assess fracture extension pressure after MICP treatment.*

Microbial Analysis. *S. pasteurii* was not cultured or detected from the sample collected prior to the experiment. The injected organism (*S. pasteurii*) was isolated and subsequently identified from the third day sample (Figure A2, Table A6). The community profile analysis confirmed the presence of *Sporosarcina* in the sample collected from the third day (Figure 9). Before inoculation, the predominant genus of bacteria in the mixing zone sample was a *Pseudomonas* species. After the experiment had progressed three days, *Sporosarcina* was the predominant genus detected. The importance of these observations was that *S. pasteurii* was successfully cultured under aseptic conditions in large volume carboys, injected into the subsurface with conventional oil field bailer delivery technologies, and was detected as the most abundant species in the mixing zone sample on the third day.

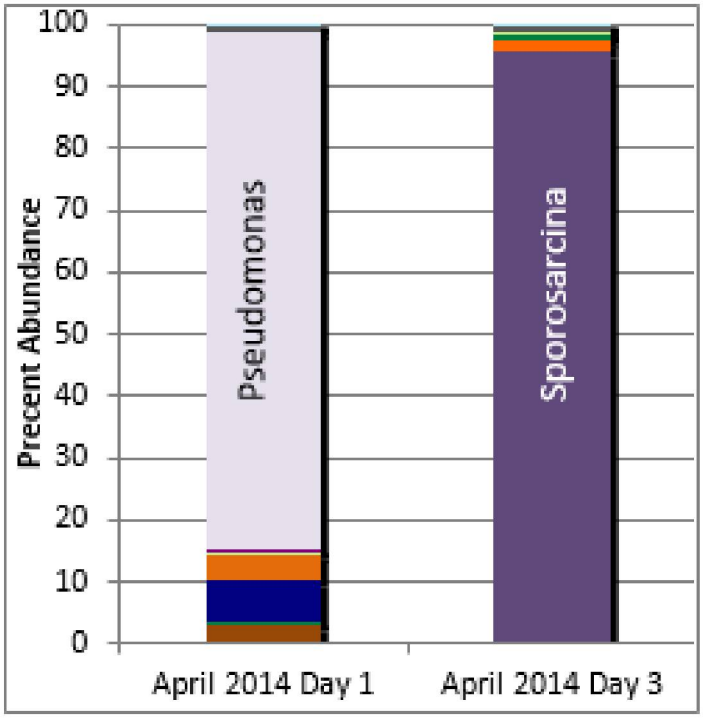


Figure 9. *The microbial community profile shifted after injection of S. pasteurii into the subsurface from a predominant Pseudomonas before injection to Sporosarcina after MICP treatment.*

Post Experiment Analysis

Sidewall Core. The side wall core shown in Figure 10 was collected 1.8 m (6 feet) above the April 2014 biomineralization fracture sealing experimental depth of 340.7 m (1118 feet) bgs. This sample was a representative piece of the casing and about 2.54 cm (1 inch) of the cement behind the casing. No formation material was retrieved from beyond the cement. X-ray computed tomography (CT) and reflect light and fluorescent stereomicroscope images of the side wall core are shown in Figure 11.The X-ray CT results show the presence of a channel through the cement material. The sample has tested positive for the presence of biomineral materials (calcite, data not shown) through X-ray diffraction (XRD) of the soft mud materials retrieved from behind the casing and by detection of auto fluorescent mineral (auto fluorescence is a property of calcite (Yoshida et al., 2010)) formed in a channel through the cement core. This suggests that even after a year, calcite is detected in an apparent flow channel present in the cement behind the casing pointing to the longevity of the mineral under these subsurface conditions. This also implies that the biomineral materials were transported not only into the fracture but also into a flow channel in the cement 1.8 m (6 feet) above the fracture zone.

Figure 10. Side wall core collected at elevation 338.9 m (1111.84 feet) bgs (approximately 1.8 m) (6 feet) above the MICP sealed fracture). The interface between the steel well casing plug (right) and the one-inch cement plug (left) contained a black, soft, mud-like material which was found to contain calcite.

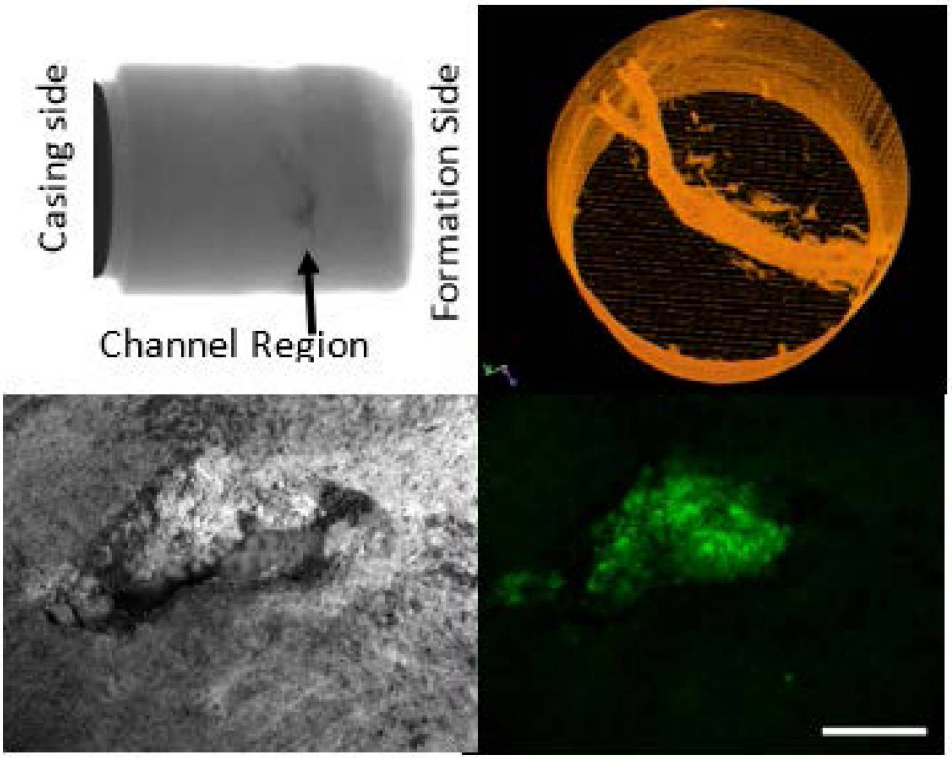


Figure 11. *X-ray CT and reflect light and fluorescent stereomicroscope images. Top Panel. A channel was detected in the one inch diameter, one inch long cement core as evidenced by the CT scan. Bottom Panel. The region of the channel was imaged with reflect light (left) and fluorescence (right). The scale bar for the stereoscope images is 2 mm (0.078 inches). Auto fluorescent mineral was detected in the flow channel suggesting calcite formed not only in the fracture but also in a cement channel.*

Microbial penetrability results summary. As indicated previously microbial penetration of rock cores was carried out as part of the post-Gorgas experimental investigation. The complete report for this activity appears in Appendix B. In summary, this work investigated transport of bacterial cells in low permeability, 2.54 CM (1 inch) diameter sandstone cores. We have determined that viable *S. pastuerii* cells can penetrate at least two inches through sandstone cores with permeability as low as 0.78 mD. Five cores with permeabilities of 0.78-31 mD were tested using a biaxial core holder with overburden pressure to eliminate bypass around the core. Bacterial cells were detected in the effluent of all cores after *S. pasteurii* was added to the influent of the core holder system. Controls indicated that the system was sterile and no bacterial cells were detected in the core effluent prior to the *S. pasteurii* addition. This research indicated that viable cells can travel into very low permeability sandstone, including the (approximately 10 mD) sandstone surrounding the fracture sealing at Gorgas. These results also demonstrate potential implications in the subsurface injection of microorganisms for applications such as enhanced oil recovery and microbially induced carbonate precipitation.

Publication of Results. Results from this project have been incorporated into a manuscript entitled "Fracture Sealing with Microbially-Induced Calcium Carbonate Precipitation: A Field Study". The manuscript was submitted to Environmental Science and Technology (ES&T), in November 2015.

CONCLUSION

This project was conducted in response to National Energy Technology Laboratory Funding Opportunity Number DE-FOA-0000652 entitled Technologies to Ensure Permanent Geologic Carbon Storage, CFDA Number: 81.089 Fossil Energy Research and Development, Area 2: Advanced Wellbore Integrity Technologies.

The goal of this project was to develop a biomineralization-based technology for sealing preferential flow pathways in the vicinity of injection wells based on field testing performed at a 24.4 cm (9.625 inch) diameter well located on the Gorgas Steam Generation facility near Jasper, Alabama. The research was aimed at (1) developing methods for delivering MICP promoting fluids downhole using conventional oil field technologies and (2) assessing the ability of MICP to seal cement and formation fractures in the near wellbore region in a sandstone formation. The target area for the MICP sealing test was a horizontal fracture extending out into sandstone of approximately 10 mD permeability and located 340.7 m (1118 feet) bgs. Protocol for the field test was developed based on results from laboratory experiments together with simulation modeling conducted by the University of Stuttgart. The field test, which was performed during the period April 1-11, 2014, resulted in complete biomineralization sealing of the fracture and surrounding wellbore region. A total of 24 injections of calcium media and six injections of microbial inoculum were delivered using an 11.4 L (3.0 gallon) wireline dump bailer over a three day period.

Key Outcomes. The key outcomes for this project are summarized below.

1. A horizontal fracture in Fayette sandstone (porosity ~10%, permeability 5-10 mD), located 340.7 m (1118 feet) below ground surface at the Gorgas well was completely sealed over a three day period in April 2014.

2. The fracture was completely plugged after 24 injections of calcium media and six injections of inoculum. Complete sealing occurred when pump pressure exceeded re-fracture pressure with zero injection flow rate. Field plugging results compared favorably with model predictions (25 Ca and six inoculation injections) made using the Stuttgart MICP model. These predictions were made prior to the field test based on planned injection strategy.

3. This project successfully integrated mesoscale laboratory experiments at CBE together with simulation modeling conducted at the University of Stuttgart to develop the protocol

for conducting the biomineralization sealing test in the field well. Effectiveness of the biomineralization seal was evaluated in the field.

4. The fracture extension pressure (i.e. the pressure necessary to re-fracture the formation) was significantly higher at 111.6 atm (1640 psi) at the termination of the experiment compared to 96.6 atm (1420 psi) prior to the experiment. This suggests the MICP seal was stronger than the formation prior to treatment.

5. Effectiveness of the biomineralization seal was evaluated in the field. Metrics indicating successful MICP sealing included: reduced injectivity during seal formation, reduction in pressure falloff, and demonstration of MICP by-products (CaCO3) in treated regions of side wall cores.

6. The 11.4 L (3.0 gallon) wireline dump bailer proved to be a cost effective method of placing the biomineralization material near the perforations.

7. The inoculum microorganism, *Sporosarcina pasteurii,* was not present in formation water and sediment samples taken prior to inoculation and biomineralization at the Gorgas site. However *S. pasteurii* was detected in formation fluids three days later at the conclusion of the biomineralization experiment. This outcome indicates the presence of an active culture of the organism responsible for ureolytic biomineralization as the field test progressed.

8. Penetration of viable *Sporosarcina pasteurii* cells through Berea sandstone core of approximately 1.0 mD permeability has been observed.

9. This study demonstrated that MICP technology can potentially seal and strengthen subsurface high permeability regions or fractures through the use of low-viscosity fluids to penetrate small aperture pores not easily reachable by traditional cement-based sealing technologies.

MICP technology evaluation. The primary success to come out of this study was development of field-ready methods to perform MICP sealing in the subsurface using conventional oil field delivery technology. The biomineralization treatment had a significant impact on key formation properties: (1) injectivity decreased significantly compared to pre-treatment levels and (2) pressure required to fracture the rock and to extend the fracture increased after treatment. The MICP treatment also resulted in a reduction in pressure decay after the pumping ceased (a measure of improved mechanical integrity). Although flow-related properties decreased and tensile strength increased, some uncertainty remains concerning how far into the formation cells were injected and where biomineralization took place. This question was addressed by way of a laboratory study which indicated that it was very likely for the injected *(S. pasteurii)* cells to penetrate at least a few centimeters into the surrounding sandstone formation. The technology developed in this project contributes to the DOE CSP effort of ensuring 99% CO_2 storage performance in the injection zone(s) for 100 years or more.

Future MICP sealing research. On-going investigations are focused on using MICP sealing to mitigate leakage pathways through compromised wellbore cement, thereby improving wellbore integrity. However, techniques must also be developed to achieve wellbore sealing under a wide range of temperature and chemical conditions to truly advance subsurface MICP sealing technology. Accordingly, our research team is now focusing on developing new (enzymatic and thermal) mineralization precipitation technologies capable of sealing near-wellbore leakage pathways under a variety of pressure, temperature conditions, and in the presence of CO_2 and brine so as to ensure CO_2 permanence within the storage formation. The processes now being explored are aimed at precipitating calcium carbonate based on either enzymatic or thermal degradation of urea resulting in precipitation which can withstand significantly greater temperature (and pressure) than the *S. Pasteurii strains* used to date. This research is being conducted under project DE-FE0026513, "Wellbore Leakage Mitigation using Advanced Mineral Precipitation Strategies". If successful enzymatic and thermal-based mineral precipitation will result in more robust biomineralization technologies capable of being applied at much greater depths than are currently possible.

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LIST OF ACRONYMS AND ABBREVIATIONS

bgs	below ground surface
CBE	Center for Biofilm Engineering
CSP	Carbon Sequestration Program
CCS	Carbon Capture and Storage
MICP	Microbially Induced Calcite Precipitation
MCDP	Minimum Capillary Displacement Pressures
CO_2	Carbon Dioxide
CBE	Center for Biofilm Engineering
DOE	U.S. Department of Energy
MSU	Montana State University
ERI	Energy Research Institute
Shell	Shell Exploration and Productions B.V.
SC	Southern Company
UAB	University of Alabama at Birmingham
SLB	Schlumberger
$scCO_2$	Supercritical Carbon Dioxide
MIP	Mercury Intrusion Porosimetry
PBS	Phosphate Buffer Solution
dH2O	Deionized Water
S. pasteurii	*Sporosarcina pasteurii*
CMM	Saturation Reducing Medium
CMM+	Calcium Medium
CMM-	Urea Growth Medium
SEM	Zeiss Supra 55VP Scanning Electron Microscope
EDS	Energy-Dispersive X-Ray Spectroscopy
N_2	Nitrogen Gas
$CaCO_3$	Calcium Carbonate
NH4+	Ammonium Cation
Ca2+	Calcium Cation
ICPMS	Inductively Coupled Mass Spectrometer
CT	Computed Tomography
XRD	X-ray Diffraction
NaCl	Sodium Chloride
ELAN	Petrophysical analysis (well log) prepared by Schlumberger
TP	Formation (fracture) threshold pressure
Micro-CT	X-ray Micro Computed Tomography
X-Ray CT	X-Ray computed tomography
MI	Mechanical Integrity of the well bore

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APPENDIX A

Supporting Information

APPENDIX A. Supporting Information

Formation water and injection water analyzed using IC and ICP-MS for analysis of cations and anions where the results were determined by comparison to calibrated standard solutions (Table A1). The subsurface samples from the mixing zone were also analyzed for urea, pH, and calcium concentration (Table A2).

Table A1. *Results from the analysis of the formation and injection (Black Warrior River) water*

		Raw formation	Filtered formation	Raw injection	Filtered injection	
Cation IC (ppm)	Lithium	na	n.d.	na	n.d.	
	Sodium	na	2140	na	42	
	Ammonium	na	n.d.	na	n.d.	
	Potassium	na	120	na	n.d.	
	Calcium	na	709	na	n.d.	
	Magnesium	na	n.d.	na	n.d.	
Anion IC (ppm)	Fluoride	na	n.d.	na		
	Chloride	na	2721	na	1.19	
	Bromide	na	38.6	na	n.d.	
	Nitrite	na	n.d.	na	n.d.	
	Nitrate	na	16.9	na	0.98	
	Phosphate	na	9.6	na	3.983	
	Sulfate	Na	12.3	na	2.27	
ICPMS (ppm)	Sodium	2436	2255	n.d.	n.d.	
	Aluminum	4.643	0.7383	0.8744	0.7339	
	Potassium	154.6	144.4	n.d.	n.d.	
	Calcium	796.9	714.6	16.54	15.76	
	Zinc	1.332	0.5909	bql	0.5326	
	Barium	2.313	2.062	bql	bql	
	Iron	5.56	bql	bql	n.d.	
Temperature(°C)		24.5		20.2		
pH		5.5		6.7		
Conductivity mS/cm		36.77		0.013		
TDS g/L		23.91		0.009		
ORP		-16		+155		
total Diss Carbon ppm			21.66 (2.45)		4.73 (1.12)	
DIC ppm			1.07 (0.03)		3.46 (0.33)	
DOC ppm			20.59 (2.42)		1.27 (0.85)	

Table A2. *Results of the sampling of the downhole mixing zone samples.*

Sample Name	Time	pH	Urea g/L	Calcium g/L
MZ#1	4/7/2014 14:30	11.6	0.0	2.1
MZ#2	4/8/2014 9:40	8.7	5.2	12.5
MZ#3	4/9/2014 19:30	9.0	11.5	8.6

Density Amendment of Sump. Tubing (2.875 inches OD) was strung down-hole with the packer unengaged. The bottom portion of the well (sump) was located between the perforations and the bottom plug which resided approximately 10 feet below. The sump was filled sodium chloride-river water solution of 17.4% NaCl (Mix-N-Fine, Cargill Salt Division, Minnesota, USA) by pumping brine through the tubing string. The purpose of filling the sump with fluid of higher density than the other injection fluids was to encourage flow into the formation instead of injected fluids sinking due to density differences into the bottom approximately 10 feet of the injection zone.

Microbial Inoculum Preparation. Frozen stocks of culture were shipped overnight and stored onsite on dry ice. Sterile BHI+ Urea medium (37 g and 20 g/L respectively) was filled to 150 ml (5.07 ounces) in 250 ml (8.45 ounces) pre-sterilized plastic screw top flasks (VWR, PA, USA) and inoculated with a thawed frozen stock. The 150 ml (5.07 ounces) overnight cultures were used to inoculate five gallon collapsible carboys (Cole Parmer) filled to four gallons with growth medium. The carboy growth medium was prepared by mixing 3 g/L Nutrient Broth (Research Products International), 10 g/L NH_4Cl (Amersco), 20 g/L Urea fertilizer (Par 4, Bridgewell Resources, OR, USA) and 24 g/L NaCl (Morton, IL, USA) to distilled water (Walmart) in the collapsible carboy prior to inoculation. A stir bar was added to the carboy and the entire carboy was placed in a heated (~70 °F) Rubbermaid tub where it was stirred and the culture allowed to grow for approximately 24 hours. No effort was made to perform the carboy culturing aseptically. Continuous overnight cultures of *S. pasteurii* were maintained throughout the experiment. Temperatures in the day were conducive to microbial growth (~65 degrees F), temperatures during the night dropped to ~45 °F requiring the heated tub to support overnight growth conditions. Periodic samples of the inoculum were drop plated on BHI+Urea agar plates to assess the microbial viability, microbial concentration, and the potential for a contamination to overtake the *S. pasteurii* culture. Cultures were started and transferred daily so that two four gallon carboys with at least 24 hour cultures were available for inoculation each day of the experiment (Table A3). Concentration and total number of cells inoculated for each of 6 bailer inoculum deliveries are shown in Table A4.

Table A3. *Inoculation and growth schedule (Note: repeat Day 1 through to Day 4)*

Action	Day -1	Day 0	Day 1	
	(Mid Day)	(Mid Day)	(Mid Day)	(Evening)
Frozen Stock	Two frozen stocks into 150 ml in two 250 flask	Two frozen stocks into 150 ml in two 250 flask	Two frozen stocks into 150 ml in two 250 flask	
Transfer		Transfer 150 ml to 4 gallons of growth medium in carboy		Transfer 150 ml to 4 gallons of growth medium in carboy
Inoculate formation			Inoculate with 4 gallons from carboy amended with urea and nutrient broth	Inoculate with 4 gallons from carboy amended with urea and nutrient broth

Table A4. *Concentration and total number of cells inoculated for each of 6 bailer inoculum deliveries.*

	Population (cfu/ml)	Total number of cells injected
Inoculation #1	5.00E+07	7.1E+11
Inoculation #2	3.25E+07	4.6E+11
Inoculation #3	3.24E+07	3.7E+11
Inoculation #4	1.56E+07	1.8E+11
Inoculation #5	4.00E+07	4.5E+11
Inoculation #6	2.40E+07	2.7E+11

All the MICP supporting substrates were pre-weighed into quart and gallon sized plastic zip closure bags at the appropriate mass to reach a desired formation substrate concentration after pumping brine following bailer delivery. Those bags of substrates were mixed with brine immediately prior to bailer delivery downhole as per the injection schedule in Table A5.

Table A5. *Injection schedule*

Date	Time	Bailer Contents	Flow Rate (gpm)	Brine pumped (gal)	Max Pressure during pumping (psi)
7-Apr	15:25	Inoculation #1	0.5	10	998
7-Apr	16:35	Calcium Bailer #1	0.5	10	1058
7-Apr	17:10	Calcium Bailer #2	0.5	10	1024
7-Apr	17:36	Calcium Bailer #2	0.5	10	1024
7-Apr	18:16	Calcium Bailer #3	0.5	10	1036
7-Apr	19:08	Inoculation #2	0.5	10	1042
8-Apr	10:17	Calcium Bailer #4	0.5	5	1056
8-Apr	11:09	Calcium Bailer #5	0.5	7.28	1051
8-Apr	11:42	Calcium Bailer #5	0.5	5	1051
8-Apr	13:43	Calcium Bailer #6	0.5	3.5	1070
8-Apr	14:41	Calcium Bailer #6	0.5	7	1076
8-Apr	14:48	Calcium Bailer #7	0.5	7	1056
8-Apr	15:17	Calcium Bailer #8	0.5	10	1092
8-Apr	15:56	Inoculation #3	0.45	9	1094
9-Apr	8:43	Calcium Bailer #9	0.5	7	1083
9-Apr	9:15	Calcium Bailer #10	0.5	7	1059
9-Apr	9:49	Calcium Bailer #11	0.45	6.3	1090
9-Apr	10:20	Calcium Bailer #12	0.4	5.6	1064
9-Apr	10:58	Calcium Bailer #13	0.5	5	1090
9-Apr	11:24	Calcium Bailer #14	0.5	10	1086
9-Apr	12:05	Inoculation #4	0.5	9	1106
9-Apr	13:47	Calcium Bailer #15	0.5	7	1091
9-Apr	14:20	Calcium Bailer #16	0.5	7	1046
9-Apr	14:48	Calcium Bailer #17	0.5	7	1067
9-Apr	15:17	Calcium Bailer #18	0.5	7	1075
9-Apr	15:46	Calcium Bailer #19	0.5	6	1076
9-Apr	16:14	Calcium Bailer #20	0.5	7	1070
9-Apr	16:42	Calcium Bailer #21	0.5	10	1058
9-Apr	17:14	Inoculation #5	0.5	5.5	1176
9-Apr	17:25	Brine	0.125	0.625	1193
10-Apr	12:22	Calcium Bailer #22	0.14	3.262	1136
10-Apr	14:00	Calcium Bailer #23	0.14	1.89	1136
10-Apr	15:23	Inoculation #6	0.14	2.023	1144
10-Apr	16:57	Calcium Bailer #24	0.14	1.8886	1138

Mud Analysis. During the sampling of mixing zone sample collected on day three it was observed to contain silty-type solids. When the sample arrived on ice at MSU, a portion of the solids were dried and analyzed with X-ray Powder Diffraction Spectrometer (XRD) (Scintag X-

GEN 4000 XRD) at the Image and Chemical Analysis Laboratory (ICAL) at MSU. The particles were scanned from 20.0 to 65.0 degrees at 1 deg/min and DMSNT analysis software (Scintag) was used to characterize the mineralogy of the samples. At MSU a portion of the sample was dried and imaged with SEM (Zeiss Supra VP, XX, XX) at 1.0 kv with a 4 mm (0.16 inch) working distance. Additionally, the sample was analyzed with energy-dispersive X-Ray spectroscopy (EDS) at 15 kv and 15 mm (49.21 feet) working distance. At the end of the experiment, the packer was set loose and the tubing string pulled from the well. When the collar stop, which was set in the mixing zone below the packer (Figure XX), was pulled from the well, it was noted that significant mud was observed to be stuck to the collar stop. The mud was scraped from the collar stop into a 50 ml (1.69 ounces) centrifuge tube, placed on ice and shipped to MSU for further analysis. A sample of this mud was also subjected to an acid digest test with 10 µL drops of 1M HCl (Fisher Scientific, New Jersey, USA). At MSU, a portion of the sample was dried and imaged with SEM (Zeiss Supra VP) at 1.0 kv with a 4 mm working distance. Additionally, the sample was analyzed with EDS at 15 kv and 15 mm (49.21 feet) working distance. Finally, a portion of the dried and collected particles were analyzed by X-ray Powder Diffraction Spectrometer (XRD) (Scintag X-GEN 4000 XRD) at MSU's ICAL. The particles were scanned from 20.0 to 65.0 degrees at 1 deg/min and DMSNT analysis software (Scintag) was used to characterize the mineralogy of the samples

Microbial Analysis. Frozen stocks were prepared of each isolate by mixing 0.2 ml (0.01 ounce) autoclaved glycerol with 0.8 ml BHI + Urea grown culture in a sterile cryovial which were stored in a -80 °C freezer. In addition, an isolated colony (Figure A1) from each culture/plate was inoculated into 5 ml (0.17 ounce) of BHI+Urea liquid media, grown for two days on a room temperature shaking table. The cultures were aliquoted into pre-sterilized cryovials, wrapped in Parafilm, and shipped to Idaho State University Molecular Research Core facility for DNA extraction and sequencing. Partial sequencing of the 16S rRNA gene sequence was performed with specific primer sets (Table A6). The sequences were returned to MSU and the sequences were manually trimmed and edited with Bioedit Sequence Alignment Editor version 7.2.5 (©Tom Hall, Carlsbad, California) software. The edited sequences (approximate sequence length of 500-700 bp) were compared with a search in the NCBI GenBank nucleotide Blast (Blastn) Microbial Genome database (http://www.ncbi.nlm.nih.gov/) against known 16s ribosomal RNA sequences. The top alignment results (top four when >97% of isolate sequence matched to the database sequences or top one if <97% alignment), the edited sequence lengths, a description of the colony morphology and microscopic cell shape observations, and the accession number of the database sequences to which the isolates were aligned and summarized (Table A6).

DNA from the filters was extracted with liquid-N2 freeze-thaw and grinding with mortar and pestle before placing in the MP Soil DNA Extraction Kit. Triplicated DNA extraction was combined for each sample in the end. Nested polymerase chain reaction (PCR) was required because the DNA concentration was very low. The first round of PCR (30 cycles) was done with universal bacterial primers (1500 bp amplicon), then a second round (25 cycles) was done with Illumina primers. Illumina are primers of interest plus Illumina adaptors which are needed for downstream adding on the indices, often referred to as "barcodes" and the term used for pyrosequencing. In this case, bacterial primers (500 bp amplicon) + Illumina adaptors were used. Five PCRs were set up for each sample and then each was combined before following Illumina's

protocol for setting up a 16S rRNA gene sequencing run. The Illumina protocol consisted of PCR clean up, adding indices, measuring DNA, diluting, and pooling samples to required DNA concentration (4nM) for sequencing run. The raw data was processed using MOTHUR's MiSEQ SOP for quality check and downstream sequence analysis with some parameter modifications.

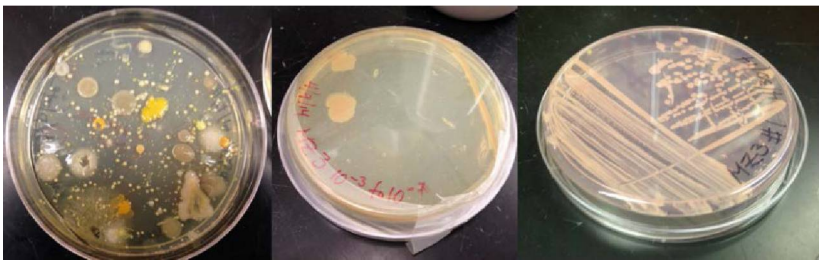

Figure A1*. Microbial isolates were isolated from the field plated mixing zone samples. From left to right: Far Left: Mixing Zone Sample #1 where many different colony types were observed and 10 isolates were cultured (two were not identified by sequencing as they were determined to be fungi). Middle: Mixing Zone Sample #3 where the tan roundish colony morphology was transferred to a fresh plate. Far Right: the isolated colony from Mixing Zone Sample #3 whose 16s rRNA partial sequence closely aligned with NCBI GenBank database sequences of S. pasteurii.*

Table A6. *Characteristics and identification of the isolated colonies from down-hole samples*

Enrich-ment Sample		Isolate	Colony Morphology	Microscopy Observation	Plate NH_3?	Primer	# BP	Genus	sp.	%	Accession #
Mixing Zone #1		MZ1-1	white, round, wrinkly	paired cocci	No	8F	690	Kocuria	rosea	99%	KC844837.1
								Kocuria	rosea	99%	KC493999.1
								Kocuria	sp.	99%	HM151657.1
								Kocuria	sp.	99%	HM151648.1
		MZ1-2	tiny, coral, round	paired cocci	No	8F	652	Kocuria	rhizophila	98%	NC_010617.1
		MZ1-3	white, fuzzy edge	small and longer joined rods	No	8F	675	Bacillus	sp.	97%	NC_021171.1
								Bacillus	infantis	97%	NC_022524.1
		MZ1-4	tiny, yellowish, round	small rods	No	8F	626	Micro-bacterium	testaceum	96%	NC_015125.1
		MZ1-5	white, round, shiny	slow motility, rods	No	8F	598	Bacillus	sp.	95%	NC_021171.1
		MZ1-6	tiny, orange-brown, round	small rods, chained with bright ends	No	8F	605	Caulobacter	segnis	96%	NC_014100.1
		MZ1-9	orange, round	rods, branched	No	8F	592	Bacillus	infantis	92%	NC-022524.1
		MZ1-10	tiny, white, round	short rods, very motile	No	8F	537	Cellulomonas	flavigena	96%	NC_014151.1
Mixing Zone #3		MZ3-1	tan, round	long rod with bright end (spore?)	Yes	1492R	567	**Sporosarcina**	**pasteurii**	**99%**	**NR_104923.1**
								Sporosarcina	soil strain	98%	NR_043527.1
								Sporosarcina	contaminans	98%	NR_116955.1
								Sporosarcina	koreensis	98%	NR_043526.1
Collar stop mud from down-hole		Mud	tan, round	rod shaped	Yes	1492R	564	**Sporosarcina**	**pasteurii**	**99%**	**NR_104923.1**
								Sporosarcina	soil strain	98%	NR_043527.1
								Sporosarcina	contaminans	98%	NR_116955.1
								Sporosarcina	koreensis	98%	NR_043526.1

Precipitation assessment under field conditions. Flasks prepared in the field with an aliquot from Mixing Zone sample #2 and calcium/growth medium along with sand resulted in precipitation of calcium carbonate and a cemented layer of sand on the bottom of the flask after 24 hours. Fifty ml (1.69 ounces) of each mixing zone sample were added to a 250 ml (8.45 ounce) flask and mixed with 50 ml (1.69 ounces) of calcium/growth solution with sand covering the bottom of the flask to assess whether the field conditions (mixing zone and calcium/growth medium) result in precipitation (Figure A2).

Figure A2. *An unfiltered portion of the Mixing Zone sample #2 was mixed with calcium and growth-containing medium in a flask that contained unconsolidated 0.5mm (0.20 inch) quartz sand. After 24 hours of incubation, the sand was observed to be cemented to the bottom of the flask and the urea in the liquid dropped from 24 g/L to 6.6 g/L indicating ureolysis-driven MICP was observed with the field condition chemical combinations and a sample from the subsurface.*

Approximately 40 ml (1.35 ounces) of the MICP promoting fluids were sampled from the spray tank after the suspension and urea/nutrient broth was mixed. A portion of the sample was filtered and put on ice, the remaining unfiltered portion of the sample was diluted into filter sterilized phosphate buffer solution (PBS) for drop plating and the pH and conductivity was assessed (Table A7). Samples of the contents of each bailer of inoculum or concentrated growth/calcium solution were collected, and the pH and conductivity were immediately measured and recorded. Additional analysis was performed on the fluids including the modified Jung Assay for urea and dissolved (0.2 μm-filtered) calcium and ammonium were measured by a Metrohm 732 IC detector.

Post Experiment Analysis

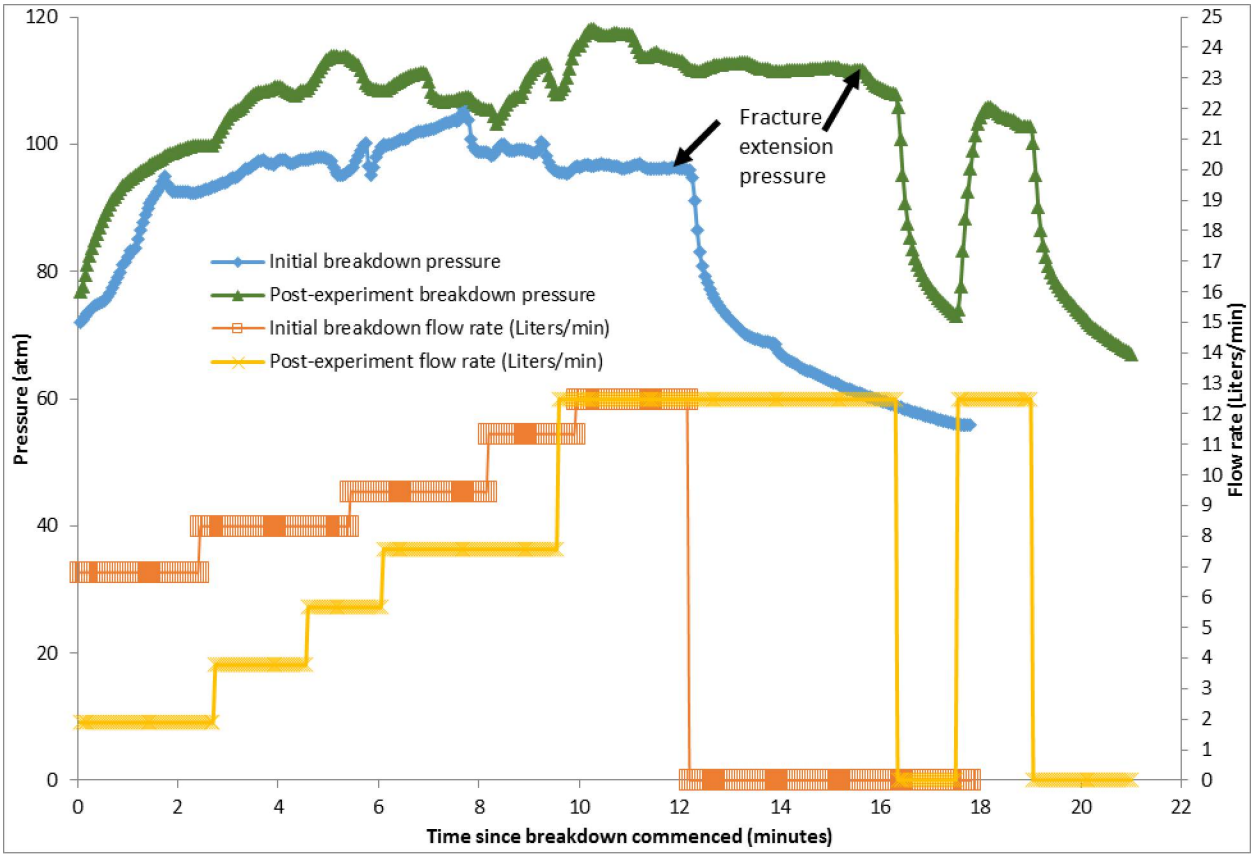

Figure A3. *The formation was stimulated before and after the experiment to assess fracture extension pressure after MICP treatment.*

In May of 2015, Schlumberger deployed a side wall coring tool into the well to retrieve samples from the fracture sealing test depth. Arriving onsite, it was noted that the well was completely full of water (as it was left after the April 2014 field experiment). The water level in the well before the experiment was estimated at 91.46 m (300 feet) bgs. The presence of an extra 91.46 m (300 feet) of water in the well suggests that over the course of the year, the biomineral seal was subject to a 91.4 m (300 feet) water column pressure challenge. This a positive result suggesting that the biomineral seal did not allow any water to enter the formation where it could equilibrate to its normal hydrostatic level.

APPENDIX B

Microbial transport through rock cores report

APPENDIX B. Determination of microbial transport through low permeability sandstone report

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Executive Summary

This work investigated transport of bacterial cells in low permeability, 1inch diameter sandstone cores. We have determined that viable *S. pastuerii* cells can penetrate at least two inches through sandstone cores with permeabilities as low as 0.78 mD. Five cores with permeabilities of 0.78 – 31 mD were tested using a biaxial core holder with overburden pressure to eliminate bypass around the core. Bacterial cells were detected in the effluent of all cores after *S. pasteurii* was added to the influent of the core holder system. Controls indicated that the system was sterile and no bacterial cells were detected in the core effluent prior to the *S. pasteurii* addition. This research indicated that viable cells can travel into very low permeability sandstone, including the (approximately 10 mD) sandstone surrounding the MICP fracture sealing at Gorgas. These results also demonstrate potential implications in the subsurface injection of microorganisms for applications such as enhanced oil recovery and microbially induced carbonate precipitation.

Goals

The aim of this work was to investigate the lower permeability limit for the transport of bacterial cells through the pore spaces of intact sandstone cores. To this end, sandstone cores 2.54 cm (1 inch) in diameter representing a range of permeability values were tested in a core system consisting of a Hassler core holder and ports for injection and sampling of bacterial cultures and fluid. The pore size distribution of a porous medium can typically be positively correlated with measured permeability, as evidenced by the mathematical models developed for prediction of these properties, including the Kozeny-Carman relationship and modifications thereof [1,2]. Thus, we hypothesized that the injection efficiency of bacterial cells into the cores would decrease with decreasing permeability due to the inability of cells to pass through smaller pore throats. However, there may be transport of cells at low permeability values due to heterogeneity in pore throat sizes.

The work determined the feasibility of microbial injection in different geologic formations for subsurface microbially induced calcium carbonate ($CaCO_3$) precipitation (MICP). MICP can be achieved by bacteria that hydrolyze urea to create favorable conditions for $CaCO_3$ precipitation [3,4]. The urea hydrolyzing organism commonly used in these applications is the bacterium *Sporosarcina pasteurii* [5,6]. Thus, in addition to investigating cell transport through cores with different permeability values and pore throat sizes, we investigated the viability and activity of transported *S. pasteurii* cells, including their ability to hydrolyze urea after transport through the cores.

Experimental Methods

High-pressure test system

A high pressure flow-through system was designed utilizing a Hassler core holder to provide overburden pressure. In this system, the permeability of rock cores and the transport of *Sporosarcina pasteurii* cells through those cores in the axial direction of flow can be measured

using 2.54 cm (1 inch) diameter cores up to 15.34 cm (6 inches) in length. The system consists of a pump capable of operation under high pressure (170 bar, 2,500 psi), a pressure transducer (Omega) to measure the differential pressure across the core, an injection port for addition of microbial cells, and a Hassler type biaxial core holder (Core Labs, Inc.) to prevent bypass of flow around the core (Figure B1). A Teledyne Isco model 500D syringe pump is used for fluid injection at a constant flow rate and the pump flow and influent pressure are monitored and recorded via computer data logging. Differential pressure across the core can also be recorded with a transducer during high pressure experiments in which the effluent is not at ambient pressure. The core holder consists of a Viton sleeve inside a Hassler core holder filled with overburden fluid and pressurized at least 3.4 bar (50 psi) above the influent pressure for experiments conducted with ambient effluent pressure. The core holder is connected to the system via stainless steel tubing with Swagelok fittings and is oriented in a horizontal position for axial flow through the core. An effluent sampling port is installed for collection of effluent fluids.

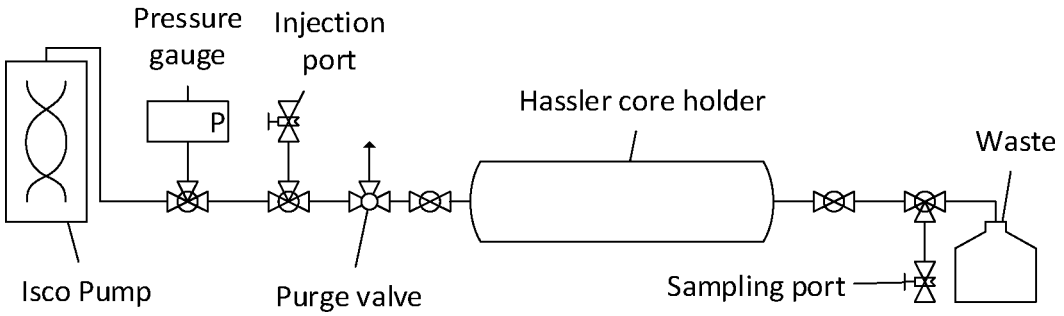

Figure B1. *Microbial injectivity system diagram.*

Cores and permeability testing

Five test cores were evaluated for microbial transport in the high pressure Hassler core holder. All cores were 2.54 cm (1 inch) in diameter and 5.08 cm (2 inches) long. Each core had to be saturated by pumping PBS through the system for 1-2 days, upon which arrival at a steady-state differential pressure indicated the core was saturated with PBS. Steady state was determined when the pressure remained within the given value +/- 10% for one hour. Pore volume in the cores was estimated to be approximately 5 mL (0.17 ounces) for the Berea cores, based on mercury porosimetry values of 11% porosity.

The Hassler core holder provided overburden pressure on the core and prevented fluid flow around the core during injection tests. Prior to performing a cell injection experiment with a particular core, the core was sterilized by baking at 200 °C for 24 hours. The remainder of the system was disinfected by pumping 25 mL (0.85 ounces) each of the following solutions in order, with 30 minutes between each solution: 5% HNO_3; 70% ethanol; sterile phosphate buffered saline (PBS). The core was placed into the Viton sleeve, inserted into the Hassler core holder and connected to the system. Overburden fluid consisting of antifreeze and water was pumped into the annulus between the inner sleeve and outer core holder. PBS was pumped through the core until the differential pressure was constant at 3.4 bar (50 psi) +/- 10% for one hour and the core was assumed to be fully saturated. The overburden pressure on the sleeve was maintained above 20.4 bar (300 psi) for all experiments with a core influent pressure of 3.4 bar (50 psi). At this point, the pressure and fluid flow rate were recorded to determine the initial

core permeability and effluent fluid was analyzed to verify that cells were not present prior to cell injection. PBS was used in all permeability characterizations and microbial transport studies and the flow rate was adjusted to keep the differential pressure across each core at 3.4 bar (50 psi).

Transport studies

At the onset of transport studies, 2 mL (0.07 ounces) of an *S. pasteurii* culture (OD_{600} = 0.07) was injected into the influent tubing of the core via the injection port. The cultures were grown by inoculating nutrient broth with 2% urea with a frozen stock culture and shaking at 30 °C overnight, then centrifuging and re-suspending the cells in phosphate buffer solution (PBS). Beginning at the time of injection, all of the effluent liquid was collected in pore volume fractions and analyzed for microbial presence and viability.

At least 50 mL (1.69 ounces) of liquid was pumped through the core and collected in 5 mL (0.17 ounce) fractions after microbial cell injection. Direct counting via 4',6-diamidino-2-pheylindole (DAPI)cell staining and microscopy were employed to determine total cells in the effluent. Additionally, samples were serially diluted, plated onto brain-heart infusion BHI agar and colony forming units were counted to determine the fraction of viable cells. A colorimetric assay for urea, the Jung assay[7], was performed after some of the experiments to determine if urea hydrolysis would occur in the effluent samples. The activity assay allowed for evaluation of the ureolytic enzyme activity during and after transport through the cores.

Results

Microbial transport in high pressure system

Experimental conditions for core tests and the resulting permeability values are listed in Table B1. The permeabilities were calculated from the given flow rate and the measured influent pressure at steady state conditions (shown in Table B1). During the permeability determination, the overburden pressure was increased above 100 psi to ensure that the differential pressure across the core remained constant and bypass was not occurring. The final overburden pressure was over 20.4 bar (300 psi) in all core experiments with the exception of the first Berea Sandstone 1. Due to the low differential pressure (1.08 bar, 16 psi) used in the first Berea core test, the overburden was maintained at 5.44 bar (80 psi).

Berea Sandstone 1 was not sterilized prior to the microbial injection test and contamination was observed on plates taken prior to injection. Thus, the four subsequent cores were sterilized prior to injection by baking for 24 hours at 200 °C. The influent and effluent samples taken before testing cores 2 through 5 did not indicate presence of any contaminating microorganisms by plating or direct counts. Therefore, the system sterilization and flushing of the core was assumed to have successfully eliminated background microbial presence.

Table B1. *Test cores used for microbial injectivity experiments.*

			Experimental conditions	
Test core	**Material**	**Permeability** (milliDarcy)	**Flow rate** (ml min^{-1})	**Differential pressure** (psi)
1	Berea Sandstone	31.4	2.0	**16**
2	Berea Sandstone	2.4	0.5	54
3	Berea Sandstone	30.6	6.0	50
4	Torrey Buff sandstone	1.4	0.25	43
5	Torrey Buff sandstone	0.78	0.15	48

At the beginning of each transport study, 2 mL (0.07 ounces) of *S. pasteurii* culture was injected into the system and pumped through the core under continuous flow of PBS. The optical density of the injected culture was adjusted to achieve the same cell density in all tests, which resulted between $2.4x10^7$ and $8.6x10^7$ colony forming units (CFU) total injected during each experiment. All fluid pumped through the core was collected via an effluent sampling port at timed intervals corresponding to an approximate pore volume of 5 mL (0.17 ounces). A total of 50 mL (1.69 ounces) or 10 samples were collected and an example breakthrough of viable cells from test core 3 using Berea sandstone is shown in Figure B2. The highest concentration of cells in the first one to two samples followed by low or no cells detected in later samples indicates that sufficient time was allowed for effluent collection of cells. The overall breakthrough of viable cells for each experiment was determined by summing the effluent viable cell counts. For the 30.6 mD Berea core, viable cell breakthrough totaled 4.2% of total injected, viable cells.

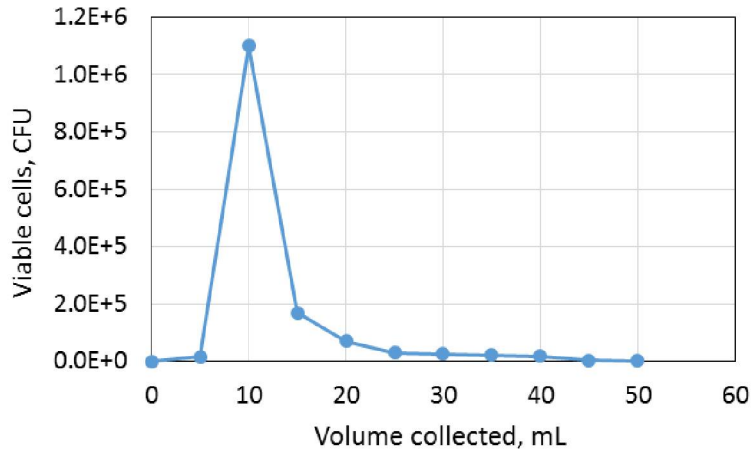

Figure B2. *Breakthrough curve of viable cells in Berea core 3 with permeability of 30.6 mD.*

The low permeability cores also indicated viable cell transport through the core, as evidenced by Figure B3, showing the results from the Berea test core 4 with permeability of 2.4 mD. In Figure B3, red symbols indicate ureolysis was detected in the effluent sample at that time point, determined by the degradation of at least 1 g/L urea overnight. Samples where viable cells were not detected but ureolysis activity was measured may indicate lysis of cells and transport of urease enzyme. Additionally, with the drop plate method used for the Berea cores, the limit of cell detection was 100 CFU/mL. For the subsequent Torrey Buff core tests, a spreadplate method was used, reducing the estimated detection limit to ~5 CFU/mL.

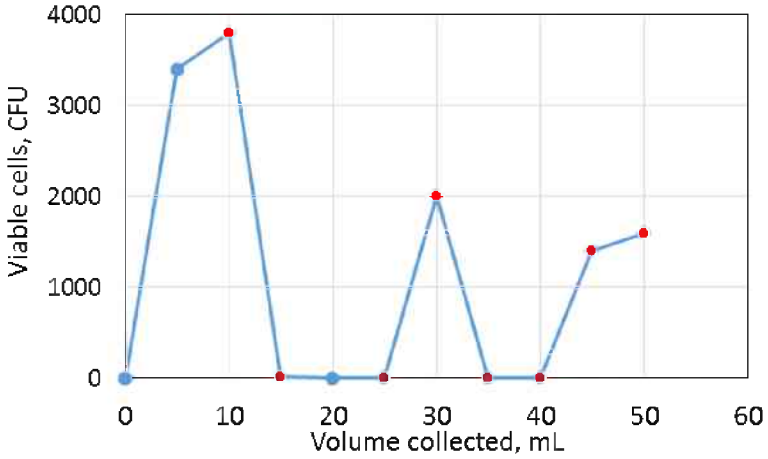

Figure B3. *Breakthrough of viable cells in Berea core 2 with permeability of 2.4 mD.*

The low permeability Torrey Buff sandstone cores exhibited similar breakthrough of viable cells, as shown in Figure B4. Though most samples did not contain effluent viable cells, the sensitive spread plating method was able to detect viable cells in at least one sample for both experiments.

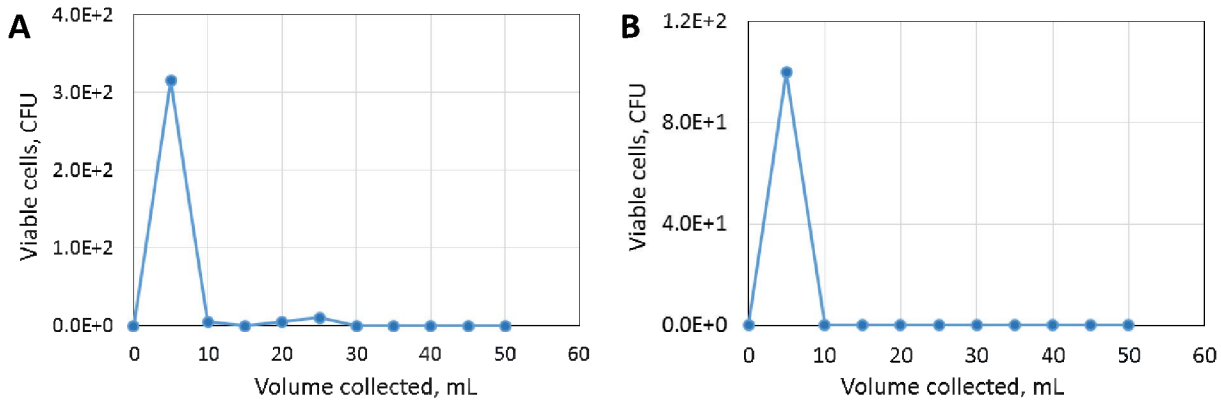

Figure B4. *Viable cell breakthrough for two Torrey Buff sandstone cores with low permeabilities of (A) 0.78 and (B) 1.4 mD.*

Total cell numbers in the inoculum and effluent samples were also determined via direct microscopic counts, which represented the presence of both viable and non-viable cells. The total number of cells detected in the core effluent for all experiments is compared to permeability in Figure B5A. The total breakthrough for the different cores range between $4x10^4$ cells to $1.3x10^7$ cells collected in the effluent, compared with 10^8-10^9 cells injected. Based on total cell counts, less than 0.1% of injected cells were transported through the 5.08 cm (2 inch) long core in all of the cores tested (Table B2). When a log-scale relationship is used the breakthrough of total cells correlates well with permeability; however, the small number of experiments suggests that more tests are needed.

The viable cells detected in the effluent for all experiments were less than the total effluent cells detected (Figure B5). The sum of viable cells transported through the cores decreased with decreasing permeability, with high variability of transport observed in cores between 0.78 and 3 mD. Regardless of this variation, viable cells were detected in at least one effluent sample for all

cores. The highest likelihood of cell detection was in the first pore volume of effluent liquid collected after each injection.

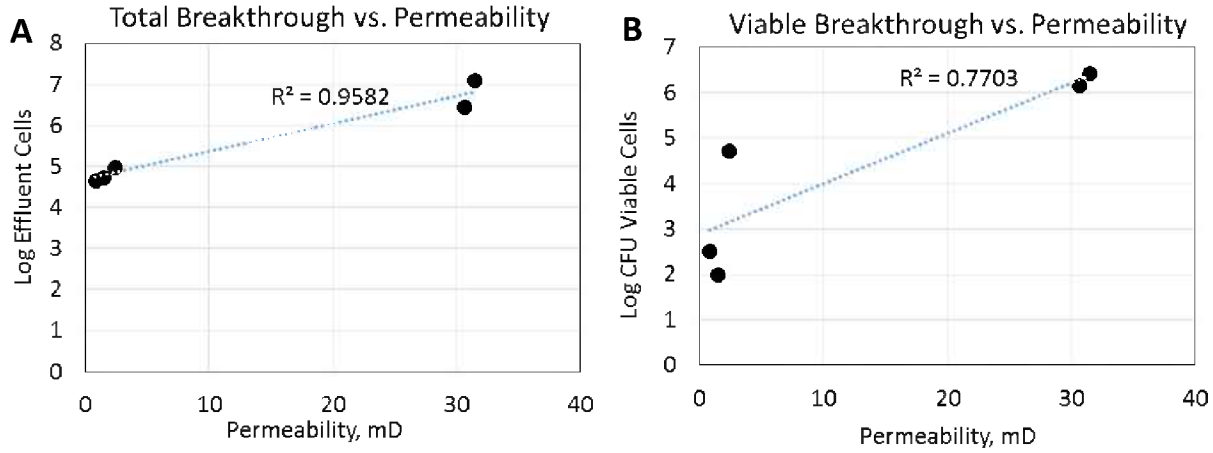

Figure B5. *Total cells transported through core in each test vs. permeability. (A) Total cells based on direct microscopic counts. (B) Viable cells transported through cores based on plate counts.*

The summary of cell breakthrough in Table B2 indicates a small percentage of both viable and total cells were transported through the cores. Test cores 1 and 3, the Berea sandstones with very similar permeability, exhibited similar viable cell breakthrough of 4.2% and 4.6% of the injected viable cells. The lower permeability Berea core (test core 2) exhibited lower fraction of viable cell transport through the core. The fraction of effluent viable cells is much lower for the Torrey Buff sandstone cores.

Table B2. *Summary of total and viable breakthrough results for microbial transport* ***experiments.***

Test core	Material	Permeability (milliDarcy)	Viable cell % breakthrough	Log reduction of viable cells	Total cell breakthrough
1	Berea Sandstone	31.4	contaminated		
2	Berea Sandstone	2.4	0.23%	2.7	0.036%
3	Berea Sandstone	30.6	4.2%	1.4	0.086%
4	Torrey Buff sandstone	1.4	0.00011%	6.0	0.017%
5	Torrey Buff sandstone	0.78	0.00044%	5.4	0.014%

The percent of total cells transported through the cores did not vary as much as viable cell counts. This indicated that although cells were able to travel through the pore spaces within the cores, they were possibly inactivated during that transport.

Mercury intrusion porosimetry analysis to determine pore size distribution has been performed with similar core materials as the Berea and Torrey Buff sandstones (Figure B6). The trendlines shown for volume intruded vs. pore diameter indicate the pore size distribution of the core sample. Porosimetry analysis indicates that Berea cores similar to test cores 1 through 3, with an estimated 30 mD permeability, contain the highest fraction of pore sizes close to 10 micrometers

(between 6-16 µm). Porosimetry analysis of the Torrey Buff sandstone indicates that the pore size distribution includes a higher fraction of smaller diameter pore sizes, between 0.1 and 5 µm. The diameter of vegetative *S. pasteurii* cells is 0.5-1.5 µm, thus pore diameters in the range detected for the sandstone samples will greatly influence the successful transport of the injected cells.

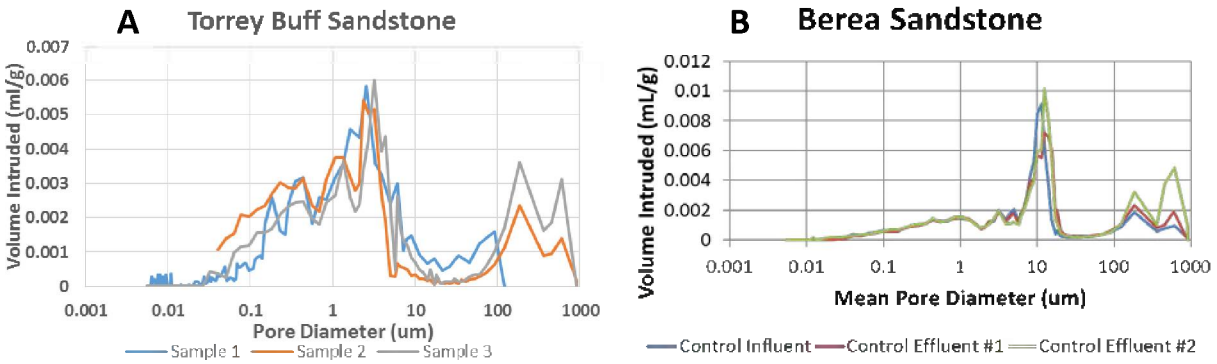

Figure B6. *Mercury intrusion porosimetry analysis of (A) Torrey Buff (~1 mD) and (B) Berea (~30 mD) samples.*

Conclusions and Future Work

Viable cells were detected in the effluent of all cores tested, down to 0.78 mD permeability. In some cases, a more sensitive plating method was required, using greater sample volume to decrease the detection limit of viable cells in the Torrey Buff effluent samples. Controls and pre-injection samples were all analyzed using the same plating methods, with no evidence of contamination. When ureolysis analyses were performed on Berea sandstone cores, ureolysis activity was detected in some, but not all, effluent samples. The extent of ureolysis detected was low, as only ~1 g/L urea was hydrolyzed overnight of the initial 20 g/L in the medium, equating to 5% hydrolysis of urea. In batch growth experiments conducted under similar conditions, growing cultures of *S. pasteurii* cells completely hydrolyze 10 g/L urea in 24 h[8]. This result supports the conclusion that the core effluent contained a low density of ureolytically active cells or enzyme.

More studies are needed to investigate the transport of bacterial cells at low permeabilities, particularly in comparison with pore size distributions of the core material. These investigations have indicated that a small fraction of bacterial *S. pasteurii* cells can travel two inches through low permeability sandstone cores. However, more studies are needed to evaluate transport of cells over greater distances in similar, low permeability geologic materials.

Additional experimental work should focus on transport of starved or sporulated cells through similar sandstone cores. Previous work in our lab developed methods to generate spore cultures of *S. pasteurii*, which may be more amenable to transport in porous media due to the absence of surface proteins and extra-cellular polymer (EPS) that promote adsorption onto solid porous media surfaces. In addition, spores may be less likely to undergo deactivation of ureolytic activity.

References

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