

VISUALIZING AND QUANTIFYING BIOMINERALIZATION IN WELLBORE
ANALOG REACTORS

by

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ABSTRACT

Subsurface fluid injection is a proposed method for the storage of hydrocarbon fuels and the mitigation of fossil fuel emissions. Concerns about leakage exist when storing fluids in the subsurface given their potential to damage functional groundwater aquifers or be emitted to the atmosphere. Defects detrimental to the integrity of subsurface storage systems can occur in and around the wellbore, thus fluid storage systems are heavily dependent on the cement surrounding the wellbore to maintain a seal.

A method proposed to seal defects in the subsurface is Microbially Induced Calcium Carbonate Precipitation (MICP). MICP is a technique that uses low viscosity fluids and microorganisms ($\sim 2 \mu\text{m}$ diameter) to seal defects troublesome to subsurface fluid storage. In the MICP process, microorganisms such as *Sporosarcina pasteurii* that contain the enzyme urease catalyze the hydrolysis of urea to produce ammonium and carbonate species. When this process occurs in the presence of dissolved calcium, calcium carbonate may precipitate.

To study MICP in defects common to the wellbore, two reactors systems were created. The first was constructed to mimic the geometry of the wellbore and allowed the visual observation of MICP formation. The second quantified MICP in a cement channel defect using X-ray computed microtomography. A reduction in apparent permeability and void fraction was observed in both systems, demonstrating the ability of MICP to restrict fluid flow in defects common to the wellbore. Observations made during these experiments will aid in improving the safety and efficacy of subsurface fluid storage systems

CHAPTER ONE

INTRODUCTION

Subsurface Leakage Potential

Subsurface fluid injection is a proposed method for the storage of hydrocarbon fuels and the mitigation of fossil fuel emissions to the atmosphere (Bai 2014, 2016). Concerns about fluid leakage are present when storing fluids (CO₂, H₂, natural gas) in the subsurface given their potential to damage functional groundwater aquifers or be emitted to the atmosphere (Dusseault et al. 2000, Bello 2014, Carroll 2014). The risk of leakage in storage systems is heavily dependent on the ability of the well cement to maintain a seal against subsurface fluids (Bai 2016, Carey et al. 2010, Watson and Bachu 2009, Dusseault 2000, Opedal et al. 2014).

The wells that are used to access the subsurface are typically made up of a steel casing nested inside a borehole drilled into the rock formation (Figure 1).

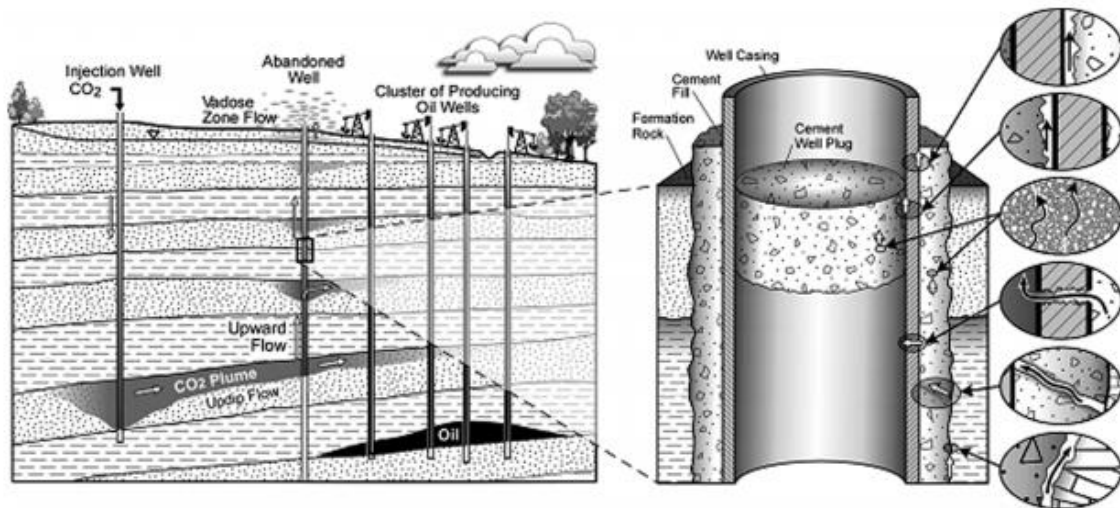


Figure 1: Representation of pathways for fluid (CO₂, natural gas) migration and possible leakage in the near wellbore environment. Leakage pathways may form in the subsurface, for example, between the casing and the cement, the cement and the formation, or through the cement (right) Reprinted with permission from Nordbotten JN, Kavetski D, Celia, MA, Bachu S. "Model for CO₂ Leakage Including Multiple Geological Layers and Multiple Leaky Wells." *Environmental Science and Technology*, vol. 43, no. 3, 2009, pp. 743-749. Copyright 2008 American Chemical Society.

A borehole is drilled through the subsurface rock formations. These formations typically make up the outer wall of the well, in some cases, a second larger steel casing is inserted to provide structural strength to the well and to isolate subsurface fluids from the surface. This casing is known as a surface casing. During installation of the casing, cement is pumped down through the inner casing to the bottom of the well and then forced up into the annular space between the inner casing and the formation or surface casing (Dusseault 2000). This cement is meant to hold the casing(s) in place and stop fluid migration between subsurface formations or to the surface, also known as zonal isolation.

Defects in the Wellbore Environment

Defects in the well may occur as a result of operational factors and physical stresses in the subsurface during and after installation of the well. Operational factors of installation, such as an elevated water content in the cement slurry or insufficient volumes of cement injected, can create areas of shrinkage or voids in the cement annulus (Dusseault 2000). During installation of the well, in order to be considered a satisfactory cement job, a return of the cement to the surface must be seen to confirm the annulus space was filled. In some cases, cement may return to the surface but may not have been evenly distributed around the well. For example, the inner casing in these systems can shift during installation, causing the casing to be unevenly spaced from the walls of the well. This can lead to pockets in the annular space of little to no cement, leading to potential fluid migration pathways (Jim Kirksey, personal communication). Physical stresses, such as geological shifts and thermal expansion or contraction have been tied to the formation of defects, which could also lead to potential leakage pathways (Watson and Bachu 2009, Dusseault 2000, Opedal et al. 2014). Bello et al. (2012) examined thermal cycling in wells and showed that increased apparent permeability and porosity can occur as a function of the number of thermal cycles experienced by a well. Models and experimental analogs have been developed to assess the risk of defect formation at interfaces and within the body of the annular cement (Carey et al. 2009, 2010; Opedal 2013). Loizzo et al. (2011) highlighted four classes of defects that have potential to compromise seal integrity (Table 1).

Table 1: Primary Defects Observed in Wellbore Cement

Defect Type	Cause	Characteristics
Mud Channels/Fluid Channels	Oil/Water based mud or fluid not replaced by cement during installation of well	Pockets of open space
Chimneys	Pressurized formation fluid/gas pushes through cement during curing	Thin channel defects
Microannuli	Mechanical failure of cement (interface or interior bonding)	Debonding/fissures
No Cement	Insufficient cement injected/loss of cement to the formation	Pockets of no cement in the annular space

The occurrence of interface defects between the cement and casing, or cement and formation, are a great risk to seal integrity. Examples of conditions that can result in interface defects are the presence of residual drilling mud, excess water in the cement paste, variable temperatures (thermal cycling), or mechanical stresses (pressure cycling) in the wellbore (Dussealt 2000, Um et al. 2014, Opedal 2014, Bai et al. 2016, Gu et al. 2016). Dussealt (2000) showed that cement shrinkage during curing and changes in stress within the subsurface can create defects at interfaces between the casing and cement. Opedal et al. (2014) investigated the bonding effects of residual drilling mud on commonly occurring rock formations in oil-bearing zones such as shale and sandstone. In this case, both water-based and oil-based drilling muds were correlated to an increase in open space at the interface between the cement and rock. Li et al. (2016) studied the impact of the confining pressure on well cement. They found that for wells under low confining pressures (~ 20 MPa), mechanical defects such as radial cracking through the

cement body occurred, but under high confining pressures (~ 70 MPa) microannuli defects at interfaces (i.e. surface debonding) were observed.

The formation of defects not only allow pathways for fluid migration but also, in the case of subsurface carbon storage, lends itself to the possibility of carbonic acid corroding well materials. (Kutchko 2008, 2009; Carey 2007, 2010; Huerta et al. 2013, 2016). Kutchko et al. (2008) investigated the degradation of well-cement cores by carbonic acid attack. This research showed that a reaction front can occur at the interface between the brine and cement, potentially leading to pathways that can act as conduits for fluid migration. Huerta et al. (2016) also researched the degradation of well cement as a result of acid attack. Results of these studies showed that as a reaction front occurs at the cement acid interface mineral precipitation can occur simultaneously and create restrictions the fluid flow paths downstream. Carey et al. (2010) investigated the impact of CO_2 saturated brine flow along a cement-steel interface. Results indicated that, while some degradation of the well cement occurred as a result of carbonic acid attack, the cement remained capable of preventing significant fluid flow.

Discharge of fluids due to leakage pathway(s) in the annular cement can reduce the efficacy of subsurface fluid storage technologies or compromise the integrity of oil and gas operations (Watson and Bachu 2009, Carrol 2016; Dussealt 2000). Current technologies to seal leakage pathways in the annular space surrounding the well include the use of cement or resins (Todorovic et al. 2016). Large defects can be sealed by injecting cement into the annular space, known as a “squeeze job.” (Todorovic et al. 2016). Limitations to this approach exist in small aperture defects, since cement slurries

are viscous and may require greater pumping pressures to inject. Fluid migration in small defects may be of small concern when storing high viscosity fluids such as crude oil. Small defects do pose a greater risk of fluid migration when attempting to store low viscosity fluids such as vapor phase hydrocarbons or gaseous CO₂. A proposed method to seal small aperture defects in the wellbore environment is the use of microbes and low viscosity fluids that promote the formation of a biocement (microbially induced calcite precipitation or MICP).

Microbially Induced Calcium Carbonate Precipitation

MICP has been proposed for a variety of engineering applications such as suppressing dust, sealing ponds or reservoirs, mitigating wellbore leakage and enhanced oil recovery (Phillips 2013a). Many studies have proposed or implemented treatment of the subsurface using MICP to restrict fluid flow (Cunningham et al. 2011, 2014; Phillips et al. 2013a, 2013b, 2015, 2016).

MICP has been showed to occur by multiple pathways such as ureolysis sulfate reduction, and photosynthesis. (Hammes & Verstraete 2002, Phillips 2013a). The work discussed in this thesis will focus on ureolysis-driven MICP. Ureolysis driven MICP utilizes the enzyme urease, produced by microbes, to hydrolyze urea, generating ammonia (NH₃) and carbonic acid (H₂CO₃), (Eq 1-2). A series of acid base reactions then equilibrate the carbonic acid into carbonate (CO₃²⁻), which in the presence of calcium can precipitate as the mineral calcium carbonate (CaCO₃), (Eq 3-5).



Microbes influence MICP two ways (1) altering the chemical state of the fluids surrounding the microbial community and (2) encouraging passive precipitation through the presence of cells (Phillips et al. 2013a, Stocks-Fischer 1999). Ureolysis driven MICP is effected by four conditions: (1) calcium concentration (2) dissolved inorganic carbon concentration (3) pH and (4) the availability of nucleation sites (Hammes & Verstraete 2002). The first three affect the saturation of dissolved calcium carbonate through the manipulation of the aqueous chemistry surrounding the cell. The precipitation of minerals results from the saturation of dissolved minerals. The saturation of calcium carbonate in solution can be described by Equation 6.

$$S = \frac{[Ca^{2+}][CO_3^{2-}]}{K_{so}} \quad \text{Eq 6}$$

In Equation 6, S is the saturation state of calcium carbonate, $[Ca^{2+}]$ and $[CO_3^{2-}]$ are the activities of the calcium and carbonate ions respectively, and K_{so} is the solubility constant of calcium carbonate. (Phillips 2013a, Benjamin 2015). Based upon Equation 6, an increase in calcium or carbonate species will increase the saturation state of solid calcium carbonate. Once the value of S becomes greater than 1 conditions are favorable for calcium carbonate precipitation. The injection of calcium ions in addition to the

production of carbonate species via ureolysis increases saturation conditions of the solution, which can lead to precipitation of calcium carbonate. As the pH of solution increases, a result of ammonia production during the MICP process, the concentration of bicarbonate and carbonate ions can increase. This is due to the shift in the carbonate buffering system. After precipitation of CaCO_3 from solution, the pH of the solution is reduced (Benjamin 2015).

The balance of fluid injection rates and reaction (precipitation) is a parameter that must be managed in order to form a homogenous layer of biocement throughout the length of the defect. The observation of heterogeneous calcium carbonate distribution as a result of MICP has been observed in previous experiments (Mortensen 2011, Phillips 2013b, Whiffin 2017). Mortensen (2011) correlated a larger precipitation gradient along the length of column with a slower fluid injection rate. A dimensionless number used to quantify the balance between reaction and transport is known as the Damkohler number (Da), (Equation 7), (Phillips 2013a).

$$Da = \frac{\text{Reaction Rate}}{\text{Transport Rate}} \quad \text{Eq 7}$$

During the injection of calcium containing solutions to a defect, the transport of fluids dominates the system leading to a low value for Da. If fluid injection is halted and a period of time without flow reaction dominates the system and the value of Da goes to infinity, By manipulating the rate of fluid injection, the Da value during flow periods can be minimized, limiting entry point plugging as a result of precipitation during injection.

This will encourage a more even distribution of precipitation throughout the defect, creating a better biocement seal.

It has also been proposed that cells act as nucleation sites for the mineral, leading to localized precipitation around the community of cells attached to the surface of the defect, known as a biofilm (Stocks-Fischer 1999, De Myunk et al. 2008). The biofilm as a mineral template makes MICP advantageous for use in the wellbore sealing.

Sporosarcina pasteurii, a urease producing microbe, is approximately 2 μm in length, allowing cells to enter small defects in the wellbore which would be difficult to seal with cement slurry injection. The localized precipitation around the biofilm can help to concentrate precipitation into defects, creating a seal capable of halting fluid flow along the wellbore in subsurface fluid storage systems. (Figure 2).

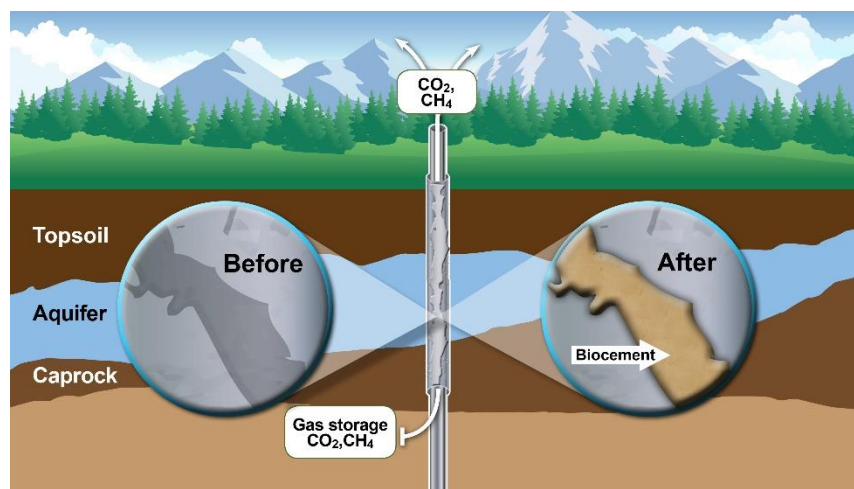


Figure 2: An illustration of MICP formation in a wellbore defect. The resulting mineral seal can mitigate fluid leakage to functional aquifers or the atmosphere.

The experiments, summarized within, utilize the ureolytic bacterium *Sporosarcina pasteurii*, to facilitate MICP. The objective of this study was to visualize

and quantify the restriction in fluid flow as a result of MICP in cement and interface defects. To do this, two novel reactor systems were designed and constructed.

In Chapter 2, MICP sealing of interface defects was visualized in a specially designed wellbore analog constructed of clear polycarbonate. The reactor was designed to mimic the subsurface geometry that would be encountered in the wellbore. The clear polycarbonate allowed for observation of MICP formation in defects, a method of study not available to researchers during field testing. By observing MICP in these defects, an understanding of how and where biomineralization forms in defects could be studied and incorporated into future applications of MICP in the wellbore.

In Chapter 3, changes in void fraction of a body defect due to MICP treatment were quantified using X-ray computed microtomography (μ -CT). CT instruments are capable of capturing three-dimensional images of the pore space within an object both non-invasively and non-destructively (Cnuddle et al. 2012, Wildenschild et al. 2012, Ittis 2013). For the investigation of MICP formation in well cement, μ -CT provides a means to quantify the change in void fraction both spatially and temporally. In CT, images are generated by mapping the attenuation of an X-ray signal as it passes through an object, a value which is correlated to the composition and density of a material (Jung 2013, Bray 2016, Wildenschild 2012). X-ray CT has been used previously to assess void fraction in cement and concrete samples (Manahilloh et al. 2012, Jung et al. 2013, Han and Sun, 2012, Jung 2013, Fukuda, 2014, Fan and Li, 2014).

CHAPTER TWO

VISUALIZING MICP FORMATION IN A WELLBORE ANALOG

Materials and MethodsReactor Design and Configuration

Previously successful demonstrations have been performed to seal fractures and channeled cement in a field test well; however, there are few ways to visually observe the MICP formation in the field (Phillips et al. 2016, 2017). To better understand how and where MICP formation occurs, the Wellbore Analog Reactor (WBR) was designed and constructed to investigate MICP sealing along the cement-casing interface (Figure 3).

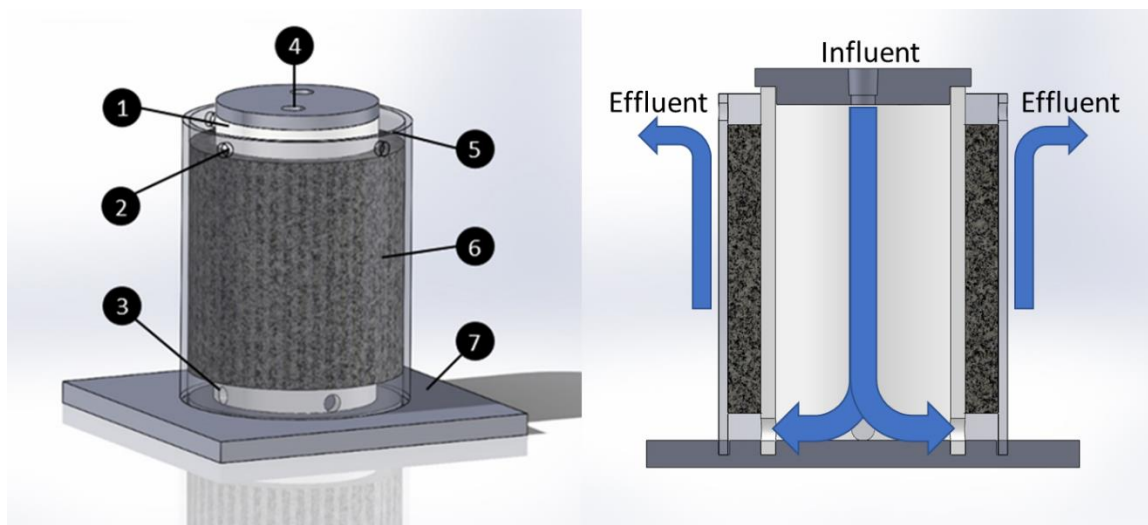


Figure 3: Left: Computerized drawing of the Wellbore Analog Reactor (WBR) created in SolidWorks (1) inner casing (2) effluent fluid ports (3) casing perforation mimicking holes drilled in the inner casing to allow fluids from the inner casing to reach the annulus space (4) injection port (5) clear polycarbonate outer casing for visualizing the mineral formation (6) cement annulus with engineered defects, for example, gaps between the cement and outer casing or channels cut into the cement (7) base plate. Right: Cross sectional image of WBR indicating the flow path which fluids take through the reactor.

The goal of the WBR was to mimic the geometry and flow characteristics of wells commonly used for the injection or extraction of fluids from the subsurface. The WBR was constructed to be 12.7 cm in height by 10.2 cm in diameter. In the annular space between the two casings, wellbore cement was cast. Engineered defects were either cut into the cement or prepared during casting. Visual observation of MICP during experiments was considered to be a highly desired attribute of the WBR, thus clear polycarbonate was selected as the outer casing material. The inner casing was constructed of PVC or metal.

Two experiments were performed in the WBR. Analog Experiment 1 (AE1) investigated the formation of MICP in an interface delamination defect, while Analog Experiment 2 (AE2) investigated a channel defect. The defect for both experiments was placed at the interface of the cement and clear polycarbonate so that MICP formation could be observed. For both experiments, the inner diameter of the polycarbonate casing was 9.40 cm. For AE1, the inner casing was constructed of PVC while AE2 was constructed with a low-carbon steel. The casing in AE2 was also shifted 0.51 cm off center to mimic a condition in the field where the inner steel casing of a well can become shifted during installation, potentially creating leakage pathways for fluids (see “Subsurface Leakage Potential,” Ch. 1) (Table 2).

Table 2: Inner Casing Parameters

Experiment	ID (cm)	Volume (mL)	Material of Construction	Alignment
AE1	6.15	380	PVC	Centered
AE2	6.65	90	1040 Carbon Steel	Off-center (0.51 cm)

Fluid Injection and Data Acquisition

Fluids were injected (Teledyne ISCO 1000D or Cole Palmer Model 270 syringe pump) through the inlet port fitting at the top of the inner casing. The fluids flowed down through the inner casing, out the perforations and up the interface between the outer casing and the cement. For both experiments, the fluid injection rate was set to achieve a 30-minute residence time within the reactor. This flowrate was maintained until the pressure in the reactor rose, requiring a reduced flowrate to avoid over pressurization of the reactor. Pressure measurements were taken using Omega Engineering Inc. PX309 series pressure transducers. Flow rate monitoring was performed continuously through the pump control operating software (LabVIEW, National Instruments).

Wellbore Cement Preparation

The annular space cement (Schlumberger Limited) was prepared using a cement congruent with what was used in the field at the Gorgas #1 well as described in Phillips et al. (2016). The cement was a blend of equal parts Class H cement and pozzolan additives with an additional 6% by weight D020 bentonite added (Jim Kirksey, personal communication). To prepare the cement slurry, water was added to the Class H cement to create a 0.4 water to cement ratio by mass (400 g water, 1000 g cement) mixture. The slurry was mixed in a blender for approximately 1 minute before pouring into the WBR for curing (Figure 4).



Figure 4: Cement casing in the wellbore mold during (left) and after (right) curing.

The WBR reactor was Teflon-foil lined along the circumference of the outer casing to: (1) allow the cast to be easily removed after curing and (2) create the desired defect mimicking a debonding defect between the casing and cement. The cement was allowed to cure in the cast for five days then was removed and immersed into water saturated with calcium hydroxide to cure for an additional 14 days at room temperature.

Defect Geometry

In AE1, the defect was formed by using a 10 mil (254 μm) Teflon liner to create a delamination along the entirety of the cement-polycarbonate interface, dimensions of this defect can be seen in Table 3.

Table 3: AE1 Defect Size and Classification

Experiment	Width (mm)	Length (mm)	Depth (mm)	Defect Classification
AE1	293	92.2	0.25	Delamination

In Table 3, depth describes the distance between the casing and cement and length is the distance that the fluid would travel within the defect. Depth and width when multiplied make up the cross-sectional area of the defect perpendicular to the direction of flow.

In AE2 the reactor was lined with a 4 mil Teflon foil, and following the 14-day curing time, a Dremel cutting tool was used to create a channel defect in the cement at the cement-polycarbonate interface (Figure 5).

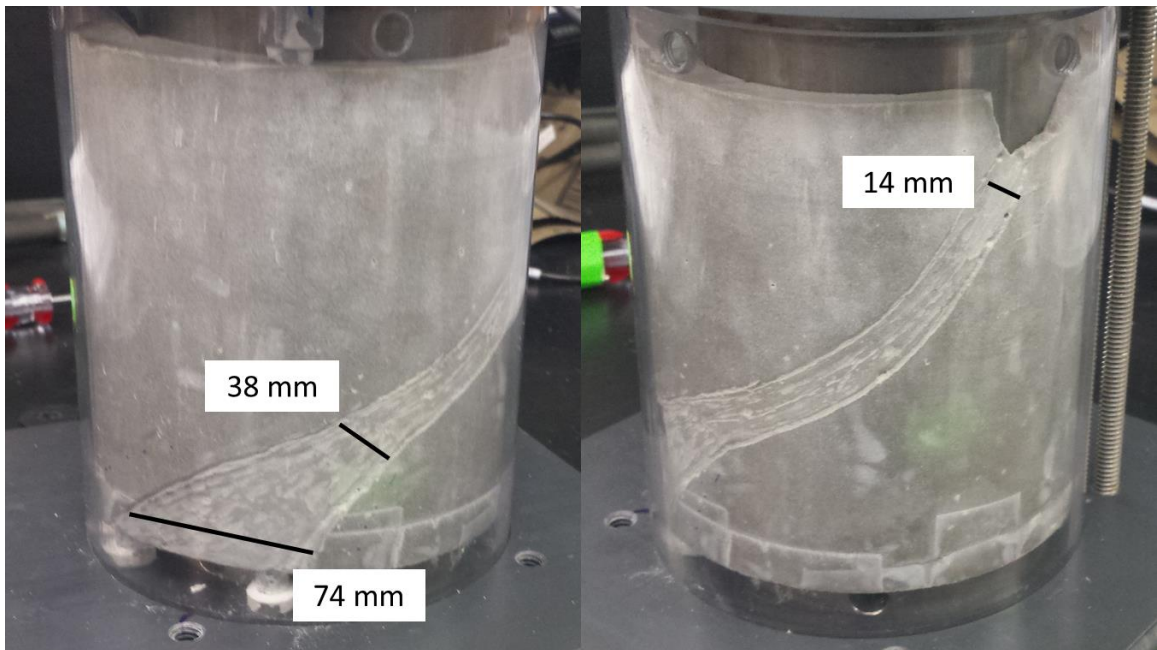


Figure 5: Channel defect for AE2. The defect was approximately 74 mm at the entrance region and tapering in width and depth along the length of the defect to an approximate width of 14 mm at the top of the cement casting.

This defect in AE2 changed in both width and depth along the direction of flow. This defect began at an approximate width and depth of 74 x 3 mm at the entry region to and tapered in both width and depth along the length of the defect. The defect traveled the length of the cement-polycarbonate interface until it reached the top of the cement.

The width of the channel at the top of the cast was approximately 14 mm. As this defect was made using a hand tool, variability in the dimensions and surface roughness of the defect are evident. The purpose of this defect was to observe how biomineralization would occur in a tortuous path through the annular space, similar to a channel style defect in a wellbore.

Fluids

The WBR experiments used three different solutions to promote MICP: (1) an inoculum which was the source of the ureolytic bacterium *Sporosarcina pasteurii*, (2) a nutrient solution to stimulate the growth of the bacteria, and (3) a source of calcium to induce the precipitation of calcium carbonate within the reactor. The growth and mineralization medias are shown in Table 4. Microbes were grown in the growth solution prior to injection (Table 4).

Table 4: WBR Experimental Media Recipes

YE- (Growth media)	Urea (Potash Brand) Sodium Chloride (Morton) Ammonium Chloride (BASF) Yeast Extract (Acros)	24 g/L (0.40M) 24 g/L (0.41M) 1 g/L (0.02M) 1 g/L
YE+ (Mineralization Media)	Urea (Potash Brand) Sodium Chloride (Morton) Ammonium Chloride (BASF) Yeast Extract (Acros) CaCl ₂ *2H ₂ O (Peladow)	24g/L (0.40M) 24 g/L (0.41M) 1 g/L (0.02M) 1 g/L 13.3 g/L Ca ²⁺ (0.33M)
Brine rinse solution	Sodium Chloride (Morton)	24 g/L (0.41M)

The YE solutions were used to promote growth of microbial cultures (YE-) or stimulate mineral precipitation (YE+). All solutions were made directly prior to their use

by mixing the chemicals in a water filled flask. A sample from each solution was taken prior to injection for testing.

Inoculum Preparation. Microbes were grown by adding 1 mL of *Sporosarcina pasteurii* (ATCC 11859) thawed frozen stock to 100 mL of autoclaved brain heart infusion (Becton Dickinson) solution (37 g/L) amended with 2% by weight urea. The organisms incubated at 30°C on a shaker at 150 rpm for 16 hours. After the incubation period, the culture was transferred to fresh YE- media at a ratio of 1 mL of culture per 100 mL of YE- media. The transferred culture was then incubated at room temperature for 24 hours prior to its use as the inoculum for the reactor. For all subsequent days following the first frozen stock culture, an aliquot of the 24-hour culture was used to inoculate fresh YE- in place of the frozen stock.

Injection Strategy

Inoculation of the reactor was performed on the first day of all MICP treatment experiments. Two reactor fluid volumes of inoculum were injected, after which a 4-hour stationary period (no flow) occurred to allow attachment of the microbes. Following the 4-hour stationary period, two fluid volumes of YE- were injected to promote microbial growth followed by an overnight (no-flow) batch period. On all days following the reactor inoculation, four calcium pulses of YE+ were injected with 90-minute stationary periods between each YE+ injection. An injection of two reactor fluid volumes of brine rinse solution was performed when transitioning to or from calcium containing media. The purpose of the brine rinse was to minimize instantaneous precipitation within the

inner casing. The reactor was re-inoculated with *S. pasteurii* at the end of the day and allowed to sit overnight prior to re-starting calcium injections.

Fluid Sampling Procedure

Influent samples were taken on all days for each unique fluid injected. For all days where calcium media was injected, effluent samples were taken at the beginning of each pulse. A volume of one mL was syringe filtered (VWR 0.2 μ m cellulose filter) and used for urea quantification.

Jung Urea Assay

All urea analysis was performed using a colorimetric absorbance based plate assay adapted from Jung et al. 1975 (as presented in Phillips et al. 2013c). Absorbance measurements were taken at 505 nm by a BIOTEK Synergy HT plate reader and the sample absorbances were compared to the absorbances of standards with known urea concentrations.

Imaging

In preparation for microscopy a section of the channel defect from AE2 was cut from the reactor using a diamond blade Dremel cutting tool. The section selected was a large formation of biomineralization approximately 20 cm from the entrance region of the channel defect. The polycarbonate outer casing was then removed from the cement-polycarbonate interface, leaving behind the wellbore cement and MICP for imaging. Some samples were washed with acid to image cells encapsulated by the mineral

deposition with electron microscopy. These samples were treated with 1% hydrochloric acid for 30 to 60 seconds, rinsed with deionized water, and left to dry before imaging.

Light microscopy images were taken using a Leica Model MDG41 Stereomicroscope at MSU's Center for Biofilm Engineering Microscopy Facility. Electron microscopy imaging was performed using a Supra 55VP Field Emission Scanning Electron Microscope manufactured by Carl Zeiss AG in the Image and Chemical Analysis Laboratory at MSU. Samples were coated with iridium at 20 mA for 30 seconds prior to imaging at 1.0 kV.

Results and Discussion

MICP Formation

The formation of calcium carbonate in both experiments was observed to be very slow during the first half of MICP treatment. While a reduction in apparent permeability was noted on the second day of calcium injection during AE1, MICP was visually observed to be more prevalent on the third day of calcium injection. In AE2, MICP formation became visually noticeable during the fourth day of fluid injection and the amount of deposited calcium carbonate increased rapidly following the fourth day (Figure 6).

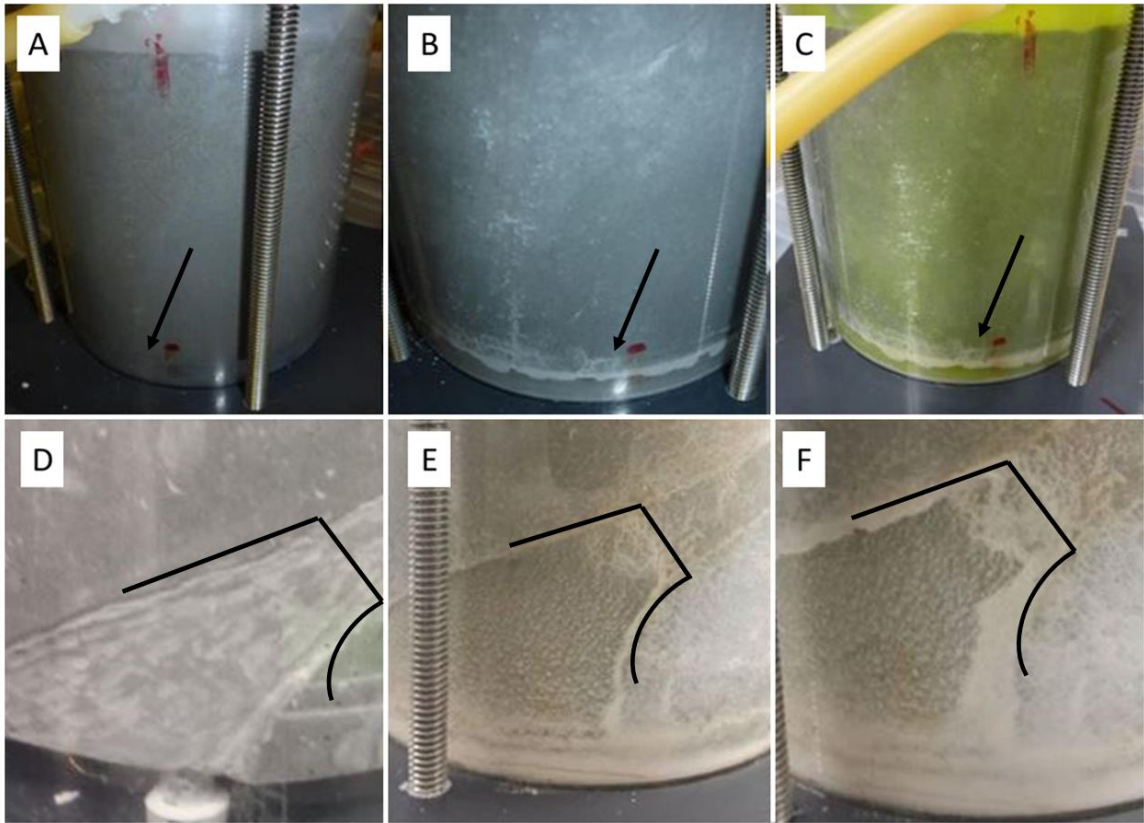


Figure 6. Calcium carbonate formation as a result of MICP treatment. A-C: Calcium carbonate formation at entrance region of 250-micron delamination in AE1. D-F: Calcium carbonate formation in channel defect constriction point during AE2. Note: The green tint C is a result of residual fluorescein tracer dye from earlier flow path tests, not a product of the microbial community. Lines and arrows were drawn to indicate areas of interest.

The majority of the MICP in AE1 formed within the entry region of the delamination defect, approximately one inch from where the fluids entered the reactor. MICP formation in the channel defect of AE2 formed at the edges of the channel first, indicated by black lines in Figure 6, then growing across the defect, and later forming a biocement plug in the channel.

Following the termination of each experiment, the reactors were deconstructed and examined. While the mineral deposit in AE1 appeared to be a solid during the experiment, it was observed to be extremely thin and brittle once the sample had dried.

While a downhole system would most likely remain wetted, these characteristics of the barrier do raise concern in regards to the integrity of the seal. Observations differed greatly for the mineral formed during AE2, where the biocement appeared solid after drying. This was confirmed when, in preparation for microscopy, the biocement was sectioned using a diamond blade dremel tool. During this process, the biocement remained intact throughout.

It was observed in both experiments that once initial calcium carbonate formation occurred in the defects, secondary deposits formed in areas surrounding these initial deposits. Once MICP formation occurred in primary defects, biomineralization began to occur in other areas of the reactor. While this observation may be obvious when considering the behavior of fluids, it does have implications for potential injection strategies if utilizing MICP in a field well. With numerous factors affecting well cement (shear forces, temperature cycling, corrosive fluids, etc.) the occurrence of complex defects comprised of multiple, hydraulically connected, smaller defects is a possibility. The observations made during these experiments suggest that if such a defect were to be encountered in a downhole system, MICP would first form in the larger primary flow paths and then in secondary flow paths. Based upon this, it is suggested that extending the runtime of the treatment would lead to a more robust seal by the formation of a biocement barrier in the smaller secondary defects in addition to the primary flow paths.

Post Experiment Imaging

Imaging was performed on the mineral formed in the channel defect of AE2. Light microscopy and Field Emission Scanning Electron Microscopy (FE-SEM) was

used to produce high resolution images of this formation, which was hypothesized to have created the flow restriction (Figure 7).

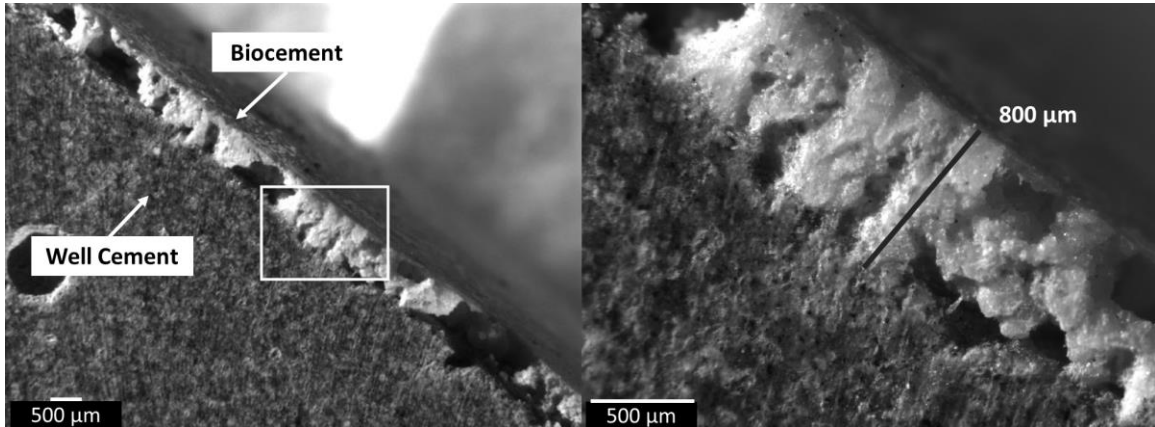


Figure 7. Light microscopy of AE2 biocement Left: Image taken of an approximately 800 μm thick layer of biocement bonded to the cement annulus at 7.81x magnification. Right: Framed portion of the left image viewed at 28.3x magnification showing the bond between the mineral formation and the cement.

From Figure 7, the layer of biocement was approximated to be 800 microns in thickness. When examined closely, the biocement and wellbore cement appeared to be bonded together, indicating attachment of the biocement to the wellbore cement rather than a superficial deposition of mineral precipitates. Images at greater magnification were performed using FE-SEM (Figure 8).

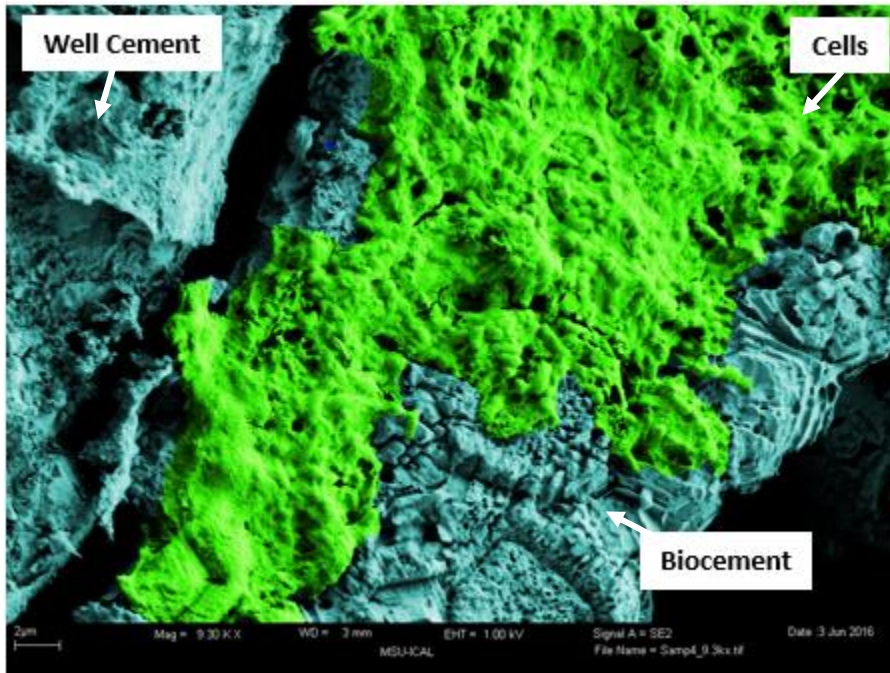


Figure 8: Bacilli (rod) shaped bacteria (false colored green) assumed to be *Sporosarcina pasteurii* observed in close proximity to the cement-biocement interface of the MICP seal in AE2. The cement appears on the left-hand side of the image while the calcium carbonate makes up the right-hand side of the interface.

Initial attempts to image the interface between the biocement and wellbore cement showed little sign of microorganisms, hypothesized to be a result of entombment during the experiment. A brief acid wash however produced an image where rod shaped cells consistent with the dimensions of *S. pasteurii* were observed. In Figure 8, a biofilm (false colored green) can be seen on the biocement side of the interface of the biocement and wellbore cement. Observing cells inside the calcium carbonate layers provided evidence that cells are associated with precipitation of calcium carbonate and therefore may have an influence on where precipitation occurs. Stocks-Fischer (1999) observed *S. pasteurii* (*Bacillus pasteurii*) acting as nucleation sites for the precipitation of calcite crystals. The hypothesis of cells acting as nucleation sites in the case of the WBR cannot

be proved or disproved however, as specific analysis of this phenomena was not performed.

Apparent permeability

Flow rate and differential pressure measurements for both experiments were collected for the final 60 seconds of each injection. This data was then used to calculate the apparent permeability of the constructed defects. All apparent permeability values were calculated using Darcy's law (Zimmerman and Bodvarsson, 1996), under the assumption that the defects could be modeled as a porous media (Equation 8).

$$k = \frac{Q}{A} * \frac{\Delta L}{\Delta P} * \mu \quad \text{Eq 8}$$

In Equation 8, k is the apparent fracture permeability, Q is the volumetric flow rate, A is the cross-sectional area of the defect tangential to flow, ΔL is the length of the defect, ΔP is the pressure drop across the reactor, and μ is the dynamic viscosity of the fluid. The dimensions used for AE1 are the approximate casting dimensions of the delamination (Table 3).

The dimensions of AE2 were chosen to be a section of the channel defect where the large formation of biocement was observed (Figure 9).

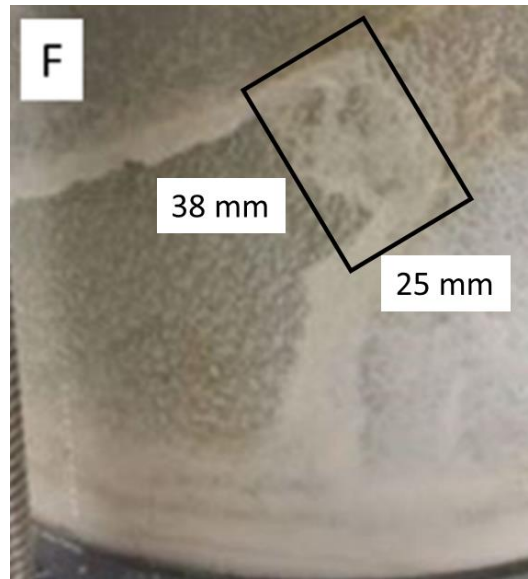


Figure 9: Representation the bio cement plug dimensions used for apparent permeability calculations in AE2.

The width and length of the section of bio cement in AE2 were physically measured following the completion of the experiment. The defect depth was approximated from light microscopy images taken of a cross section of the bio cement plug. While these dimensions do not represent the entire defect, the complex geometry of the channel made determining dimensions representative of the entire defect difficult. Apparent permeability calculations using these dimensions still provide a measure of the flowrate restriction as this is where the majority of the biomineralization, occurred during AE2.

The apparent permeability decreased in both experiments as a function of the number of calcium pulses delivered (Figure 10).

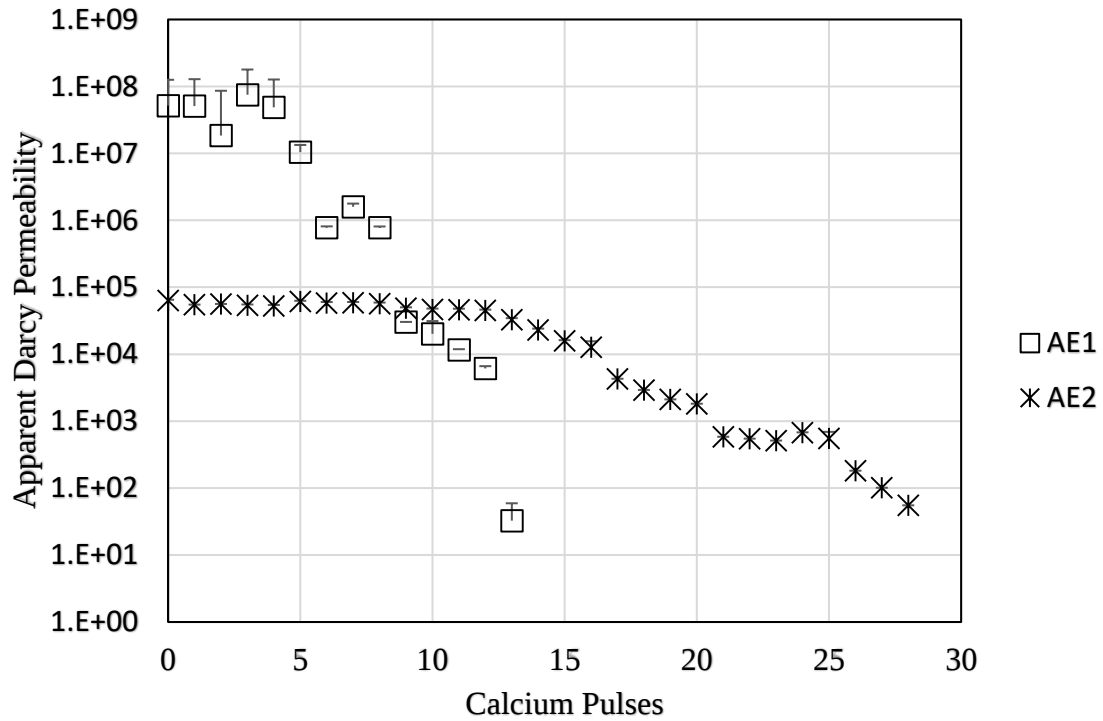


Figure 10: Apparent Darcy permeability of AE1 and AE2 as a function of calcium pulses delivered. Only positive error bars are depicted since negative error bars in a log plot will appear to extend below the x-axis and therefore cannot be plotted.

The final apparent permeability of the defects in AE1 and AE2 were calculated to be 33 and 55 millidarcy (mD) respectively. While a difference in the initial apparent permeability was noted, it is important to understand that the defects were of different geometries and experienced different flowrates in each respective experiment. This will alter parameters in Equation 8, leading to different calculated values for apparent permeability. As these are apparent permeability measurements and not absolute, the overall trend of decreasing apparent permeability is the most important observation in Figure 10. The decrease in apparent permeability observed shows that over the course of the experiment it became more difficult for fluids to travel through the defects, indicating that biomineralization altered fluid flow in these defects.

An attempt to determine aperture size was made using Cubic's law for fracture flow (Equation 9) (Zimmerman and Bodvarsson, 1996).

$$b = \left(\frac{12 * \mu * Q * L}{w * \Delta P} \right)^{\frac{1}{3}} \quad \text{Equation 9}$$

In Equation 9, b is the aperture depth, Q is the volumetric flow rate, L is the fracture length, w is the aperture width, μ is the dynamic viscosity of the fluid, and ΔP is the pressure drop across the reactor. The initial aperture size for AE1 and AE2 were known to be 250 and 800 μm , respectively from casting conditions and microscopy imaging. Cubic's law approximations for the final aperture size in AE1 and AE2 were 0.004 mm and 0.009 mm, correlating to a 98.3% and 98.9% reduction in aperture size respectively.

It is hypothesized that the difference in time required to seal the defect during each experiment was a result of the defect depth. The formation of MICP requires the growth of the seal from the cement surface. Assuming similar rates of MICP growth, the discrepancy in depth of 250 vs. 800 μm for AE1 and AE2 may have caused the extended time required to seal AE2 as compared to AE1. A reactor focused specifically on the comparison of different aperture sizes would be required to determine the effect that a changing aperture size has on MICP formation.

Ureolysis

Throughout the experiment, urea concentration was the metric used to quantify microbial activity within the reactor. The concentration of urea remaining inside the

reactor after each batch period was determined and plotted as the ratio of effluent urea concentration to influent urea concentration as a function of the fluid pulse from which those fluids were injected (Figure 11). Influent concentrations were measured at the beginning of each day and effluent concentrations were measured at the beginning of each fluid pulse.

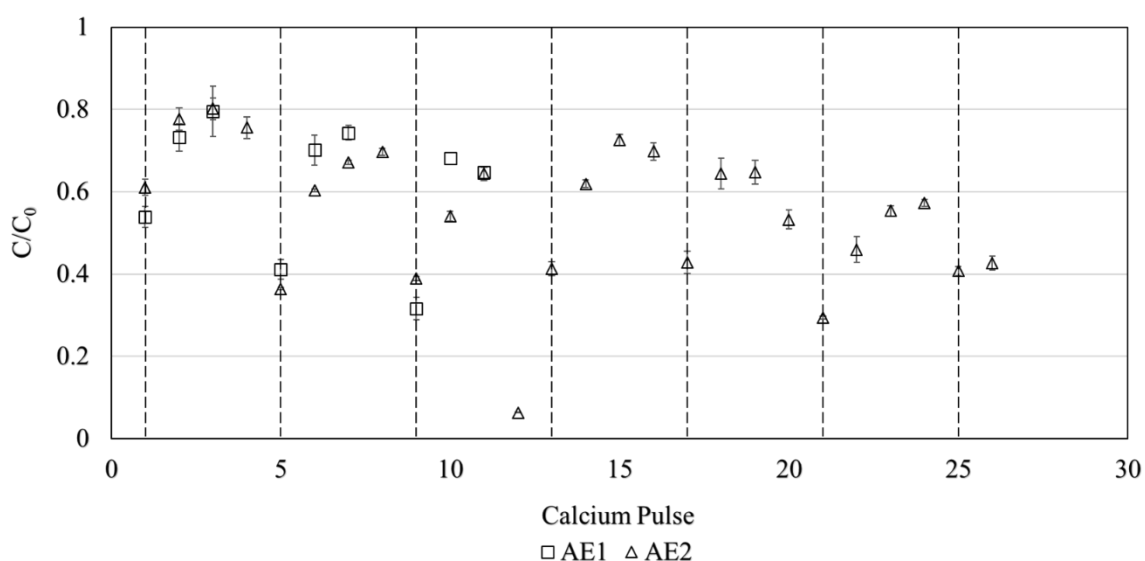


Figure 11: Ratio of effluent to influent urea concentration as a function of calcium pulse for AE1 and AE2. Dashed vertical lines indicate the first pulse of each day.

Overall, the effluent to influent ratio was observed to increase throughout each day as the number of calcium pulses delivered increased. After nightly inoculation of the reactor, a decrease in the ratio was seen during the first pulse of the subsequent day. The decrease in this ratio indicated that more urea hydrolysis occurred post inoculation. Factors such as cell washout (Doran 2013), reaction product inhibition (Fidaleo & Lavecchia 2003, Lauchnor et al. 2015), or entombment (Stocks Fisher, 1999) could be contributors to the loss of urea hydrolysis over the course of the day. None of these

potential factors were specifically investigated by the experiments, therefore conclusions in regards to these parameters cannot be made.

Regardless of the mechanism for the inhibition of ureolysis an overall conclusion can be made that there exists an inverse relationship between urea hydrolysis (activity) and an increasing number of calcium pulses delivered without resuscitation of the microbial community. A reduction in urea hydrolysis would lead to less dissolved inorganic carbon being produced, potentially leading to less mineral precipitation in the defect. Proper spacing of microbial inoculations or resuscitation pulses could minimize the loss of ureolytic activity, maintaining the rate of MICP formation, and reduce the time needed to seal subsurface leakage pathways.

CHAPTER THREE

QUANTIFICATION OF MICP USING X-RAY COMPUTED TOMOGRAPHY

Materials and MethodsReactor Design and Configuration

A reactor, hereafter referred to as the Core Experiment Reactor (CER) was designed to accommodate the requirements of the Skyscan 1173 X-ray Microtomograph available through the Subzero Science and Engineering Research Facility at Montana State University – Bozeman (Figure 12).

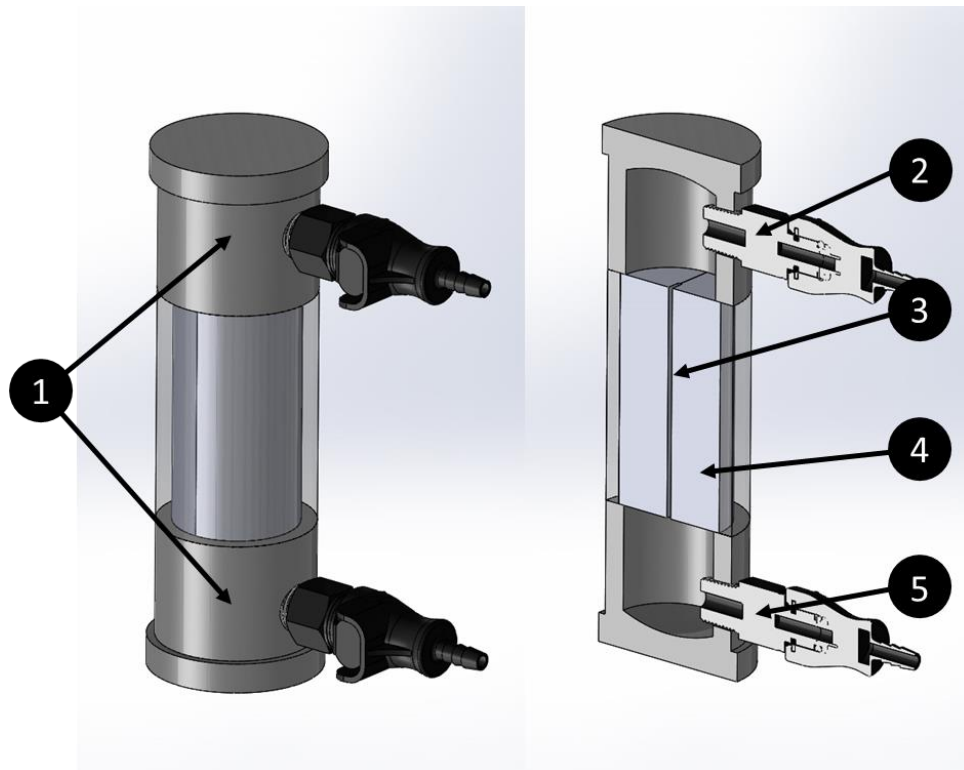


Figure 12: Schematic of the Core Experiment Reactor (CER) created in SolidWorks (1) end caps (2) effluent fluid port (3) cement space defect 0.25 cm x 0.05 cm x 5 cm (4) cement core (5) influent fluid port.

The reactor was designed so that a cement core (Class H well cement as described in Chapter 2) with a channel defect at the center could be housed inside PVC that was μ -CT compatible. The reactor was equipped with quick-release valve fittings at the influent and effluent ports so that the reactor could be detached from the pumps and tubing and brought to the μ -CT scanner periodically throughout the experiment. The reactor was 11.4 cm in height and 3.4 cm in diameter. The cement core within the reactor was cut to a length of 5 cm.

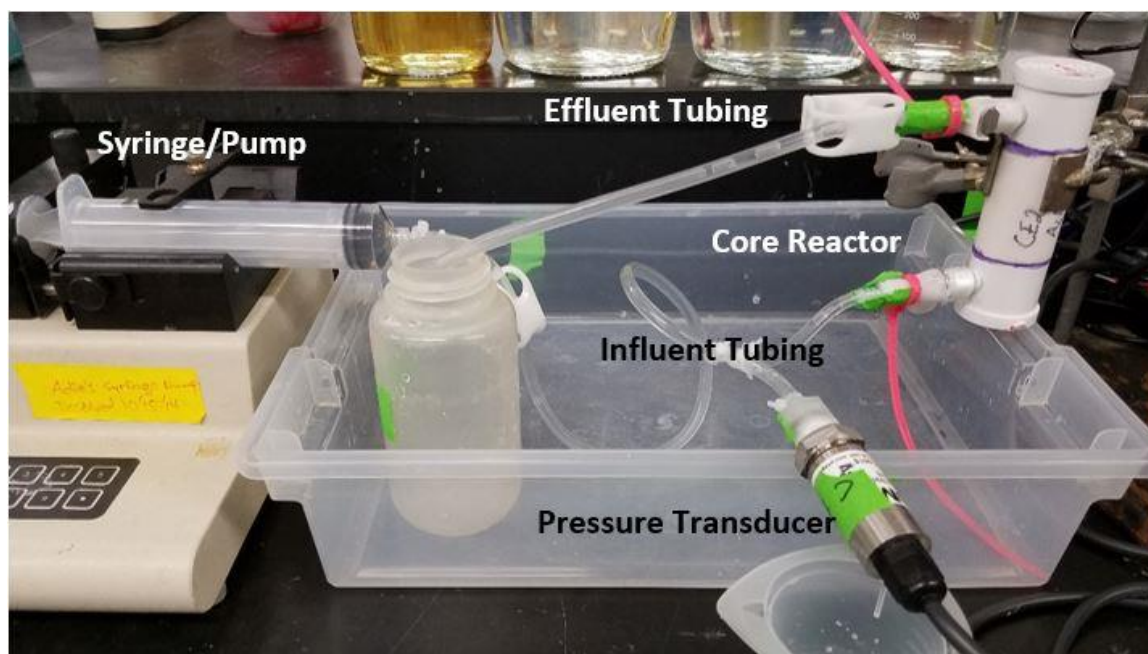


Figure 13: Assembled reactor system for the CER experiments.

Pressure measurements were taken using Omega Engineering Inc. PX309 series pressure transducers and flow rate monitoring was performed through the pump control operating software (LabVIEW, National Instruments). Fluids were delivered to the reactor using a Cole Palmer 210 syringe pump. Injection of fluids entered through the inlet port, traveled up the defect, and out the effluent port (Figure 13). Fluids were

delivered at a flowrate of 1 mL/min, which corresponded to a 30-minute fluid residence time during periods of flow.

Cement Sample Preparation

Cement cores were cast as two separate half-cylinders and then joined together in the reactor to create a circular core with a defect at the center. The mold used to cast these half-cylinders was machined from Teflon and was comprised of a baseplate and a half round outer shell. The baseplate was machined to imprint a rectangular defect onto the half-cylinder so that when the two half-cylinders were joined, the channel defect was created (Figure 14).

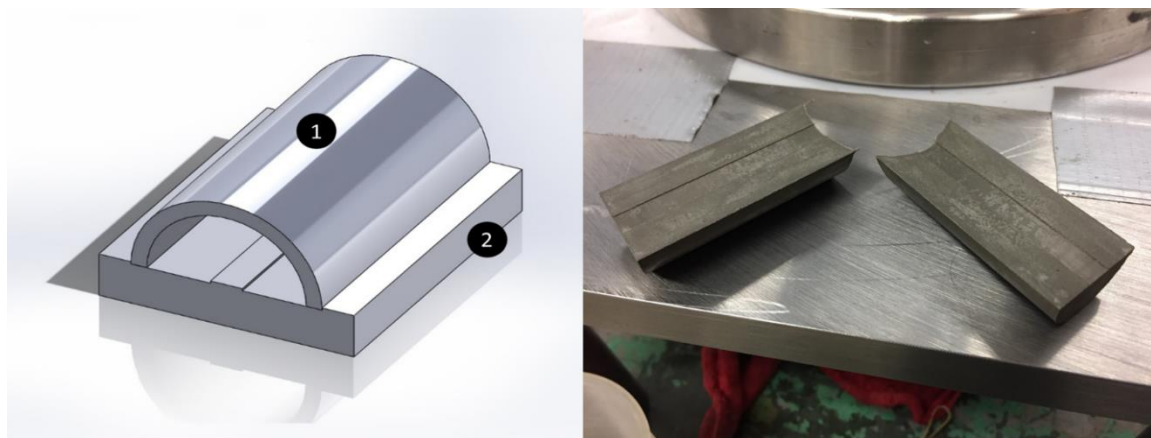


Figure 14: Cement cast for CER experiments Left: Schematic of the mold used to cast the half-cylinders of the cement core. (1) outer shell (2) baseplate. Right: Cement cores following extraction from the mold.

The cement cores were prepared using a cement and method previously described in Chapter 2. Once poured the cement was allowed to set at room temperature in the mold for a total of four days. The cores were removed from the cast and immersed in water

saturated with $\text{Ca}(\text{OH})_2$ for a minimum of 7 days. After curing, the reactor was assembled and end caps were attached to seal the reactor.

Fluid Injection

Three experiments in total were performed in the CER. Two biomineralization experiments where inoculation of the reactor with *S. pasteurii* occurred (CE1 and CE2) and one negative control experiment without reactor inoculation (CEC). All media and inoculation cultures were prepared in the manner described in Chapter 2. Similar to Chapter 2, inoculation was performed first with a 4-hour stationary period (no flow) to allow attachment of the microbial community. Then, YE- was injected to the reactor to promote microbial growth followed by the injection of calcium using YE+ with 60-minute stationary periods between each YE+ injection. The periods of no-flow were included in the injection strategy to allow additional time for the MICP process to occur once fluids had been introduced. Also, as described in Chapter 2, the reactor was re-inoculated with *S. pasteurii* at the end of each day and was allowed to sit overnight to resuscitate the microbial activity.

Whenever transitioning to or from calcium containing medium two fluid volumes of brine rinse solution were injected. This brine rinse was performed to prevent large amounts of precipitation upon the introduction of calcium species or microbial rich fluids by diluting extra calcium and inorganic carbon species inside the reactor. All fluids during the control experiment (absent of ureolytic bacteria) were amended with 25 mg/L (ppm) Chloramphenicol (Fischer-Scientific), an antibiotic known to inhibit growth and protein production of *S. pasteurii* (Martin et al. 2012).

Sampling Procedure

For all days where calcium media was injected, effluent samples were taken at the beginning of each pulse. Approximately three mL of fluid was taken for all samples. A volume of one mL was syringe filtered and refrigerated for urea quantification (see Chapter 2), the remaining unfiltered sample was used for pH measurement with a VWR Symphony SB70P pH meter, which was calibrated daily.

Viable Cell Counts

Viable cell counts were performed using the drop plate method (Herigstad et al. 2001). When performing suspended cell counts, one mL of unfiltered sample was transferred into nine mL of Phosphate Buffer Solution (PBS). Samples were serially diluted and 10 μ L of sample was plated on BHI agar plates amended with 20g/L urea. Plates were incubated at 30 degrees C for 16-24 hours, after which colonies (cells) were counted.

CT Imaging

Imaging was performed using a Skyscan 1173 X-ray Microtomograph before and after MICP treatment as well as on days during which calcium media was injected. During the experiment, images were taken between the final calcium injection and nightly inoculation of the reactor.

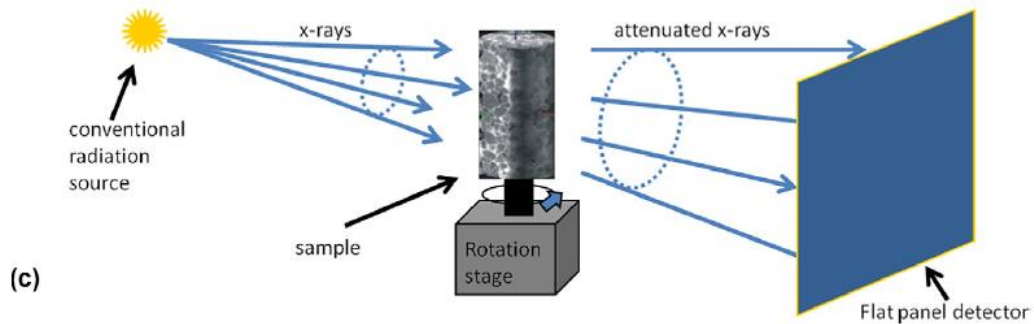


Figure 15: Schematic of X-ray CT imaging setup. Reprinted from Advances in Water Resources, volume 51, Wildenschild D, Sheppard AP, X-Ray Imaging and Analysis Techniques for Quantifying Pore-Scale Structure and Processes in Subsurface Porous Medium Systems, pp. 417-423 No. 5, 2012, with permission from Elsevier

In X-Ray CT, an x-ray beam is generated and passed through an object which is being rotated. As the beam passes through the object, the intensity of the beam is altered based upon what material it passes through. Once the beam has passed through the object, the intensity of the x-ray beam is measured by a detector (Figure 15). Based upon the known intensity of the original beam and the measured intensity of the altered beam, a reconstruction of the object can be created (Wildenschild 2012). Flat field calibration was performed prior to each scan to determine the initial intensity of the beam. Scans were performed every 0.7° for 180° to create a full image of the reactor. All scans were performed at a voltage of 130 kV and a current of 60 μA with an unfiltered beam. Image resolution and step size was determined by the computer software to be 25 μm .

CT Image Processing

From the X-ray CT scans, a 2D series (stacks) of projection radiographs (images) were produced from signal attenuation through the reactor. The data was reconstructed in commercially available NRecon (Bruker Software), using the Feldkamp algorithm, to produce a series of 2D images divided into an array of pixels. A linear attenuation coefficient was assigned to each pixel based upon the change in X-ray signal intensity and thresholding of the images was performed in CTAn (Bruker Software, NRecon Manual 2016, Feldkamp et al. 1984).

Raw data from scans of the reactor were pre-processed to remove noise using a Gaussian kernel smoothing and then a collection of 2D reconstructions divided into pixels was produced. Once reconstructed, a rectangular region of interest (ROI) 2mm x 9mm was drawn around the defect at the center of the core for each 2D slice (Figure 16).

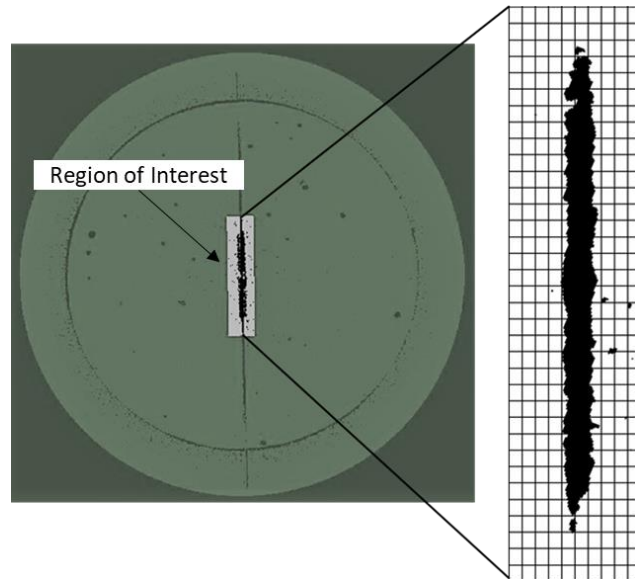


Figure 16: Two-dimensional radiograph and representative pixilation of the region of interest

Each pixel was then assigned an attenuation coefficient, a value which corresponds to the material density through which the beam passes. The change in signal intensity can be translated into a linear attenuation coefficient using Lambert-Beer's law (Equation 10), (Wildenschild 2012).

$$\mu = \frac{1}{x} \ln \left[\frac{I_0}{I} \right] \quad \text{Equation 10}$$

Where μ is the linear attenuation coefficient, x is the path length, I_0 is the flat field intensity, and I is the beam intensity measured at the detector. To distinguish the cement from the water and air within the reactor, the data was segmented into two regions: 1.) open pixels (fluids in the channel) 2.) closed pixels (cement and MICP) based upon the attenuation of each voxel (Figure 17) (Fan and Li 2015).

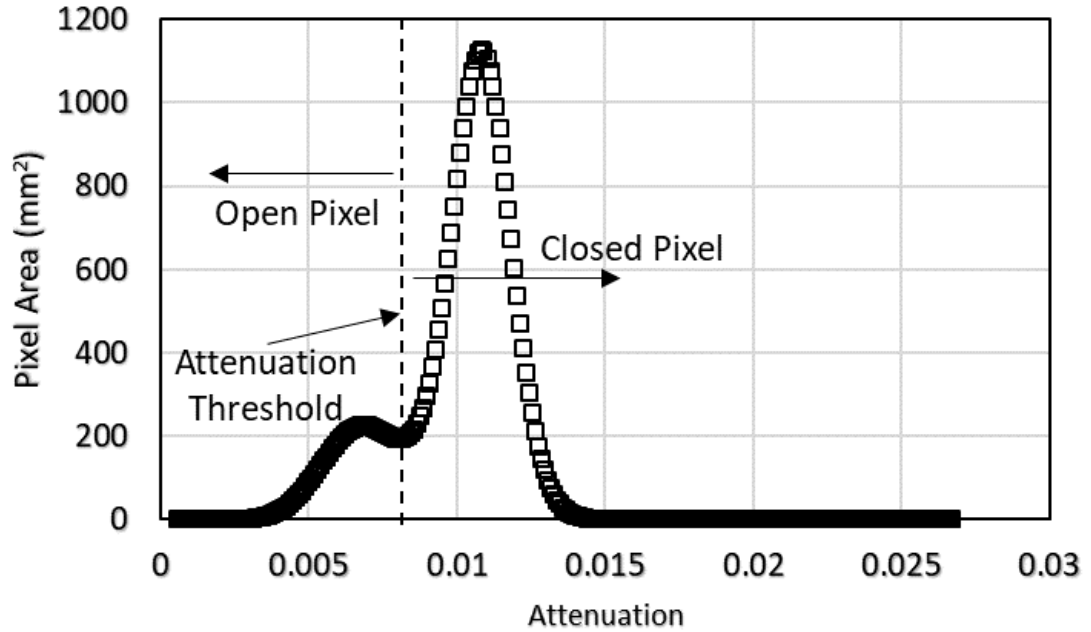


Figure 17: Distribution of voxel areas as a function of signal attenuation. The quantification of signal at each specific attenuation values is based upon the summation of all voxels found at each attenuation value throughout the entire reactor scan.

The binarization threshold was determined from scans performed prior to MICP treatment (Day 0) by first adjusting the threshold to approximate the void fraction of the region of interest based upon the dimensions of the defect cast into the cement. The thresholding value was then finely adjusted to approximate the local minima between the two peaks (Figure 16). This threshold was then applied to scans for all subsequent days of the experiment to determine the change in the void fraction of the reactor. Finally, the images (2D) were stacked together to create a 3D reconstruction of the channel.

Post-Experimental Imaging

At the conclusion of each experiment, the reactor was deconstructed and the cement cylinder was separated into two halves for imaging. Light microscopy images were taken using a Leica Model MDG41 Stereomicroscope.

Result and Discussion

Apparent permeability

The apparent permeability of the defect was calculated to quantify the flow restriction resulting from MICP. Apparent permeability was calculated using Darcy's law (Chapter 2, Equation 8). The values for each pulse were taken as an average of the final 60 seconds of flow and pressure data from each pulse. This averaging was done to minimize the impact of the signal noise from the pressure transducer (Figure 18).

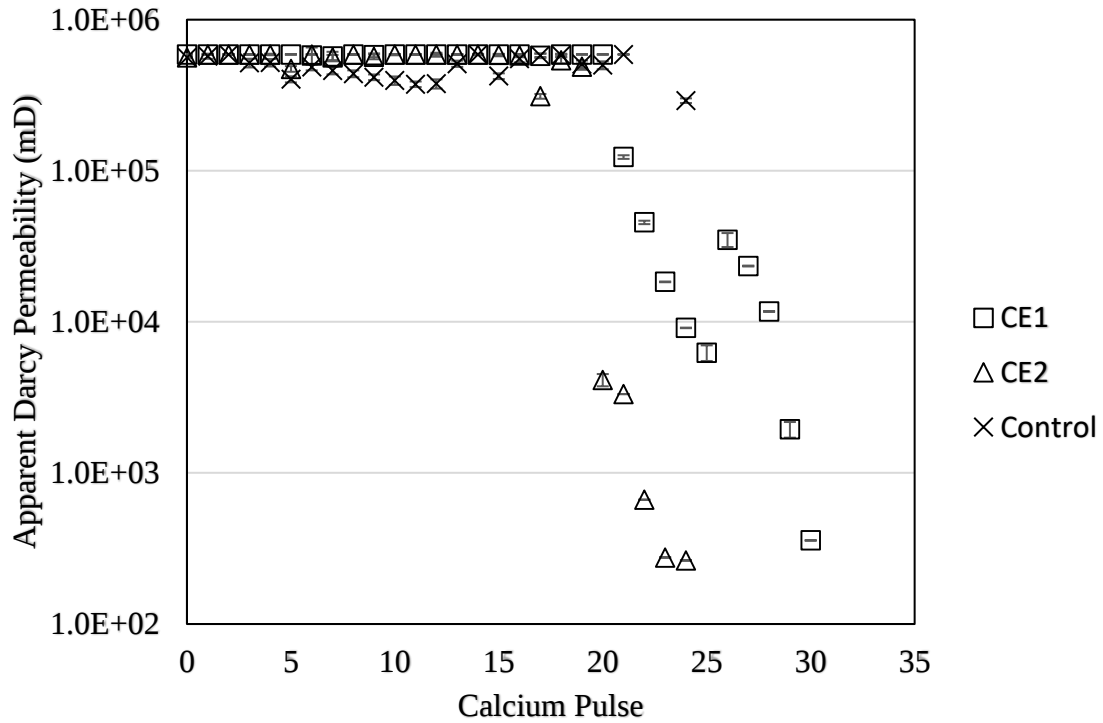


Figure 18: Apparent Darcy permeability of CER experiments as a function of calcium pulses delivered. Apparent permeability values for each pulse were calculated as an average of the calculated apparent permeability from the final 60 seconds of each pulse.

The initial apparent permeability measured prior to MICP treatment for all experiments was between 5.6×10^5 and 5.8×10^5 mD respectively. A minimum pressure for the pressure transducer was set to 5 cm of H_2O due to the geometry and orientation of the reactor. This value was set as the minimum because the sensitivity of the transducer did not allow for accurate measurements of pressure at values near this 5 cm of H_2O value. Any error created by the baseline pressure was assumed to be less than what was associated with the pressure transducer's inability to accurately measure pressures near the 5 cm H_2O value.

A reduction in apparent permeability greater than one order of magnitude was first seen during the 21st calcium pulse for CE1 and the 20th calcium pulse for CE2. The

final apparent permeability of CE1 and CE2 was 357 and 263 mD respectively. Minimal change in apparent permeability was measured in the control experiment (CEC). The slight decrease in apparent permeability seen in CEC was attributed to the noise in the pressure transducer readings. The similarity between the final apparent permeability values for CE1 and CE2 was most likely a result of system limitations for flow and pressure being the same for both experiments. Leading to similar minimum values for apparent permeability.

Aperture depth was approximated using Cubic's law for fracture flow (Chapter 2, Equation 8). The initial aperture depth was 0.5 mm. The final calculated aperture sizes, approximated from Cubic's law, for CE1 and CE2 were 0.013 and 0.012 mm respectively. The final aperture size approximations for CE1 and CE2 represent a 97.4% and 97.6% change from the cast aperture size of 0.5 mm respectively. No change in the calculated aperture size was observed for CEC. Approximated aperture size values taken at the end of the experiments were more likely to be accurate as pressures measured at the end of CE1 and CE2 were within the accuracy of the pressure transducer.

Void Fraction

The goal of the core experiments was to quantify the MICP by measuring changes in the void fraction of the reactor using μ -CT. The void fraction of a 2mm x 9mm region of interest (ROI) surrounding the fracture was quantified for each slice and plotted as a function of distance along the fracture flow path for each day. The change in the ROI void fraction was determined by comparing the pre-MICP scans to the all other scans for

the experiment. ROI void fraction plots were compared visually to light microscopy imaging performed following the termination of each experiment for CE1 (Figure 19).

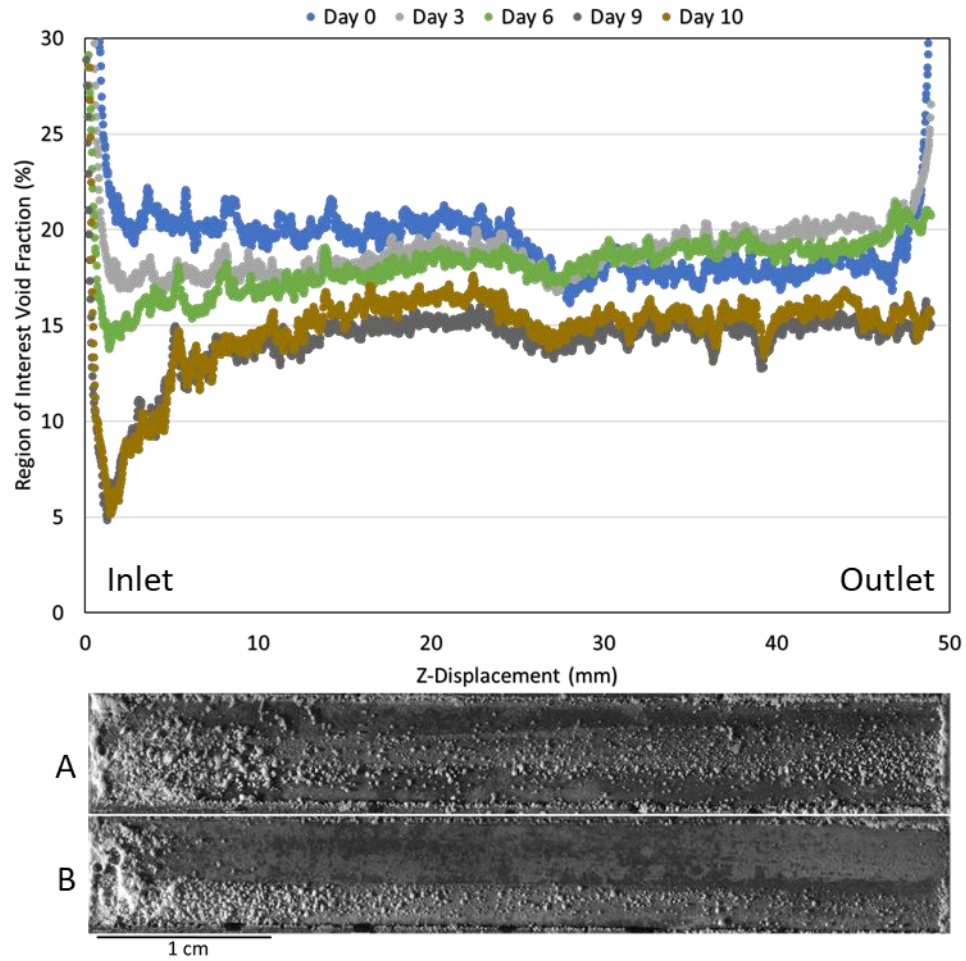


Figure 19: Void fraction vs. light microscopy imaging CE1. TOP: CE1 void fraction distribution as a function of axial distance from the inlet, calculated from a 2mm x 9mm rectangular region of interest. BOTTOM: Light-microscopy image of the defect taken during the post-experimental deconstruction stage. A and B each represent separate half-cylinders created when the reactor was deconstructed.

In CE1, the greatest reduction in void fraction was seen within 10 mm from the fracture inlet. This observation correlated well with post experimental light microscopy

imaging of the reactor, where a large quantity of calcium carbonate deposition was observed within 10 mm from the fracture inlet.

An overall shift in all attenuation coefficients during Day 0 of CE2 and CEC was observed. These scans of different reactors were performed on the same day using the same parameters. It was hypothesized from these observations that the flat field calibration was the cause of the error for this day. As a result, the original thresholding value of 0.00849 for Day 0 was adjusted to a value of 0.00100 to compensate for the shift in attenuation coefficients. Justification of this attenuation shift can be seen in Appendix D. The resulting analysis for CE2 (Figure 20) and CEC (Figure 21) was plotted in similar fashion to CE1.

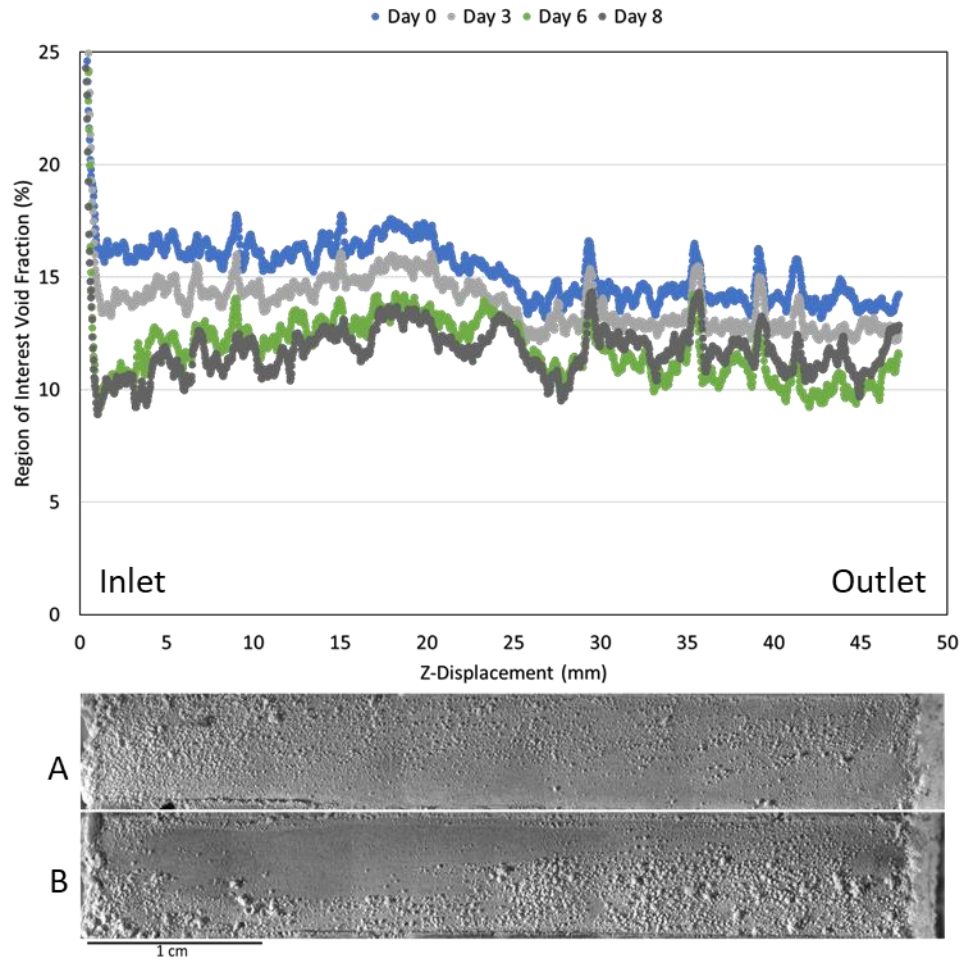


Figure 20: Void fraction vs. light microscopy imaging CE2. TOP: CE2 void fraction distribution as a function of axial distance from the inlet, calculated from a 2mm x 9mm rectangular region of interest. BOTTOM: Stereoscope images of the flow path defect, taken as two separate half-cylinders. A and B each represent separate half-cylinders created when the reactor was deconstructed.

Again, it was observed that the greatest change in void fraction reduction was seen within 10 mm of the fracture inlet. A region of similar void fraction reduction was noted between 35 mm to 45 mm from the inlet. These observations appeared to correlate with the visual calcium carbonate deposition observed through light microscopy imaging.

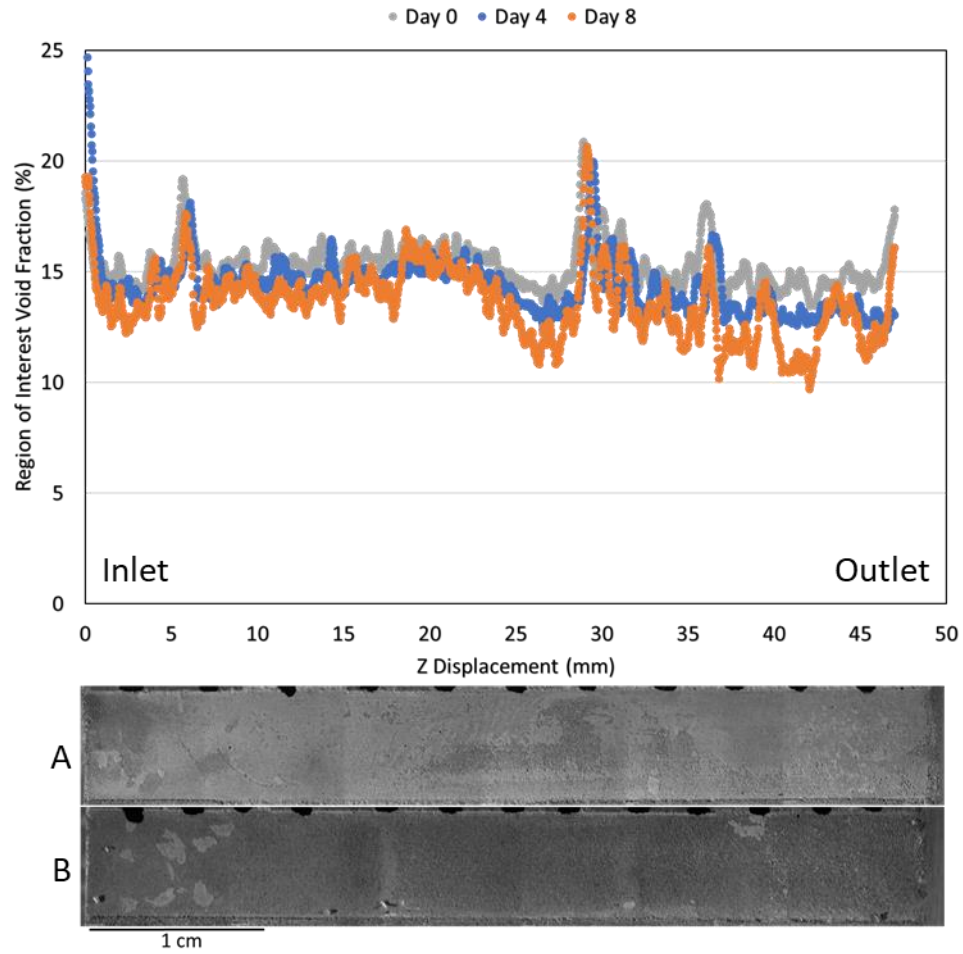


Figure 21: Void fraction vs. light microscopy imaging CEC. TOP CEC void fraction distribution as a function of axial distance from the inlet, calculated from a 2mm x 9mm rectangular region of interest. Day 0 was adjusted to correct for an apparent shift in the baseline intensity measurement of the scanner. BOTTOM: Stereoscope images of the flow path defect, taken as two separate half-cylinders. A and B each represent separate half-cylinders created when the reactor was deconstructed.

In the uninoculated and antibiotic treated solution control (CEC), little to no change in void fraction was observed in the first half of the fracture, 0 to 22.5 mm. Regions of variable void fraction were observed past 30 mm from the inlet in the Day 8 scan. This could have been a result signal noise caused by air pockets which became trapped in the defect during injection since the light microscopy imaging shows no

evidence of mineral precipitation in the defect. The ROI void fraction for CE2 and CEC appears to have an overall value lower than that of CE1, this could have been a result of expansion or contraction of the cement core creating a slightly different defect size. However, the overall change in void fraction was similar for CE1 and CE2, which was the metric used to quantify the effect of biomineralization on the flow path.

The observation of heterogeneous calcium carbonate distribution similar to that seen in CE1 and CE2 has been observed in previous works and thus is not unique to this experiment (Mortensen et al. 2011, Phillips et al. 2013, Whiffin 2007). Mortensen et al. (2011) correlated a larger precipitation gradient along the length of column with a reduction in fluid injection rate, which provides a possible explanation for the heterogenous distribution of mineral deposits observed. Flow rates in these experiments were slow which could have led to increased precipitation in the beginning of the flow path.

3D Void Fraction

Analysis of the core experiments in three spatial dimensions (3D) was performed based upon the 2D void fraction data and the slice height from CT imaging. The total void fraction of the ROI for each day was calculated as a summation of the open voxel area divided by the total voxel area for all slices. These values were then plotted as a function of time (Figure 22).

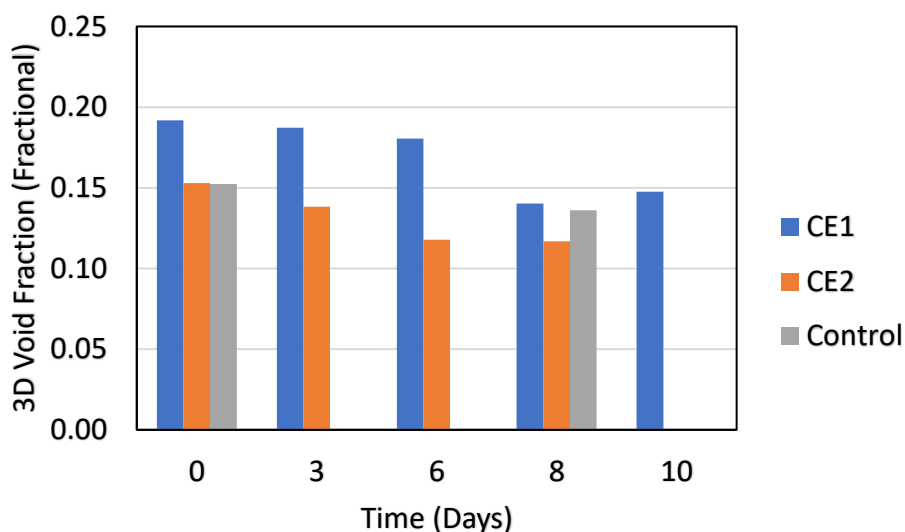


Figure 22: Three-dimensional void fraction for all core experiments as a function of time.

The reduction in void fraction between the initial and final measurements for CE1 and CE2 was 23.1% and 23.6% respectively, while the reduction in void fraction for CEC was 10.7%. The reduction in void fraction for CEC is most likely a measure of the error associated with scanning and data analysis methods, although this value is still less than the reduction seen in CE1 and CE2. The observation of greater flow restriction for the biomineralization experiments (CE1 and CE2) as compared to the control experiment coincide with the observations for both the apparent permeability and 2-dimensional analysis made previously.

Ureolysis

Throughout all experiments, urea hydrolysis was the metric used to quantify microbial activity within the reactor. The concentration of urea remaining inside the reactor after the batch period of each pulse was determined by analyzing the effluent

fluids at the beginning of the subsequent pulse. Influent concentrations were measured at the beginning of each day. Urea concentrations were plotted as the ratio effluent to influent urea concentration and plotted as a function of calcium pulse number (Figure 23).

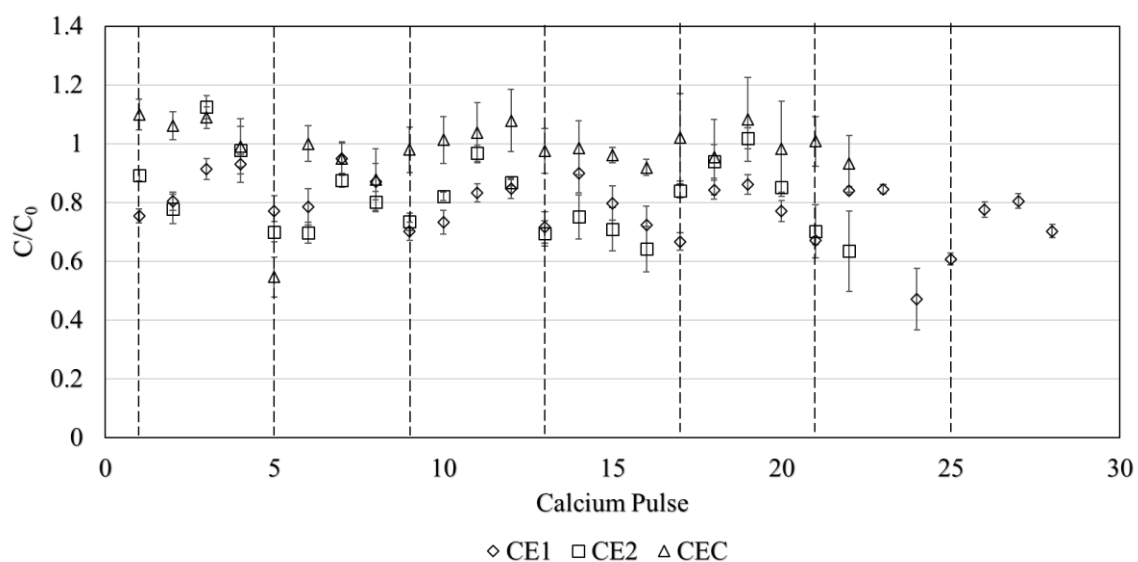


Figure 23: Ratio of effluent to influent urea concentration as a function of calcium pulse for CER experiments. Dashed vertical lines indicate the first pulse of each day.

An increasing ratio of effluent to influent urea concentrations was observed for the majority of days as a function of calcium pulses delivered. The rise in this ratio indicated a loss in ureolytic activity from the beginning to end of each day. The pH in the influent and effluent during calcium injection ranged from 5.8-6.5 and 7.7-9.6, respectively. As discussed in Chapter 2, a loss in ureolytic activity could have resulted from factors such as product inhibition, washout, or entombment. The cell populations measured during the first calcium pulse for CE1 and CE2 averaged 1.58×10^7 and 8.01×10^6 CFUs/mL respectively. For both experiments, the suspended viable cell

concentration during the final calcium pulse of the day was determined to be less than the detection limit of 10 CFUs/mL. The cell population was restored overnight due to the nightly inoculations with *S. pasteurii*. Inoculation pulse cell concentrations for CE1 and CE2 averaged 4.75×10^7 and 2.61×10^7 CFU/mL, respectively.

The fraction of effluent over influent urea concentration for CEC remained near the value of 1 for the entirety of the experiment. Influent urea concentrations for all days of CEC were between 22 and 24 g/L. The average influent urea concentration measured during calcium injection was 23.2 g/L (Appendix C). While there was a slight difference in the influent and effluent concentrations, this difference was minimal and most likely a result of errors associated with sample pipetting or variability in the Jung urea assay.

Influent and effluent pH measurements for CEC during calcium injection varied from 6.2-6.7 and 8.1-10.8 respectively. When compared to pH measurements from CE1 and CE2, the effluent pH values were elevated in the control (CEC). A possible explanation of the elevated pH values for CEC would be the leaching of hydroxide species into solution from the cement coupled with lack of calcium precipitation normally seen as a result of MICP. This would lead to an overall increase in pH from the hydroxide species without the typical reduction in pH associated with calcium carbonate precipitation.

CHAPTER FOUR

CONCLUSIONS & SUGGESTIONS FOR FUTHER WORK

Wellbore Reactor Experiments

The formation of MICP appeared to take time to develop; however, once mineral deposition began to occur rapid growth of the biocement followed. Nucleation of biocement appeared to preferentially occur at points where the flow constricted rapidly. While the change in apparent permeability seen in AE1 was much greater in a shorter period of time than AE2, the seal formed in AE2 was observed to have a greater resilience to stress and generally appeared to be a more stable seal. Overall, the WBR in combination with the current MICP injection strategies showcases MICP's ability to form barriers to fluid flow within potential leakage pathways of the wellbore.

Core Reactor Experiments

The apparent permeability and void fraction of each defect was successfully measured for all three experiments. MICP treatment was observed to reduce the void fraction and apparent permeability as compared to the control. The reduction of urea hydrolyzed per pulse as a function of the amount of calcium delivered indicates that optimization of the injection strategy, such as decreasing the number of calcium pulses between inoculation pulses, could help to improve the efficiency of the MICP process. The core style reactor and its compatibility with the μ -CT system at Montana State

University – Bozeman, provides a means to correlate MICP with the reduction in void fraction and apparent permeability over time and space.

Future Work

Both experimental systems show great promise for improving the efficacy of MICP in the wellbore. Further research utilizing these reactor systems should concentrate on the effects of defect size, attachment of microbes and biofilm growth on cement and steel surfaces and refinement of injection strategies to promote MICP formation. MICP may not be an effective tool for all defects, but changing the size and shape of defects in these systems could lead to an understanding of the defect sizes where MICP is an effective tool. Manipulation of the injection strategies will aid in understanding the best method for the delivery of fluids to promote biofilm attachment and growth, as a healthy and active biofilm will create optimum conditions for the MICP process to occur. Determination of the limitations of the MICP technology will provide those in the oilfield a valuable resource when selecting the treatment strategy that suits each unique system.

APPENDICIES

APPENDIX A

EFFECTS OF IRON ON THE UREOLYSIS BY *S. PASTEURII*

Due to the use of carbon steel pipe as the material of choice for well casings, investigation of influence of iron on the ureolytic activity of *Sporosarcina pasteurii* was performed. Urea hydrolysis batch studies were carried out in iron amended media were compared to that of media absent of iron.

Materials and Methods

Two sets of batch studies were performed aimed at investigating the effect of iron and other precipitating species on *S. pasteurii*. A summary of the experimental parameters can be seen in Table A-1.

Table A-1: Iron Inhibition Experimental Summary

Experiment	Duration	Medias	Parameters Sampled
Iron Experiment 1 (IE1)	24 hours	CMM- CMM+Fe	Urea Iron pH
Iron Experiment 2 (IE2)	72 hours	CMM- CMM+Fe CMM+Ca CMM+Fe+Ca	Urea pH

Negative controls were performed for all medias in IE1 but not in IE2. Batch studies of iron containing medias were performed in triplicate while non-iron containing medias were performed as single batch studies. Measurement of pH and urea were performed in the same manner as Chapters 3. Iron was measured using a colorimetric plate assay adapted from Lovely and Phillips 1987. A summary of the medias used, utilizing Calcium Mineralizing Media (CMM-) as the base media, can be seen in Table A-2.

Table A-2: Iron Inhibition Experimental Medias

CMM=	Ammonium Chloride (Amresco Inc.) Difco Nutrient Broth (Becton Dickinson & Co)	10 g/L (0.19 M) 3 g/L
CMM-	Urea (Fischer) Ammonium Chloride Difco Nutrient Broth	20 g/L (0.33 M) 10 g/L (0.19 M) 3 g/L
CMM+Fe	Urea Ammonium Chloride Difco Nutrient Broth Ferric Chloride (Sigma)	20 g/L (0.33 M) 10 g/L (0.19 M) 3 g/L 0.356 g/L (100 ppm Fe ²⁺)
CMM+Ca	Urea Ammonium Chloride Difco Nutrient Broth CaCl ₂ *2H ₂ O (Fischer)	20 g/L (0.33 M) 10 g/L (0.19 M) 3 g/L 0.370 g/L (100 ppm Ca ²⁺)
CMM+Fe+Ca	Urea Ammonium Chloride Difco Nutrient Broth Ferric Chloride CaCl ₂ *2H ₂ O	20 g/L (0.33 M) 10 g/L (0.19 M) 3 g/L 0.356 g/L (100 ppm Fe ²⁺) 0.370 g/L (100 ppm Ca ²⁺)

Inoculation

Microbes were grown by adding 1 mL of *Sporosarcina pasteurii* (ATCC 11859) thawed frozen stock to 100 mL of autoclaved brain heart infusion (Becton Dickenson) solution (37 g/L) amended with 2% by weight urea. The organisms incubated at 30°C on a shaker at 150 rpm for 16 hours. After 16 hours of growth the bacterial culture was centrifuged at 6000 RPM for 15 minutes at 4°C then resuspended in CMM= media to an optical density (600 nm) of 0.4. To inoculate, 1 mL of suspended culture was added to 100 mL of media.

Results and Discussion

Iron Experiment 1

Urea concentration was the primary metric to evaluate microbial activity during the batch experiment. Measurements were taken for the duration of the experiment and plotted as function of time (Figure A-1).

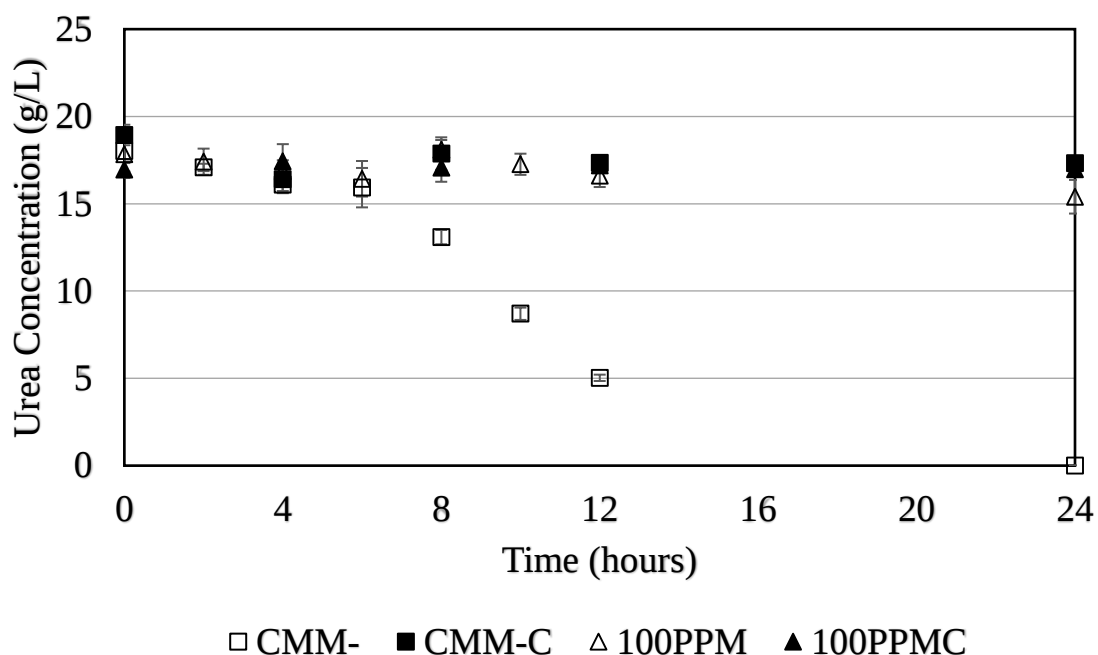


Figure A-1: Urea concentration as a function of time for IE1. Open symbols indicate experiments in which the media was inoculated with *S. pasteurii*. Closed symbols indicate negative control experiments where no bacterial inoculation of the media occurred.

The base media, CMM-, showed complete hydrolysis of urea between 12 and 24 hours. Little to no ureolysis was observed for the media amended with 100 ppm iron during the 24-hour experiment. Negative controls of both the base media and the iron amended media showed no urea hydrolysis. The lack of urea hydrolysis observed in the iron amended media indicated a potential inhibition of the ureolytic bacteria by the iron.

Approximately two days following the termination of the experiment however, urea measurements of the inoculated iron amended media were taken and were found to have experienced complete urea hydrolysis. This lead to the conclusion that while no ureolysis was observed in the presence of iron for the first 24-hours, viable cells did still exist and perhaps were only experiencing a lag period in their activity.

Measurements of pH were taken throughout the experiment as changes in pH are associated with both urea hydrolysis and iron precipitation. These values were plotted as a function of time (Figure A-2).

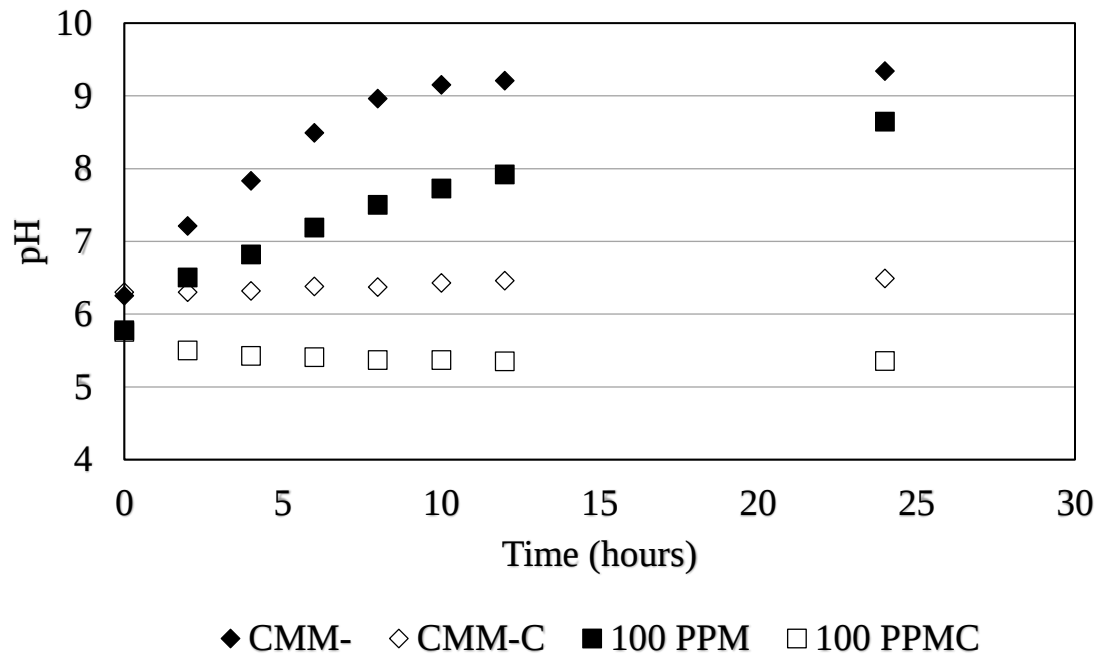


Figure A-2: pH as a function of time for IE1. Open figures indicate experiments in which the media was inoculated with *S. pasteurii*. Closed figures indicate negative control experiments where no bacterial inoculation of the media occurred.

While an increase in pH of approximately 3 units was noted for 100 PPM batch tests, the rate at which the pH increased as noticeably slower than that of the CMM-

positive control. Increasing pH observed in the iron amended media could explain the precipitation of iron as elevated pH values will cause the precipitation of iron hydroxide species (Figure A-3).



Figure A-3: Left: CMM- Right: CMM+Fe media 24 hours after inoculation.

Even though the iron was added as ferrous iron (Fe(II)Cl_2), iron is easily oxidized in aerobic conditions, quickly converting ferrous iron to ferric iron (Fe(III)), which is highly insoluble at pH values seen during this experiment (US Geological Survey). The reddish color observed in the iron containing medias during IE1 was indicative of iron species precipitating from solution. The rapid precipitation of iron leading to the removal of ureolytic microorganisms from solution provides a possible explanation for the loss in ureolytic activity. Ferrous iron species were measured through the experiment and plotted as a function of time (Figure A-4).

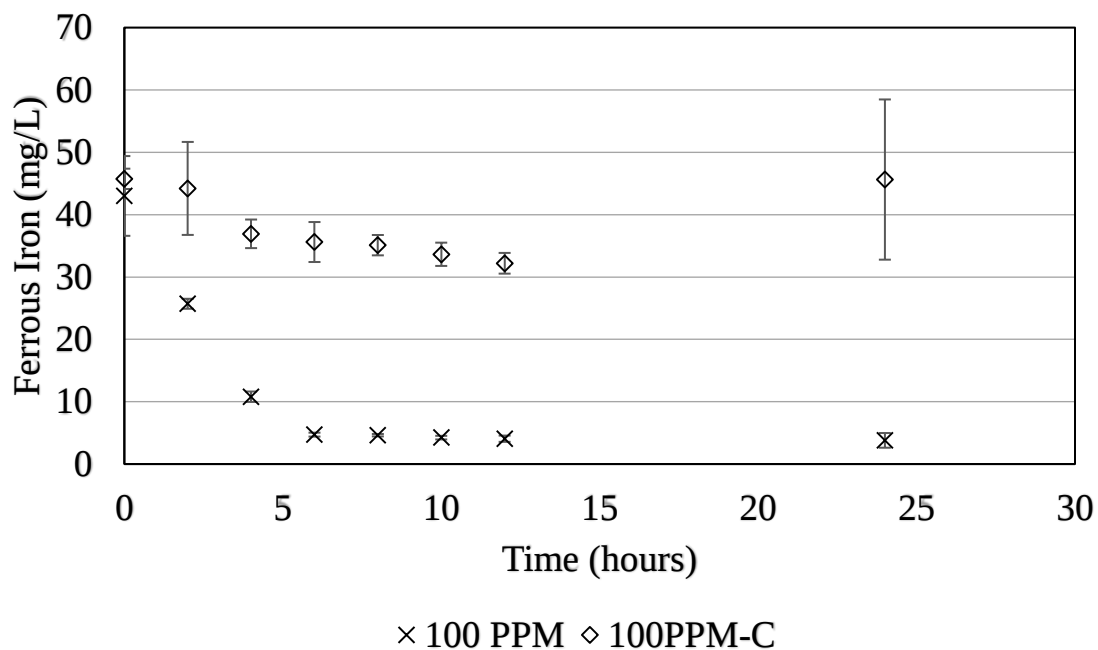


Figure A-4: Aqueous ferrous iron (Fe(II)) concentration as a function of time for IE1.

A decrease of approximately 40 ppm Fe^{2+} was observed within the first 6 hours for the iron amended media following the inoculation of *S. pasteurii*. This change was not observed however in the negative control even after 24 hours. This observation could have been a result of the three-pH unit increase of the inoculated media, which was not seen in the negative control.

Iron Experiment 2

In IE2, the study of precipitation of iron and calcium species on *Sporosarcina pasteurii* was carried out to evaluate whether precipitation of suspended solids could have a negative effect on the rate of urea hydrolysis. Urea concentration was the primary metric used to evaluate the effect of varying the media composition on the microbial

community. Samples were collected for a total of 72 hours and plotted as a function of time (Figure A-5).

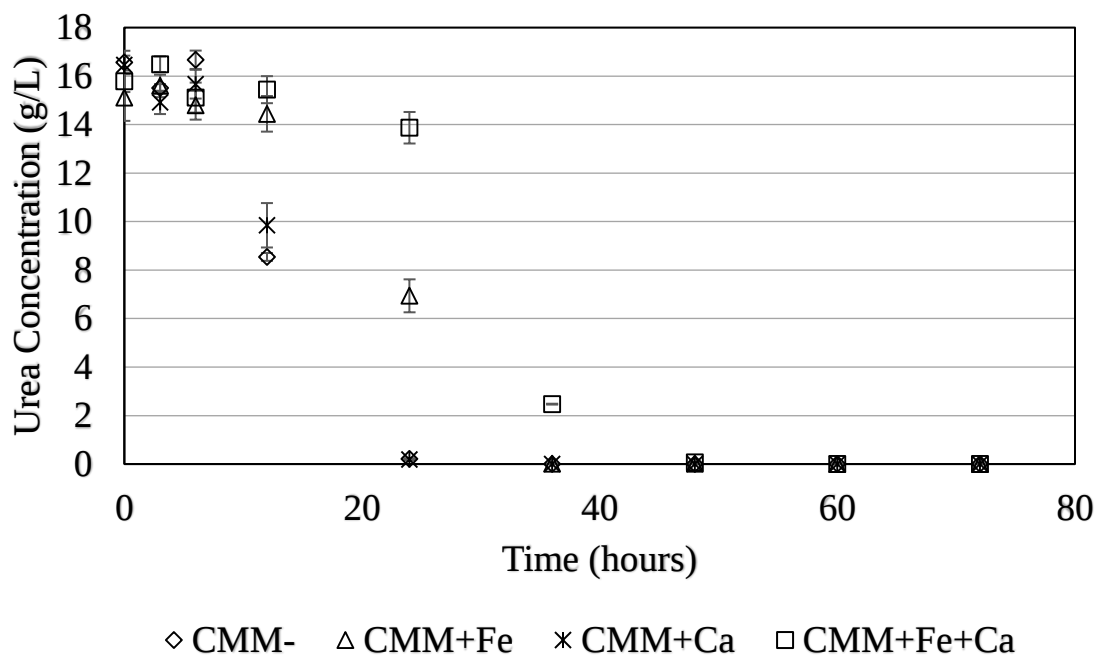


Figure A-5: Urea concentration as a function of time for the precipitation study.

Little difference was seen between the CMM- and CMM+Ca medias however, experiments which contained iron were observed to have a slower rate of urea hydrolysis. The longest time required to hydrolyze all urea present was observed for the CMM+Fe+Ca media, which required between 36 and 48 hours. Measurements of pH indicate similar trends to that of those observed for the urea hydrolysis data (Figure A-6).

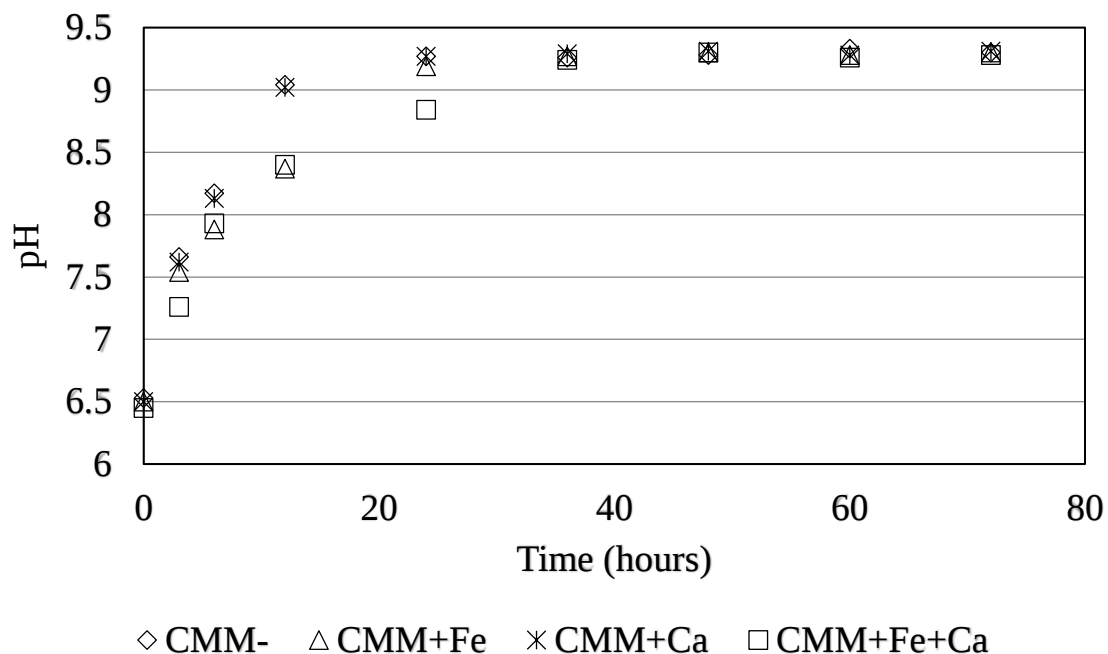


Figure A-6: pH as a function of time for IE2.

Minimal difference in pH was observed in the CMM+Ca media when compared to the base media of CMM-. The CMM+Fe and CMM+Fe+Ca medias differed similarly from the base media, however these differences were not nearly as drastic as those observed from the urea data. All media reached a final pH of 9.3 by the 36-hour time point after which values of pH remained constant from hours 36 to 72.

Conclusion

A reduction in ureolysis rate was observed in iron containing media for the first 24 hours after inoculation. Complete urea hydrolysis was observed by 48 hours for all media during IE2 and all medias reached the same pH value of 9.3. The oxidation of Fe(II) as a result of performing these experiments in an aerobic environment creating precipitation makes the determination of the inhibition mechanisms difficult. To

definitively determine the effect of iron on the ureolytic activity of *S pasteurii*, anaerobic experiments would be required.

APPENDIX B

FLUID DYNAMIC MODELING OF THE WELLBORE ANALOG REACTOR

As a result of experiments in the Wellbore Analog Reactor (Chapter 1), an interest arose into computational modeling of the fluid dynamics in the reactor. To create a preliminary model, the dimensions of the WBR were used to build a fluid dynamics model in COMSOL Multiphysics (Figure B-1).

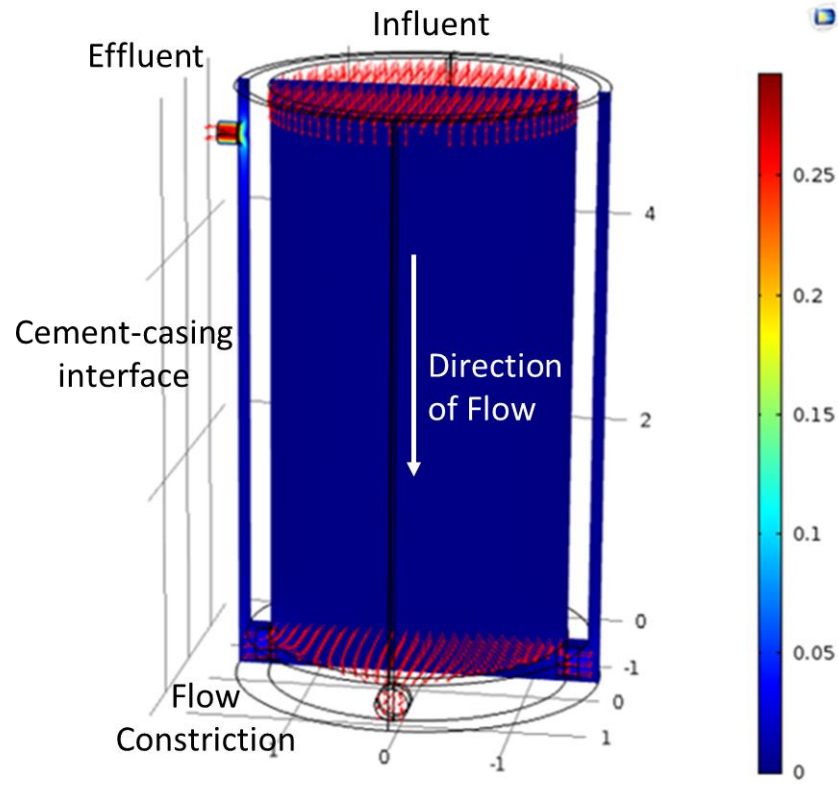


Figure B-1: Cross sectional image of the flow velocity model. The scale bar (right) indicates the linear fluid velocity (m/s).

Red arrows at the top and bottom of the reactor indicate the fluid profile at the boundary conditions of the fluid injection and flow constriction at the entrance to the cement-outer casing interface. A uniform, low-velocity profile was observed for the

majority of the reactor. The highest velocities were observed at the effluent where the greatest flow constriction was observed.

This working fluid model of the WBR will provide researchers insight into how fluids react when encountering defects common to the wellbore. Integration of reactive transport modeling can be added to this model to provide researchers a test space to evaluate potential injection strategies aimed at optimizing the distribution of mineral precipitation throughout the cement-casing interface.

APPENDIX C

CT THRESHOLDING SHIFT JUSTIFICATION

In CE2 and CEC, void fraction measurements from the Day 0 scan appeared to be abnormally low when compared to scans from other days of the experiment. Analysis of the attenuation values for each scan led to conclusion that an overall shift had occurred in the attenuation values (Figure C-1).

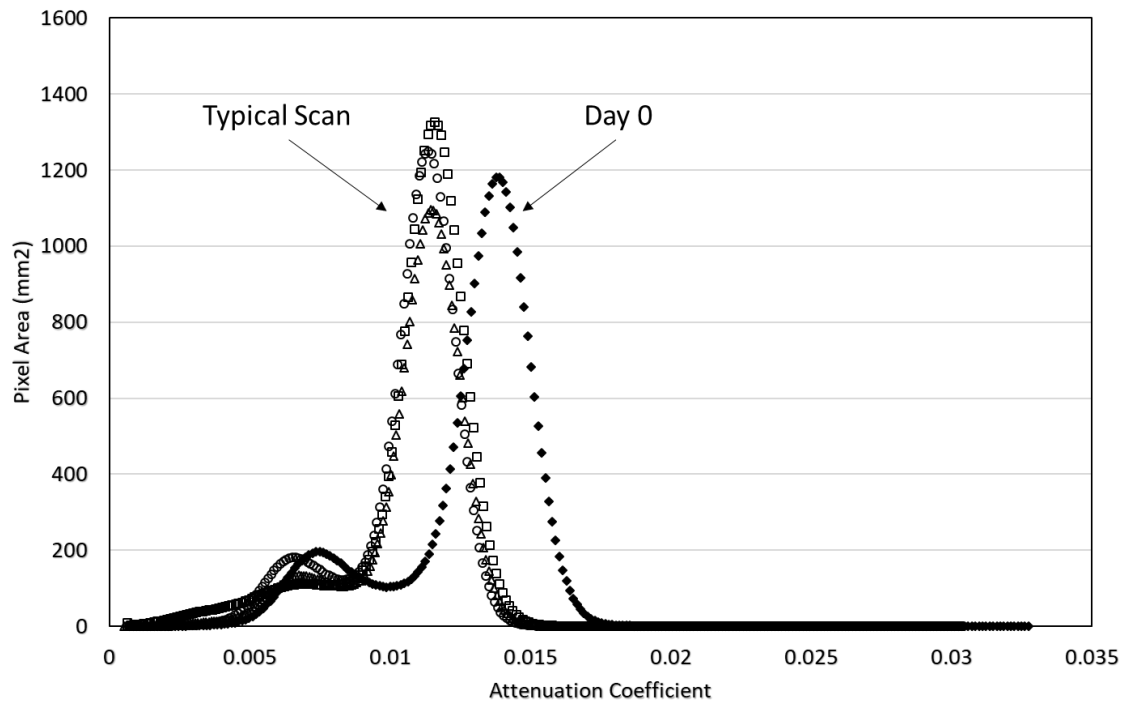


Figure C-1: Attenuation value distribution of selected days from CE2. Closed diamonds represent attenuation values from the Day 0, open figures represent attenuation values from all days not including Day 0.

A similar plot of pixel area vs attenuation coefficient was seen for Day 0 of CEC. Since μ -CT scans for CE2 and the CEC were both performed at the same time using the same parameters, it was assumed that the source of the error in both experiments resulted from a common source. This shift in attenuation was hypothesized to be an error in the

flat field calibration performed prior to scanning the reactor. To quantify the shift in the data sets, comparison of common points between the histograms was performed (Figure C-2).

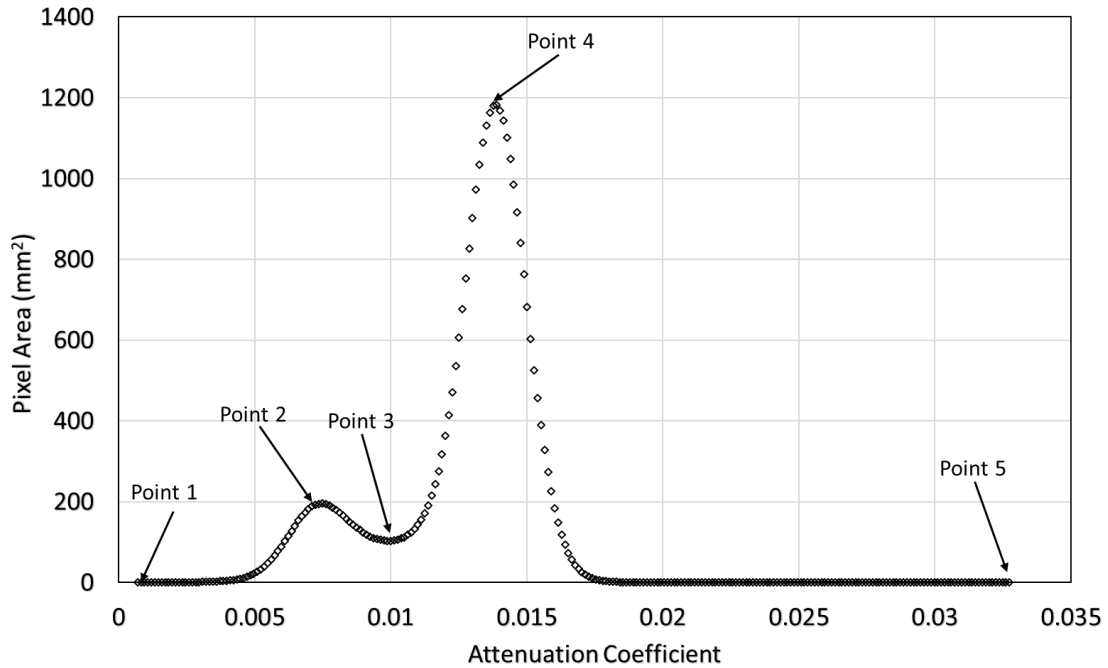


Figure C-2: Significant points used to quantify the attenuation coefficient data shift.

For the Day 0 scan in question, the attenuation value at Peak 2 was 0.01389, a 0.00244 change from the average value of 0.011446 from Days 2-6. A single shift for the entirety of the data set is hard to justify however given that the program uses Lambert-Beer's law (Chapter 3, Equation 9), which is not a linear function with respect to signal intensity. As a result, all five points of the Day 0 scan were compared to the average of their corresponding points from scans on Day 2-6 (Table C-1).

Table C-1: Significant Points Comparison

Point	Day 0 Attenuation Coefficient	Days 2-6 Average Attenuation Coefficient	Difference
1	0.007	0.0006	0.0001
2	0.0075	0.0068	0.0007
3	0.0099	0.0084	0.0015
4	0.0139	0.0114	0.0025
5	0.0327	0.0271	0.0056

The attenuation values from Table C-1 were plotted against each other and a linear correlation was determined between the two data (Figure C-3).

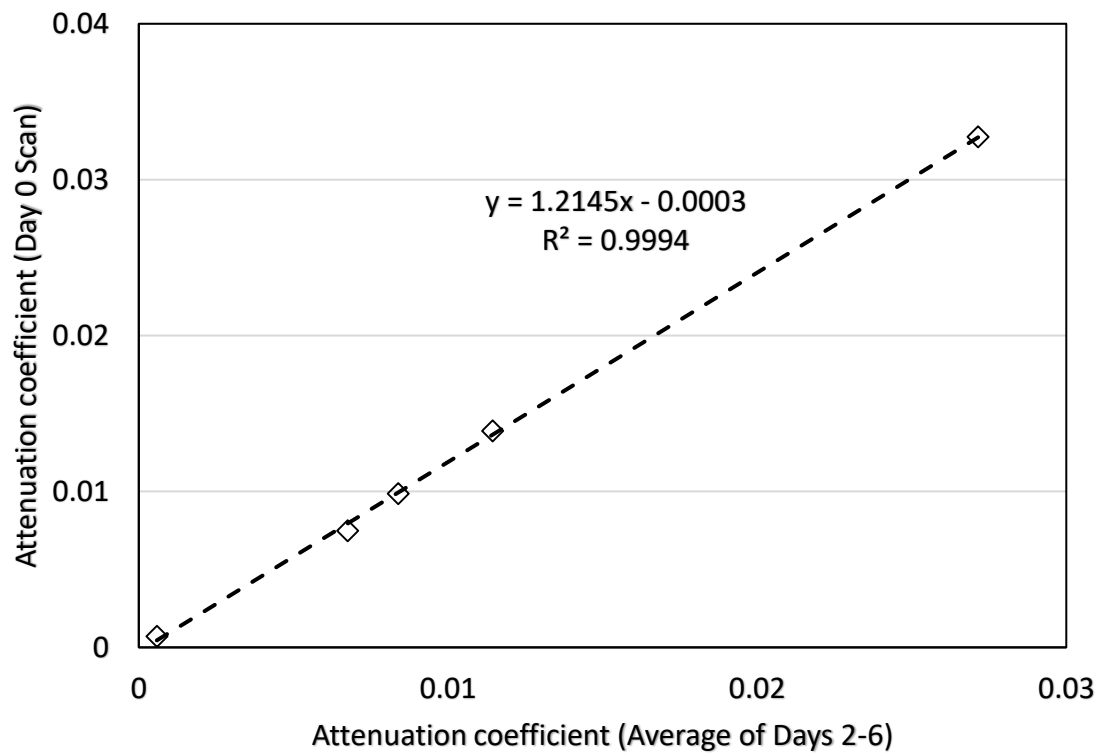


Figure C-3: Attenuation coefficient of Day 0 as a function of the average attenuation value of Days 2-6. A linear trendline was fit to the data to determine a relationship between the data sets.

A linear relationship between these values was observed with a R^2 value of 0.9994. If it assumed that an error in the data resulted from a constant shift in the flat-field calibration intensity between Day 0 to the other data sets, the error in attenuation values can be explained using Lambert-Beers law.

The error in the flat-field intensity value (I_0) can be represented by an error constant ϵ . The attenuation coefficient of the Day 0 scan is notated as u_j (Equation C-1) and the attenuation coefficient for the typical scan is noted as u_i (Equation C-2).

$$u_j x = \ln \left[\frac{\epsilon I_0}{I} \right] \quad \text{Equation C-1}$$

$$u_i x = \ln \left[\frac{I_0}{I} \right] \quad \text{Equation C-2}$$

$$\frac{u_j}{u_i} = \frac{\ln \left[\frac{\epsilon I_0}{I} \right]}{\ln \left[\frac{I_0}{I} \right]} \quad \text{Equation C-3}$$

$$\frac{u_j}{u_i} = \frac{\ln[\epsilon] + \ln \left[\frac{I_0}{I} \right]}{\ln \left[\frac{I_0}{I} \right]} \quad \text{Equation C-4}$$

$$\ln \left[\frac{I_0}{I} \right] = c \quad \text{Equation C-5}$$

$$\frac{u_j}{u_i} = \frac{\ln[\epsilon]}{c} + \frac{c}{c} \quad \text{Equation C-6}$$

$$\frac{\ln[\epsilon]}{c} + 1 = k \quad \text{Equation C-7}$$

$$u_j = u_i k \quad \text{Equation C-8}$$

By dividing Equation C-1 by Equation C-2 a ratio of the two attenuation values can be determined (Equation C-3). Following log rules, the Equation C-3 can be expanded to the result shown in Equation C-4. Assuming the error of the flat-field

intensity is completely contained in the constant error ϵ , the values of I_0 and I can be assumed to have remained constant across all days. As a result, the natural log of the ratio between I_0 and I is a constant (Equation C-5). The value of ϵ was initially defined to be a constant error coefficient and therefore the summation on the right-hand side of Equation C-6 can be assumed to be a new constant, “k” (Equation C-7). The final result is a relationship between the two attenuation coefficients that fits the form of a line with slope k, as was shown in the curve fit of Figure C-3. Using the linear fit from Figure C-3, the attenuation threshold for Day 0 was set to 0.00100, as this was the closest attenuation available from the system to the desired value of 0.009968.

APPENDIX D

FLUID INJECTION WITHOUT NACL BRINE FLUSH

Prior to AE1 and AE2 an experiment was performed in the WBR without an NaCl brine flush when transitioning to or from calcium media injection, seen during AE1 and AE2. This experiment investigated the formation of MICP in a delamination defect at the cement-outer casing interface cast to have the same dimensions as AE1. Fluids were delivered in the same manner as AE1 however daily inoculation of the reactor was performed during the day between the second and third calcium pulse. The primary result of this experiment was the observation of large amounts of instantaneous precipitation formed upstream of the desired defect. This precipitation continued throughout the entirety of the experiment and ultimately lead to the termination of the experiment.

Almost immediately after the first injection of calcium containing media (YE+) to the reactor, precipitation was observed. An abundance of white floc appeared in most fluid areas, which can be seen in Figure D-1.



Figure D-1: Instantaneous precipitation, seen as a white flock, occurring during the first injection of calcium containing media.

The precipitation documented in Figure D-1 persisted over the course of the six-day experiment. No pressure increase was seen at any point of the experiment until the final day, when a large pressure spike stopped fluid injection and caused the shutdown of the reactor. Pressure data for this portion of the experiment is not available however since the pressure transducer which was located upstream of the reactor ruptured and failed to produce data as a result of the pressure spike. Upon deconstruction of the reactor, a large amount of precipitation was found in and around the inner casing of the reactor (Figure D-2).



Figure D-2: Post experimental deconstruction of the Wellbore Analog Reactor used for Analog Experiment 1. A large amount of precipitation was found in and around the perforations at the bottom of the reactor.

The precipitation seen in Figure D-2 formed in a densely-packed manner at the bottom of the inner casing and the region just below the cement annulus. This precipitation was attached to the PVC and polycarbonate casings at points, however the majority of the precipitation was not attached and possessed a texture more to the liking of wet beach sand than that of a hard-mineral deposit.

The instantaneous precipitation was hypothesized to be a result of calcium ions in the injected media immediately encountering a high dissolved inorganic carbon (DIC) concentration present at the influent of the reactor as a result of urea hydrolysis. To counteract the high concentration DIC at the influent of the reactor, a brine rinse step was added to the injection protocol to flush the influent of DIC. This would allow the calcium containing media to travel reach the intended defect in the reactor before the majority precipitation occurred.

APPENDIX E

EFFLUENT AND INFLUENT UREA PLOTS

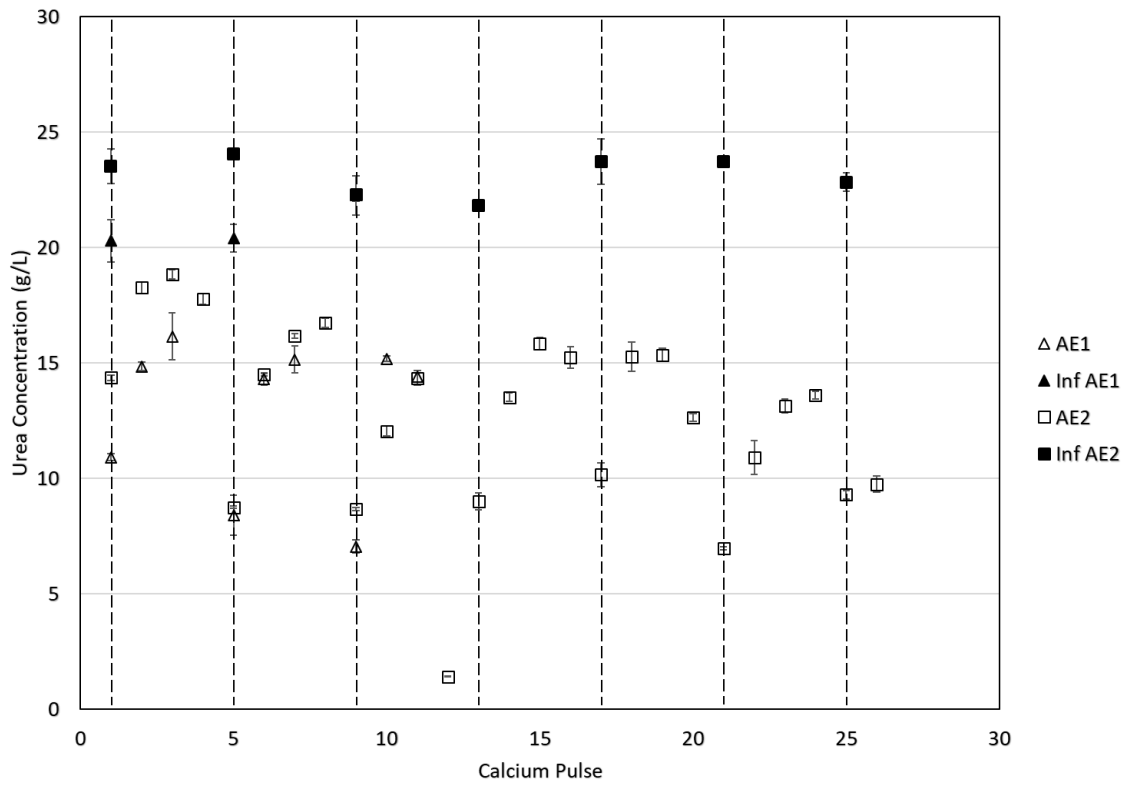


Figure E-1: Influent and effluent urea concentrations for AE1 (Delamination) and AE2 (Channel). Open figures indicate effluent measurements while closed figured indicate influent measurements.

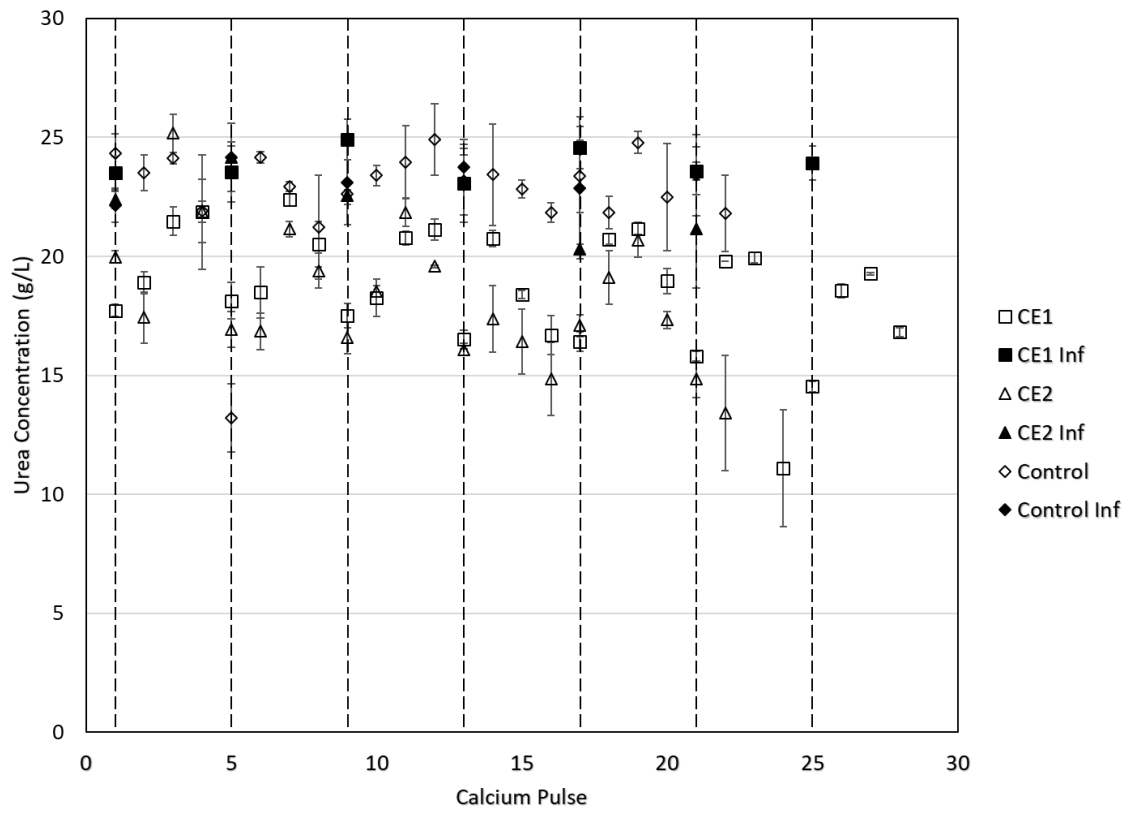


Figure E-2: Influent and effluent urea concentrations for CER experiments. Open figures indicate effluent measurements while closed figured indicate influent measurements.

REFERENCES CITED

1. Bai M, Song K, Sun Y, He M, Li Y, Sun J. "An Overview of Hydrogen Underground Storage Technology and Prospects in China." *Journal of Petroleum Science and Engineering*, vol. 124, 2014, pp 132-136.
2. Bai M., Zhang Z., Fu X. "A Review on Well Integrity Issues for CO₂ Geological Storage and Enhanced Gas Recovery." *Renewable and Sustainable Energy Reviews*, vol. 59, 2016, pp. 920-926
3. Bello, Kolawole S. "Experimental Assessment of Cement Integrity under Thermal Cycle Loading Conditions in Geopressured Geothermal Reservoirs." MS Thesis. Louisiana State University, 2014.
4. Benjamin, Mark M. *Water Chemistry*. 2nd ed. Long Grove: Waveland Press, Inc., 2015. Print.
5. Bray JM, Lauchnor EG, Redden GD, Gerlach R, Fujita Y, Codd, S, Seymour JD. "Impact of Mineral Precipitation on Flow and Mixing in Porous Media Determined by Microcomputed Tomography and MRI." *Environmental Science and Technology*, vol. 51, no. 3, 2017, pp. 1562-1569.
6. Carey JW, Wigand M, Chipera SJ, WoldeGabriel G, Pawar R, Lichtner PC, Wehner SC, Raines MA, Guthrie Jr. GD. "Analysis and Performance of Oil Well Cement with 30 Years of CO₂ Exposure from the SACROC Unit, West Texas, USA." *International Journal of Greenhouse Gas Control*, vol. 1, no. 1, 2007, pp. 75-85.
7. Carey JW, Svec Robert, Grigg Reig, Zhang J, Crow W. "Experimental Investigation of Wellbore Integrity and CO₂-Brine Flow along the Casing-Cement Microannulus." *International Journal of Greenhouse Gas Control*, vol. 4, no. 2, 2010, pp. 272-282.
8. Carroll SA, Keating E, Mansoor K, Dai Z, Sun Y, Trainor-Guitton W, Brown C, Bacon D. "Key Factors for Determining Groundwater Impacts due to Leakage from Geologic Carbon Sequestration Reservoirs." *International Journal of Greenhouse Gas Control*, vol. 29, 2014, pp. 153-168.
9. Cnuddde V, Boone M. "High-Resolution X-ray Computer Tomography in Geosciences: A Review of the Current Technology and Applications." *Earth-Science Review*, vol. 123, 2013, pp. 1-17.
10. Cunningham A.B., Gerlach R., Spangler L., Mitchell A.C., Parks S., Phillips A. "Reducing the Risk of Wellbore Leakage of CO₂ Using Engineered Biomineralization Barriers." *Energy Procedia*, vol. 4, 2011, pp. 5178-5185

11. Cunningham AB, Phillips AJ, Troyer E, Lauchnor E, Hiebert R, Gerlach R, Spangler L. "Wellbore Leakage and Mitigation Using Engineered Biomineralization." *Energy Procedia*, vol. 63, 2014, pp. 4612-4619.
12. Doran, Pauline M. *Bioprocess Engineering Principles*. 2nd ed. Amsterdam: Elsevier/Academic, 2013. Print.
13. De Myunk W, Debrouwer D, De Belie N, Verstraete W. "Bacterial Carbonate Precipitation Improves the Durability of Cementitious Materials." *Cement and Concrete Research*, vol. 38, no. 7, 2008, pp. 1005-1014
14. Dusseault MB. "Why Oilwells Leak: Cement Behavior and Long-Term Consequences." *Society of Petroleum Engineers* 64733, 2000.
15. Fan S, Li M. "X-ray Computer Microtomography of Three Dimensional Microcracks and Self-Healing in Engineered Cementitious Composites." *Smart Materials and Structure*, vol. 24, no 1, 2015, 015021.
16. Feldkamp LA, Davis LC, Kress JW. "Practical Cone-Beam Algorithm." *Optical Society of America*, vol. 1, no. 6. 1984, pp. 612-619.
17. Fidaleo M, Lavecchia R. "Kinetic Study of Enzymatic Urea Hydrolysis in the pH Range 4-9." *Chemical and Biological Engineering Quarterly*, vol. 17, no. 4, 2003, pp. 311-318.
18. Fukuda D, Maruyama M, Nara Y, Hayashi D, Ogawa H, Kaneko K. "Observation of Fracture Sealing in High-Strength and Ultra-Low Permeability Concrete by Micro-Focus X-ray CT and SEM/EDX." *International Journal of Fracture*, Vol 188, Issue 2, 2014, pp.159-171.
19. Gu J, Zhong P, Shao C, Bai S, Zhang H, Li K. "Effect of Interface Defects on Shear Strength and Fluid Channeling at Cement-Interlayer Interface." *Journal of Petroleum Science and Engineering*, vol. 100, 2012, pp. 117-122.
20. Hammes F, Verstraete W. "Key Roles of pH and Calcium Metabolism in Microbial Carbonate Precipitation. *Reviews in Environmental Science and BioTechnology*, vol. 1, no. 1, 2002, pp. 3-7.
21. Han J, Sun W, Pan G. "In Situ Dynamic XCT Imaging of the Microstructure Evolution of Cement Mortar in Accelerated Carbonation Reactor." *Magazine of Concrete Research*, vol. 64, no. 12, 2012, pp. 1025-1031.

22. Herigstad B, Hamilton M, Heersink J. "How to Optimize the Drop Plate Method for Enumerating Bacteria." *Journal of Microbiological Methods*, vol. 44, no. 2, 2001, pp. 121-129.
23. Huerta NJ, Hesse M, Bryant SL, Strazisar B, Lopano C. "Experiment Evidence for Self-Limiting Reactor Flow Through a Fractured Cement Core: Implications for Time-Dependent Wellbore Leakage." *Environmental Science and Technology*, vol 47, no. 1, 2013, pp. 269-275.
24. Huerta NJ, Hesse M, Bryant SL, Strazisar B, Lopano C. "Reactive Transport of CO₂-Saturated Water in a Cement Fracture: Application to Wellbore Leakage During Geologic CO₂ Storage." *International Journal of Greenhouse Gas Control*, vol. 44, 2016, pp. 276-289.
25. Iltis, Gabriel C. "Visualization and Characterization of Biofilm Spatial Distribution in Porous Media using X-ray Computer Microtomography." PhD Dissertation. Oregon State University, 2013.
26. Jung D, Biggs H, Erikson J, Ledyard PU. "New Colorimetric Reaction for End-Point, Continuous-Flow, and Kinetic Measurement of Urea". *Clinical Chemistry*, vol. 21, no. 8, 1975, pp. 1136-1140.
27. Jung HB, Jansik D, Um W. "Imaging Wellbore Cement Degradation by Carbon Dioxide under Geologic Sequestration Conditions using X-ray Computed Microtomography." *Environmental Science and Technology*, vol. 47, no. 1, 2013, pp. 283-289.
28. Kirksey, J (Principal at Loudon Technical Services) in discussion with the author, February 2016.
29. Kutchko BG, Strazisar BR, Lowry GV, Dzombak DA, Thaulow N. "Rate of CO₂ Attack on Hydrated Class H Well Cement under Geologic Sequestration Conditions." *Environmental Science and Technology*, vol. 42, no. 16, 2008, pp. 6237-6242.
30. Kutchko BG, Strazisar BR, Huerta N, Lowry GV, Dzombak DA, Thaulow N. "CO₂ Reaction with Hydrated Class H Well Cement Under Geologic Sequestration Conditions: Effect of Flyash Admixtures. *Environmental Science and Technology*, vol. 43, no. 10, 2009, pp. 3947-3952.
31. Lauchnor EG, Topp DM, Parker AE, Gerlach R. "Whole Cell Kinetics of Ureolysis by *Sporosarcina Pasteurii*." *Journal of Applied Microbiology*, vol. 118, no. 6, 2015, pp. 1321-1332.

32. Li Z, Zhang K, Guo X, Liu J, Cheng X, Du J. "Study of the Failure Mechanisms of a Cement Sheath Based on an Equivalent Physical Experiment." *Journal of Natural Gas Science and Engineering*, vol. 31, 2016, pp. 331-339.
33. Loizzo M, Akemu AP, Jammes L, Desroches J, Lombardi S, Annunziatellis A. "Quantifying the Risk of CO₂ Leakage Through Wellbores." *SPE Drilling and Completion*, vol. 26, no 3, 2011, pp. 324-331.
34. Lovely DR, Phillips EJP. "Rapid Assay for Microbially Reducible Ferric Iron in Aquatic Sediments." *Applied Environmental Microbiology*, vol. 53, 1987, pp. 1536-1540.
35. Martin D, Dodds K, Ngwenya BT, Butler IB, Elphick SC. "Inhibition of *Sporosarcina pasteurii* under Anoxic Conditions: Implication for Subsurface Carbonate Precipitation and Remediation via Ureolysis." *Environmental Science and Technology*, vol. 46, no. 15, 2012, pp. 8351-8355.
36. Mortensen BM, Haber MJ, DeJong JT, Caslake LF, Nelson DC. "Effects of Environmental Factors on Microbial Induced Calcium Carbonate Precipitation." *Journal of Applied Microbiology*, vol. 111, no. 2, 2011, pp. 338-349.
37. Nordbotten JN, Kavetski D, Celia, MA, Bachu S. "Model for CO₂ Leakage Including Multiple Geological Layers and Multiple Leaky Wells." *Environmental Science and Technology*, vol. 43, no. 3, 2009, pp. 743-749
38. NRecon User Manuel. Bruker micro CT, 2016.
39. Opedal NT, Torsaeter M, Vrålstad T, Cerasi P. "Potential Leakage Paths along Cement-Formation Interface in Wellbore; Implications for CO₂ Storage." *Energy Procedia*, vol. 51, 2014, pp. 56-64.
40. Manahiloh KN, Muhunthan B, Kayhanian M, Gebremariam S. "X-ray Computer Tomography and Nondestructive Evaluation of Clogging in Porous Concrete Field Samples." *Journal of Materials in Civil Engineering*, vol. 24, no. 8, 2012, pp. 1103-1109.
41. Peters EJ, Hardham WD. "Visualization of Fluid Displacements in Porous Media Using Computer Tomography Imaging." *Journal of Petroleum Science and Engineering*, vol. 4, no. 2, 1990, pp. 155-168.
42. Phillips AJ, Gerlach R, Lauchnor E, Mitchell AC, Cunningham AB, Spangler L. "Engineered Applications of Ureolytic Biomineralization: A Review, *Biofouling*, vol. 29, no. 6, 2013(a), pp. 715-733.

43. Phillips AJ, Lauchnor E, Eldring J, Esposito R, Mitchell A, Gerlach R, Cunningham AB, Spangler LH. "Potential CO₂ Leakage Reduction through Biofilm-Induced Calcium Carbonate Precipitation." *Environmental Science and Technology*, vol. 47, no. 1, 2013(b), pp. 142-149.
44. Phillips AJ. "Biofilm-Induced Calcium Carbonate Precipitation: Application in the Subsurface." PhD Dissertation. Montana State University, 2013.
45. Phillips AJ, Eldring J, Hiebert R, Lauchnor E, Mitchell A, Cunningham A, Spangler L, Gerlach R. "Design of a Meso-Scale High Pressure Vessel for the Laboratory Examination of Biogeochemical Subsurface Processes." *Journal of Petroleum Science and Engineering*, vol. 126, 2015, pp.55-62.
46. Phillips AJ, Cunningham AB, Gerlach R, Hiebert R, Hwang Chiachi, Lomans BP, Mantilla C, Kirksey J, Esposito R, Spangler L. "Fracture Sealing with Microbially-Induced Calcium Carbonate Precipitation: A Field Study." *Environmental Science and Technology*, vol. 50, no. 7, 2016, pp. 4111-4117.
47. Stocks-Fischer S, Galinat JK, Bang SS. "Microbiological Precipitation of CaCO₃." *Soil Biology and Biochemistry*, vol. 31, no. 11, 1999, pp. 1563-1571.
48. Todorovic J, Røphaug M, Lindeberg E, Vrålstad T, Buddensiek ML. "Remediation of Leakage through Annular Cement Using a Polymer Resin: a Laboratory Study." *Energy Procedia*, vol. 86, 2016, pp. 442-449.
49. Um W, Jung H, Kabilan S, Fernandez CA, Brown CF. "Geochemical and Geomechanical Effects on Wellbore Cement Fractures." *Energy Procedia*, vol. 63, 2014, pp. 5808-5812.
50. US Geological Survey. "Chemistry of Iron in Natural Water." Washington, U.S. Govt. print. Off., 1962.
51. Watson TL, Bachu S. "Evaluation of the Potential for Gas and CO₂ Leakage Along Wellbores." *SPE Drilling and Completion*, vol. 24, no. 1, 2009, pp. 115-126.
52. Whiffin SV, van Paassen LA, Harkes MP. "Microbial Carbonate Precipitation as a Soil Improvement Technique." *Geomicrobiology Journal*, vol. 24, no. 5, 2007, pp. 417-423.
53. Wildenschild D, Sheppard AP. "X-ray Imaging and Analysis Techniques for Quantifying Pore-Scale Structure and Processes in Subsurface Porous Media Systems." *Advances in Water Resources*, vol. 51, 2012, pp. 217-246.

54. Zimmerman RW, Bodvarsson GS. "Hydraulic Conductivity of Rock Fractures." *Transport in Porous Media*, vol. 23, no. 1, 1996 pp. 1-30.