

A STUDY OF BIO-MINERALIZATION FOR THE APPLICATION OF REDUCING
LEAKAGE POTENTIAL OF GEOLOGICALLY STORED CO₂

by

Ryanne Leigh Daily

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ABSTRACT

A primary concern of carbon capture and storage (CCS) is leakage of the stored carbon dioxide (CO₂) from the subsurface back to the surface. To ensure long term storage of the CO₂, mitigation strategies are being developed to seal high permeability regions, such as fractures present in the caprock or the near wellbore environment. Ureolysis induced calcium carbonate precipitation (UICP) is a widely investigated technology utilizing the enzymatically driven process of ureolysis to alter the properties of porous media. The advantage of this technology over traditional fracture sealing methods, such as well cement, is the use of low-viscosity aqueous fluids enabling access to smaller fractures. However, CCS reservoirs provide a problematic environment for microbial activity due to the acidity of dissolved CO₂, high pressures, and elevated temperatures. A flow-through pressurized reactor experiment and batch high-pressure ureolysis rate experiments were conducted to investigate the application of UICP technology to mitigate CO₂ migration. First, UICP was induced in two composite rock cores in an environment simulating a CCS reservoir, using a high-pressure axial flow reactor, with an initial and final exposure of the rock cores to a carbonated brine. As a result of UICP, the apparent permeability of the rock cores were reduced by 5-orders of magnitude. The CO₂ challenge increased apparent permeability by 4-orders of magnitude, likely due to a preferential flow path created through the calcium carbonate (CaCO₃) seal, which was found with X-ray microcomputed tomography (μ -CT) imaging. The porosity of the composite rock cores was assessed throughout the experiment with two non-invasive technologies, μ -CT and nuclear magnetic resonance (NMR), both reported a significant decrease in porosity due to UICP and a slight increase after the CO₂ exposure. Second, ureolysis kinetics were assessed in the presence of a pressurized carbonated brine at pressures between 0 and 4 MPa. The kinetic studies were performed in a high-pressure batch reactor connected to high-pressure pH and conductivity probes. Samples could not be taken from the batch reactor without losing pressure; thus, conductivity was used as a surrogate measurement for urea concentration. It was found that, for the pressures tested, JBM urease was capable of hydrolyzing urea in the presence of a pressurized carbonated brine. It was also hypothesized that the rate observed at each experimental pressure may have been dependent on the buffered pH of the system. The combination of these studies suggests that, if the challenge of dissolution could be overcome, bio-mineralization may be used to enhance CCS by reducing the permeability of CO₂ leakage pathways.

1

CHAPTER ONE

INTRODUCTION

Background

The goal of this thesis is to investigate the application of bio-mineralization technology for reducing potential leakage pathways of geologically stored carbon dioxide (CO₂). The two studies presented are (1) bio-mineralization of composite rock cores in a high-pressure axial flow reactor in the presence of a carbonated brine and (2) assessing the kinetics of urea hydrolysis in the presence of a pressurized carbonated brine (0-4 MPa).

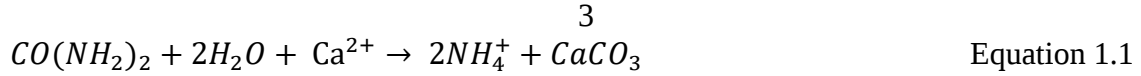
With atmospheric CO₂ concentrations on the rise and the Paris Climate Agreements goal of keeping the mean temperature rise of the Earth under 2° C, CO₂ mitigation strategies are being explored. In the last 200 years, anthropogenic emissions have increased the partial pressure of CO₂ in the atmosphere from 280ppm to 407ppm (Stocker et al. 2013). For the Earth to stay within 2° C of the current mean temperature, the atmospheric concentration of CO₂ must stay under 450 ppm. Carbon capture and storage (CCS) is one mitigation strategy being explored to reduce emissions of greenhouse gases and reach this goal. CCS captures CO₂ emissions created from the combustion of fossil fuels, removes, and stores them away from the atmosphere. According to the 5th Assessment Report by the Intergovernmental Panel on Climate Change (IPCC), CCS is a vital technology to stay within the 450 ppm limit (Stocker et al. 2013). Researchers estimate that CCS alone has the potential to reduce CO₂ emissions by

19% by 2050, but in order to do so, 3,600 CCS operations would either have to be retrofitted or built worldwide (DECC 2012).

A commonly utilized storage method is structural trapping, where low permeability layers trap the CO₂ within the subsurface (DOE). However, there is leakage potential associated with this method, such as cracks within the low permeability layers or leaks within the CO₂ injection wells. The efficacy of CCS depends on ensuring that the CO₂ will remain safely within the subsurface for geological time periods (thousands of years). The research presented here investigates applying bio-mineralization to enhance CCS by mitigating potential CO₂ leakage in order to ensure safe storage.

Bio-mineralization

Bio-mineralization is commonly used to describe the process of ureolysis induced calcium carbonate precipitation (UICP) (Eqn. 1.1). This process begins with the hydrolysis of urea (ureolysis) which is typically induced with the enzyme urease (Ferris et al. 2004; Stocks-Fischer, Galinat, and Bang 1999; Mobley and Hausinger 1989; Phillips et al. 2013), but can also happen spontaneously at high temperatures (Mahalik et al. 2010; Sahu et al. 2009; Rahimpour 2004). Once the reaction occurs, the ammonia (NH₃) equilibrates with the water, resulting in a pH increase. This pH increase shifts the carbonate equilibrium toward bicarbonate (HCO₃⁻) and carbonate ions (CO₃²⁻). If ureolysis occurs in the presence of a sufficient amount of calcium ions (Ca²⁺), the saturation index could be exceeded and the chemical conditions could be favorable for calcium carbonate (CaCO₃) to precipitate out of solution (Ferris et al. 2004; Stocks-Fischer, Galinat, and Bang 1999; Mobley and Hausinger 1989; Phillips et al. 2013).



Certain microorganisms (Mobley and Hausinger 1989) and plant sources (Krajewska 2009) produce the urease enzyme. *Sporosarcina pasteurii*, formerly known as *Bacillus pasteurii* (Yoon et al. 2001), is a commonly used microbial source due to its highly active urease production (Mobley and Hausinger 1989; Ferris et al. 2004). The seeds from the Jack Bean plant are also known to contain large amounts of urease compared to other plant sources (Mateer and Marshall 1916). For the studies presented here both *S. pasteurii* along with Jack Bean meal (JBM) were used as urease sources.

Thesis Overview

Chapter 2 investigates reducing leakage potential of geologically stored CO₂ with ureolysis induced calcium carbonate precipitation (UICP) by mineralizing composite rock cores under pressure. The porosity of the cores was assessed with both microcomputed tomography (μ-CT) and nuclear magnetic resonance (NMR). CCS storage reservoirs provide a harsh acidic environment due to high pressures and high temperatures, making microbial activity difficult. If bio-mineralization technology is going to be applied to CCS reservoirs, the technology needs to be studied under those conditions. Hence, a high-pressure axial flow reactor system, with the capability of making and flowing carbonated brines, was developed. Composite (sandstone/cement) rock cores were bio-mineralized in the high-pressure axial flow reactor in the presence of a carbonated brine. Two trials were performed. The objectives of this study were to assess bio-mineralization in an environment that simulates a geological CO₂ storage

reservoir and to assess the seal's durability once it was formed by challenging it with a carbonated brine. Two non-invasive imaging techniques (NMR and μ -CT) were used to analyze the porosity and pore volume of the composite cores throughout the experiments. The apparent permeability of the rock cores were decreased by 5-orders of magnitude as a result of bio-mineralization. The CO₂ challenge increased the apparent permeability by 4-orders of magnitude, likely due to a preferential flow path created through the CaCO₃ seal. NMR and μ -CT produced complementary data about the porosity change at each stage of the experiments. Both methods reported a decrease in porosity after bio-mineralization and a slight increase after the CO₂ challenge.

With the same goal of further investigating the application of bio-mineralization to mitigating CO₂ leakage during CCS, the kinetics of ureolysis in the presence of pressurized carbonated brines were evaluated in Chapter 3. Ureolysis batch experiments were performed under pressure using change in conductivity as a surrogate measurement for change in urea concentration (Whiffin, van Paassen, and Harkes 2007). A surrogate measurement was needed because samples could not be taken without depressurizing the reactor system. First, the pH of carbonated brines without ureolysis was established. Rates of ureolysis and changes in pH conditions in the presence of urea, JBM and CO₂ were then assessed. The carbonated brines were near CO₂ saturation for each pressure tested (0-4 MPa) and amended with 330 mmol/L urea. It was shown that JBM urease was capable of hydrolyzing urea in the presence of a pressurized carbonated brine at all pressures tested. It was also hypothesized that the rate observed at each experimental pressure may have been dependent on the buffered pH of the system. Chapter 4

summarizes the results of Chapters 2 and 3 and suggests future work to further investigate the studies presented.

CHAPTER TWO

REDUCING LEAKAGE POTENTIAL OF GEOLOGICALLY STORED CO₂ WITH
UREOLYSIS INDUCED CALCIUM CARBONATE PRECIPITATION: ASSESSING
THE MINERAL SEAL WITH MICROCOMPUTED TOMOGRAPHY (μ -CT) AND
NUCLEAR MAGNETIC RESONANCE (NMR)

IntroductionCarbon Capture and Storage

With anthropogenic CO₂ levels on the rise, greenhouse gas mitigation methods are being researched (Stocker et al. 2013). One proposed method is carbon capture and storage (CCS) (Aminu et al. 2017), where CO₂ emissions created from the combustion of fossil fuels are removed from flue gas streams or captured from the atmosphere (Lackner 2014) and stored away from the atmosphere. Multiple storage options for the CO₂ are being explored, including geological storage, deep ocean storage, and mineral carbonation. In the case of geological storage, the CO₂ is injected into deep subsurface saline reservoirs. A significant concern involved is the migration of the CO₂ back to the surface through leakage pathways (Aminu et al. 2017). Figure 2.1 is a conceptual diagram of a subsurface saline reservoir used in geological CO₂ storage. It depicts potential CO₂ leakage pathways associated with the injection well and the caprock of the reservoir.

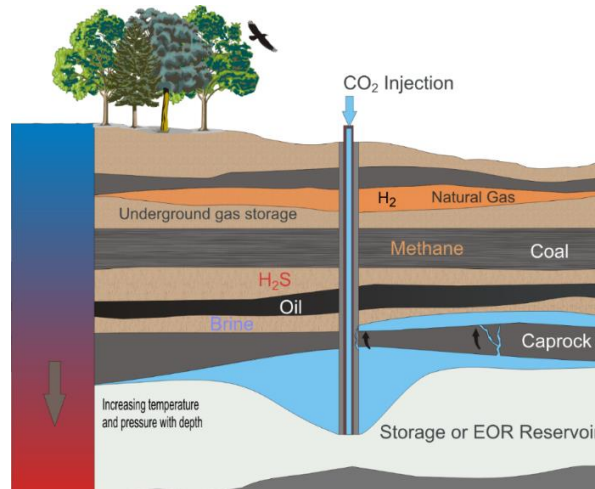


Figure 2.1: A conceptual diagram of a subsurface saline reservoir used for geological storage of CO₂. Potential CO₂ leakage pathways in the caprock and near well bore environment are displayed. Increasing temperatures and pressures are seen with increasing depth, as well as, acidic conditions due to dissolved CO₂.

In order to implement CCS on large scales, it is necessary to assess the efficacy of the process and its potential environmental consequences. The introduction of a potentially reactive solution of dissolved CO₂ into an environment that has never interacted with large amounts of CO₂ is a concern (Moore et al. 2003; Gaus, Azaroual, and Czernichowski-Lauriol 2005; Hemme and van Berk 2017). CO₂ injected into geologic formations has the potential to impact the chemistry of the brine solutions present, the mineralogy of the caprock, and could react with the cement present around the injection wells (Moore et al. 2003; Gaus, Azaroual, and Czernichowski-Lauriol 2005; Hemme and van Berk 2017). Some of the resulting geochemical reactions, such as carbonate precipitation, may aid in the trapping of the CO₂. Other geochemical reactions, such as mineral dissolution, may create leakage pathways (Mitchell et al. 2010). The reactions that occur are highly dependent on the initial geological and fluid chemical conditions of the storage reservoir. Failures can also occur in multiple locations of the

injection wells, including the casing-cement interface, the cement matrix, cement-defect channels, and cement-cap rock interface. It is crucial, therefore, to assess the performance of the 'engineered' cement seals and the behavior of borehole cement in the presence of CO₂. This work focuses on a technology, called bio-mineralization, to help ensure safe storage of CO₂ within geological storage sites. Bio-mineralization technology has the potential to encourage reactions that aid in the trapping of CO₂ as solid carbonate minerals, provide a protective barrier between the reactive solutions and the rock formations or well cement, and seal leakage pathways.

Bio-mineralization and CCS

Bio-mineralization, or carbonate precipitation, has been widely researched for a range of engineering applications including soil stabilization (van Paassen et al. 2010), dust suppression, cement remediation, pond/reservoir sealing, and groundwater remediation (Phillips et al. 2013; Mitchell and Ferris 2005). Bio-mineralization is commonly used to describe the process of ureolysis-induced calcium carbonate precipitation (UICP). UICP begins with the hydrolysis of urea (ureolysis), typically induced with the enzyme urease (Ferris et al. 2004; Stocks-Fischer, Galinat, and Bang 1999; Mobley and Hausinger 1989; Phillips et al. 2013). Eqn. 2.1 shows the overall urea hydrolysis reaction. Once the urea is hydrolyzed, the NH₃ equilibrates with water and increases the pH of the system (Eqn. 2.2), which shifts the carbonate equilibrium towards bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) (Eqn. 2.3 and 2.4). If ureolysis occurs in the presence of a sufficient amount of calcium ions (Ca²⁺), the chemical conditions could be favorable for calcium carbonate (CaCO₃) to precipitate out of solution (eqn. 2.4).



This study investigates applying the bio-mineralization technology to prevent CO₂ leakage from CCS reservoirs. The use of bio-mineralization for this application is advantageous for many reasons. Bio-mineralization uses low viscosity fluids such as dissolved urea and calcium and suspended urease in aqueous solutions. The low viscosity enables the fluids to penetrate smaller apertures than traditional sealing methods, such as viscous cement, can influence. Additionally, a calcium carbonate (CaCO₃) mineral is created and left behind, which may fill leakage pathways and protect the cement and rock formation from carbonation (Phillips et al. 2012). Moreover, utilizing a pulsed injection strategy, alternating solutions containing urea and calcium with urease solutions, a gradual reaction is encouraged and a wider, more homogeneous spatial distribution of CaCO₃ may be achieved (Tobler, Maclachlan, and Phoenix 2012; Ebigbo et al. 2012). The disadvantage is that the acidic environment created by dissolved CO₂ has the potential to influence the rate of ureolysis, the rate of mineral formation, and can cause the dissolution of the CaCO₃.

Previous research has been done on applying bio-mineralization to CCS (Phillips

et al. 2012; Mitchell et al. 2013; Mitchell et al. 2009; Cunningham et al. 2011; Dupraz, Ménez, et al. 2009). Past research has shown that bio-mineralization can seal both fractures and porous media (Phillips 2013; Cunningham et al. 2011). Mitchell et al. (2013) investigated both bio-mineralization at high pressures and how the mineralized seal responds to exposure to CO₂. A synthetic rock core was bio-mineralized in a high-pressure flow reactor at 8.27 MPa (1200 psi). After mineralization, the core was exposed to both dry and water-saturated supercritical CO₂ (scCO₂). The calcite precipitates produced during bio-mineralization were resilient to the dry scCO₂, but mass loss was seen in the water-saturated scCO₂ (Mitchell et al. 2013). Mineral trapping has been studied by Dupraz et al. in artificial groundwater (AGW) with a range of salinities and partial pressures of CO₂. Their observations showed that MICP could promote a substantial pH increase due to the ureolysis reaction in AGW equilibrated with CO₂ pressures of up to 1 bar (0.1 MPa) and successfully precipitate CaCO₃ from the dissolved CO₂ (Dupraz, Ménez, et al. 2009). Mitchell et al (2010) also demonstrated ureolysis was effective at precipitating initially gaseous CO₂ originating from a headspace. They also observed that the increase of pH, due to ureolysis, enhanced the solubility-trapping capacity of CO₂ within the brine and a flux of CO₂ into the brine occurred (Mitchell et al. 2010). Phillips et al. have shown that the bio-mineralization process can happen at industrial scales by promoting MICP in a fractured sandstone formation 340.8 m below the ground surface using conventional oil field fluid delivery technologies (Phillips et al. 2016). The combination of this research gives promise to the application of bio-mineralization to CCS reservoirs, however more research needs to be performed in an environment simulating that of a CCS reservoir, which is presented here.

The use of NMR for characterizing fluids in porous media is well-established (Callaghan 2011), and has previously been used to characterize mineralization (Bray et al. 2017) and bio-mineralization (Handley-Sidhu et al. 2013; Kirkland et al. 2017; Fridjonsson et al. 2011) within porous media. The NMR signal results from the response of protons in water, or "spins," to applied perturbations in the static magnetic field. The initial amplitude of the NMR signal is proportional to the amount of water in an excited state due to the perturbations, thus reflects the volumetric water content in the sampled region. This initial amplitude was used to estimate the pore volume of the composite rock cores in this study. The T_2 relaxation time can be used to determine pore size distribution within porous media. The T_2 relaxation time is comprised of three terms, the relaxation time of the bulk pore fluid, the surface relaxation time, and the diffusion relaxation time (Kirkland et al. 2017). Typically, a decrease in pore size would result in a faster relaxation time, and in the case of heterogeneous pore sizes, multiple relaxation times may be observed.

X-ray microcomputed tomography (μ -CT) has been applied to analyzing properties of porous media and has been used to quantify CaCO_3 mineralization (Kirkland et al. 2019; Bray et al. 2017). X-ray μ -CT images are generated by mapping the X-ray attenuation at each location in a sample. The attenuation corresponds to the material properties, such as density, through which the X-ray beam has passed (Wildenschild and Sheppard 2013). Thresholding methods can be applied to these images to distinguish between pore space and solid material.

The combination of μ -CT and NMR techniques were used by Bray et al. (2017) to evaluate the influence of mineralization on the flow and mixing of fluids within porous

media. Spatial maps of changing local velocity fields and dispersion in the flow cell were generated from MRI (NMR), while high resolution μ -CT imaging visualized the precipitate formed in the porous media (Bray et al. 2017). During the current study, both μ -CT and NMR techniques were used to quantify the CaCO_3 mineral formation within the rock cores. Both methods are non-invasive and non-destructive means of quantifying the change in pore volume. Using X-ray μ -CT, the spatial distribution of the CaCO_3 precipitates formed within the composite cores was determined and correlated to the pore size distribution, and total pore volume found with NMR.

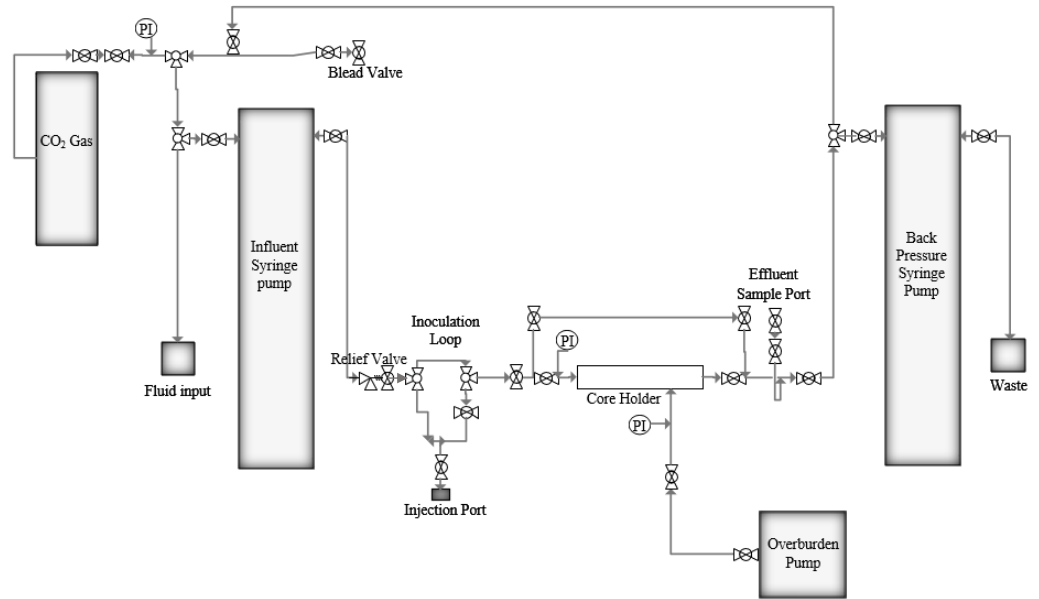
Here, a defect in the cement-cap rock interface of a geological CO_2 storage reservoir was simulated with cylindrical composite rock cores that consisted of a Berea sandstone sleeve and a cement center with a 100 μm gap between them. The bio-mineralized seal was induced within the gap, and the behavior of the seal in the presence of a carbonated brine was assessed. Two trials were done in a high-pressure axial flow reactor. Both started with soaking the composite rock core in a carbonated brine at 8.27 MPa (1200 psi) and 55 °C. Bio-mineralization was then induced in the cores until a 5-log reduction in apparent permeability was observed. Heat treated *S. pasteurii* was used as the urease source in these experiments. Once the mineral seal was formed, the cores were exposed to a carbonated brine to assess the seals' durability. NMR and μ -CT were used to analyze the porosity and pore volume of the rock cores throughout the experiments.

High-pressure flow reactor design

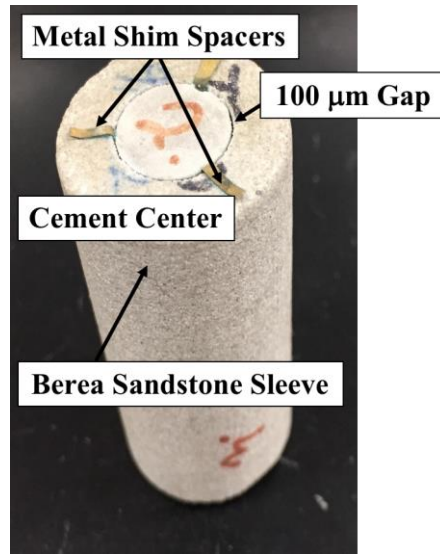
Modifications were made to a previously used high-pressure flow reactor (Phillips 2013; Mitchell et al. 2010; Mitchell et al. 2013) to simulate the environment in a subsurface reservoir used for geologically stored CO₂. The system allowed for axial flow through a composite rock core at a pressure of 8.27 MPa (1200 psi) with an overburden pressure of 11.03 MPa (1600 psi) and a temperature of 55° C. These conditions are a good representation of pressures and temperatures that could be seen in a CO₂ storage reservoir (DOE).

The high-pressure flow reactor consisted of a biaxial Hassler-type core holder (Temco, Tulsa, OK) connected to three Teledyne Isco syringe pumps for influent flow, back pressure control, and overburden pressure. A compressed CO₂ gas tank was connected to the influent pump, enabling the pump to make a carbonated brine and inject the brine through the flow reactor. A 25 mL inoculation loop was also added between the pump and the core holder in which bacteria could be injected without contaminating the influent pump. All components of the system were connected with ¼" stainless steel tubing and Swagelok fittings and valves (Swagelok, ID, USA) (Fig. 2.2a). A heated water jacket, which consisted of tubing connected to a constant temperature heated water bath, was fitted to the core holder. Insulation wrap was used to insulate the core holder and heated water jacket in order to keep a constant temperature of 55°C throughout the extent of the experiments.

Prior to loading the core into the reactor, the entire reactor system, minus the core holder, was disinfected. This was done by pumping each of the following fluids through the reactor system: (1) 5mL bleach and 3.5 g Tween 80 dissolved into 500 ml DI water, (2) 500 ml of autoclaved DI water, (3) an autoclaved solution of 1.26 g sodium thiosulfate dissolved into 500 mL DI water, (4) 0.2 μm filter sterilized (Thermo Scientific, NJ, USA) 70% ethanol solution and (5) 500 ml of autoclaved PBS in DI water (Mitchell et al. 2009; Phillips 2013). Each fluid was allowed a 30 minute residence period. The core holder was rinsed separately with DI water. After disinfection, the rock cores were loaded into a Buna-N sleeve (TEMCO Inc., Tulsa, OK), and the core holder was assembled. The core holder was then connected to the rest of the assembly, and the overburden annulus was filled with water. The designated Isco pump provided an overburden pressure of 11.03 MPa (1600 psi). The rest of the system was then pressurized to 8.27 MPa (1200 psi) with 10g/L NH_4Cl , using both the influent and back pressure Isco pumps.



(a)



(b)

Figure 2.2. (a) Schematic diagram of the high-pressure flow reactor system consisting of the core holder containing the sandstone/cement composite core, three Teledyne Isco syringe pumps (influent, backpressure, and overburden pressure), CO₂ gas tank, injection loop, and effluent sample port. The core holder was wrapped with heated tubing connected to a constant temperature flowing water bath and insulated, in order to heat the core to 55°C. (b) Picture of a sandstone/cement composite rock core prior to mineralization. The core consisted of Berea sandstone sleeve and cement center with 100 μm gap between. The cement core was held with three metal shims on each end.

Composite Rock Core

The composite rock core was designed to represent a leakage pathway that could occur between the injection well cement and the formation rock (Fig. 2.2b). The core consisted of a Berea sandstone sleeve and a cement center with a 100 μm gap. The cylindrical core was 5.08 cm (2") long with a 2.54 cm (1") diameter.

The Berea Sandstone cores were obtained from Cleveland Quarries, Ohio. The center of each core was drilled out on a lathe using a 1.27 cm ($\frac{1}{2}$ ") masonry drill bit. After boring, the cores were cut to 5.08 cm (2") length. The core hole diameter was measured with a digital caliper at the entry and exit side. Several measurements were taken at each end of the core in order to determine the diameter and roundness of the borehole.

For the making of the cement cores, a casting form was fabricated from an acetal polymer rod of 5.08 cm (2") diameter and 6.35 cm (2.5") length. The 5.08 cm (2") diameter was chosen in order to assure form stability during the hole boring process. According to the hole diameter data, a 12.2 mm center hole was machined into the acetal rod using a lathe. The cement rods were cast from a well casing fine cement classified as 50/50, Poz H, 6% Dozo, a proprietary blend received from Slumberger, using a water:cement mix volume ratio of 5:9. The cement was cured inside the acetal casting form for 72 hours at room temperature, pressed out of the cast form and post-cured for seven days at room temperature in a calcium saturated aqueous solution. The cement rods shrank to approximately 5.84 cm (2.3") length while curing in the cast form and were cut to 5.08 (2.0") final length before the composite core assembly. There was a 2.5

cm long crack or channel, presumably formed during curing of the cement, present on the inside of the cement core used for Trial 2.

Figure 2.2b illustrates how the cement rods were positioned and affixed in the sandstone cores holes using stainless steel shim stock. Shims were used to assure a consistent annular gap size of 100 μm between the two components during the bio-mineralization studies.

The pore volumes for each section of the composite rock cores were estimated. The volume within the gap was calculated from the volume of cylinder equation using the inner dimension of the sandstone sleeve and the outer dimension of the cement core. To calculate the pore volume within the sandstone sleeve, the total volume of the sleeve was calculated and multiplied by 18.9% (assumed porosity of Berea sandstone)(Phillips 2013). For these calculations it was assumed that the pore volume within the cement core was negligible.

Table 2.1: Estimated pore volumes within composite rock cores

Section of Composite Rock Core	Estimated Pore Volume (mL)
Sandstone Sleeve	3.7
Gap	0.3
Total	4.0

Apparent Permeability Calculation

Flow rate and differential pressure were measured throughout the experiment with the influent Teledyne Isco pump and used to calculate the hydraulic conductivity (Eqn. 2.5), which was then used to calculate permeability (k) (Eqn. 2.6) (Todd and Mays 2005).

$$K = -\frac{Q L}{\Delta P A} \quad \text{Equation 2.6}$$

$$k = \frac{K \mu}{\rho g} \quad \text{Equation 2.7}$$

In Equation 2.6, K is hydraulic conductivity, Q is the recorded fluid flow rate, Δp is the recorded differential pressure, A is the cross section area of the composite core excluding the cement center, assuming no flow through the cement, (3.7 cm^2), and L is the length of the composite core (5.08 cm). In Equation 2.7, k is the permeability (mD), μ is the dynamic viscosity of the fluid ($1.051 \times 10^{-3} \text{ kg/(m*s)}$), ρ is the density of the fluid (1013 kg/m^3), and g is gravity (9.81 m/s^2) (Phillips 2013). In this study the permeability is referred to as an apparent permeability because the cross sectional area in the calculation uses the gap and the sandstone combined. The gap between the sandstone and the cement is not porous media, however, it is being treated as porous media in these calculations.

Experimental Protocol

Two trials of the experiment, both done under the same conditions, were performed as described below. Table 2.2 describes the injection strategy for both experiments.

Saturation and Initial Permeability. Before each experiment, the core was saturated by flowing 500 mL of 10 g/L NH_4Cl through the core. The initial permeability

was then determined by recording differential pressure data while increasing the flow rate until a stable pressure reading was reached, which was at a flow rate of 120 mL/min for both trials and a differential pressure of 0.08 MPa (1.2 psi) and 0.012 MPa (1.8 psi) for Trials 1 and 2 respectively.

Initial CO₂ Soak. In order to simulate a subsurface reservoir storing CO₂, the experiment started with soaking the core in a carbonated brine overnight. To prepare the carbonated brine, 250 mL of 10 g/L NH₄Cl was added to the influent pump. The pump was then drawn down to have a 300 mL headspace which was pressurized to 4.01 MPa (580 psi) with CO₂ gas from the compressed CO₂ gas tank. The pump was then isolated from the CO₂ tank and slowly pressurized to 8.27 MPa (1200 psi). 250mL of this brine was injected into the core at 5 mL/min; the core was then isolated and allowed to soak overnight.

Inoculum Preparation. A culture of *S. pasteurii* (ATCC 11859) was prepared by inoculating 100 mL of growth media (37 g/L Brain Heart Infusion (BHI), and 20 g/L urea) with 100 µL of a thawed frozen stock in an Erlenmeyer flask. The culture was shaken at 30°C and 150 rpm overnight. Cultures for each day following the first were prepared by inoculating 100 mL of growth media with 1 mL of the previous days' culture. These cultures were also shaken at 30°C and 150 rpm overnight. Each culture was centrifuged, resuspended in fresh nutrient media (3 g/L Difco Nutrient Broth, 20 g/L urea and 10 g/L NH₄Cl), and adjusted to an optical density at an absorbance of 600 nm (OD₆₀₀) of 0.4 by putting 200 µL in a 96 well plate in triplicate (Synergy plate reader

Biotech). The OD was adjusted in order to stay consistent between days and pulses, not for kinetic analysis.

To inoculate the core, a 30 mL sterile syringe was filled with 25 mL of inoculum. The syringe was attached to the Swagelok fitting connected to the inoculation loop, and the inoculum was injected into the system. The inoculation loop was then pressurized, and the inoculum was injected into the rock core and allowed to remain in the core for 15 minutes.

Mineralization. Mineralization pulses began after the overnight carbonated brine soak. Each mineralization pulse consisted of an inoculum period and a mineralization period. During the inoculation period, 25 mL of inoculum was injected, according to the procedure above, followed by a 15-minute no-flow period. After the no-flow period, the mineralization period began. This started with 5 mL of 10 g/L NH_4Cl , to separate the inoculation and mineralization fluids, followed by 60 mL of the mineralization media (20 g/L urea, 49 g/L $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ and 10 g/L NH_4Cl). The mineralization media was allowed a 1-hour no-flow period. The inoculation/mineralization pulse strategy was repeated four times a day at the beginning of the experiment with decreasing frequency throughout the experiment as the flow rate slowed due to decreasing permeability. A flow rate of 5 mL/min was initially set until a differential pressure of 1.03 MPa (150 psi) was reached. At this point the influent pump was set to constant pressure mode at 9.31 MPa (1350 psi), with the back pressure (effluent) pump set to 8.27 MPa (1200psi) to establish and maintain a constant differential pressure of 1.03 MPa (150 psi). In constant pressure mode, the pumps adjust the flow rate accordingly. Mineralization pulses continued until

a 5-log reduction in apparent permeability was achieved. Effluent samples were taken and analyzed every pulse using the procedure described below.

Final CO₂ Challenge. After the 5-order of magnitude reduction in apparent permeability was achieved, a CO₂ challenge was performed on the core. Carbonated brine was made using the same methods described in the initial carbonated brine soak section. As with the initial carbonated brine soak, 250 mL of the carbonated brine was injected through the core with a 1.03 MPa (150 psi) pressure differential. Once all the brine was injected, the core was isolated and soaked overnight. After the CO₂ challenge, the apparent permeability was tested again, with 10 g/L NH₄Cl solutions.

Porosity Analysis and Experimental Termination. After both the mineralization period and the final CO₂ challenge, the system was depressurized, and the core was removed from the core holder and placed in a glass beaker filled with 10 g/L NH₄Cl. The cores porosity were then assessed with both the NMR and μ -CT techniques described in sections below.

Table 2.2: Injection Strategy of Each Stage of Experiments

Experimental Step	Fluid	Duration (hr)	Volume (mL)	Flowrate (mL/min)	Differential Pressure (MPa)
Pre-Min Porosity Scans	<i>NMR</i> <i>μ-CT</i>	24			
Saturation	10 g/L NH ₄ Cl	1.67	500	5	< 1.03
Initial CO ₂ Soak	Carbonated Brine No-flow	0.88 16	250	5	< 1.03
Mineralization	Inoculum No-flow Min-media No-flow Inoculum No-flow Min-media No-flow Inoculum No-flow Min-media No-flow Inoculum No-flow Min-media No-flow	0.08 0.25 0.1 1 0.08 0.25 0.1 1 0.08 0.25 0.1 1 0.08 0.25 0.1 1	25 60 25 60 25 60 25 60 25 60 25 60	5 5 5 5 5 5 5 5 5 5 5 5	< 1.03 < 1.03 < 1.03 < 1.03 < 1.03 < 1.03 < 1.03 < 1.03 < 1.03 < 1.03 < 1.03 < 1.03 < 1.03 < 1.03
Reached Max Differential Pressure <i>(continued until apparent permeability reduced 5-orders of magnitude)</i>	Inoculum No-flow Min-media No-flow Inoculum No-flow Min-media No-flow	> 0.08 0.25 > 0.1 1 > 0.08 0.25 > 0.1 1	25 60 25 60	< 5 < 5 < 5 < 5	1.03 1.03 1.03 1.03
Post-Min Porosity Scans	<i>NMR</i> <i>μ-CT</i>	24			
Final CO ₂ Challenge	Carbonated Brine No-flow	0.88 16	250	< 5	1.03
Final Apparent Permeability Test	10 g/L NH ₄ Cl	> 1.67	500	5	< 1.03
Post-CO ₂ Porosity Scans	<i>NMR</i> <i>μ-CT</i>	24			

Effluent Sample Analysis

For every pulse, a sample of the fluids present in the reactor during the mineralization period was collected through the sample port and prepared for sample analysis. One mL of this sample was filtered through a 0.2 μm polycarbonate membrane (VWR International). The filtered portion of the sample was diluted in deionized water and analyzed with the colorimetric modified Jung assay to determine the concentration of urea in the sample (Jung et al. 1975; Phillips 2013). The non-filtered portion was used to test pH and determine the number of colony forming units (CFU's) as a measure of cell culturability. Samples were serially diluted in phosphate buffered saline (PBS) from 10^{-1} to 10^{-5} , and 10 μL of the dilutions was dropped onto an agar plate amended with 37g/L BHI and 20g/L urea. The plates were incubated at 37°C for 24 hours, and plates were assessed for the presence of colonies (Herigstad, Hamilton, and Heersink 2001).

Porosity Analysis

X-ray microtomography ($\mu\text{-CT}$). Images were collected with a SkyScan 1173 $\mu\text{-CT}$ system at three time points in the experiment: 1) pre-biomineralization, 2) post-biomineralization but pre- CO_2 -challenge, and 3) post- CO_2 -challenge. 2D images were taken as the core rotated around its vertical axis every 0.7° . Scans were performed at an X-ray production voltage of 130 kV and a current of 60 μA , and the resolution of the image pixels were 22.68 μm .

From the raw vertical images, 2D horizontal stacks of projection radiographs were reconstructed using NRecon software which uses the Feldkamp algorithm (Feldkamp, Davis, and Kress 1984; "NRecon User Manual" 2016). The reconstructed images are

made up of pixels, each assigned a linear attenuation coefficient corresponding to the X-ray signal intensity received at that location. The X-ray signal intensity is a function the material properties such as density (Wildenschild and Sheppard 2013). Thresholding of the images was performed in ImageJ using the MaxEntropy algorithm (Kapur, Sahoo, and Wong 1985) to distinguish solid material from the pore space within. The data was separated into two categories dependent on the attenuation coefficient at each pixel. Above the threshold limit, all material was considered to be in the solid phase (rock core or CaCO_3) and below, everything was considered to be pore space. The percent of each image that was below the threshold was determined to represent the pore space of the core. Several thresholding algorithms were tested, with MaxEntropy having the best results for the images in this study. The MaxEntropy algorithm accounted for the pore space present and added the least false porosity, confirmed by comparing results to the calculated pore space present within the gap, 0.37 mL (Table 2.1).

NMR. For this study, a 2 MHz Rock Core Analyzer (RCA) was used because it is well suited for NMR measurements of samples with high magnetic susceptibilities, such as, rocks and natural sediments, due to its low magnetic field strength. It operates at a large bandwidth which enables measurements of fast T_2 relaxation rates, also making it suitable for the rock cores in this study due to the small pores sizes present within the sandstone. The NMR signal results from the response of protons in water to applied perturbations in the static magnetic field. The initial amplitude of the NMR signal is proportional to the total water volume in the sampled region. This measurement was used to calculate the porosity of the rock core.

The T_2 relaxation time was measured to observe how pore size distribution in the rock core changed due to bio-mineralization and the CO_2 challenge. T_2 relaxation time is comprised of three terms, the relaxation time of the bulk pore fluid, the surface relaxation time, and the diffusion relaxation time (Kirkland et al. 2017). For this work, there were no expected changes in fluid properties between scans, so changes in the bulk pore fluid relaxation time were assumed to be negligible and were neglected. The diffusion relaxation component was also neglected because of the low magnetic field. Therefore, the changes in the T_2 relaxation time was assumed to only depend on the surface relaxation time (Kirkland et al. 2017). Long T_2 relaxation times are typically associated with larger pores, while shorter T_2 relaxation times are typically associated with smaller pores. A decrease in pore size typically results in a faster relaxation time. The relative amplitude of each peak in the T_2 distribution is proportional to the volume of water with that T_2 relaxation time and can be interpreted as a pore size distribution. A pore size distribution is often represented by multiple peaks on the T_2 relaxation time distribution; in this work, two peaks were observed.

Measurements were performed at three time points in the experiment: 1) pre-bio-mineralization, 2) post-bio-mineralization but pre CO_2 challenge, and 3) post-bio-mineralization and CO_2 challenge. Cores were treated with 10 g/L NH_4Cl in a vacuum flask before all measurements. The T_2 measurements were executed using the standard Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence with an echo time of $\tau_e = 150 \mu\text{s}$, 10000-30000 echoes, depending on the stage of the experiment, $25 \mu\text{s}$ and $50 \mu\text{s}$ pulse durations for the $\pi/2$ and π excitation pulses respectively, and a dwell time of $1 \mu\text{s}$. The minimum signal to noise ratio (SNR) was set to 100 for the T_2 measurements so that the

number of averages performed depended on the liquid volume in the core.

Results and Discussion

Apparent Permeability

In both trials, the overall apparent permeability was reduced as a result of bio-mineralization by 5-orders of magnitude. In Trial 1, the apparent permeability reduced from 1.84×10^4 to 8.0×10^{-2} mD with 21 mineralization pulses. In Trial 2 the reduction was from 1.10×10^4 to 6.0×10^{-2} mD with 18 pulses. A negative control was not performed, but it has previously been observed with similar urea and calcium solutions that permeability reduction was not seen without the addition of ureolytic bacteria (Wheeler 2009) or with the addition of fluids amended with antibiotics to reduce microbial growth (Kirkland et al. 2017; Kirkland et al. 2019).

After the CO₂ challenge, the apparent permeability increased to 4.79×10^2 and 4.59×10^2 mD in Trials 1 and 2, respectively. While the apparent permeability increased 4-orders of magnitude, it remained below the pre-mineralization apparent permeability in both trials.

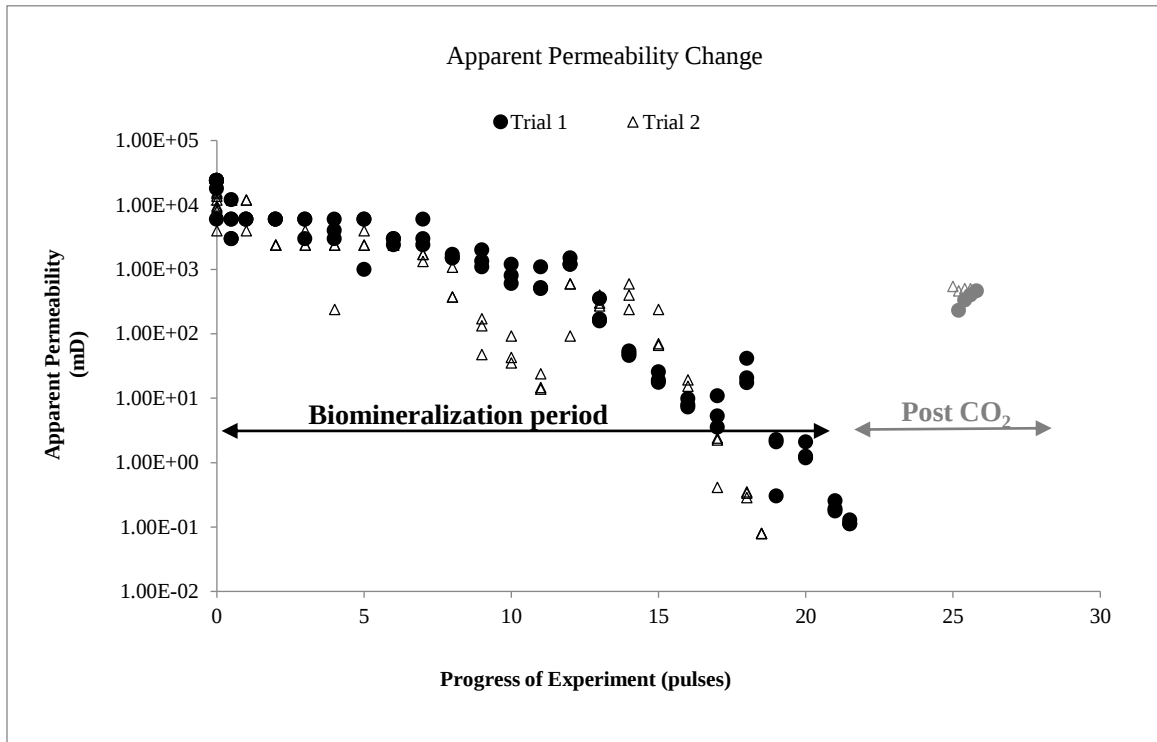


Figure 2.3: Apparent permeability change throughout the experiment. If the permeability changed throughout the pulse, multiple data points are presented for that pulse. Bio-mineralization decreased the apparent permeability by 5-orders of magnitude. The CO₂ challenge increased the apparent permeability by 4-orders of magnitude.

Contribution to Apparent Permeability by “Mineral Cap”

Significant reduction in apparent permeability was seen starting at pulse 13 in Trial 1. The reduction is hypothesized to be due to the void space within the gap and influent volume of the core holder being filled with CaCO₃ and diverting the flow through the Berea sandstone, which was previously shown to have an average initial permeability of 30 mD (Phillips 2013). The CaCO₃ filling the influent volume of the core holder created a "mineral cap" on the cores that can be seen in Figure 2.4. A similar effect was seen in Phillips 2013, where a “skin” was seen at the influent of the cores. They observed that the skin influenced the permeability of the core (Phillips 2013). During Trial 2, the significant drop in apparent permeability was seen at pulse 9, which is earlier

than Trial 1. This could suggest that the seal or “mineral cap” formed earlier in Trial 2. Also, during Trial 2, after the apparent permeability decrease was seen at pulse 9, an apparent permeability increase was observed at pulse 12, this could have been a result of the seal breaking or being dislodged due to the high differential pressure. After pulse 14, the apparent permeability began to reduce again, and it is hypothesized the seal was reformed (Fig. 2.3).

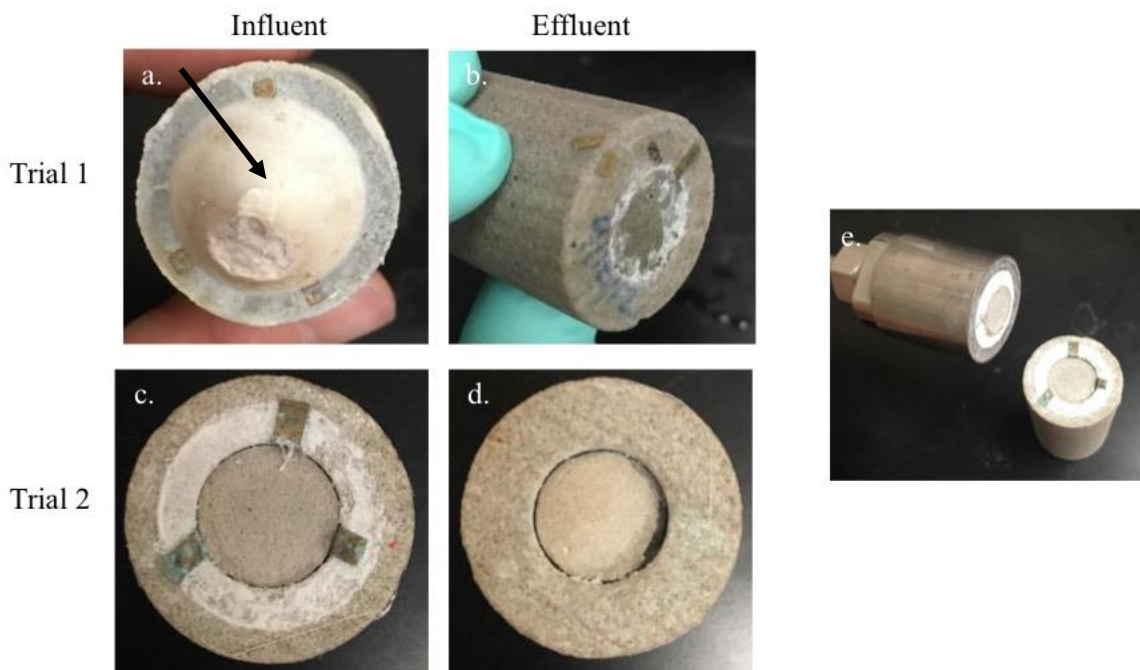


Figure 2.4: Influent and effluent regions of the mineralized cores. (a) Influent of Trial 1. The black arrow is pointing to the “mineral cap” of CaCO_3 (the white cone). (b) The effluent of Trial 1. The CaCO_3 mineral can be seen filling the gap. (c) Influent of Trial 2. The “mineral cap” broke off inside of the core holder and is not shown in the picture but can be seen in picture e. (d) Effluent of Trial 2. Note less mineral was present in the gap. e.) The influent core holder rod with the “mineral cap” broken off inside, along with the influent side of the composite core. The picture was taken after the mineralization period of Trial 2.

A preferential flow path formed in the “mineral cap” of Trial 1 during the CO_2 challenge was found with μ -CT images (Fig. 2.5). The significant apparent permeability increase observed after the CO_2 challenge could be attributed to the preferential flow

path. The "mineral cap" from Trial 2 broke off in the core holder and could not be imaged. However, because of the similarity of the apparent permeability data from Trial 1 and 2, it is suspected that a preferential flow path also formed in Trial 2.

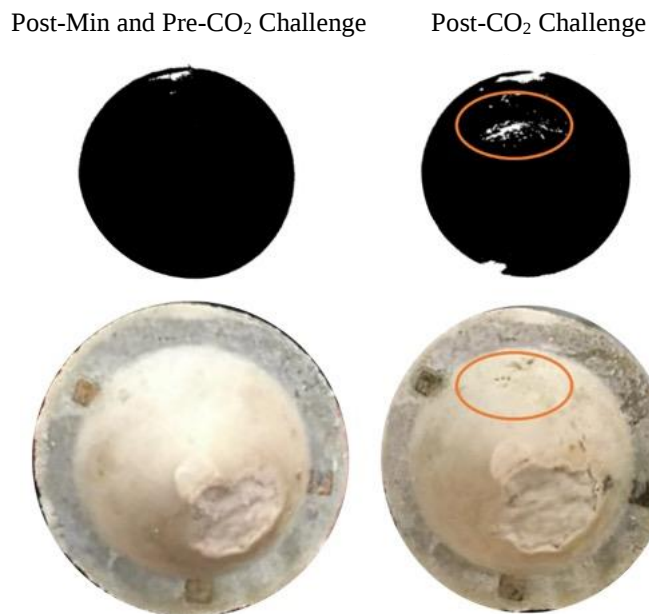


Figure 2.5: Images showing the flow path formed in the "mineral cap" of CaCO_3 from Trial 1. (Top) μ -CT images of the "mineral cap." The binary images were created in ImageJ using the MaxEntropy threshold algorithm. (Bottom) Pictures taken of the "mineral cap" before and after the CO_2 challenge. The orange circles seen in the post CO_2 challenge images indicate the presumed preferential flow path.

Effluent Sample Analysis

Figure 2.6 shows the fraction of urea in the effluent samples compared to the initial urea concentration for each pulse. There is variability between pulses, which could be attributed to the sampling method. Even with assuming plug flow it was difficult to time the opening of the sample port when the 3 mL that were present within the core holder during the mineralization period were passing by the sampling tubing apparatus. The average percent of effluent urea concentration to the initial urea concentration

observed for Trials 1 and 2 was 49% and 28%. The timing of sample withdrawal was improved in Trial 2, resulting in samples with less urea due to more of the sampled fluids having resided within the core and not in the tubing of the reactor during the reaction period. The pH of effluent samples varied between 7.4 and 8.5. The influent inoculation had an average cell concentration of 1.5×10^8 CFU/mL. However, there were no observed CFUs observed, with a detection limit of 10^3 CFU/mL, in the effluent samples that were analyzed, suggesting that the cells were inactivated while in the reactor held at 55°C.

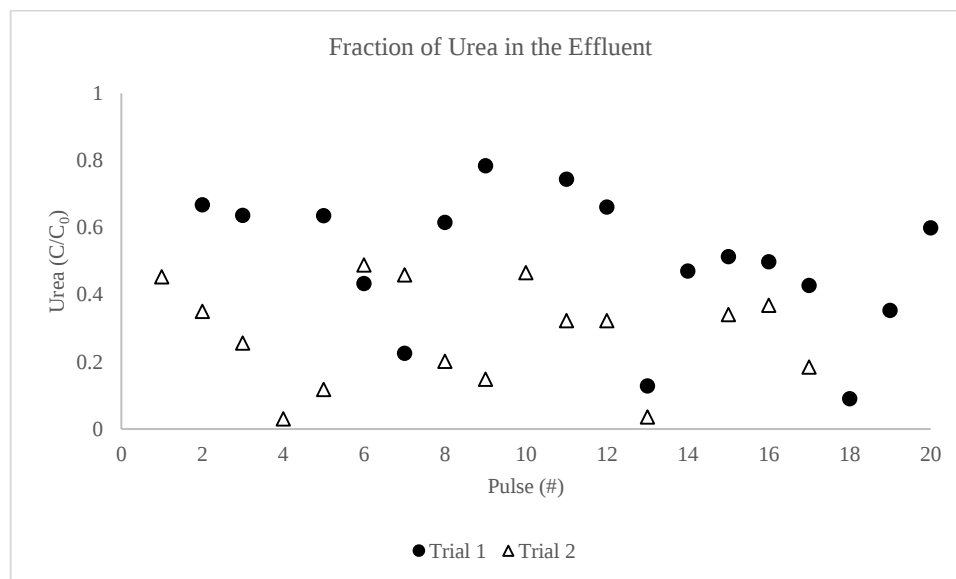


Figure 2.6: Fraction of effluent urea concentration to the initial urea concentration for each pulse. In Trial 1, an average of 49 % of the urea was detected in the effluent, and in Trial 2, an average of 28% of urea was detected in the effluent. This difference could be attributed to sampling methods, the timing of sample withdrawal was improved in Trial 2.

Porosity

X-ray microtomography (μ -CT). The μ -CT image resolution was 22.68 μ m per pixel. This is larger than the average 6-16 μ m pore size found with mercury intrusion porosimetry (MIP) from a piece of similar Berea sandstone (Phillips 2013). For this

reason, it was assumed that the porosity measurements from the μ -CT images accounted for the 100 μm gap between the cement and the sandstone, but not for most of the porosity within the sandstone. The results of μ -CT imaging are shown in Figures 2.7, 2.8 and Table 2.2.

According to μ -CT, the initial pore volumes of the cores were, 0.57 mL for Trial 1 and 0.71 for Trial 2. As a result of bio-mineralization the pore volume in Trial 1 decreased by 65% to 0.20 mL and the pore volume in Trial 2 decreased by 27% to 0.54 mL (Figure 2.7). As discussed in the apparent permeability section, it is suspected that the CaCO_3 seal may have formed earlier in Trial 2 than in Trial 1. This could have prevented precipitation from occurring further in the flow path accounting for the smaller reduction in pore volume observed in Trial 2 than in Trial 1. The CO_2 challenge increased the remaining pore volume after mineralization by 27% and 6% in Trials 1 and 2, respectively (Fig 2.7). This corresponds to a volume increase of 0.05 mL in Trial 1 and 0.03 mL in Trial 2 (Fig. 2.7). The percentage value was greater in Trial 1 because the change was relative to pore space after mineralization, which was smaller in Trial 1. However, the small volume increase was comparable between the trials.

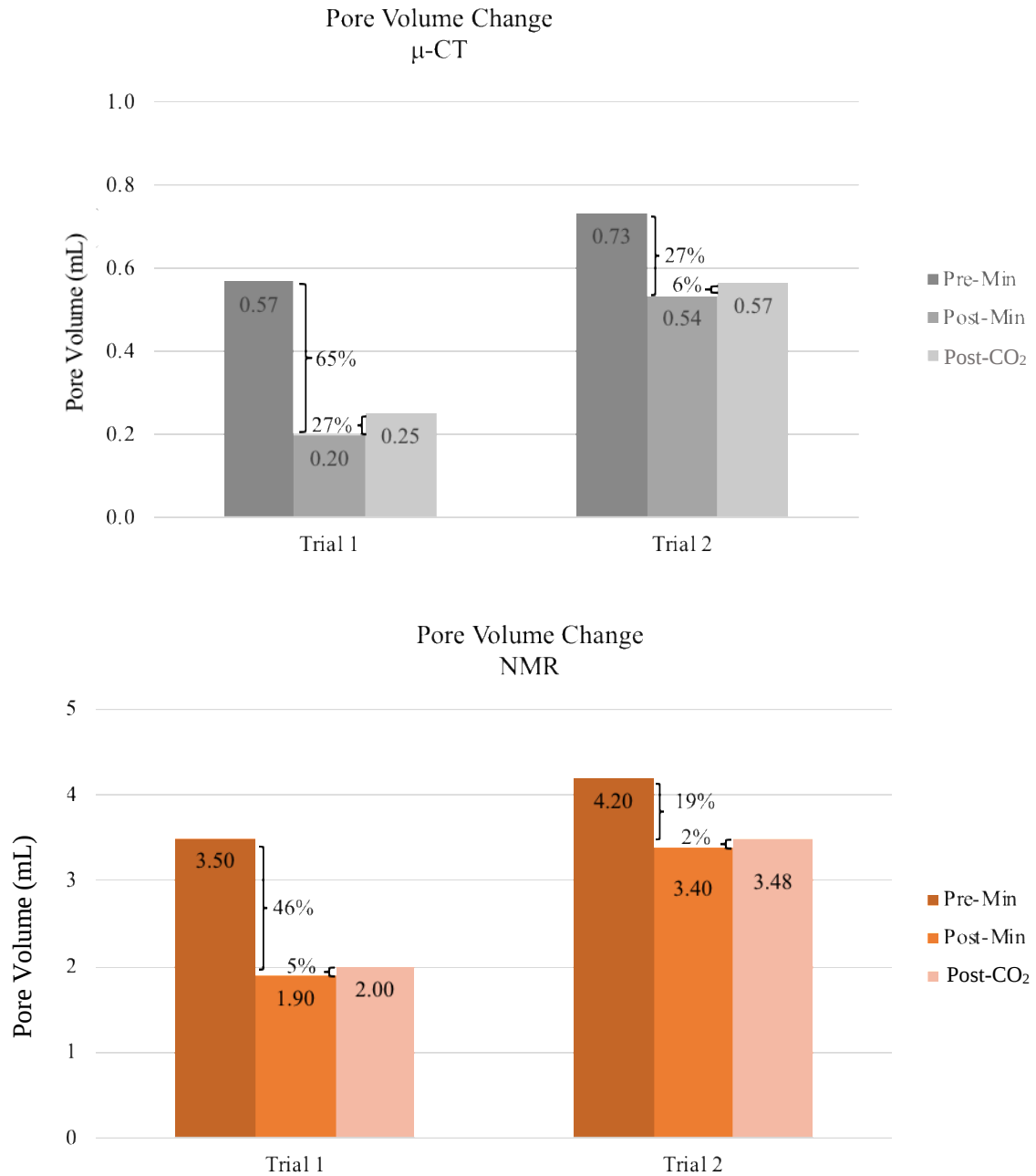


Figure 2.7: The pore volumes for both trials measured with (top) μ -CT and (bottom) NMR. The numbers within the bars are pore volumes (mL) at each stage. The percent values are the percent change from the adjacent bars. The initial pore volume is larger, and the pore volume reduction is smaller in Trial 2 than in Trial 1, measured by both μ -CT and NMR.

Table 2.3: Pore Volume and Average Porosity Measured with NMR and μ -CT

	Trial 1				Trial 2			
	NMR		μ -CT		NMR		μ -CT	
	Pore Volume (mL)	Porosity (%)	Pore Volume (mL)	Porosity (%)	Pore Volume (mL)	Porosity (%)	Pore Volume (mL)	Porosity (%)
Pre-Min	3.50	13.30%	0.57	2.21%	4.20	17.40%	0.73	2.84%
Post-Min	1.90	7.00%	0.20	0.64%	3.40	14.00%	0.54	2.08%
Post-CO₂	2.00	7.40%	0.25	0.98%	3.48	14.05%	0.57	2.55%

In Trial 1, the reduction of porosity, as measured by μ -CT, appeared to be more uniform across the entire length of the core (Fig. 2.8 a), suggesting a uniform spatial distribution of the CaCO_3 precipitates within the gap. The porosity reduction was not as uniform in Trial 2 (Fig. 2.8 b), even though the injection strategies and reactor operation were consistent between trials. In Trial 2, mineralization had a greater influence on the reduction of porosity in the first 30 mm of the core, while the effluent half of the core was minimally affected (Fig. 2.8 b). It appears that a plug or seal may have formed within the gap around 30 mm, hindering mineralization from occurring past that point. The difference between the spatial distribution of the porosity reduction due to mineralization between trials, along with mineralization having a greater influence on the influent than the effluent of the rock core in Trial 2, supports the hypothesis that the CaCO_3 seal formed closer to the influent in Trial 2 than in Trial 1. The cement core in Trial 2 also started with a crack that extended through the first 25mm, increasing the initial porosity measurement in the corresponding length of the core.

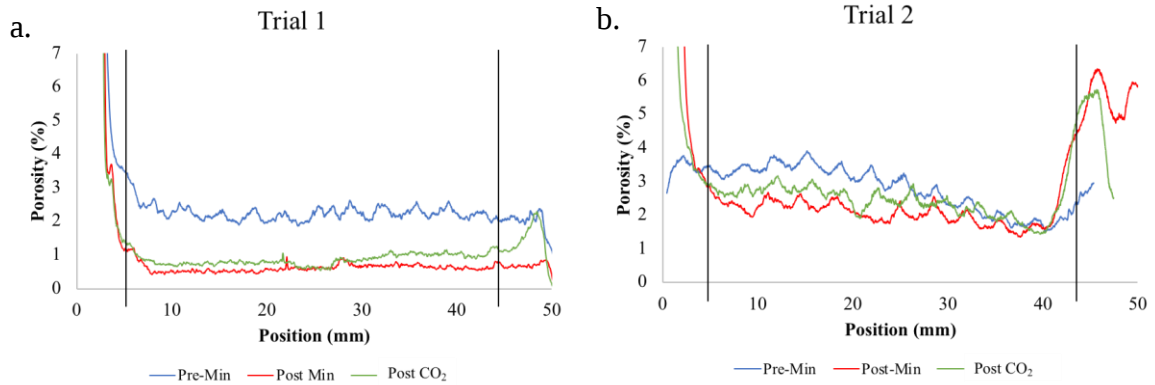


Figure 2.8: The porosity over the position in the composite rock cores measured with μ -CT at each stage of the experiment. (a.) In Trial 1, post mineralization, a uniform reduction in porosity across the length of the core was observed. After the CO₂ challenge, a slight increase was observed across the length of the core. (b.) In Trial 2, the larger porosity in the first 25 mm of the core, in the initial measurement, was due to a crack present in the cement. The porosity reduction was not as uniform in Trial 2 as in Trial 1, there was a great reduction observed in the influent 30 mm. In all images, the color of the line represents the stage of the experiment, (blue) pre-bio-mineralization (red) post-bio-mineralization but pre-CO₂-challenge and (green) post-CO₂-challenge. The black lines are located at the first and last 7 mm. This region was not included in the average porosity or pore volume calculations because the metal shims interfered with the images and therefore the thresholding.

Nuclear Magnetic Resonance (NMR) The results of the NMR scans are shown in Figures 2.7 and 2.9 and Table 2.3. The rock core analyzer (RCA-NMR) was used to measure two aspects of the rock core: (1) the pore volume, calculated from the initial amplitude of the signal, and (2) the pore size distribution, calculated from the distribution of T_2 relaxation times.

The pore volumes of the rock cores in the initial measurement were 3.5 mL and 4.2 mL in Trials 1 and 2, respectively. Both values are close to the estimated total pore volume within the composite rock cores, 3.69 mL (Table 2.1). The larger pore volume in Trial 2 could be attributed to the crack present within the cement core of Trial 2. In Trial 1, after bio-mineralization, the initial pore volume, found with NMR, was reduced by

46% (1.6 mL); Trial 2's initial pore volume was reduced by 19% (0.8mL). The post mineralization pore volume of the cores increased after the CO₂ challenge by 5% and 2% relative to the post-mineralization pore volume, in Trials 1 and 2 respectively. This corresponds to a 0.1 mL and 0.08 mL pore volume increase (Fig. 2.7 and Table 2.3).

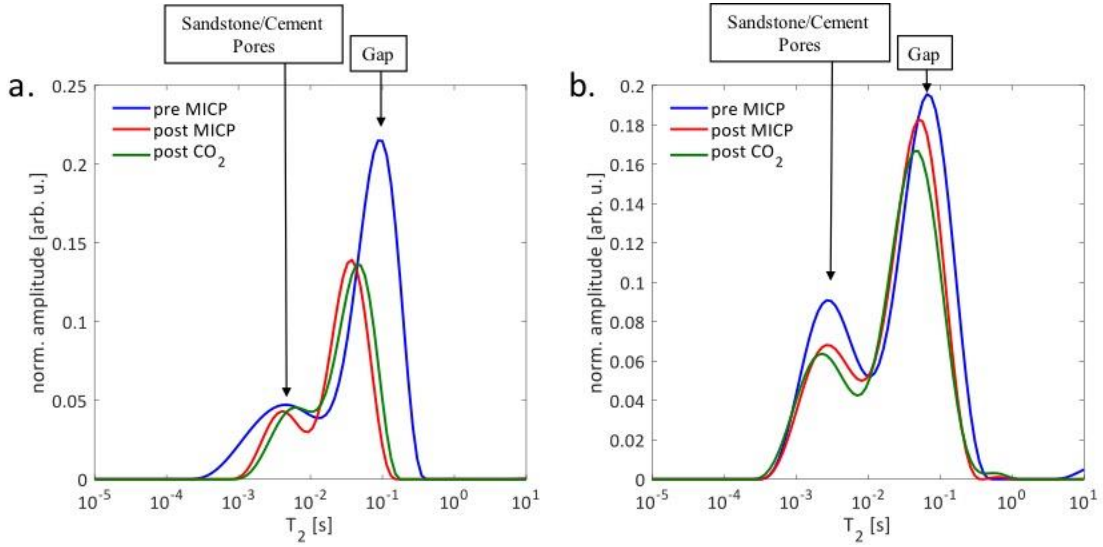


Figure 2.9: The T_2 relaxation time measured with the NMR-RCA. (a.) Trial 1; two populations were present, the larger was assumed to be the gap, and the smaller was assumed to be the pores within the sandstone and cement. After mineralization the gap population shifted to smaller T_2 relaxation time, suggesting smaller pores were present. After the CO₂ challenge, there was a slight shift to a larger time, suggesting the pore size increased. (b.) Trial 2; again, two populations were present. In all images, the colors represent the stage of the experiment, (blue) pre-bio-mineralization (red) post-bio-mineralization but pre CO₂ challenge and (green) post CO₂ challenge.

For both trials, two populations of T_2 relaxation times were observed (Fig. 2.9), which can be interpreted as a pore size distribution. The longer T_2 time population was attributed to water located in the 100 μ m gap and will be referred to as the gap population. The shorter T_2 time population was attributed to the water trapped in the pores of the sandstone and cement and will be referred to as the pore population. In the pre-bio-mineralization scans, the pore population was larger in Trial 2 than in Trial 1.

This was likely due to the thinner sections of the crack present in Trial 2's cement core, also found with μ -CT imaging.

After bio-mineralization (Fig. 2.9, red line) in both trials, the gap population got smaller and shifted to a shorter T_2 relaxation time. This suggested that the pore volume in the 100 μ m gap was reduced and the "pore size" of the gap got smaller. The size reduction of the gap population was more pronounced in Trial 1 than 2, indicating that less mineralization occurred in the gap during Trial 2.

After the CO₂ challenge (Fig. 2.9, green line) in Trial 1, there was a slight shift back to longer T_2 relaxation times in both populations, suggesting a slight increase in pore size occurred in both the gap and the sandstone pores. In Trial 2, a shift was not seen in the gap population, and a shift in the opposite direction, to a smaller T_2 relaxation time, was seen in the pore population. A possible explanation of this result was that during Trial 2 less mineral precipitation was present in the gap, and therefore less protection for the cement core, allowing the carbonated brine to react with the cement, creating small pores that were not previously present. This could cause the shift to a smaller T_2 relaxation time that was observed in that population. Degradation of effluent side of cement core after the CO₂ challenge was also observed physically, where a rounded edge that was previously a sharp edge was found (Fig. 2.4 d) supporting this hypothesis.

Comparison of NMR and μ -CT. The results from the NMR and μ -CT analyses were compared because they produce complementary data about the porosity change. As discussed above, the porosity found with μ -CT likely only accounted for the 100 μ m gap between the sandstone and cement. However, it was a useful tool for investigating the

spatial distribution of the mineral precipitate within that gap (Fig 2.8). The NMR signal resulted from protons in water; thus, if the core was fully saturated, was capable of assessing the porosity of the entire composite rock core, including the pore space within the sandstone and cement. By combining the information found with each instrument some assumptions could be made about the location of the pore volume changes that occurred within in the composite rock cores (within the gap or within the pores of the sandstone and cement), and thus where the mineral precipitation/dissolution may have occurred.

The volume difference between the total pore volume (found with NMR) and the volume within the gap (found with μ -CT) was attributed to the pore space within the sandstone and the cement. This was 3 and 3.5 mL in the pre-biomineralization scans in Trials 1 and 2, respectively. The additional volume in Trial 2 could be a result of the crack present in the cement core of Trial 2.

A larger reduction in pore volume reported by NMR versus μ -CT may be due to mineralization occurring within the pore space of the sandstone and cement, thus reducing the pore volume within. The difference between the total pore volume reduction (found with NMR) and the volume reduction within the gap (found with μ -CT) was attributed to the pore volume reduction within the sandstone and the cement. In both trials NMR reported a larger reduction in pore volume, due to bio-mineralization, than μ -CT (Table 2.3). This could suggest that bio-mineralization successfully created a plug or seal within the gap and the fluids were then forced to flow through the sandstone, resulting in bio-mineralization reducing the pore volume within the sandstone. In Trial 1, NMR reported a 1.6 mL pore volume reduction after bio-mineralization and μ -CT

reported a pore volume reduction of 0.371 mL (Table 2.3). This could indicate that there was 1.23 mL pore volume reduction within the 3.69 mL of estimated pore space (Table 2.1) within the sandstone. In Trial 2, there was a smaller reduction in pore volume due to bio-mineralization reported by both NMR and μ -CT. NMR measured a reduction of 0.8 mL, and μ -CT measured a 0.20 mL reduction. This suggests a 0.6 mL pore volume reduction occurred in the sandstone and cement of Trial 2 (Table 2.3).

Both NMR and μ -CT measured a small increase in pore volume after the CO₂ challenge (Fig. 2.7 and Table 2.3). A small increase in volume suggests that only a small amount of mineral was influenced/dissolved by the carbonated brine. This, along with the preferential flow path found with μ -CT in the "mineral cap," supports the hypothesis that the preferential flow path was the primary cause of the apparent permeability increase seen in both experiments.

Conclusions and Future Work

A novel high-pressure axial flow reactor system enabled bio-mineralization experiments to be performed in an environment simulating that of subsurface reservoirs used in CCS. Additionally, the two non-invasive techniques, NMR and μ -CT, produced complementary data of porosity change throughout the experiments. It was shown that bio-mineralization was capable of filling a gap in a composite rock core after soaking in a carbonated brine, and the apparent permeability and porosity were reduced in the composite rock cores as a result.

In both trials, the apparent permeability decreased by 5-orders of magnitude due to bio-mineralization and increased by 4-orders of magnitude due to the CO₂ challenge. A

“mineral cap” of CaCO_3 formed at the influent of both cores due to bio-mineralization. It was hypothesized, but cannot be confirmed, that this was the primary cause of the apparent permeability decrease of the cores. After the CO_2 challenge, a preferential flow path was observed with μ -CT imaging. The preferential flow path was assumed, but also cannot be confirmed, to be the cause of the apparent permeability increase. This was supported by the small pore volume increase observed with both μ -CT and NMR after the CO_2 challenge, suggesting that the acidic carbonated brine influenced only a small amount of the CaCO_3 .

While the apparent permeability data of the two trials was similar, the porosity data between the trials differed. It is hypothesized that this was due to the CaCO_3 seal forming closer to the influent of the rock core in Trial 2 than in Trial 1. This hypothesis was supported by both, the with mineralization having a greater influence on the influent of rock core in Trial 2 than the effluent (Fig. 2.8). As well as, the lack of mineral seen in the effluent of the gap in the rock core of Trial 2 after mineralization (Fig. 2.4). The mineral plug or seal could have resulted in the smaller pore volume reduction seen in the composite core within both the gap and the sandstone pores of Trial 2.

The ability to displace the carbonated brine and reduce the porosity and apparent permeability along with the small volume loss of mineral observed during the CO_2 challenge suggest that bio-mineralization could be used to enhance CCS by reducing CO_2 migration. A small amount of dissolution of the mineral was observed during the CO_2 challenge, creating a preferential flow path (found with μ -CT) which could have been the cause of the increase in apparent permeability. Dissolution of the mineral is the most significant challenge associated with applying bio-mineralization to CCS, but the small

volume loss of mineral from the carbonated brine gives reason to believe that this challenge could be overcome. However, this does need to be further investigated. Huerta et al.'s and Brunet et al.'s previous work suggested that if a lower differential pressure, resulting in a slower flow rate, was applied during the CO₂ challenge, a preferential flow path may not have occurred (Brunet et al. 2016; Huerta et al. 2012). The pressure gradient in the subsurface can be approximately $0.00022 \frac{\text{MPa}}{\text{cm}}$ ($1 \frac{\text{psi}}{\text{in}}$) (Dahlberg 1982). If this gradient were applied to the 5.08 cm rock cores used in this study it would result in a differential pressure of 0.0011 MPa. This is 3 orders of magnitude smaller than the 1.03 MPa differential pressure applied during the CO₂ challenge in this study. Based on this, it is reasonable to assume that in a typical storage reservoir environment the differential pressure would be lower than the 1.03 MPa performed in these experiments.

Further research should also concentrate on an injection strategy that encourages less mineralization in the inlet space, in order to prevent the "mineral cap" from forming. A larger volume of a urea and Ca²⁺ free solution between the inoculum and the mineralizing fluid could prevent the two fluids from interacting upstream of the core (Phillips 2013). This may encourage more mineralization within the gap and less within the inlet and the tubing. It has previously been shown that a faster flow rate of bio-mineralization fluids may also be beneficial (Kirkland et al. 2019; Qin, Hassanizadeh, and Ebigbo 2016; Ebigbo et al. 2012). An increased flow rate would favor transport and minimize the reaction/precipitation process while fluids are flowing. Once fluid flow is stopped the reaction may occur within the entire length of the gap.

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CHAPTER THREE

UREOLYSIS IN THE PRESENCE OF
PRESSURIZED CARBONATED BRINES (0-4 MPa)

Introduction

As discussed in the previous chapters, the fundamental concept of CCS is to remove CO₂ from waste streams typically released to the atmosphere and instead pump the CO₂ into subsurface saline reservoirs. These reservoirs are under great amounts of pressure, as in the subsurface increasing pressures are encountered with increasing depths. In order for bio-mineralization to be an applicable technology for sealing CO₂ leakage pathways from CCS reservoirs, it must be demonstrated that ureolysis can occur in the proximity of a pressurized carbonated brine, which presents challenges. The two central obstacles involved are the acidity of the carbonated brine and the pressure of the system. Both can influence the activity of an enzyme, and therefore, the rate of ureolysis (Fidaleo and Lavecchia 2003; Krajewska 2009; Krajewska, van Eldik, and Brindell 2012; Qin and Cabral 1994; Dixon et al. 1980).

Previous research has shown that pH can influence urease's enzyme activity (Fidaleo and Lavecchia 2003; Krajewska 2009; Qin and Cabral 1994; Lauchnor et al. 2015). Enzymes are amphoteric molecules containing a large number of acid and base groups typically located on their surface (Chaplin 2014). Variation in the pH of the fluid surrounding an enzyme will influence the acid and base groups and may alter the ionic form of the active site or change the three-dimensional shape of the enzyme (Chaplin

2014). These alterations may impact the binding of the substrate, the catalytic efficiency, and the amount of active enzyme, and therefore, the rate of the reaction. The pH dependence of ureolysis kinetics has been described by Equation 3.1 (Moynihan et al. 1989; Qin and Cabral 1994; Fidaleo and Lavecchia 2003; Lauchnor et al. 2015):

$$\frac{d[urea]}{dt} = \left(\frac{V_{max}[urea]}{(K_m + [ura]) \left(1 + \frac{[H^+]}{K_{es,1}} + \frac{K_{es,2}}{[H^+]} \right)} \right) \quad \text{Equation 3.1}$$

where $[H^+]$ is the hydrogen concentration, $[urea]$ is the concentration of urea, V_{max} is the maximum rate of reaction (without pH control), K_m is the half-saturation coefficient, and $K_{es,1}$ and $K_{es,2}$ are the acid-base dissociation constants for protonation and deprotonation of the enzyme/substrate complex (Lauchnor et al. 2015; Fidaleo and Lavecchia 2003).

This equation assumes that there is only one active form of the protonated enzyme and that the K_m is not dependent on pH (Dixon et al. 1980; Fidaleo and Lavecchia 2003). The equation results in a "bell-shaped" curve of reaction rate dependent on pH. The maximum and minima depend on the values of $K_{es,1}$ and $K_{es,2}$. Equation 3.1 can be used to predict theoretical pH optima of enzymes. However, knowledge of the active site characteristics, such as the acid/base groups present, is required, which may be difficult to obtain. For this reason, the optimal pH range for an enzymatic reaction is typically determined experimentally. These experiments have been done for the urease enzyme by several research groups with various conclusions (Fidaleo and Lavecchia 2003; Krajewska 2009; Qin and Cabral 1994; Lauchnor et al. 2015; Moynihan et al. 1989).

Fidaleo and Lavecchia studied ureolysis with purified urease from jack bean seeds over the pH range 4-9 with buffers. They found that a modified Michaelis-Menten equation described the reaction rate data with a pH-dependent rate coefficient and a

product inhibition term for NH_4^+ . The results also indicated that pH has more influence on the $V_{\max}$ than pH has on the K_m . The fastest rate ($V_{\max}$) was observed at pH 7, with slower rates at pH 6 and 8, and the lowest rates at pH 4, 5 and 9 (Fidaleo and Lavecchia 2003). Qin et al. investigated JBM urease kinetics in the presence of CO_2 and at a pH range of 5.5-9.5 by bubbling CO_2 into solutions to control the pH of the reactors. Their experiments resulted in the commonly observed “bell-shaped” curve for ureolysis rate dependent on pH, with pH 7.2 exhibiting the fastest $V_{\max}$ (Qin and Cabral 1994). Moynihan et al. studied JBM urease immobilized onto ion-exchange resins, finding the optimal pH value to be 7.6 in a dialysate solution and pH of 8.0 in a phosphate buffer (Moynihan et al. 1989). Dixon et al. performed rate analysis studies of purified urease from jack bean seeds between pH 3.4 and 7.8 with buffers and observed the fastest rate occurred at a pH of 7.5 (Dixon et al. 1980).

Lauchnor et al. studied ureolysis rates with whole cells of *S. pasteurii* in order to determine the relationship of the reaction rate with urea, cell, and NH_4^+ concentrations, along with pH in the range of 5-10. With qualitative comparison, it was observed that the ureolysis rate was slightly dependent on pH with pH 9 being the fastest. However, based on multiple t-test comparisons of the rates at different pH values, the highest rate at pH 9 was only different from the lowest rates at pH 5 and 10, and the intermediate rates were not statistically different.

Most studies observed a “bell-shaped” pH-dependent rate curve with the optimal pH in the range 7-9. Significantly slower rates of ureolysis were observed at pH values below 4.5 and above 10 (Fig. 3.6) (Qin and Cabral 1994; Lauchnor et al. 2015; Dixon et al. 1980; Fidaleo and Lavecchia 2003). All the above pH-dependent rate studies used

Michaelis-Menten kinetic models, which represent enzymatic reactions. First-order rate kinetics have also been successfully applied to a range of experimental ureolysis data (Lauchnor et al. 2015; Dupraz, Parmentier, et al. 2009; Ferris et al. 2004; Handley-Sidhu et al. 2013). However, no studies were found that applied first-order rate kinetics to describe pH-dependent ureolysis rates. Due to the single initial substrate concentration used in this study and an adequate fit, a first-order rate expression was used to fit the data.

Elevated pressure may influence enzymatic reaction rates in two ways: 1.) by changing the structure of the enzyme itself, and thus, its' function, or 2.) affecting the volume change at the molecular level as the reaction occurs (Balny 2004; Krajewska, van Eldik, and Brindell 2012; Masson and Balny 2005). Molecular volume changes are associated with both steps of an enzymatic reaction, substrate binding to the enzyme ($E \rightarrow ES$), as well as, production/release of the product from the enzyme-substrate complex ($ES \leftrightarrow P$). Depending on the manner and extent of the volume change, pressure could either negatively or positively influence the reaction (Masson and Balny 2005; Krajewska, van Eldik, and Brindell 2012).

Krajewska and van Eldik studied the steady-state kinetic parameters of JBM urease at a range of pressures (5-132 MPa). They observed little variation in K_M values at elevated pressures, suggesting that pressure had a limited effect on the enzyme-substrate binding step of the reaction. However, increased pressure did reduce the V_{max} , indicating that pressure indeed had an effect on the enzymatic reaction step that produced/released the product. There was a 94% reduction in V_{max} up to 40 MPa and a threefold reduction in V_{max} from 40 MPa to 130 MPa (Krajewska, van Eldik, and Brindell 2012). These studies

determined that pressure could influence the rate of an enzymatic reaction, including ureolysis. However, it must be considered that the pressures used in Krajewska and Eldik's study were higher than most of the pressures used in the study presented here.

So far, ureolysis has been studied at a range of pH values, at high pressures, and in the presence of CO₂. However, no studies were found that combined all of these components. The objective of the experiments presented was to assess JBM ureolysis in the presence of pressurized carbonated brines to simulate the CCS storage reservoir environment. It must be noted that the pressures in this study are lower than what could be observed in a typical CCS reservoir but were near the limits of the reactor system. First, the pH of carbonated brines at various pressures without ureolysis occurring was established, then ureolysis in the presence of a pressurized carbonated brine at increasing pressures was assessed.

Materials and Methods

High-Pressure Batch Reactor

The high-pressure batch reactor consisted of high-pressure pH and conductivity probes (Barben Analyzer Technologies) and a high-pressure Teledyne Isco syringe pump. A stainless-steel cross, with a 50 mL reservoir, connected all components (Fig. 3.1). The high-pressure probes were connected to a data logger (National Instruments) and a LabVIEW platform capable of logging data at set time points. A pressurized CO₂ gas tank was connected to the influent Isco pump enabling the system to make carbonated brines. All components of the reactor system were connected via flexible ¼" stainless-

steel tubing and Swagelok fittings and valves (Swagelok, ID, USA). The tubing between the reactor and the influent pump had a volume of 50 mL (Fig. 3.1).

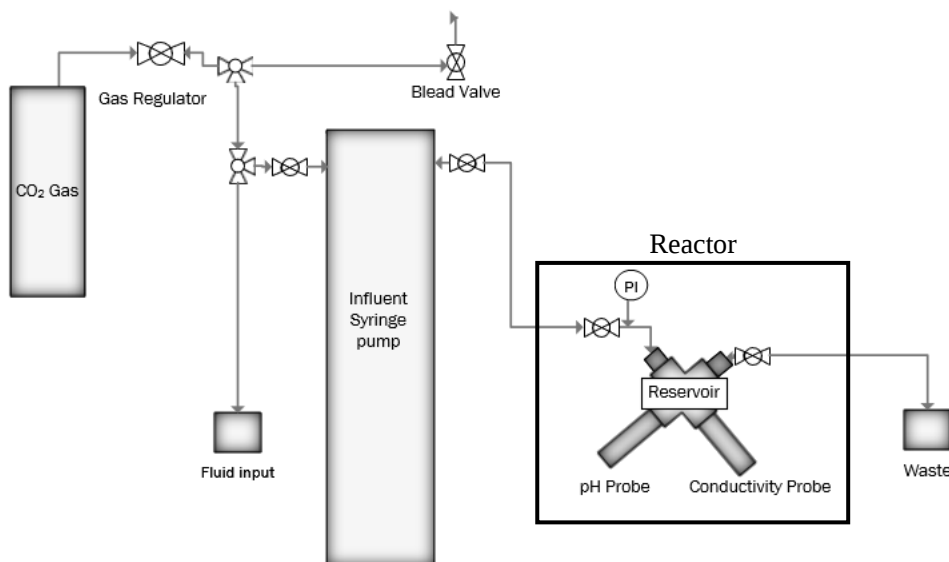


Figure 3.1: Schematic diagram of the high-pressure batch reactor system consisting of an influent pump, CO₂ tank, stainless steel cross, high-pressure pH and conductivity probes, and Swagelok tubing and valves. The box is around the reactor, which consisted of the stainless-steel cross and both high-pressure probes. The tubing from the influent syringe pump to the reactor reservoir had a volume of 50 mL, and the reactor reservoir had a volume of 50 mL.

Reactor Operation

First, the initial pH conditions of the carbonated brines were established without the urease enzyme present. Brines were carbonated at a range of pressures (Table 3.1), and the pH was monitored until equilibrium was reached. Next, ureolysis rate experiments were performed. During ureolysis rate experiments, the same process was repeated, except with JBM urease was added to the brine prior to carbonation, and the pH and conductivity were monitored. Urea concentration samples could not be taken while

the reactor was under pressure; thus, conductivity was used as a surrogate measurement for urea concentration during the rate experiments (Whiffin, van Paassen, and Harkes 2007). All experimental conditions tested are shown in Table 3.1. Experiments were also done at 6.89 and 8.27 MPa, with inconclusive results (Appendix A).

Table 3.1: Experimental Matrix: Two sets of experiments; brines with/without urease

Pressure (MPa)	No Urease	Urease
0	X	X
0.15	X	
0.23	X	
0.89	X	X
1.89		X
2.89	X	X
4	X	X

Experimental Solutions

For the initial pH of carbonated brines experiments, the brine consisted of 330 mmol/L urea and 187 mmol/L NH_4Cl . For the ureolysis rate experiments, the brine was amended with 2.5 g/L JBM. This was done by mixing 5 g/L JBM in DI water in an Erlenmeyer flask on a stir plate overnight. The following morning 60 mL of the 5 g/L JBM was mixed with 60 mL of 2x brine (660 mmol/L urea and 374 mmol/L NH_4Cl), resulting in 120 mL of brine (330 mmol/L urea and 187 mmol/L NH_4Cl) amended with 2.5 g/L JBM.

Reactor Operation

Experiments started with flowing CO_2 gas through the entire system at 0.1 MPa (15 psi) to flush out the air present; then the effluent valve was closed. The appropriate amount of CO_2 gas, calculated for each pressure condition (calculation shown in a later section), was injected into the reactor system. The reactor system was isolated from the

pump, and the pump was filled with brine, either amended with or without JBM urease. The pump was pressurized to the reactor system pressure (equal to the pressure of CO₂ gas previously injected into the system). The brine was injected into the reactor system, pushing the CO₂ gas and experimental solutions into the 50 mL reactor reservoir. Solutions were injected at 20 mL/min until the desired experimental pressure was reached. At this point, the pump was switched to constant pressure mode set at the experimental pressure for the extent of the experimental period. Depending on the rate of reaction, experiments lasted between 1 and 5 hours. Conductivity and pH were recorded every minute via the data logger (National Instruments) and LABview software.

Two physical samples were taken during each experiment, an initial and a final. The initial sample was taken prior to the brine injection, and the final sample was taken at the end of the experiment once the system had been depressurized. Samples were diluted 1:10 in 0.5 mol/L sulfuric acid to stop the enzymatic reaction, and the colorimetric modified Jung assay was used to assess the urea concentration (Jung et al. 1975; Phillips 2013).

CO₂ Calculations

The following procedure was used to estimate the amount of CO₂ gas required for each experimental pressure.

1. Determine the desired experimental pressure.
2. Calculate the CO₂ saturation concentration for the experimental pressure and temperature (Henry's Law (Eqn. 3.2, below)).
3. Calculate the pressure of CO₂ gas to inject into the 100 mL reactor system that equals the calculated amount of moles needed for the CO₂ saturation concentration (ideal gas law).

The initial injection of CO₂ gas filled the entire volume of the system and based on the calculation should have resulted in a nearly saturated solution within the reactor reservoir once the system was pressurized to the experimental pressure.

To calculate the saturation concentration, Henry's Law was used:

$$H = \frac{c_a}{p} \quad \text{Equation 3.2}$$

where c_a is the concentration of the species in the aqueous phase, p is the partial pressure of the species in the gas phase, and H is Henry's constant. The Henry's constant used was $0.032 \frac{\text{mol}}{\text{L atm}}$, which assumes the CO₂ is dissolving into DI water at 25°C. (Perry and Green 1984). For calculations, it was assumed that the brines used would have comparable CO₂ saturation concentrations as DI water. The temperature, volume, and number of moles (calculated with Henry's Law) were all known and the ideal gas law was then used to calculate the pressure of gas to inject into the system.

Probe Calibration

The reactor probes were calibrated at the beginning of each day of experiments. The pH probe was calibrated with pH 4.01 and pH 7 buffers. The conductivity probe was calibrated with air for 0 mS/cm and a 36 mS/cm standard solution made of 37.28 g/L potassium chloride (KCl) (Adamson 2006).

Urea Concentration/Conductivity Correlation Curve

Change in conductivity was used as a surrogate measurement to monitor the change in urea concentration during the experiments because samples could not be taken while the reactor system was under pressure. The production of ionic species (NH₄⁺ and

HCO_3^{1-}) from non-ionic substrates (urea and H_2O) results in an increase in overall conductivity of the solutions, and the rate at which conductivity increases is proportional to the rate at which the urea concentration decreases (Whiffin, van Paassen, and Harkes 2007).

A change in conductivity to change in urea concentration correlation curve was made by performing four separate experiments at 2.89 MPa (420psi), which were terminated at four different time points (1, 2, 3, and 5 hr), resulting in different residual urea concentrations. The conductivity was recorded directly before the system was depressurized, and the final samples were analyzed for urea concentration. The change in urea concentration with respect to the change in conductivity was then plotted and a linear regression was used to fit a correlation curve. (Fig. 3.2). Equation 3.3 is the equation for the correlation line seen in Figure 3.2:

$$\Delta U = 7.5207 \times \Delta \kappa - 0.735 \quad \text{Equation 3.3}$$

where ΔU is the change in urea concentration (mmol/L) and $\Delta \kappa$ is the change in conductivity (mS/cm). This equation was used to convert the conductivity measurements recorded throughout the high-pressure experiments to urea concentrations for rate analysis. The R^2 value for this line was 0.98, indicating an adequate correlation.

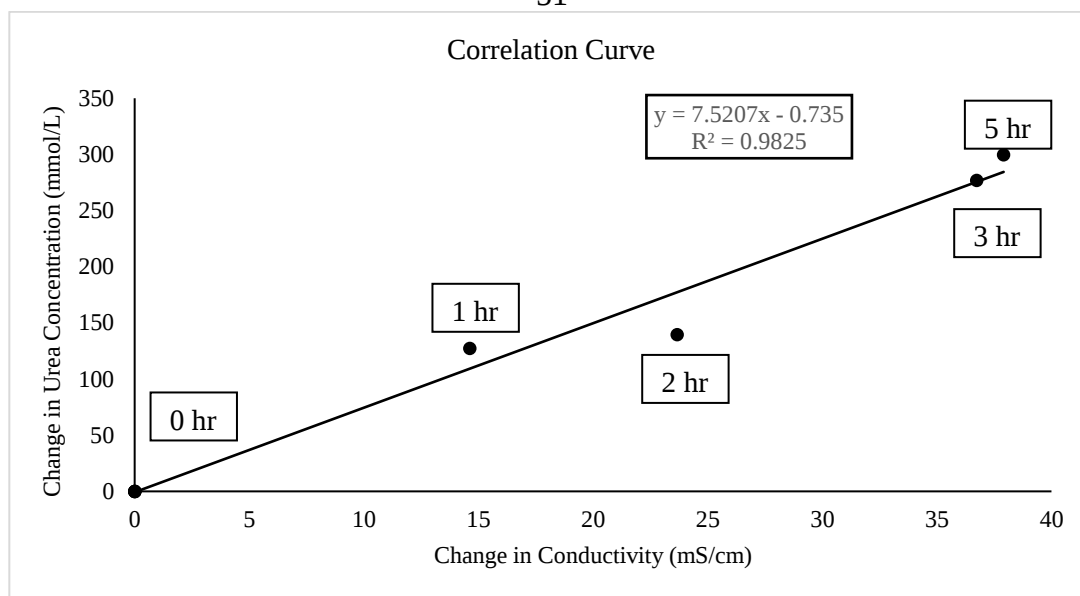


Figure 3.2: Change in conductivity to change in urea correlation curve made at 2.89 MPa. The equation of this line was used to convert conductivity to urea concentration.

Ureolysis Rate Model

Both the apparent first-order ureolysis rate coefficients (k_1) and the initial rates (1st hour) were calculated and used to compare results found in this study to the literature. The apparent k_1 was used to assess the kinetics for the full length of the experiments. While, the initial rate was used to compare these results to other pH dependent ureolysis rate studies, due to the fact that there were no studies found that analyzed k_1 values dependent on pH. The initial ureolysis rate for the first hour of each experiment was calculated from the slope of the urea concentration over time data (Fig. 3.5a). An assumption involved with the initial rate method is that a linear rate can be determined at the early time points of an experiment (1st hour) before the substrate concentration has changed significantly (Shuler and Kargi 1992). The apparent k_1 was calculated using a linear regression fitted to the urea concentration over time data for the total extent of the

experiments (Fig. 3.5a). Equation 3.4 represents a first-order reaction rate model, where k_1 is the first-order rate coefficient (Lauchnor et al. 2015).

$$\frac{d[urea]}{dt} = -k_1[urea] \quad \text{Equation 3.4}$$

The averages and standard deviations of the triplicate runs were calculated from the individual apparent k_1 values and initial rates for each condition. A multiple comparison t-test was performed to determine the statistical significance of the rate results.

Apparent k_1 values normalized to the concentration of urease were calculated for the apparent k_1 values found in this study along with apparent k_1 values found in the literature. For studies using *S. pasteurii* as a urease source, first, a cell dry weight (CDW) was estimated from the OD600 reported in the studies. A conversion of 0.39 CDW(g/L)/OD600 was used (Ren et al. 2013). This conversion was found for *Escherichia coli*, however, for these calculations it was assumed *S. pasteurii* had the same conversion. The concentration of urease was then found by assuming that the CDW of *S. pasteurii* contained 1% ureases (Bachmeier et al. 2002). For the studies that used JBM as the urease source it was assumed that JBM contained 0.14% urease (Krajewska 2009). Once the urease concentration was estimated for each study, the apparent k_1 values with respect to urea concentration were normalized to urease concentration by dividing the apparent k_1 values by the estimated urease concentration.

pH of Carbonated Brines

Experiments were done without urease present to assess the pH of a carbonated brine without the influence of ureolysis. As CO_2 dissolved into solution, carbonic acid was produced, thereby lowering the pH. At higher pressures, more CO_2 was able to dissolve into solution, and therefore, as expected, the pH decreased as the pressure increased for the pressure range 0.15-4 MPa (Fig. 3.3). The pH values found here are slightly higher than the results found in Haghi et al. (Haghi et al. 2017), which may be due to the solutions in this work not being fully saturated with CO_2 , or because of the difference in brines tested, NaCl vs. NH_4Cl containing urea.

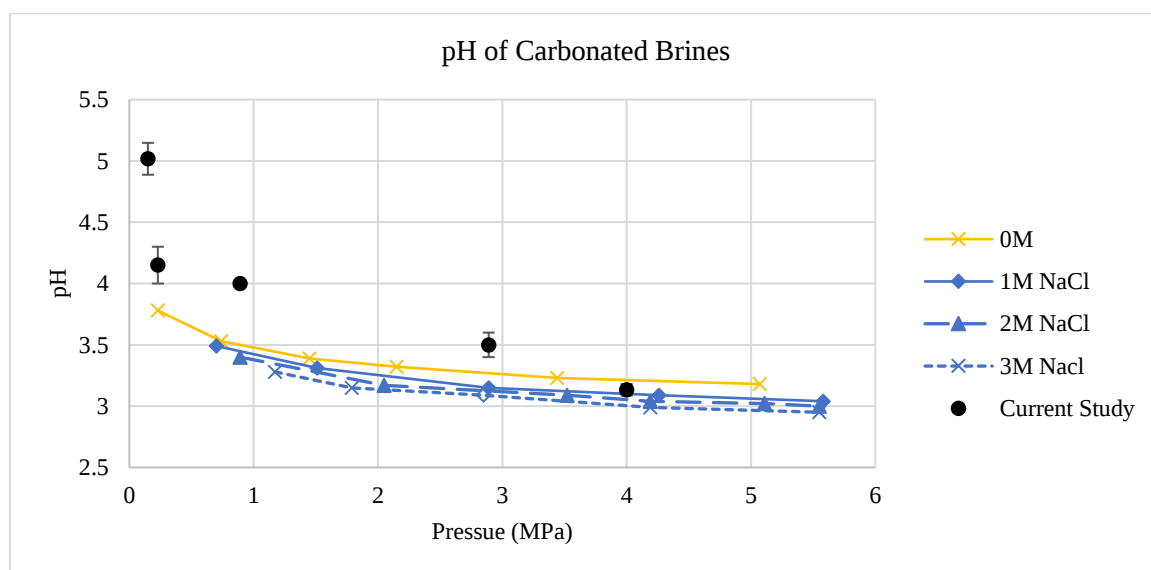


Figure 3.3: The pH of carbonated brines over pressure. Black circles are carbonated brines for the current study in the high-pressure batch reactor. The colored data is from Haghi et al. (2017) CO_2 saturation data. The yellow is DI water with no NaCl. Blue lines have increasing concentrations of NaCl.

Ureolysis in the Presence of CO₂

The raw data collected from the high-pressure probes within the reactor are presented in Figure 3.4. A pH plateau, where a steady pH was reached, was observed during each experiment. The plateau is presumably where the system buffered and a dynamic equilibrium was reached between the dissolution of CO₂ and the production of ammonium. This pH plateau was also observed by Dupraz et al. (2009) (Dupraz, Parmentier, et al. 2009). The final pH values agreed with the predicted pH values calculated from pC-pH diagrams (Appendix B). The final or buffered pH value decreased with increasing experimental pressure (Fig. 3.4a), likely due to the increase in CO₂ concentration.

All pressure conditions had an initial pH of around 5, which was higher than the pH found for the carbonated brines without ureolysis, pH 3.2-4 (Fig. 3.3). The higher initial pH could be a consequence of mixing the JBM and urea solutions before coming in contact with the CO₂. Thus, ureolysis was underway and may have increased the pH of the solution before the dissolution of the CO₂ could decrease the pH.

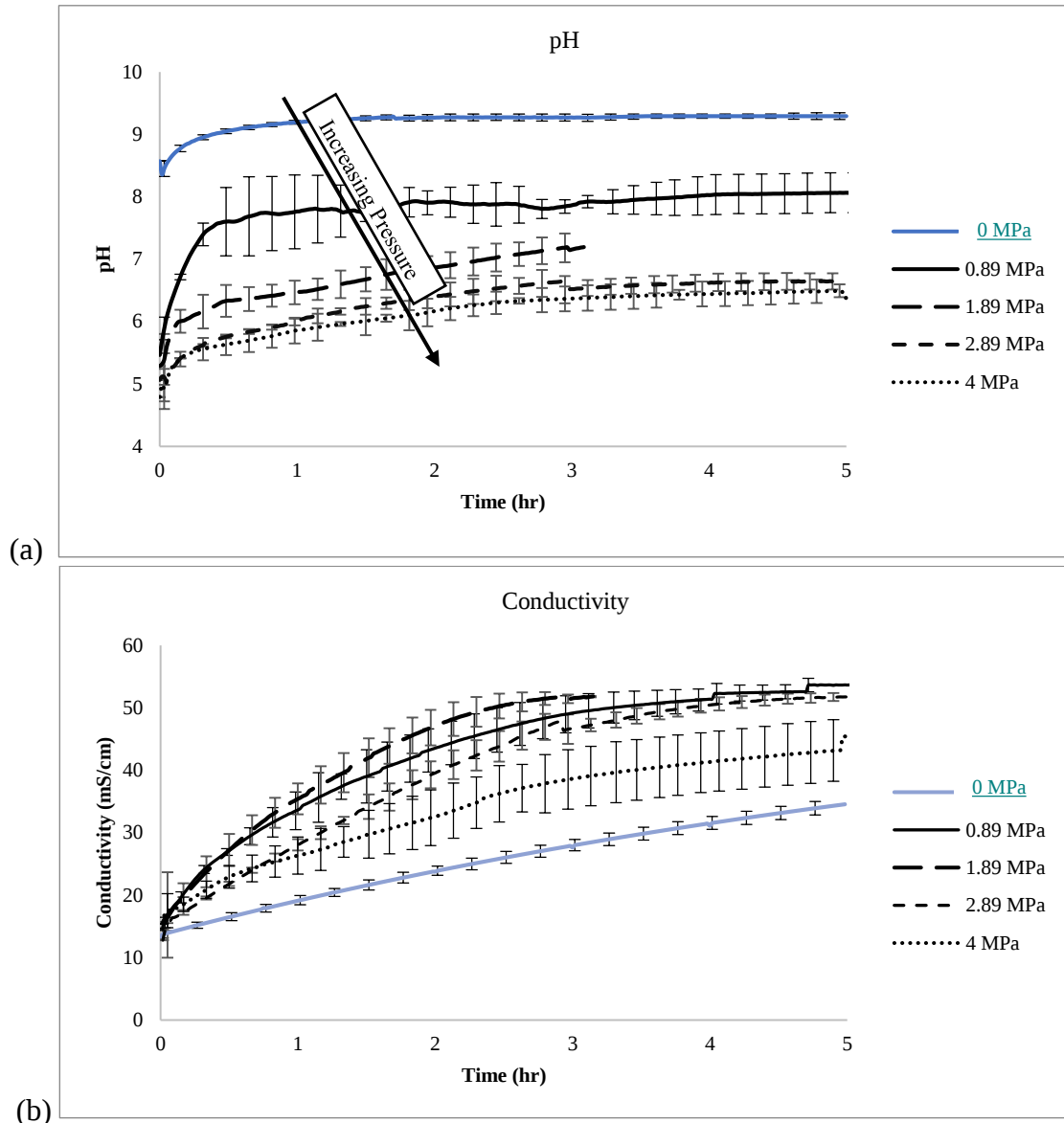


Figure 3.4: (a) The pH and (b) the conductivity change throughout the extent of the experiments. For both figures, the black lines represent experimental conditions with CO_2 at increasing pressures, and the blue line is at 0 MPa and no CO_2 present.

The change in conductivity to change in urea concentration correlation curve (Eqn. 3.3) was used to convert the conductivity data (Fig. 3.4b) to the urea concentration for the 0, 0.89, 1.89, 2.89 and 4 MPa experimental conditions (Fig. 3.5a black lines). The urea concentration found from the final sample with the Jung assay on average was 51% different from the concentration calculated from the conductivity. This appears to be a

large difference. However, due to the urea concentrations being so low at the end of the experiments, a small difference could result in a high percent difference. For example, the concentration found with the Jung assay in Trial 2 of the 2.89 MPa experiments was 4.17 mmol/L (the Jung assay lower detection limit) and the concentration predicted by the change in conductivity was 2.2 mmol/L, which results in a percent difference of 20%.

The first-order reaction rate models found in Figure 3.5a (orange lines) all had an R^2 value of above 0.95 when compared to the data, indicating an adequate correlation. For the pressures tested, a qualitative comparison of the apparent first-order rate constants (k_1) indicated the rate changed with experimental pressure with the highest rate at 1.89 MPa (Fig. 3.5b). However, based on multiple t-test-comparison, the apparent k_1 for the 1.89 MPa experiment only differed significantly from the apparent k_1 values for the 0 MPa and the 4 MPa experiments. Because of the small sample base of only three trials for each condition, the statistical significance was low. The apparent k_1 values found in this study are higher than apparent k_1 values reported in most previous studies except for those reported by Lauchnor et al. (2015) (Table 3.2). However, when all of the apparent k_1 values were normalized to urease concentration the values found in this study were comparable to the values found in previous studies (Table 3.2).

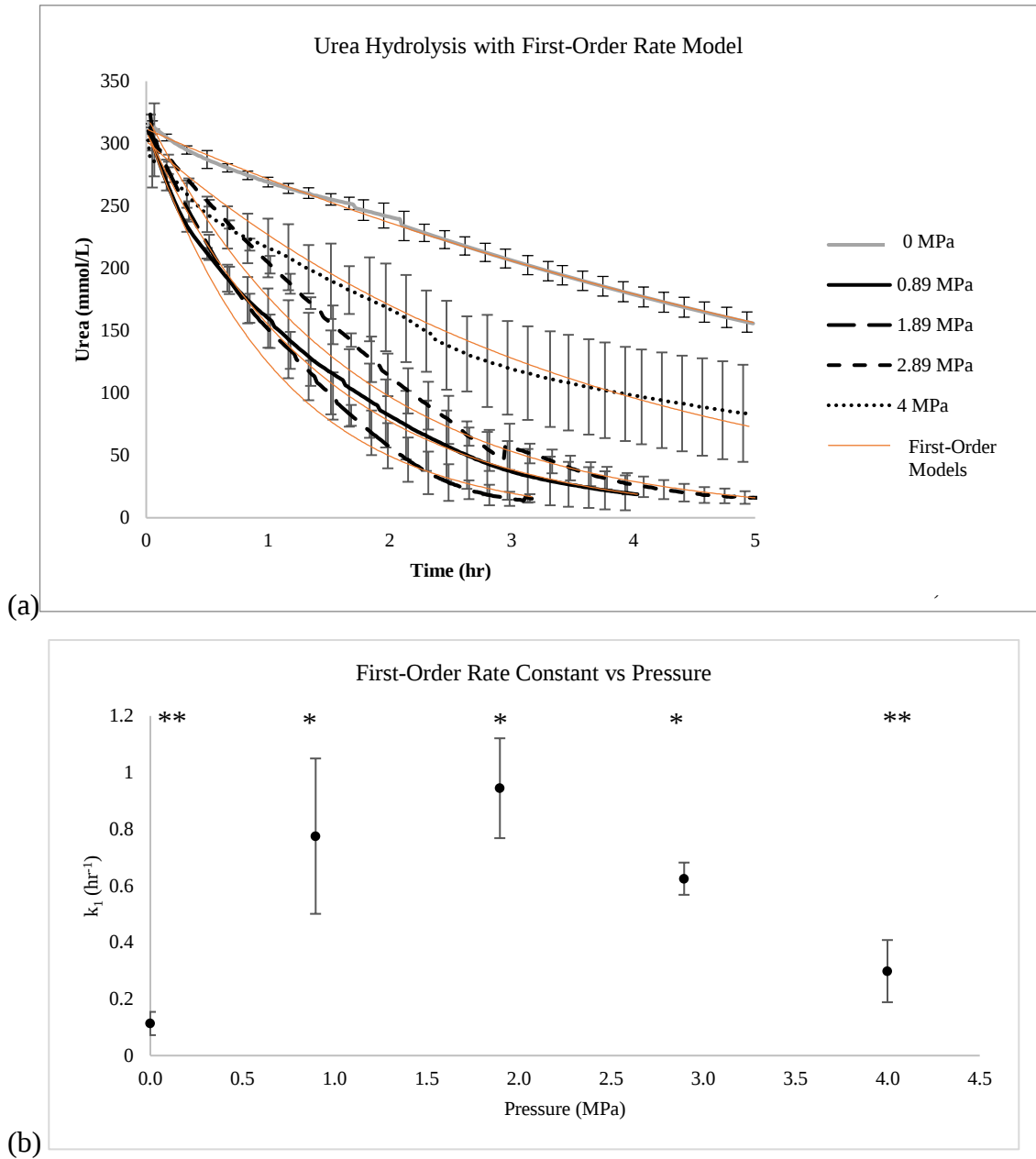


Figure 3.5: First-order rate models (a) Urea concentration data calculated from conductivity vs. time for all pressure conditions (black lines) and first-order rate models (solid orange lines). All models had a strong correlation with an R^2 value of more than 0.95. (b) First-order rate constants vs. experimental pressure conditions. The stars above represent statistical significance. Data points with the same number of stars are not statically different, points with a different number of stars are statically different from each other.

Table 3.2: Experimental and reported apparent first order rate coefficients (k_1) for ureolysis

Urease Source	OD ₆₀₀ /JBM conc.	Urea (mmol/L)	Final pH	Apparent k_1 (hr ⁻¹)	Normalized Apparent k_1 (hr ⁻¹ * (g/Lurease) ⁻¹)	Reference
<i>S. pasteurii</i>	0.07	333	9.3	0.038	151 *	Ferris et al. (2004)
<i>S. pasteurii</i>	0.05	33-333	8.7-9.5	0.027-0.035	150-350 *	Dupraz et al. (2009)
<i>S. pasteurii</i>	0.1	1-722	9.3	0.31	861 *	Lauchnor et al. (2015)
<i>S. pasteurii</i>	0.07	250	8.0	0.095	376 *	Tobler et al. (2012)
JBM	0.5 g/L	200	8.5	0.011	15.7 **	Handley-Sidhu et al. (2013)
JBM	2.5 g/L	330	6.1-9.3	0.13-0.94	37-269 **	Current Study

*assumes 0.36 CDW(g/L)/OD600 (Ren et al. 2013) and 1% urease in *S. pasteurii* (Bachmeier et al. 2002)

**assumes 0.14% urease in JBM (Krajewska 2009)

The “bell-shaped” curve of the apparent k_1 values dependent on experimental pressure was unexpected. It was originally hypothesized that as the pressure and CO₂ concentration increased the ureolysis rate and apparent k_1 values would decrease. The “bell-shaped” rate curve may be due to the different buffered pH conditions observed at each pressure tested. As discussed in the introduction, the rate of ureolysis has been found to be dependent on pH, and numerous research groups have applied Equation 3.1 to describe this dependence (Moynihan et al. 1989; Lauchnor et al. 2015; Qin and Cabral 1994; Fidaleo and Lavecchia 2003). To test the hypothesis that the different rates found in this study were a result of the different pH conditions in the system at each pressure condition, the average initial ureolysis rates were plotted against the pH values of the system, for each experimental condition tested. These results were compared to other pH

dependent ureolysis rates found in the literature (Fig. 3.6) . Each study had varying $V_{\max}$ and K_m values, which may have been due to varying urease concentrations. The varying $V_{\max}$ and K_m values resulted in different maximum rates. However, all studies found a “bell shaped” pH ureolysis rate dependent curve with the fastest rate occurring between 7.2 and 7.8. The initial rates vs. pH found in this study resulted in a similar shaped curve as found in the other studies, with the fastest rate occurring during the 1.89 MPa experiment, which buffered at a pH of 7.15. The data from this study fitting within other ureolysis pH dependent data found in the literature, supports, but does not confirm, the hypothesis that the variation in ureolysis rates observed in this study, may be due to the different pH conditions of the system at each pressure tested.

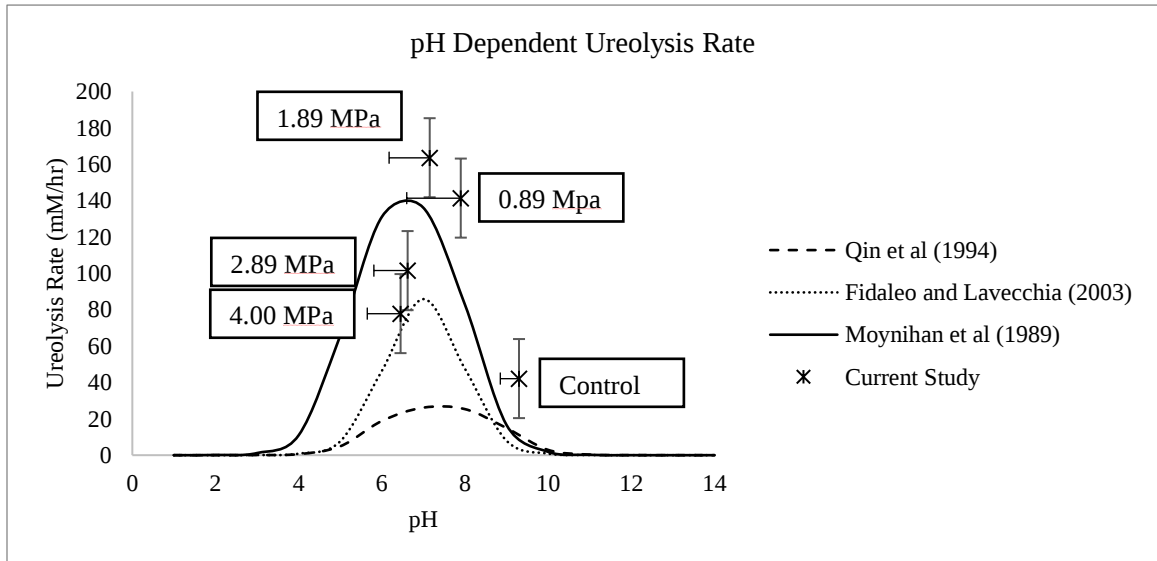


Figure 3.6: Ureolysis rates vs. pH values. Initial rates of experimental results for carbonated brines at 0-4 MPa (x). Error bars are the standard deviations of the initial ureolysis rates. The initial rates from this study are compared to the models made with Equation 3.1 using the values of $K_{es,1}$, $K_{es,2}$, $V_{\max}$ and K_m from Qin et al. (1994) (dashed), Fidaleo and Lavecchia (2003) (dotted), and Moynihan et al. (1989) (solid). Kinetic parameter values are reported in Appendix C.

Conclusion

Methods were developed to assess ureolysis kinetics under pressure and in the presence of a carbonated brine. Initial experiments were done at 0-4 MPa. It was shown that JBM urease was capable of hydrolyzing urea in the presence of a pressurized carbonated brine up to 4 MPa. It was also shown that the rate of the experiments may be dependent on the buffered pH of the reactor. A “bell-shaped” distribution with the initial rate dependent on pH, consistent with previous studies in the literature, was observed. The initial experiments of this work give reason to believe that bio-mineralization for the use of reducing CO₂ leakage pathways in shallow CCS reservoirs could be possible. However, it is necessary to perform more experiments that better represent CCS reservoir conditions.

These experiments need to be done at higher pressures along with increased temperature to test more realistic CCS reservoir conditions. Experiments were attempted at 6.89 and 8.27 MPa, however, under those conditions the CO₂ could be a liquid, which resulted in inconclusive results due to liquid solubility not being taken into account (Zhao et al. 2015; Zhao 2015) (Appendix A). For this reason, a more accurate model for estimating liquid CO₂ saturation concentration needs to be used for the higher pressure conditions. It would also be beneficial to use a brine mixture that better represents the brine chemistry present in CCS reservoirs. Adding calcium to the system and assessing precipitation rates also would be an essential step to determine the validity of applying bio-mineralization to CCS reservoirs.

CONCLUSIONS

The research presented in this thesis provides evidence that suggests the application of bio-mineralization to reduce permeability and seal potential CO₂ leakage pathways during CCS could be applicable. It was shown that ureolysis could be induced with heat-treated *S. pasteurii* cell suspensions and CaCO₃ minerals could precipitate within a simulated leakage pathway in an environment that resembled a CCS reservoir at elevated temperature and pressure. In another study, it was observed that JBM urease could hydrolyze urea in the presence of a pressurized carbonated brine up to 4 MPa.

In the high-pressure core experiments in Chapter 2, the porosity and apparent permeability were reduced by bio-mineralization in composite rock cores that simulated leakage pathways between the formation rock and well cement in a CCS environment (high pressure, temperature and CO₂ present). Upon a carbonated brine challenge, a small amount of dissolution of the mineral was observed, creating a preferential flow path that increased the apparent permeability. The dissolution of the mineral is the most significant challenge associated with applying bio-mineralization to CCS. However, the small amounts of mineral loss from the CO₂ challenge gives reason to believe that solutions could exist, but this needs to be further investigated. It was also shown in Chapter 2, that NMR and μ -CT produce complementary porosity data, with both methods reporting a decrease in porosity after bio-mineralization and a slight increase after the CO₂ challenge.

In Chapter 3, methods were developed to assess ureolysis rates in the presence of pressurized carbonated brines. It was shown that JBM urease is capable of hydrolyzing

urea in the presence of a carbonated brine up to 4 MPa. It was hypothesized that the apparent first-order rate constants and the initial rates may have been dependent on the buffered pH of the system at each pressure tested. The initial ureolysis rates plotted against the pH values resulted in a curve consistent with other pH-dependent ureolysis rate studies found in the literature, supporting the hypothesis.

Future work

Bio-mineralization of Composite Rock Cores: In the future, a method to create more consistent results between trials needs to be developed. To do this, it is necessary to improve the mineralization pulse technique in order to create less mineralization in the inlet space, preventing the “mineral cap” from forming. One method that could be tested is to run a larger volume of a Ca^{2+} and urea free solution between the inoculum and the mineralization solution. This would prevent the urease from coming in contact with Ca^{2+} and urea prior to entering the cores. With dissolution being the most significant challenge involved in this process, it is also necessary to attempt to reduce mineral dissolution and prevent preferential flow paths. Huerta et al.’s and Brunet et al.’s previous work suggest that if a lower differential pressure, resulting in a slower flow rate, was applied during the CO_2 challenge, a preferential flow path may not have occurred (Brunet et al. 2016; Huerta et al. 2012). The pressure gradient in the subsurface is approximately $0.00022 \frac{\text{MPa}}{\text{cm}}$ ($1 \frac{\text{psi}}{\text{in}}$) (Dahlberg 1982). This would result in a differential pressure of 0.0011 MPa across the 5.08 cm composite rock cores, which is 3 orders of magnitude smaller than the 1.03 MPa differential pressure applied during the CO_2 challenge in this study. Based on this it is

reasonable to assume that in a typical reservoir storage environment the differential pressure would be lower than the 1.03 MPa performed in these experiments.

Ureolysis in the Presence of Pressurized Carbonated Brines: These experiments need to be performed at higher pressures along with adding increased temperature to test more realistic CCS reservoir conditions. A more accurate model for estimating saturation concentration needs to be used for the higher pressure conditions. It would also be beneficial to use a brine mixture that better represents the brine chemistry present in CCS reservoirs. Adding calcium to the system and assessing precipitation rates would also be an essential step to determine the validity of applying bio-mineralization to CCS reservoirs. Another interesting piece of data to add to this would be to repeat the experiments with *S. pasteurii* and compare how the two urease sources respond to the challenging conditions. This reactor system also has the capability to inject other gases into the system. For instance, experiments could be done with methane to assess bio-mineralization for the use of sealing leakage pathways in natural gas wells.

REFERENCES CITED

- Adamson, John. 2006. 'Preparation of Conductivity and pH Solutions', UK Environmental Change Network. <http://www.ecn.ac.uk/measurements/technical-notes/preparation-of-conductivity-and-ph-solutions/view>.
- Aminu, Mohammed D, Seyed Ali Nabavi, Christopher A Rochelle, and Vasilije Manovic. 2017. 'A Review of Developments in Carbon Dioxide Storage', *Applied Energy*.
- Bachmeier, Keri L, Amy E Williams, John R Warmington, and Sookie S Bang. 2002. 'Urease Activity in Microbiologically-Induced Calcite Precipitation', *Journal of Biotechnology*, 93: 171-81.
- Balny, Claude 2004. 'Pressure Effects on Weak Interactions in Biological Systems', *Journal of Physics: Condensed Matter*, 16: S1245.
- Bray, Joshua M., Ellen G. Lauchnor, George D. Redden, Robin Gerlach, Yoshiko Fujita, Sarah L. Codd, and Joseph D. Seymour. 2017. 'Impact of Mineral Precipitation on Flow and Mixing in Porous Media Determined by Microcomputed Tomography and MRI', *Environmental Science & Technology*, 51: 1562-69.
- Brunet, Jean-Patrick Leopold, Li Li, Zuleima T Karpyn, and Nicolas J Huerta. 2016. 'Fracture Opening or Self-Sealing: Critical Residence Time as a Unifying Parameter for Cement–CO₂–Brine Interactions', *International Journal of Greenhouse Gas Control*, 47: 25-37.
- Callaghan, Paul T. 2011. *Translational dynamics and magnetic resonance: principles of pulsed gradient spin echo NMR* (Oxford University Press).
- Chaplin, Martin. 2014. 'Enzyme Technology: Effect of pH and ionic strength', London South Bank University, Accessed May 2019. <http://www1.lsbu.ac.uk/water/enztech/ph.html>.
- Cunningham, Alfred B, Robin Gerlach, L Spangler, AC Mitchell, S Parks, and A Phillips. 2011. 'Reducing the Risk of Well Bore Leakage of CO₂ Using Engineered Biomineralization Barriers', *Energy Procedia*, 4: 5178-85.
- Dahlberg, Eric C. 1982. *Applied Hydrodynamics in Petroleum Exploration* (Springer-Verlag New York Inc: New York).
- DECC, CCS. 2012. 'Roadmap: Supporting deployment of Carbon Capture and Storage in the UK', *UK Department of Energy and Climate Change*.

- Dixon, Nicholas E, Peter W Riddles, Carlo Gazzola, Robert L Blakeley, and Burt Zerner. 1980. 'Jack bean urease (EC 3.5. 1.5). V. On the Mechanism of Action of Urease on Urea, Formamide, Acetamide, N-methylurea, and Related Mompounds', *Canadian Journal of Biochemistry*, 58: 1335-44.
- DOE. 'Carbon Storage FAQs', National Energy Technology Laboratory, Accessed July 2019. <https://www.netl.doe.gov/coal/carbon-storage/faqs/carbon-storage-faqs>.
- Dupraz, Sébastien, Bénédicte Ménez, Philippe Gouze, Richard Leprovost, Pascale Bénézeth, Oleg S Pokrovsky, and Francois Guyot. 2009. 'Experimental Approach of CO₂ Biomineralization in Deep Saline Aquifers', *Chemical Geology*, 265: 54-62.
- Dupraz, Sébastien, Marc Parmentier, Bénédicte Ménez, and François Guyot. 2009. 'Experimental and Numerical Modeling of Bacterially Induced pH Increase and Calcite Precipitation in Saline Aquifers', *Chemical Geology*, 265: 44-53.
- Ebigbo, Anozie, A Phillips, Robin Gerlach, Rainer Helmig, Alfred B Cunningham, Holger Class, and Lee H Spangler. 2012. 'Darcy-Scale Modeling of Microbially Induced Carbonate Mineral Precipitation in Sand Columns', *Water Resources Research*, 48.
- Feldkamp, Lee A, LC Davis, and James W Kress. 1984. 'Practical Cone-Beam Algorithm', *Josa a*, 1: 612-19.
- Ferris, FG, V Phoenix, Y Fujita, and RW Smith. 2004. 'Kinetics of Calcite Precipitation Induced by Ureolytic Bacteria at 10 to 20 C in Artificial Groundwater', *Geochimica et Cosmochimica Acta*, 68: 1701-10.
- Fidaleo, Marcello, and Roberto Lavecchia. 2003. 'Kinetic Study of Enzymatic Urea Hydrolysis in the pH range 4-9', *Chemical and Biochemical Engineering Quarterly*, 17: 311-18.
- Fridjonsson, Einar O, Joseph D Seymour, Logan N Schultz, Robin Gerlach, Alfred B Cunningham, and Sarah L Codd. 2011. 'NMR Measurement of Hydrodynamic Dispersion in Porous Media Subject to Biofilm Mediated Precipitation Reactions', *Journal of Contaminant Hydrology*, 120: 79-88.
- Gaus, Irina, Mohamed Azaroual, and Czernichowski-Lauriol. 2005. 'Reactive Transport Modelling of the Impact of CO₂ Injection on the Clayey Cap Rock at Sleipner (North Sea)', *Chemical Geology*, 217: 319-37.

- Haghi, Reza K, Antonin Chapoy, Luís MC Peirera, Jinhai Yang, and Bahman Tohidi. 2017. 'pH of CO₂ Saturated Water and CO₂ Saturated Brines: Experimental Measurements and Modelling', *International Journal of Greenhouse Gas Control*, 66: 190-203.
- Handley-Sidhu, S, E Sham, MO Cuthbert, S Nougazol, M Mantle, ML Johns, LE Macaskie, and JC Renshaw. 2013. 'Kinetics of Urease Mediated Calcite Precipitation and Permeability Reduction of Porous Media Evidenced by Magnetic Resonance Imaging', *International Journal of Environmental Science Technology*, 10: 881-90.
- Hemme, Christina, and Wolfgang van Berk. 2017. 'Change in Cap Rock Porosity Triggered by Pressure and Temperature Dependent CO₂–Water–Rock Interactions in CO₂ Storage Systems', *Petroleum*, 3: 96-108.
- Herigstad, Becky, Martin Hamilton, and Joanna Heersink. 2001. 'How to Optimize the Drop Plate Method for Enumerating Bacteria', *Journal of Microbiological Methods*, 44: 121-29.
- Huerta, Nicolas J, Marc A Hesse, Steven L Bryant, Brian R Strazisar, and Christina L Lopano. 2012. 'Experimental Evidence for Self-Limiting Reactive Flow Through a Fractured Cement Core: Implications for Time-Dependent Wellbore Leakage', *Environmental Science Technology*, 47: 269-75.
- Jung, D., H. Biggs, J. Erikson, and P. Ledyard. 1975. 'New Colorimetric Reaction for End-Point, Continuous-Flow, and Kinetic Measurement of Urea', *Clinical Chemistry*, 21: 1136-40.
- Kapur, Jagat Narain, Prasanna K Sahoo, and Andrew KC Wong. 1985. 'A New Method for Gray-Level Picture Thresholding Using the Entropy of the Histogram', *Computer Vision, Graphics, Image Processing*, 29: 273-85.
- Kirkland, Catherine M, Drew Norton, Olivia Firth, Joachim Eldring, Alfred B Cunningham, Robin Gerlach, and Adrienne J Phillips. 2019. 'Visualizing MICP with X-ray μ -CT to Enhance Cement Defect Sealing', *International Journal of Greenhouse Gas Control*, 86: 93-100.
- Kirkland, Catherine M, Sam Zanetti, Elliot Grunewald, David O Walsh, Sarah L Codd, and Adrienne J Phillips. 2017. 'Detecting Microbially Induced Calcite Precipitation in a Model Well-Bore using Downhole Low-Field NMR', *Environmental Science and Technology*, 51: 1537-43.
- Krajewska, Barbara. 2009. 'Ureases I. Functional, Catalytic and Kinetic Properties: A Review', *Journal of Molecular Catalysis B: Enzymatic*, 59: 9-21.

- Krajewska, Barbara, Rudi van Eldik, and Małgorzata Brindell. 2012. 'Temperature-and Pressure-Dependent Stopped-Flow Kinetic Studies of Jack Bean Urease. Implications for the Catalytic Mechanism', *JBIC Journal of Biological Inorganic Chemistry*, 17: 1123-34.
- Lackner, Klaus S. 2014. 'The Use of Artificial Trees', *Geoengineering of the Climate System*, 38: 80.
- Lauchnor, Ellen G, DM Topp, AE Parker, and Robin Gerlach. 2015. 'Whole cell kinetics of ureolysis by *Sporosarcina pasteurii*', *Journal of Applied Microbiology*, 118: 1321-32.
- Mahalik, K, JN Sahu, Anand V Patwardhan, and BC Meikap. 2010. 'Kinetic studies on hydrolysis of urea in a semi-batch reactor at atmospheric pressure for safe use of ammonia in a power plant for flue gas conditioning', *Journal of Hazardous Materials*, 175: 629-37.
- Masson, Patrick, and Claude Balny. 2005. 'Linear and Non-Linear Pressure Dependence of Enzyme Catalytic Parameters', *Biochimica et Biophysica Acta -General Subjects*, 1724: 440-50.
- Mateer, JG, and EK Marshall. 1916. 'The Urease Content of Certain Beans, with Special Reference to the Jack Bean', *Journal of Biological Chemistry*, 25: 297-305.
- McBride-Wright, Mark, Geoffrey C Maitland, and JP Martin Trusler. 2014. 'Viscosity and Density of Aqueous Solutions of Carbon Dioxide at Temperatures from (274 to 449) K and at Pressures up to 100 MPa', *Journal of Chemical & Engineering Data*, 60: 171-80.
- Mitchell, Andrew C, Knud Dideriksen, Lee H Spangler, Alfred B Cunningham, and Robin Gerlach. 2010. 'Microbially Enhanced Carbon Capture and Storage by Mineral-Trapping and Solubility-Trapping', *Environmental Science and Technology*, 44: 5270-76.
- Mitchell, Andrew C, and F Grant Ferris. 2005. 'The Coprecipitation of Sr into Calcite Precipitates Induced by Bacterial Ureolysis in Artificial Groundwater: Temperature and Kinetic Dependence', *Geochimica et Cosmochimica Acta*, 69: 4199-210.
- Mitchell, Andrew C, Adrienne Phillips, Logan Schultz, Stacy Parks, Lee Spangler, Alfred B Cunningham, and Robin Gerlach. 2013. 'Microbial CaCO₃ Mineral Formation and Stability in an Experimentally Simulated High Pressure Saline Aquifer with Supercritical CO₂', *International Journal of Greenhouse Gas Control*, 15: 86-96.

- Mitchell, Andrew C., Adrienne J. Phillips, Randy Hiebert, Robin Gerlach, Lee H. Spangler, and Alfred B. Cunningham. 2009. 'Biofilm Enhanced Geologic Sequestration of Supercritical CO₂', *International Journal of Greenhouse Gas Control*, 3: 90-99.
- Mobley, HL, and RP Hausinger. 1989. 'Microbial Ureases: Significance, Regulation, and Molecular Characterization', *Microbiological Reviews*, 53: 85-108.
- Moore, J, M Adams, R Allis, S Lutz, and S Rauzi. 2003. "CO₂ mobility in natural reservoirs beneath the Colorado Plateau and southern Rocky Mountains: An example from the Springerville-St. Johns field, Arizona and New Mexico." In *Arizona and New Mexico: Proceedings of the Second Annual Conference on Carbon Sequestration*, 5-8.
- Moynihan, HJ, CK Lee, W Clark, and N-HL Wang. 1989. 'Urea hydrolysis by immobilized urease in a fixed-bed reactor: Analysis and kinetic parameter estimation', *Biotechnology and Bioengineering*, 34: 951-63.
- "NRecon User Manual." In. 2016. edited by Bruker micro CT.
- Perry, RH, and DW Green. 1984. 'Perry's Chemical Engineer's Handbook Pdf'.
- Phillips, Adrienne. 2013. 'Biofilm-Induced Calcium Carbonate Precipitation: Application in the Subsurface', Montana State University
- Phillips, Adrienne J, Alfred B Cunningham, Robin Gerlach, Randy Hiebert, Chiachi Hwang, Bartholomeus P Lomans, Joseph Westrich, Cesar Mantilla, Jim Kirksey, and Richard Esposito. 2016. 'Fracture sealing with microbially-induced calcium carbonate precipitation: A field study', *Environmental Science and Technology*, 50: 4111-17.
- Phillips, Adrienne J, Ellen Lauchnor, Joachim Eldring, Richard Esposito, Andrew C Mitchell, Robin Gerlach, Alfred B Cunningham, and Lee H Spangler. 2012. 'Potential CO₂ leakage reduction through biofilm-induced calcium carbonate precipitation', *Environmental Science and Technology*, 47: 142-49.
- Phillips, Adrienne J., Robin Gerlach, Ellen Lauchnor, Andrew C. Mitchell, Alfred B. Cunningham, and Lee Spangler. 2013. 'Engineered applications of ureolytic biomineralization: a review', *Biofouling*, 29: 715-33.
- Qin, Chao-Zhong, S Majid Hassanizadeh, and Anozie Ebigbo. 2016. 'Pore-scale network modeling of microbially induced calcium carbonate precipitation: Insight into scale dependence of biogeochemical reaction rates', *Water Resources Research*, 52: 8794-810.

- Qin, Yingjie, and Joaquim MS Cabral. 1994. 'Kinetic studies of the urease-catalyzed hydrolysis of urea in a buffer-free system', *Applied Biochemistry Biotechnology and Bioengineering*, 49: 217-40.
- Rahimpour, MR 2004. 'A non-ideal rate-based model for industrial urea thermal hydrolyser', *Chemical Engineering Processing: Process Intensification*, 43: 1299-307.
- Ren, Qun, Bernhard Henes, Michael Fairhead, and Linda Thöny-Meyer. 2013. 'High level production of tyrosinase in recombinant Escherichia coli', *BMC Biotechnology*, 13: 18.
- Sahu, JN, KK Mahalik, AV Patwardhan, and BC Meikap. 2009. 'Equilibrium studies on hydrolysis of urea in a semi-batch reactor for production of ammonia to reduce hazardous pollutants from flue gases', *Journal of Hazardous Materials*, 164: 659-64.
- Shuler, Michael, and Fikret Kargi. 1992. *Bioprocess Engineering Basic Concepts* (Prentice Hall P T R: Englewood Cliffs, New Jersey).
- Stocker, Thomas F, Dahe Qin, Gian-Kasper Plattner, Melinda Tignor, Simon K Allen, Judith Boschung, Alexander Nauels, Yu Xia, Vincent Bex, and Pauline M Midgley. 2013. "Climate change 2013: The physical science basis." In.: Cambridge University Press Cambridge.
- Stocks-Fischer, Shannon, Johnna K Galinat, and Sookie S Bang. 1999. 'Microbiological precipitation of CaCO_3 ', *Soil Biology Biochemistry*, 31: 1563-71.
- Tobler, Dominique J, Erica Maclachlan, and Vernon R Phoenix. 2012. 'Microbially mediated plugging of porous media and the impact of differing injection strategies', *Ecological Engineering*, 42: 270-78.
- Todd, David, and Larry Mays. 2005. *Groundwater Hydrology* (Jon Wiley and Sons Inc.).
- van Paassen, Leon A, Ranajit Ghose, Thomas JM van der Linden, Wouter RL van der Star, and Mark CM van Loosdrecht. 2010. 'Quantifying biomediated ground improvement by ureolysis: large-scale biogrout experiment', *Journal of Geotechnical and Geoenvironmental Engineering*, 136: 1721-28.
- Wheeler, Laura Allison. 2009. 'Establishment of ureolytic biofilms and their influence on the permeability of pulse-flow porous media column systems', Montana State University-Bozeman, College of Engineering.
- Whiffin, Victoria S., Leon A. van Paassen, and Marien P. Harkes. 2007. 'Microbial carbonate precipitation as a soil improvement technique', *Geomicrobiology Journal*, 24: 417-23.

- Wildenschild, Dorte, and Adrian P Sheppard. 2013. 'X-ray imaging and analysis techniques for quantifying pore-scale structure and processes in subsurface porous medium systems', *Advances in Water Resources*, 51: 217-46.
- Yoon, Jung-Hoon, Keun-Chul Lee, Norbert Weiss, Yung Hee Kho, Kook Hee Kang, and Yong-Ha Park. 2001. 'Sporosarcina aquimarina sp. nov., a bacterium isolated from seawater in Korea, and transfer of *Bacillus globisporus* (Larkin and Stokes 1967), *Bacillus psychrophilus* (Nakamura 1984) and *Bacillus pasteurii* (Chester 1898) to the genus *Sporosarcina* as *Sporosarcina globispora* comb. nov., *Sporosarcina psychrophila* comb. nov. and *Sporosarcina pasteurii* comb. nov., and emended description of th', *International Journal of Systematic Evolutionary Microbiology*, 51: 1079-86.
- Zhao, Haining. 2015. 'PennState EMS Energy Institute', Pennsylvania State Univeristy, Accessed 05/25/2019. http://www.energy.psu.edu/tools/PSUCO2_v2/index.php.
- Zhao, Haining, Robert Dilmore, Douglas E Allen, Sheila W Hedges, Yee Soong, and Serguei N Lvov. 2015. 'Measurement and modeling of CO₂ solubility in natural and synthetic formation brines for CO₂ sequestration', *Environmental Science & Technology*, 49: 1972-80.

APPENDICES

APPENDIX A

UREOLYSIS IN THE PRESENCE OF CARBONATED BRINES: (6.89 AND 8.27
MPa)

Ureolysis in the presence of a pressurized carbonated brine experiments were attempted at 6.89 and 8.27 MPa with inconclusive results. For these experiments, the slope of the conductivity lines was steeper, and the maximum conductivity was lower than the experiments done at lower pressures (Fig. A.1). The inconsistent behavior between the experiments is hypothesized to be due to the CO₂ being in the liquid phase. After the experiments were performed, experimental conditions were plotted on a CO₂ phase diagram and it was realized at these conditions the CO₂ might have been in the liquid form. Liquid solubility was not accounted for and may have resulted in two phases within the reactor, an aqueous phase, and a CO₂-rich phase (McBride-Wright, Maitland, and Trusler 2014; Zhao et al. 2015). Each phase would have had a different conductivity, consequently, making it challenging (and maybe impossible) to correlate the conductivity to the urea concentration. Therefore, the kinetics of the ureolysis reaction for the 6.89 and 8.27 MPa experiments could not be assessed from the conductivities measured. However, the urea concentration (found with the Jung assay) was 0 mmol/L at the end of the 5-hour experiments for both condition tested, suggesting that the ureolysis rate was faster than the 42 mM/hr rate found during the 4 MPa experimental condition.

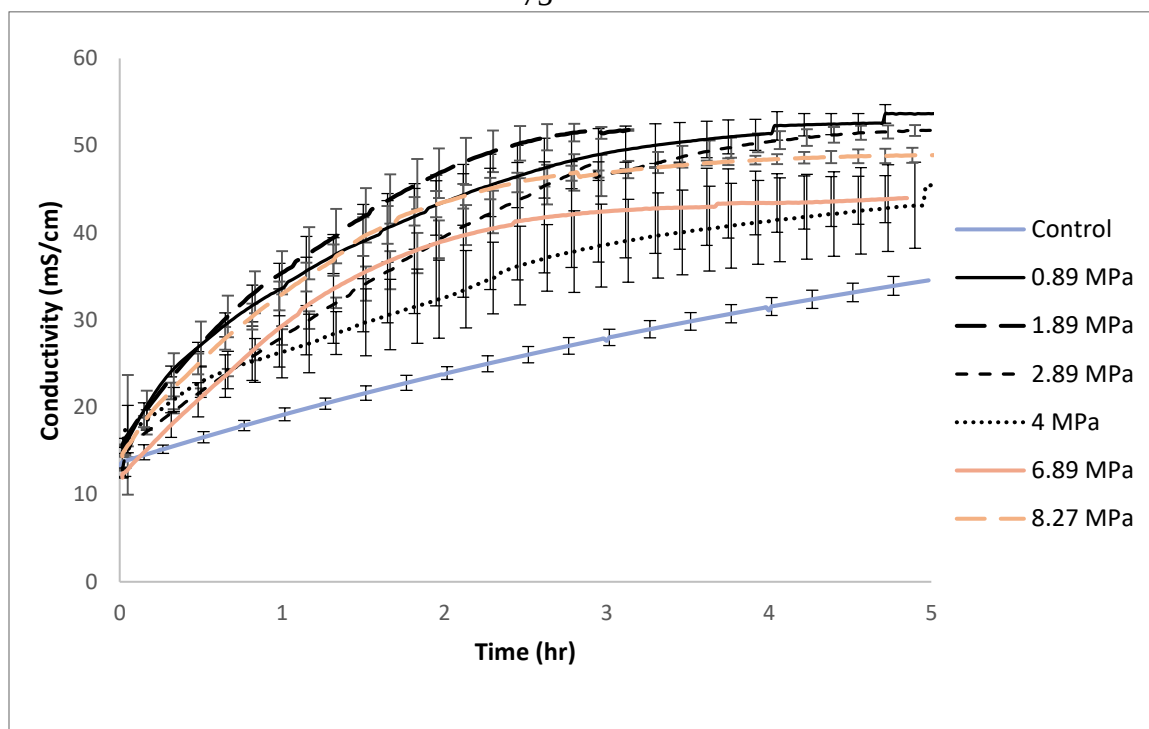


Figure A.1: Conductivity change over time for all pressure conditions tested. Black lines represent experiments in which the CO₂ was in a liquid phase at the experimental pressure, the pink lines are experiments in which the CO₂ was likely in the liquid phase at experimental pressure, and the blue line is at 0 MPa with no CO₂.

APPENDIX B

pC-pH DIAGRAMS

The theoretical final pH for each experimental condition was calculated with pC-pH diagrams that accounted for the pKa values of carbonate and ammonia (Table B.1). Figures B.1 and B.2 are examples of the pC-pH diagram for the 6.89 and 0.89 MPa conditions. Complete hydrolysis was assumed; hence, a concentration of 0.66 M of ammonia was used during calculations. The CO₂-Brine Phase Equilibrium Model from Pennsylvania State University was used to calculate the concentration of CO₂ at each pressure (Table B.1) (Zhao 2015). All final experimental pH values were within 0.3 of the predicted pH.

Table B.1 Predicted and Experimental pH

Pressure (MPa)	Molarity of CO₂ at Saturation (mol/L)	Theoretical pH	Average Experimental pH
0	0	9.3	9.28 ± 0.03
0.89	0.28	8	7.99 ± 0.28
1.89	0.93	6.9	7.18 ± 0.23
2.89	1.24	6.4	6.65 ± 0.13
4	1.56	6.25	6.40 ± 0.11
6.89	2.18	6.2	6.15 ± 0.23 *
8.27	2.61	6.15	6.17 ± 0.03 *

*CO₂ could be in liquid phase, causing uncertainty in these values

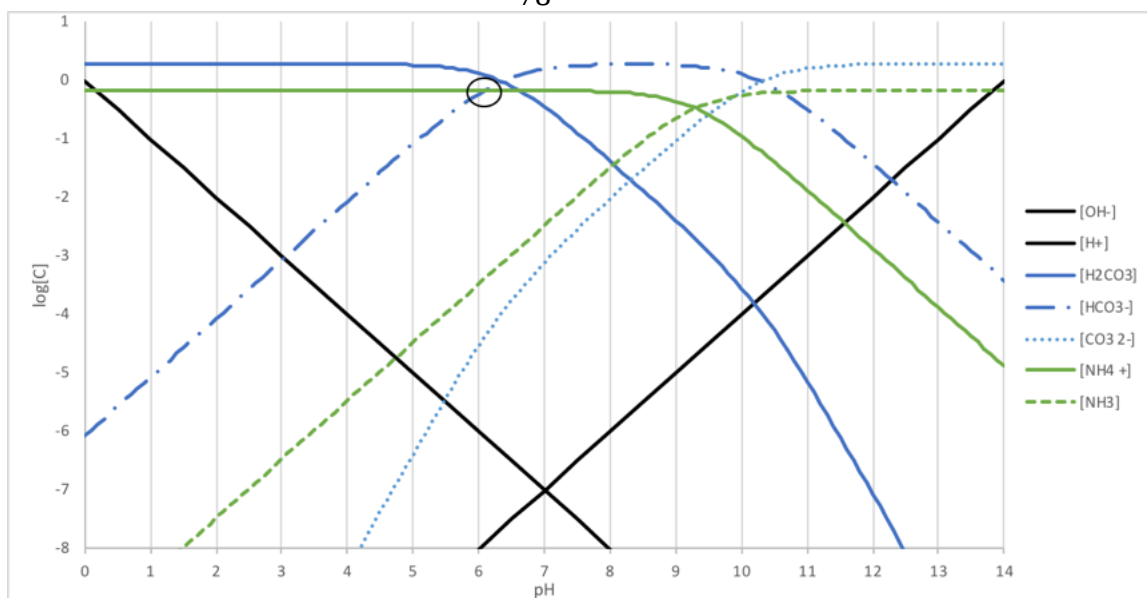


Figure B.1: pC-pH diagram for the aqueous solution within the reactor for the 6.89 MPa experimental condition: The black circle is around the point in which the system's charge balance is in equilibrium and, therefore, corresponds with the theoretical pH of the system (pH 6.2) This is close to the experimental pH of 6.15 ± 0.23 .

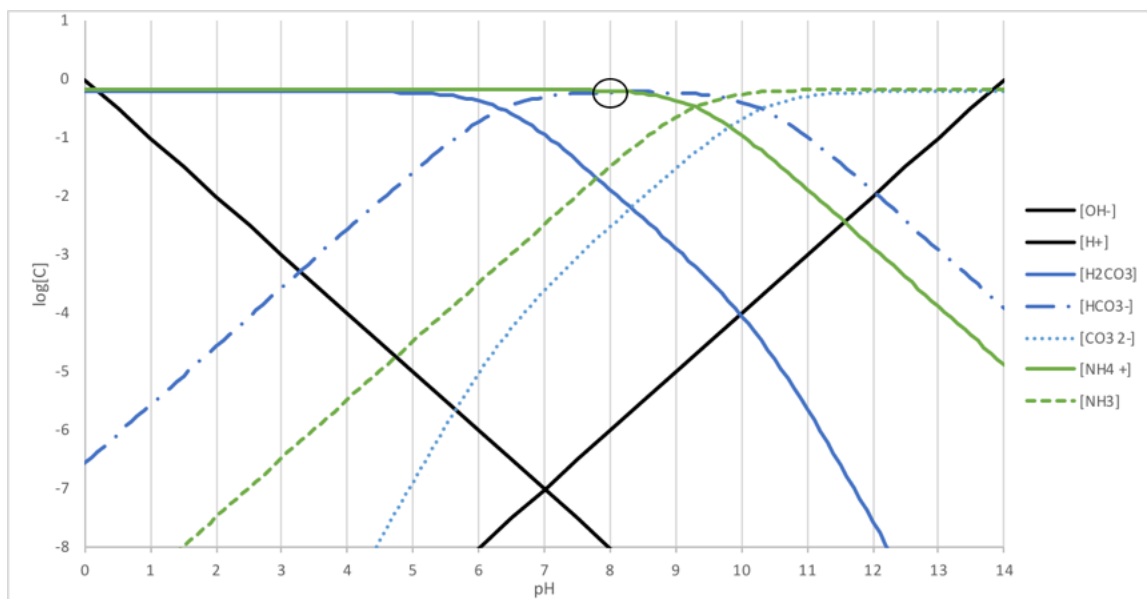


Figure B.2: pC-pH diagram for the aqueous solution within the reactor for the 0.89 MPa experimental condition: The black circle is around the point in which the system's charge balance is in equilibrium and, therefore, corresponds with the theoretical pH of the system (pH 8.0) This is close to the experimental pH of 7.99 ± 0.28 .

APPENDIX C:

PARAMETERS FOR PH-DEPENDENT UREOLYSIS RATE

Table C.1: Reported kinetic parameters for pH-dependent ureolysis

Urease Source	$V_{\max}$ (mmol l ⁻¹ h ⁻¹)	K_m (mmol/L)	$K_{es,1}$ (pK _{es,1})	$K_{es,2}$ (pK _{es,2})	References
0.1 g/L JBM	109	3.21	7.59×10^{-07} (6.12)	1.26×10^{-08} (7.9)	Fidaleo et al. (2003)
0.010 g/L JBM	27.81	2.47	2.14×10^{-06} (5.67)	8.51×10^{-10} (9.02)	Qin et al. (1994)
Immobilized JBM	150	3.21	7.94×10^{-06} (5.81)	7.94×10^{-09} (8.1)	Moynihan et al. (1989)
<i>S. pasteurii</i>	200	305	1.58×10^{-08} (7.8)	6.61×10^{-10} (9.18)	Lauchnor et al. (2015)

APPENDIX D

LOW AND HIGH-PRESSURE BATCH EXPERIMENTS AT VARIOUS PH
VALUES WITH BUFFERS

This appendix presents batch experiments performed to investigate ureolysis under varying pH conditions at high and low pressures. At low pressure, the pH range 3-12 was assessed. Buffers were prepared at 300 mM concentrations in DI water amended with 3 mM urea and 0.25 g/L JBM. At high pressures, it was attempted to use conductivity as a surrogate measurement for urea concentration, considering as discussed above, samples could not be taken without depressurizing the reactor system. However, the buffers interfered with the conductivity measurements, resulting in inconsistent data. In order to create the correlation curve for urea concentration/conductivity, it was necessary to decouple the ureolysis reaction from the reactions with the buffers, which was challenging. For this reason, and because of the relevance to CO₂ in the subsurface, CO₂ gas dissolved into a brine solution was used instead of buffers to change the pH and mimic subsurface condition (Chapter 3).

Materials and Methods

Low-Pressure Batch Experiments.

Buffers were prepared at a total concentration of 300mM concentrations in DI water, which was amended with 66 mmol/L urea. The buffers used at each pH condition were as follows: pH 3: citric acid/sodium citrate, pH 4: acetic acid/sodium acetate, pH 5: citric acid/sodium citrate, pH 6: MES buffer (2-morpholin-4-ylethanesulfonic acid), pH 7: monosodium phosphate/disodium phosphate, pH 8: TRIS/TRIS-HCl (2-amino-2-(hydroxymethyl)propane-1,3-dio), pH 9: CHES (2-(cyclohexylamino)ethanesulfonic acid)

buffer, pH 10 and 11: sodium carbonate/sodium bicarbonate and pH 12: disodium phosphate. 10 mL of the amended buffer was mixed with 10 mL 0.5 g/L JBM in 30 mL glass vials, resulting in a final concentration of 33 mmol/L urea, 0.25 g/L JBM and 150 mM buffer. The batch experiments were run in triplicate in a water bath set to 30°C and agitated at 70 rpm. Previous experiments were performed where lower concentrations of the buffers were used, and the buffer capacity was not enough to counter the increase in pH as ureolysis occurred. However, with 150 mM buffer concentrations, the pH was noted to be relatively stable over the 150 minutes at each respective pH value. The pH values for the 3 and 9-12 stayed within +/- 0.15 of the desired pH throughout the extent of the experiment. The pH values 4-8 increased slightly, throughout the extent of the 2.5-hour experiment, to 4.5, 5.5, 6.7, 7.7, and 8.3 respectively.

The batch systems were sampled at 15-minute intervals, over 150 minutes. Samples were tested for pH and then diluted 1:10 in 0.5 mol/L sulfuric acid to stop the enzymatic reaction. The colorimetric modified Jung assay was used to assess the urea concentration (Phillips 2013; Jung et al. 1975).

High-Pressure Batch Studies with Buffers

The same high-pressure batch reactor with high-pressure pH and conductivity probes, described in Chapter 3, was used for these experiments. Along with similar experimental methods, *S. pasteurii* was used as a urease source vs. JBM, as seen in Chapter 3. Buffer solutions amended with urea were mixed with the inoculum and injected into the reactor system. Only pH 5 and 7 were attempted at high pressures. For pH 7 studies, a 300 mM phosphate buffer, and pH 5 studies, a 100 mM citric acid buffer were used.

First, low-pressure tests were run to construct a urea concentration/conductivity correlation. The conductivity was measured, and a 0.3 mL sample was taken every 15 minutes. The samples were filtered and analyzed for urea concentration using a modified Jung assay (Jung et al. 1975; Phillips 2013). These data were used to create the correlation. This correlation was to be used to track the progress of the reaction under high pressure, where conductivity was the only measurement available.

Low-pressure tests were run with pH 7 buffer, pH 5 buffer, and non-amended growth media. The non-amended growth media experiment was done as a proof of concept and was successful, resulting in a good urea/conductivity correlation (Fig. D.1).

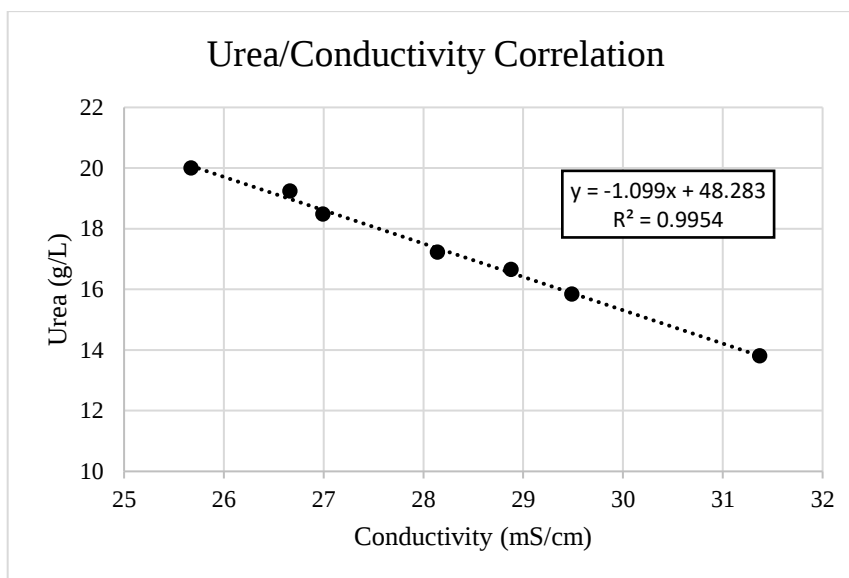


Figure D.1: Urea Concentration/Conductivity correlation done with non-amended CMM-

Low Pressure

Results from the low-pressure urea hydrolysis batch experiments at pH values 3-12 are shown in Figure D.2. Complete hydrolysis of urea was observed for pH values 6-9 in the 150-minute experiments. Ureolysis occurred fastest at pH 6, where all urea was hydrolyzed in 90 minutes. Lower hydrolysis rates were observed at pH values 4, 5, and 10. A residual urea concentration of 0.5 mmol/L for pH 10 and 1.7 mmol/L for pH values 4 and 5 were observed. No decrease in urea was observed at pH values of 3, 11, and 12, which could be attributed to inhibition of the enzyme over the time frame tested. The high concentration of buffer compared to the urea concentration must be noted, 300 mmol/L vs. 3.3 mmol/L. The high concentration differential of buffer to the substrate was necessary in order for the ureolysis reaction not to exceed the buffer capacity, which would result in a significant pH change during the experiment.

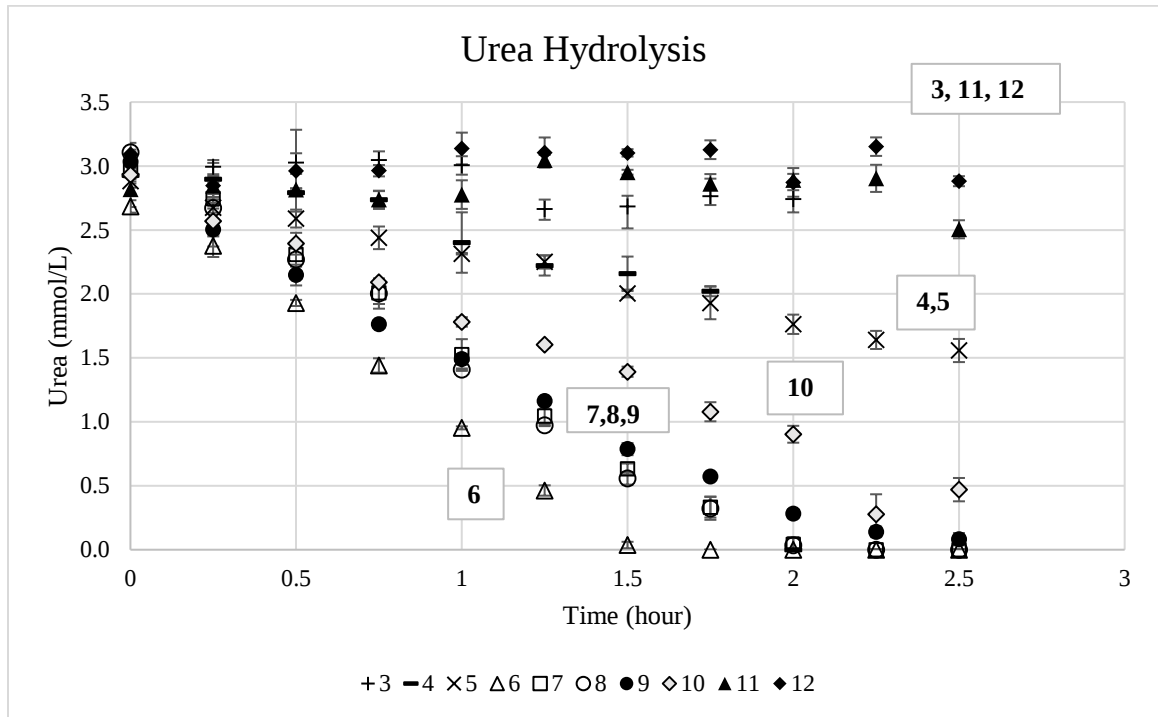


Figure D.2: Urea concentration (mmol/L) over time (hours) for all pH values tested. pH ranged from 3 to 12. The fast reaction was observed at 6, while no urea hydrolysis was seen at pH values 3, 11, and 12

The initial rate method was used to calculate the rate of each experiment. This method assumes that a linear rate can be determined at the early time points of an experiment before the concentration of the substrate changes significantly (Shuler and Kargi 1992). Figure D.3 is a box plot of the initial rates over the pH range tested. A qualitative comparison of the ureolysis rates indicated a dependence on pH, with pH 6 being the fastest. However, based on multiple t-test comparison, the rates in the pH range 6-10 did not differ significantly from each other. The rates at pH values 3, 4, 11, and 12 also did not differ significantly from each other. However, the pH group 6-10 did differ from the pH group 3, 4, 11 and 12. The rate at pH 10 only differed from the rates at pH values 3, 11, and 12. Because of the small sample base of only three replicates, there was low statistical significance between the rates at different pH values.

REACTION RATE FOR PH 3-12

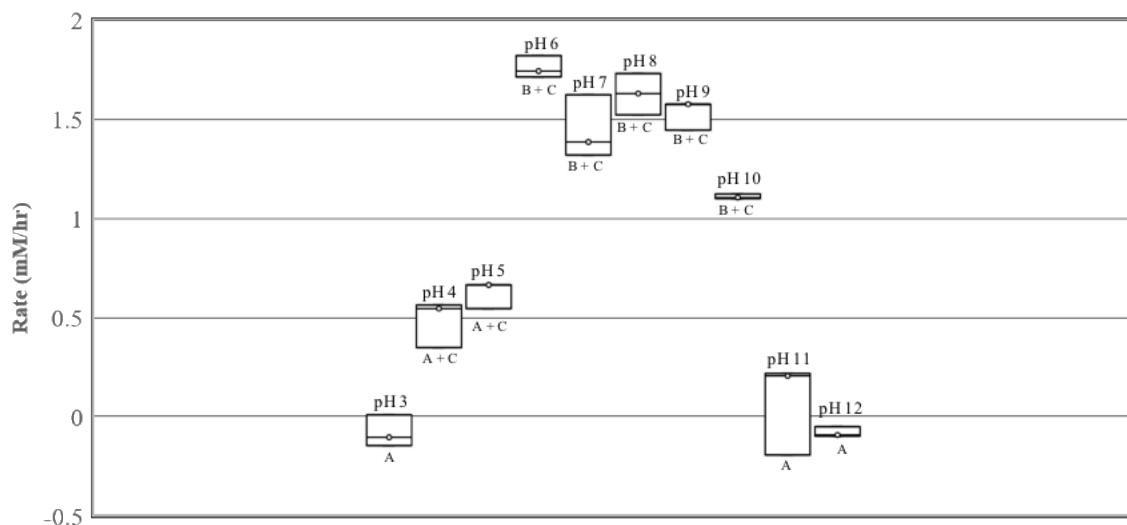


Figure D.3: A box and whisker plot of the ureolysis rates found for pH 3-12. The letters under each box represent the results from the multiple comparison t-test. The rates of ureolysis at pH values with the same letter did not significantly differ from each other.

High Pressure

Because the buffers add ions to the media, a different urea/conductivity correlation was needed for every pH. It was attempted to make these correlations at low pressure with two trials at pH 7 (Fig D.4 and D.5) and one trial at pH 5 (Fig. D.7). A high-pressure trial at pH 7 (Fig. D.6) was also attempted. It can be seen from the figures that the results were very inconsistent between trials.

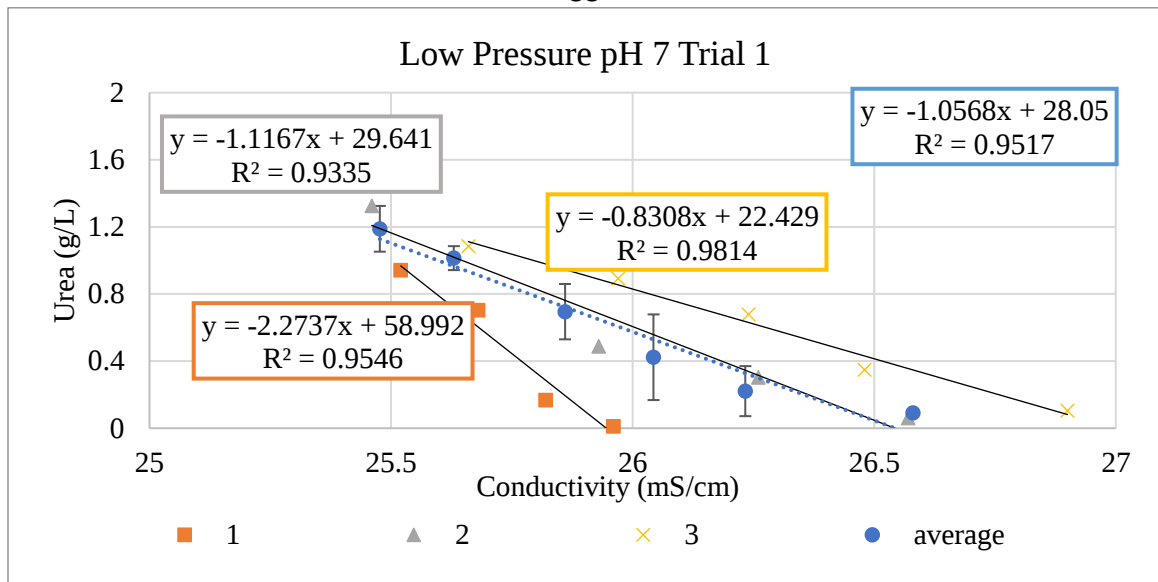


Figure D.4: Low-pressure pH 7 Trial 1

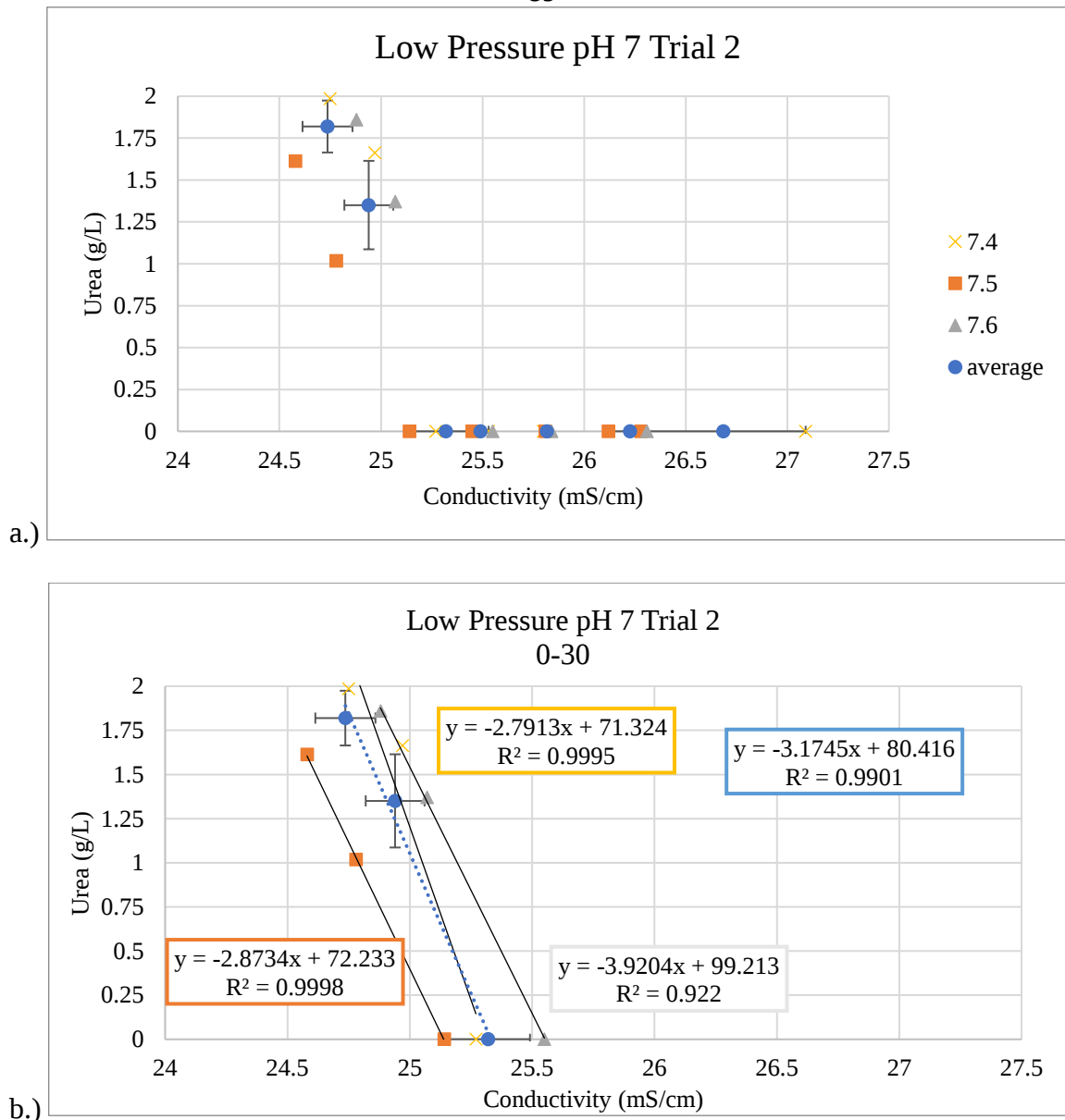


Figure D.5: Trial 2 low-pressure pH 7: (a) All time points throughout the two-hour experiment. (b) The first 30 minutes of the experiment, which were linear.

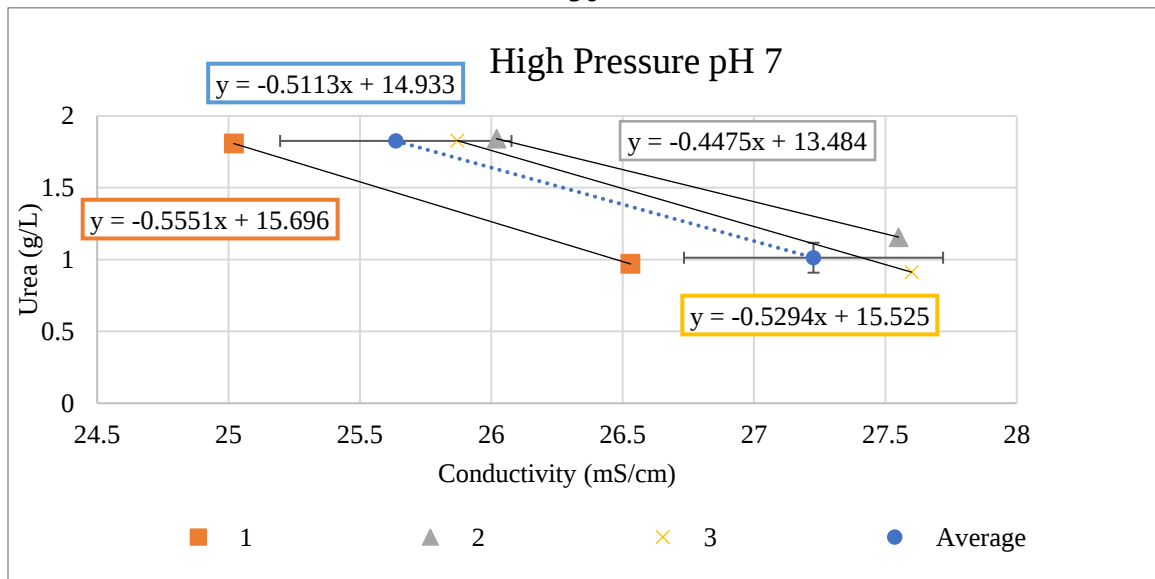


Figure D.6: High-Pressure pH 7

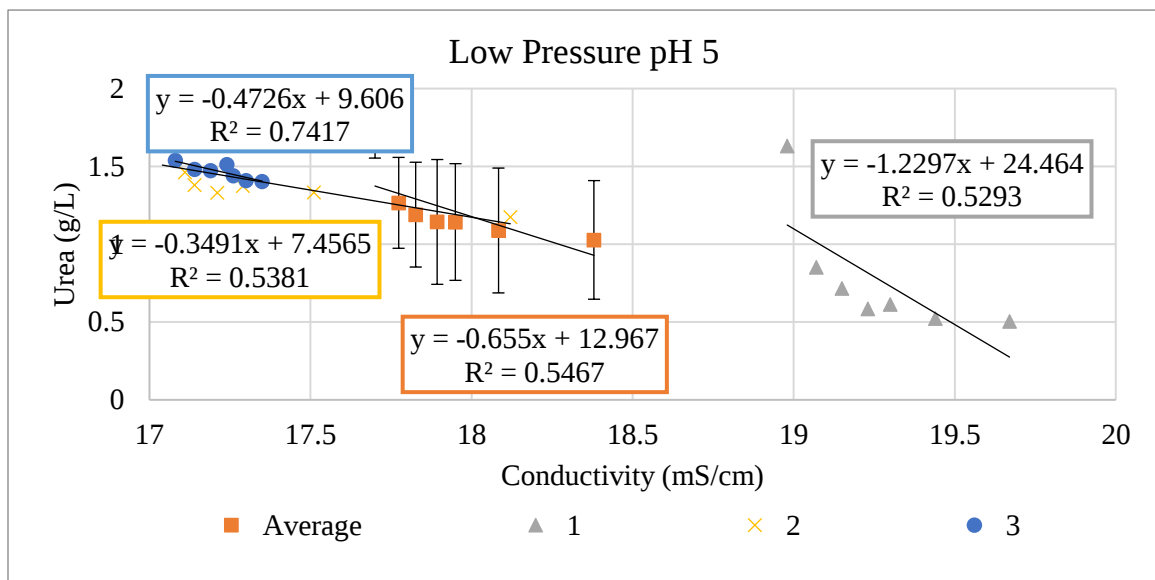


Figure D.7: Low-Pressure pH 5

Conclusion

The low-pressure experiments the rate resulted in a “bell-shaped” curve dependent on pH (Fig A.3), seen in past studies. Based on qualitative comparisons, pH 6 had the fastest

rate. However, based on multiple t-test comparisons, there was no significant difference between the rates at pH 6-9.

All experiments done in the high-pressure batch reactor had inconsistent and unpredictable results, making it extremely difficult if not impossible to create a reliable urea concentration/conductivity correlation curve. It was observed that if the reaction did not exceed the buffer capacity, the correlations were better but still unpredictable. Consistent results were never produced; thus, it was concluded that the use of buffer to adjust the initial pH conditions was not a feasible method to test ureolysis at different pH conditions under pressure. For this reason, experiments were done by dissolving CO₂ to alter the pH conditions (Chapter 3).