Bio-Inspired Self-Healing Infrastructure Materials

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BIO-INSPIRED SELF-HEALING INFRASTRUCTURE MATERIALS

By

Yara Wehbe

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of the University of Miami
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BIO-INSPIRED SELF-HEALING INFRASTRUCTURE MATERIALS

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Bio-inspired Self-healing Infrastructure Materials

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The current state of our nation’s infrastructure was given a grade of D+ in 2013, which warrants immediate remedial actions to improve structural integrity and ensure public safety. This has motivated intensive research aimed at enhancing the sustainability of infrastructure with the goal of reducing maintenance cost. Concrete is the most widely used infrastructure materials primarily due to its low cost and wide applicability. However, concrete is brittle and prone to crack formation due to mechanical loads and environmental conditions during its service life. Thus, innovative materials with self-healing capability provide a viable path towards mitigating crack related issues facing concrete infrastructure. In this dissertation, an overview of a bio-inspired self-healing methodology is presented. This methodology is based on microorganism induced calcium carbonate (CaCO₃) precipitation filling and binding cracks in the cementitious materials. The effect of addition of microorganisms and related materials on the hydration, compressive strength, transport, and microstructure of cementitious materials is evaluated. The influence of parameters affecting the morphology and chemical structure of CaCO₃ is investigated, using microscopy and analytical techniques, to establish the process-microstructure relations of CaCO₃.
Acknowledgments

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Chapter 1

Introduction
Concrete is the most widely used construction material worldwide. The popularity of concrete is the result of its low cost, wide applicability and availability. However, concrete also faces challenges such as sustainability and crack formation. The production of Portland cement, an essential element of concrete, requires an enormous amount of energy to be produced and releases a significant amount of CO₂. On the other hand, concrete is strong in compression but weak in tension, which makes it susceptible to cracking; internal stresses induce micro-cracks, which ultimately propagate into macro-cracks when additional load is applied. The formation of cracks, not only reduces the strength of the concrete, but also allows the ingress of harmful substances such as chloride ions into the concrete matrix. Traditional repair methods such as cement grouting and maintenance are being used to heal the cracks, but these techniques are difficult and expensive [1]. A grade of D+ is attributed to the current state of our infrastructure. This means that our infrastructure is at the risk of failure and so the building industry can no longer ignore these issues. The pictures below show the failing infrastructure[2],[3].

Figure 1: Collapse of a bridge [2].
A novel technique that could maximize the life span of the infrastructure while minimizing maintenance, repair cost and the consumption of non-renewable resources is required. A self-healing concrete can achieve these goals. Ordinary concrete is capable of self-healing but the process is very slow. Concrete has some unreacted cement particles that are unhydrated and so when cracks occur, water as well as carbon dioxide from air penetrate the concrete. The unhydrated cement particles will be hydrated and carbonation of calcium hydroxide (a component of hydration product) results in the formation of calcium carbonate; both continued hydration and carbonation contribute to crack filling [4], [5]. A self-healing concrete can achieve these goals by mimicking the process of self-healing in broken bones. Recent research showed that it might be possible to develop a smart cement-based material that is able to self-heal by the mean of biomineralization. Biomineralization is a process in which microorganisms stimulate the formation of

Figure 2: Corrosion of steel reinforcement bars [3].
minerals such as calcium carbonate. The precipitate formed can bind particles together; therefore, allowing autogenous healing of cracks in the concrete matrix. This technique has many challenges, the choice of microorganisms and their viability in the cement matrix, the incorporation of the microorganisms in the cement matrix, the formation of calcium carbonate and its ability to heal the cracks.

The aim of this study was to investigate the effect of self-healing microorganisms on the mechanical properties, the hydration, the transport and the microstructure of the cementitious materials. We wanted to study also the effect of using different salt types and the addition of polymeric additives on the calcium carbonate morphology and chemical structure. Finally, we wanted to look at the effect of the encapsulation of microorganisms in the hydrogel on its properties. We investigated the swelling and deswelling of the hydrogel in distilled water as well as in synthetic pore solution in order to simulate the environment in the cement paste.

1.1-Bio-Mineralization Mechanism

Biomineralization is the chemical alteration of an environment by microbial activity that will lead to the precipitation of minerals [6]. The biomineralization process is divided into two main groups: biologically induced precipitation and biologically controlled precipitation [7]. In the Biologically induced precipitation, the precipitation of minerals is due to the interaction between the biological activities and the environment. In this process, cells act as agents for nucleation and mineral growth. [7]. On the other hand, in biologically controlled biomineralization, the organisms use cellular activities to direct the nucleation, growth and the morphology in an isolated and controlled environment. [7].
Microbially induced CaCO₃ precipitation or biocalcification is a biochemical process in which microorganisms stimulate the formation of minerals such as carbonate in natural systems, such as soils, sediments and minerals [8]. Microbial induced calcium carbonate precipitation (MICCP) is one form of bio-mineralization process. This process involves microorganisms either in the vegetative form or spore (dormant) form. The spores are metabolically inactive until they are exposed to a suitable environment, and then they become vegetative again, producing urease and inducing precipitation. [8]. Organisms that use CO₂ as their carbon source induce precipitation of carbonates by removing the CO₂ from bicarbonate solutions in the presence of a calcium source (Ca²⁺) [9]. Ureolysis or urea hydrolysis is one of the bio-mineralization processes and is represented by the following equation:

\[
NH₂CONH₂ + 3H₂O \xrightarrow{Urease} 2NH₄⁺ + CO₂ + 2OH⁻HCO₃⁻ + OH⁻ \tag{1}
\]

\[
2NH₄⁺ + CO₂ + 2OH⁻HCO₃⁻ + OH⁻ \leftrightarrow H₂O + CO₃²⁻. CO₃²⁻ + H₂O \tag{2}
\]

\[
H₂O + CO₃²⁻. CO₃²⁻ + H₂O \leftrightarrow 2OH + CO₂ \tag{3}
\]

The urea hydrolysis releases NH₄⁺ and CO₂ as shown through equation 1. The release of ammonium and carbon dioxide leads to an increase in pH and alkalinity making the environment suitable for the formation of carbonate [9]. Therefore, in environments containing adequate amount of Ca²⁺, calcium carbonate (CaCO₃) precipitation will occur according to the following reaction

\[
CaCO₃ \leftrightarrow Ca²⁺ + CO₃²⁻ \tag{4}
\]

In the biomineralization process, bacteria serve as nucleation sites through which calcium carbonate precipitates with the bacteria. [6]. Bacterial cells are negatively charged and so attract divalent cations and bind them into their cells [6]. In the presence of a sufficient
concentration of $\text{Ca}^{2+}$ and $\text{CO}_3^{2-}$. $\text{CaCO}_3$ precipitation occur at the bacterial cell according to the following reactions:

\begin{align*}
\text{Ca}^{2+} + \text{cell} & \rightarrow \text{cell} - \text{Ca}^{2+} \\
\text{Cell} - \text{Ca}^{2+}\text{CO}_3^{2-} & \rightarrow \text{Cell} - \text{CaCO}_3
\end{align*}

1.2-Calcium Carbonate Morphology

Calcium carbonate is a mineral found in nature and the precipitation of $\text{CaCO}_3$ crystals can be formed by the mean of a chemical reaction, and biochemical reactions [10]. The precipitation rate of $\text{CaCO}_3$ depends mainly on the concentrations of $\text{Ca}^{2+}$ and $\text{CO}_3^{2-}$ ions as well as the microorganism type. The microorganisms can influence the rate of $\text{CaCO}_3$ precipitation, controlling therefore the polymorph of the calcium carbonate crystals [10]. Although the type of microorganisms is mainly responsible for the morphology of the

Figure 3: Crack repair by biomineralization [6].
crystals, calcium concentration, pH and availability of nucleation sites also influence the CaCO3 crystals’ morphology [8]. The biomineralization process allows the formation of different phases of CaCO3; anhydrous polymorphs such as calcite, aragonite and vaterite, as well as hydrated crystalline phases and amorphous calcium carbonate (ACC) [6]. Calcite is the most stable polymorph of CaCO3 and the primary product of microbially induced calcite precipitation (MCIP) [6]. Calcium carbonate minerals are the most abundant biogenic minerals in terms of quantities produced [7] and according to Winer et al., [3] different calcium sources lead to the production of CaCO3 crystals with different shapes. Calcium chloride creates rhombohedral crystals while calcium acetate produces lamellar shape crystals made out of vaterite. On the other hand, calcium lactate and calcium gluconate induce a complex form of crystals vaterite with a spherical shape [7]. The bonding and cementing capacity of microbial induced calcite precipitation, inspired the researchers to use MCIP in the consolidation of sand and soil and in several geotechnical engineering applications such as the use of biogrout in order to increase the shear strength of the soil to enhance foundation bearing capacity and slope stability [6].

Microbial mineral precipitation has different pathways, involves many microorganisms and can happen in different environments [11]. S. pasteurii a bacterium known for its capability of producing calcite and so the calcium carbonate precipitated has a potential of sealing fractures and surface fissures in granite and consolidating sand at the same time [11]. Microorganisms produce ammonia through the decomposition of urea and create an alkaline micro-environment around the cell and this increase in pH around the cells allows the commencement of the CaCO3 crystal growth [12].
Binding functions of microorganisms have been studied through sand consolidation experiment and *S. Pasteurii* was the microorganism chosen to conduct the experiment because of its ability to precipitate calcite and consolidate the sand in the presence of a calcium source and urea.

Gorospe et al., [13] investigated the effect of different calcium salt on calcium carbonate crystal formation using *Sporosarcina pasteurii*. *S. pasteurii* was grown in yeast extract medium of pH 9 and tightness test was conducted [13]. Three calcium salts: calcium chloride, calcium acetate and calcium lactate were used in this experiment. An overnight night culture of bacteria was harvested and re-suspended in a solution containing 2% urea and 50 mM of calcium salt. The cells were inoculated for 3 hours in a shaking incubator until precipitation occurred and then precipitate were collected and dried overnight at a temperature of 50°C and then the crystals were observed using SEM [13]. Calcium chloride caused the formation of rhombohedral calcium carbonate which characterizes calcite.
On the other hand, the use of calcium acetate induced lamellar shape crystals while calcium lactate induced spherical shaped crystals that both represent vaterite [13]. Also it was noticed that calcium lactate led to the formation of the largest crystals followed by calcium acetate and then calcium chloride [13]. Different calcium salts can induce different crystal sizes and so can affect bioconsolidation.

Figure 4: Morphology of calcium carbonate crystals.
1.3-Choice of Microorganisms and Applications

Bacteria can be found everywhere on earth; they grow in soil, water as well as in the live bodies of plants and animals [11]. Bacteria are capable of producing calcium carbonate precipitation in the form of calcite through metabolic activities [11]. A primary role of bacteria in precipitation is to create an alkaline environment to induce precipitation [11]. Microbially induced calcite precipitation (MICCP) results from a chain of metabolic reactions initiated by the hydrolysis of urea into carbonate and ammonium [8]. The ammonia produced increases the pH and trigger the precipitation of calcium carbonate [14]. Therefore, the choice of microorganisms is important. Ureolytic bacteria and non-ureolytic bacteria can be used. Ureolytic bacteria have a high urease enzymatic activity [15] and are able to decompose urea to produce calcium carbonate. On the other hand, non-ureolytic bacteria use the respiration process to convert the organic calcium sources provided with calcium carbonate [14]. Ureolytic bacteria are characterized by the release of ammonia which can have negative effects on the environment as well as on human health [16], and so in order to avoid these drawbacks, calcium precipitation, by the mean of non-ureolytic bacteria, has been proposed [16]. Different species of bacteria have been used in the biomineralization process and in concrete remediation applications and some species have been found to have a larger surface area compared to some other species, therefore allowing the attraction of more divalent cations making the microorganism more prone to calcium carbonate precipitation [15]. Due to its alkaliphilic and spore forming nature, urease and binding capacity, S. pasteurii is the most common microorganism used for sand consolidation as well as for concrete remediation. S. pasteurii are alkali-resistant, have high urease and binding capacities and are negatively charged, they can therefore attract
divalent cations and bind them into their cells to precipitate calcium carbonate \( \text{CaCO}_3 \). Because of these characteristics, \textit{S. pasteurii} have been used in sand consolidation experiments and biomineralization in cementitious materials.[9],[15],[17]. Even though \textit{S.pasteurii} is the most common microorganism for bio-remediation, other type of microorganisms has been used in concrete remediation. Although \textit{S.pasteurii} is the bacteria specie mainly used in biomineralization applications, other species have also been used for self-healing of cement and concrete. Jonkers et al., [18] used \textit{Bacillus pseudofirmus} and \textit{Bacillus cohnii}, two aerobic, alkaliophilic, spore forming bacteria and were incorporated into cement stone specimens in order to investigate the viability of incorporated bacteria at different ages. Achal et al.,[19], used \textit{B. megaterium} and grew them in NBU media and studied the viability and the compressive strength of cement mortar. \textit{Bacillus sphaericus} another ureolytic, alkali-tolerant spore forming strain has been encapsulated in hydrogel and used for simulating, self-healing in concrete [20]. On the other hand Xu et al., [21], used \textit{B. cohnii} a non-ureolytic bacteria as a surface treatment for mortar.

The table below summarizes the list of bacteria that has been used, their characteristics and their application.
<table>
<thead>
<tr>
<th>Bacteria type</th>
<th>Characteristics</th>
<th>Applications</th>
<th>Viability in cement based materials</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>B. cohnii</td>
<td>-aerobic</td>
<td>-treatment of concrete surface</td>
<td>-spores were viable up to 4 months</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>-spore forming</td>
<td>-incorporation in mortar to achieve self-healing</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-non-ureolytic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. megaterium</td>
<td>-aerobic</td>
<td>-incorporation into cement to investigate crack remediation</td>
<td>-cells were viable up to 28 days beyond that age the cells were still viable but below the detection limit</td>
<td>[19]</td>
</tr>
<tr>
<td></td>
<td>-spore forming</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>bacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-ureolytic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-alkali-resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. sphaericus</td>
<td>-aerobic</td>
<td>-encapsulation into hydrogel and crack remediation</td>
<td>-the cells were after encapsulation in hydrogel and after being incorporated in the cement matrix</td>
<td>[15]</td>
</tr>
<tr>
<td></td>
<td>-spore forming</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-alkali-resistant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. pasteurii</td>
<td>- aerobic</td>
<td>-sand consolidation</td>
<td>-S. pasteurii can survive up to 330 days in cement paste and mortar</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td>- Spore forming</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-alkaliphilic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- ureolytic</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.4-Nutrient Medium Selection

Calcium carbonate precipitation depends on the microorganism type, the availability of sites where divalent ions can attach too as well as the bacteria concentration and nutrients availability [15]. To keep microorganisms alive and in the metabolic state carbon and nitrogen source are needed [22]. Yeast is usually used as the carbon source and provides at the same time all the vitamins and amino acids required for the survival of the bacteria [19]. Although a carbon and nitrogen source is required for the bacteria to have an active metabolic state, it is also necessary for the microbial induced calcium carbonate precipitation to occur to have a urea and calcium source. De Muynck et al., [23] investigated the concentration of urea and calcium on the effectiveness of microbial induced carbonate precipitation on limestones. For this investigation, the bacterial strain B. sphaericus has been used and were grown in a media that contains 20 g/l urea and 20 g/l yeast. The limestone specimens 1 cm$^3$ were at the beginning submerged in growth media solution for 1 day and then transferred to solutions of varying composition of urea and calcium chloride CaCl$_2$.H$_2$O salt [23]. It was shown that the urea was not completely hydrolyzed in the presence of calcium source, and this is due to the fact that the precipitation of calcium carbonate affects the surface availability for nutrient uptake and eventually leads to the death of the cells [23]. Therefore, for a certain amount of cells there is an optimum urea and calcium dosage, if this dosage is exceeded, it can lead to accumulation of salts and urea on the pores [23] reducing the surface availability for nutrient uptake, and so the cells will start to die. So it is very important to have a nutrient medium that has the optimum amount of carbon, nitrogen, and urea and calcium source to allow the growth of bacteria as well as bio-mineralization.


1.5-Incorporation of Bacteria in Cement-based Materials and Viability of Cells

As described in section 2.3 many microorganisms are capable of precipitating calcium carbonate. However, since these microorganisms are embedded in the cement, it is very important to find a microorganism that can survive in high pH environment, resist the mixing process and survive having a limited access to nutrients [15]. There are different methods of incorporating the bacteria or spores into the cement matrix. The bacteria can be added immediately or using encapsulation methods both synthetic and natural Wang et al., [20] used the synthetic technique and grew B.sphaericus in yeast and urea solution, allowed them to sporulate and then encapsulated them and their corresponding nutrients into hydrogel. It was shown that the spores were still viable after the encapsulation and they were able to decompose urea and precipitate CaCO3 in the hydrogel matrix. Bang et al., [14] immobilized Bacillus pasteurii in polyurethane as yet another synthetic encapsulation approach. The PU membrane provided protection for the microorganisms from the high pH environment in the cement paste and acted as a nucleation site for calcite precipitation; therefore, improving the urease activity of the cells and allowing a better biomineralization process.[24] On the other hand, Jonkers et al., [18] used the resilient nature of B. cohnii, the spore form and incorporated them into the cement matrix without any additional nutrients [15]. These spores survived up to 4 months in the matrix. However, Wiktor et al., [25] adopted a hybrid approach, a natural-synthetic encapsulation method and used Bacillus alkalinitrilicius an alkali-resistant, spore forming bacteria and encapsulated them in expanded clay particles impregnated twice under vacuum with calcium lactate in order to preserve the spores and increase its service life functionality.
The encapsulated spores were then incorporated into the mortar specimens and then cracks were induced and monitored for self-healing. Cracks up to 0.46 mm width were completely healed in bacteria based specimens while cracks up to 0.18 mm were only completely healed in control samples [25].

As stated previously, one of the key challenges in incorporating microorganisms in cement-based materials is to find a microorganism that is capable of surviving the mixing process, a high pH environment and survive having limited access to nutrients [15]. It has been shown that \textit{S. pasteurii} are capable of surviving in cement for up to 4 months under the spore form while \textit{B. megaterium} can only survive up to 28 days according to [19]. On the other hand, it was shown that when microorganisms were encapsulated in clay particles impregnated with calcium lactate, their lifetime service increased and they were able to survive longer in the cement matrix [20].

\textbf{1.6-Remediation of Cracks by Bio-Mineralization}

Concrete is strong, durable and inexpensive and is a material used worldwide [25]. However, a major drawback is that concrete is subjected to cracks caused by mechanical and environmental factors [26]. The presence of micro-cracks do not result in a significant loss of strength of the concrete, but they allow the ingress of water and chloride, therefore enhancing the corrosion of reinforced steel bars [26]. According to Jonkers et al., [18], 4 billion dollars are spent in the United States alone as a direct cost for maintenance and repair of concrete due to steel bars corrosion.

To improve durability and decrease maintenance cost, it would be ideal if cracks could be healed autonomously after formation by releasing self-healing agents inside the matrix when cracks occur [27]. Various techniques could be used for crack remediation.
Traditional repair consists of injecting a two-component epoxy resin into the cracks [28]. However, this technique have some disadvantageous aspects such as different thermal expansion coefficient coupled with environmental and health hazard [28].

An alternative technique to traditional repair methods is to use microbiologically induced calcium carbonate precipitation [21]. The concept consists of incorporating ureolytic bacteria that hydrolyses urea and therefore precipitate calcium carbonate in the micro-crack region [21], therefore resulting in self-healing of cracks.

Many researchers have investigated the self-healing of cracks using microbially induced calcium carbonate precipitation (MICCP).

Ramachandran et al., [29], used *Bacillus pasteurii* to examine healing of cracks. For this purpose, cement mortar beams with no cells were cast and cured in water for 28 days and left exposed to air afterwards. The width of the crack generated in the mortar beams was maintained at 3.175 mm and 9.175 mm. The cracks were filled with *B. pasteurii* and sand and cured in Urea-CaCl₂ medium for 28 days [29]. *B. pasteurii* showed more remediation in shallower cracks than deeper ones leading to a significant increase in the stiffness of the beam [29]. The sand particles were held together by CaCO₃ indicating that the precipitation of calcium carbonate filled the voids between the particles and allowed its consolidation [29].

On the other hand, Jonkers et al., [25], used *Bacillus alcalinitrilicus*, an alkali-resistant and spore forming bacteria and embedded both the bacteria and the nutrients in expanded clay particles [25]. The spores are therefore protected during the production and hardening phase of concrete allowing the microorganisms to survive longer; until the moment when self-healing is needed [30]. To investigate crack remediation, Jonkers et al., [25], prepared
cracked mortar specimens: control and bacteria based with a high number of cracks having different crack widths and submerged them in water for 100 days in a container and leaving the container exposed to the atmosphere. The specimens were removed weekly and crack healing was monitored using a stereomicroscope and photographic imaging [25]. Cracks of 0.46 mm width were completely healed in the bacteria based specimens while cracks of 0.18 mm were healed in control specimens [25]. Bacteria in and around the crack surface will be activated and will precipitate CaCO$_3$ in situ to heal cracks [31]. However, the size of the bacteria is in the range of 1 µm-3 µm, while the pore size in the concrete matrix are smaller than 0.5 µm, therefore it is likely that the bacteria inside the matrix will be squeezed and crushed [31]. Water on the other hand is an essential element for bacterial activities and so sufficient and continuous water supply is needed to keep bacterial active [31]. To provide constant water supply, specimens were submerged in water. However, in the case of hydrogel a wet-dry cycle was conducted because the hydrogel absorbs the water during the wet phase and releases it gradually to support bacterial activity during the dry stage [31]. For these reasons, Wang et al., [20] encapsulated $B$. sphaericus spores into the hydrogel and then the hydrogel was dried and crushed and added to the mortar specimens. After 28 days, prisms were subjected to multiple cracking by tensile test and then subjected to wet-dry cycles for 4 weeks [20]. Crack filling was observed under a light microscope and initial and final images were taken and crack healing was evaluated by crack width decrease [20]. In the specimens embedded with hydrogels and spores, more crack healing occurred in comparison with the specimens containing pure hydrogel. Specimens with bio-hydrogels were able to heal cracks up to 0.5 mm of width [20].
Finally, Van Tittelboom et al., [28], investigated the self-healing of standardized and realistic cracks. A thin copper plate of 0.3 mm of thickness was introduced in the fresh mortar paste up to a depth of 20 mm and then the plate was removed after 24 h. On the other hand, splitting tests were performed on concrete cylinders wrapped in fiber reinforced polymers (FRP) to create realistic cracks [28]. Samples were treated by different methods. Some of the cracks were injected using a syringe with *Bacillus sphaericus* encapsulated in Levasil sol gel and then submerged in an equimolar urea-CaCl$_2$ solution. Other specimens were submerged in an overnight culture of bacterial solution for 24 h and then submerged in an equimolar urea-CaCl$_2$ solution [28]. When bacteria was immobilized in Levasil sol gel, complete filling of cracks occurred as if the samples were treated using epoxy resin. However, when specimens were only submerged in bacteria solution and then in Urea-CaCl$_2$, no CaCO$_3$ crystals were detected through the microscope. This could be due to the fact that the bacteria died because they were not protected against the high pH of concrete and therefore no calcium carbonate was produced.
Chapter 2

Experimental Methods

2.1-Choice of Microorganisms

As described in section 1.3 many microorganisms are capable of precipitating calcium carbonate. However, it is important to find a microorganism capable of surviving high pH environment. *S. pasteurii* is the most common bacteria used for biomineralization and self-healing applications [12]. *S. pasteurii* is a spore forming bacteria and is non-pathogenic [15], have a high urease activity, so it is capable of producing calcium carbonate and finally is capable of surviving high pH environment. *S. pasteurii* was also capable of self-healing in cement based materials. For these reasons, *S. pasteurii* was purchased from the American Type Culture Collection (ATCC 6453) and research was conducted using this microorganism. A microscopic image of *S. pasteurii* is shown in Figure 5.

2.2-Microorganism Growth

Figure 5: Micrograph showing *S. Pasteurii*. 
Carbon, nitrogen and other nutrients are required to keep the bacteria in an active metabolic state [22]. Urea is considered to be the nitrogen source in MCIPP and yeast extract is used as the carbon source for *S. pasteurii* [15]. *S. pasteurii* ATTC (6453) was grown in a medium consisting of 1 L of distilled water, 20 g of urea, 5 g of peptone, 5 g NaCl, 2 g yeast extract and 1 g beef extract. The pH of the medium was adjusted to 9.00 by adding NaOH. The mixture was then autoclaved at 121°C. When the mixture was cooled down, a colony from the plate was inoculated into the flask containing the mixture. *S. pasteurii* cells were grown aerobically at 37°C at 250 rpm shaking conditions in a 500 ml of the corresponding medium for 24 hours (see Figure 6).

![Image of microorganism growth setup.](image)

**Figure 6: Image of microorganism growth setup.**

### 2.3-Determination of Bacterial Concentration

15 g of Agar was added to the medium described in the previous section and then autoclaved. The medium was cooled down up to 55°C and then poured in petri dishes to get agar plates. A serial dilution technique has been used in order to determine the bacterial
concentration. A sample from the liquid culture is inoculated and spread on an agar plate using a glass rod to ensure a uniform sample distribution. However, the number of cells in a liquid culture are too high to count, so the solution is diluted into tubes and then plated on agar plates according to the procedure described in [32]. The cells were counted according to the following formula:

\[
\text{Original cell density} = \frac{\text{Colonies counted} \times \text{dilution factor}}{\text{volume plated}}
\]  

(5)

2.4-Precipitation of Calcium Carbonate

In order to confirm the formation of calcium carbonate precipitation we followed the experiment described in [15]. \textit{S. pasteurii} cells were allowed to grow in a 75 mL of nutrient medium described previously supplemented with 0.1M of CaCl2 in a 125 mL flask on the shaker at 37°C for 24 h. The precipitate was later collected and centrifuged at a speed of 3000 x g for 10 min and then the supernatant was later removed under vacuum. The precipitate collected was washed with distilled water and then centrifuged again at the same speed for 5 min. Thermogravimetric analysis (TGA) was then conducted on the precipitate.

2.5-Experimental Methods

2.5.1-Sample Preparation and Compressive Strength

Four different mix designs with a water to cement ratio (w/c) of 0.5 were prepared for this experiment using ordinary Portland cement type I having the following composition:
Table 2: Portland cement composition

<table>
<thead>
<tr>
<th>Composition</th>
<th>Formula</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silica</td>
<td>SiO₂</td>
<td>20.82</td>
</tr>
<tr>
<td>Alumina</td>
<td>Al₂O₃</td>
<td>4.98</td>
</tr>
<tr>
<td>Iron Oxide</td>
<td>Fe₂O₃</td>
<td>3.68</td>
</tr>
<tr>
<td>Calcium Oxide</td>
<td>CaO</td>
<td>64.34</td>
</tr>
<tr>
<td>Magnesium Oxide</td>
<td>MgO</td>
<td>0.91</td>
</tr>
<tr>
<td>Sodium Oxide</td>
<td>Na₂O</td>
<td>0.19</td>
</tr>
<tr>
<td>Potassium Oxide</td>
<td>K₂O</td>
<td>0.41</td>
</tr>
<tr>
<td>Sulfur Trioxide</td>
<td>SO₃</td>
<td>2.79</td>
</tr>
<tr>
<td>Titanium dioxide</td>
<td>TiO₂</td>
<td>0.24</td>
</tr>
<tr>
<td>Loss of Ignition</td>
<td>LOI</td>
<td>2.03</td>
</tr>
</tbody>
</table>

- Control paste: cement, water.
- Nutrient paste: cement, water and *S. pasteurii* medium described previously.
- Bacterial paste: cement, water and *S. pasteurii* culture.
- Bacterial paste with addition of CaCl₂:

Cubes of (508 mm x 508 mm x 508 mm) were prepared according to ASTM C305 procedure. The ASTM C305 was modified by replacing the mixing water with bacterial or nutrient medium. For bacterial paste, *S. pasteurii* was grown in the medium described in the previous section and then the bacterial solution was mixed with cement. The final concentration of *S. pasteurii* in the liquid media was estimated to be 2 x 10⁶ CFU/mL by the mean of the serial dilution. The cubes were demolded after 3 days and submerged into liquid media solution saturated with lime until testing. Compressive strength testing was conducted at 4, 14 and 28 days.
2.5.2-Electrical Resistivity

The electrical resistivity measurement was used in order to get an insight into the microstructure of the cement paste. The electrical resistivity of the cement paste was measured using the electrochemical impedance spectroscopy (EIS) method. The cubes were submerged in bacterial media and stored in a room with a relative humidity of 95% and a temperature of 23 °C until the day of testing. Testing occurred at 4 days, 14 days and 28 days. The setup consisted of two metallic sheets connected to a Gamry 600 potentiostat for the EIS measurements. A piece of foam wetted in 1 M NaCl solution was placed on both surfaces of the cube and the cube is then compressed between the two metallic plates. A weight was placed on top of the metallic plate to ensure a good electrical contact between the cube and the plate. The EIS was carried out using a 250 mV AC signal and frequency range of $10^6$ to 10 Hz. The electrical resistivity was then measured using the following formula:

$$\rho = \frac{RA}{l}$$

$\rho$: resistivity in $\Omega\cdot m$

R: measured resistance

A: Area of the surface of the cube in $m^2$

$l$: distance between the two metallic plates in m.

The electrical resistivity setup is shown in the picture below.
2.5.3- Non-evaporable Water Content

The non-evaporable water content is used in order to evaluate the degree of hydration of cementitious materials. The non-evaporable water content has been used in order to explore the effect of nutrients and bacteria on the hydration of cement and was conducted at 4, 14, 28 days.

The cement paste cube was broken during the compressive test, and then a small piece from the middle of each cube was ground and sieved using sieve #60. In porcelain crucibles, about 6 g of cement powder collected from each cube was placed in them and dried for 24 h in the oven at 105°C. After 24h, the mass of the samples were measured using a high precision scale, and then the samples were transferred to a furnace at a temperature of 1050°C for 5 h and then their ignited mass was measured. The non-evaporable water content was calculated according to the formula used in [33].

\[
W_n(\%) = 100 \times \left[ \frac{\text{dried mass} - \text{ignited mass}}{\text{ignited mass}} \right] - LOI
\]  

(1)

\( LOI \) is assumed to be 2.03 %
2.5.4- Thermogravimetric Analysis

TGA is a technique that measures the change in the mass of a sample over a range of temperature. As the sample is heated, its mass changes. This change can be used to determine the composition of a material. TGA tracks the change in mass as a function of temperature. TGA has been used in order to detect, measure and quantify the presence of calcium hydroxide and calcium carbonate in the cement paste at the ages of 3 days, 14 days and 28 days. A small piece from the center of the cement paste was ground and passed through the sieve #60. The ground samples were vacuum dried for 24 h at 60°C and the cement powder was placed in a crucible of capacity 50 mg and analyzed using TG 209F3. The initial temperature was set at 20°C while the final temperature was set to be 1000°C. The heating rate was set to be 20K/min. The loss in mass in the temperature range between 400°C and 500°C is attributed to the decomposition of Ca(OH)₂ and the calcium hydroxide content is computed using the following equation:
\[ CH = \frac{74.1 \Delta m}{18 \ m} \]

On the other hand the mass loss between 600°C and 800°C is attributed to the decomposition of calcium carbonate and the CaCO\textsubscript{3} content is computed using the following equation

\[ CH = \frac{100.0 \Delta m}{44.0 \ m} \]

\(\Delta m\): mass loss related to the decomposition of Ca(OH)\textsubscript{2} or CaCO\textsubscript{3} (mg)

\(m\): Initial mass of cement paste sample (mg).

Figure 9: Image of TGA analyzer.
2.5.5-Scanning Electron Microscopy (SEM) of Cement Paste

Microscopic examination on cement paste and cement paste containing bacterial solution cured at 14 days was conducted in order to look at the microstructure of the cement paste. After mechanical testing, the samples were soaked in acetone for two hours in order to stop the hydration process. The samples were then left to dry at 60 °C for two days. A small piece from the middle of each sample was set in epoxy overnight, the samples were then polished using sand papers with 180, 320, 600 and 1200 grit sizes using ethanol for lubrication. For a smooth finish the samples were further polished using a 1µm diamond paste and then placed in an ultrasonicator for 10 min. The samples were then examined in SEM. The following picture shows the prepared samples.

Figure 10: Image of samples set in epoxy for SEM examination.
2.6-Calcium Carbonate Characterization

2.6.1-Sample Preparation

We wanted to investigate the effect of using different calcium salts, different polymeric additives and bio-precipitation on the morphology of the calcium carbonate crystals. The purpose of adding polymeric additives was to simulate the extracellular polymeric substance (EPS). EPS establish the functional and structural integrity of biofilms, and are considered the fundamental component that determines the physiochemical properties of a biofilm. Calcium carbonate precipitation was conducted using three different methods: chemical precipitation, chemical precipitation with addition of two types of polymers Polyvinyl alcohol (PVA) and PolyAcrylic Acid (PAA) were added to the solution. Finally calcium carbonate was precipitated by the mean of microorganisms. Three different calcium salts: calcium chloride, calcium acetate and calcium lactate have been used.

The purpose of using different calcium salts is to determine the effect of these salts on crystal morphology and chemical structure. For the chemical precipitation 50 mL of calcium salts were titrated with 50 mL of sodium carbonate both having a concentration of 50 mM and then filtered so that the precipitate is collected. On the other hand, for the chemical reaction that includes polymers, the procedure was exactly the same as the chemical reaction except that 50 mL of PVA was mixed with 50 mL of calcium salts and then titrated with 50 mL of sodium carbonate; while 50 mL of PAA was mixed with 50 mL of sodium carbonate and then titrated with calcium salts. Finally, for the bio-precipitation, the procedure was the same as the one described in the previous section except that three calcium salts were used at a concentration of 0.1 M. The precipitate collected from all the samples was stored in petri dishes and dried in the oven at 60°C for
2 days. The dried samples were then crushed into powder for the Fourier transform infrared spectroscopy (FTIR) and SEM analysis.

2.6.2-SEM Imaging

The powdered samples were mounted on studs and then images were taken using SEM in order to determine the morphology as well as the particle sizes of the crystals formed. The particle sizes was determined using ImageJ software.

2.6.3-Fourier Transform Infrared Spectroscopy (FTIR)

In Infrared spectroscopy, IR radiation is passed through a sample. Some of these radiations are absorbed by the sample while the other passes through. The spectrum obtained represents the molecular absorption and transmission, this spectrum creates a molecular fingerprint of the sample and no two unique molecular structures produce the same infrared spectrum [34]. The method is very popular because it is very easy, quick, doesn't require a large amount of material and is capable of identifying unknown materials and determines the amount of components in a mixture.

The spectral characterization of the precipitated powder was carried out by PerkinElmer Fourier Transform Infrared Spectroscopy (see Figure 11). 1g of the precipitated powder was placed over a diamond crystal and the FTIR spectrum was recorded over the range of 600 cm\(^{-1}\) and 4000 cm\(^{-1}\).
2.7-Potential Use of Hydrogel Encapsulating Medium

2.7.1-Preparation of Poly(acrylamide-co-acrylic acid hydrogel)

Poly(acrylamide-co-acrylic acid) hydrogels were prepared following the procedure described in [35]. These hydrogels all contain 40% acrylamide and 60% acrylic acid. The hydrogels were synthesized by free radical polymerization mechanism. The monomer acrylic acid was dissolved in distilled water and neutralized with NaOH. Then the acrylamide monomer and the crosslinking agent N, N’-Methylene bisacrylamide (BIS) were dissolved in the solution. The solution was then purged under Argon gas for 10 min and then the ammonium persulfate initiator was added. The solution is then casted into glass molds separated by a rubber separator of 0.5 mm. The top of the mold is sealed using parafilm and then put in the oven to dry for 2h to 3 h at 60°C. For the hydrogel with bacterial solution, the same procedure was followed except that the initiator as well as bacterial solution were added before purging.
2.7.2-Hydrogel Absorption

It is important to determine the swelling of hydrogel and determine how the addition of the bacterial solution affects the swelling properties of the hydrogel. For this purpose and in order to simulate the condition in cement paste, the hydrogel is swelled in synthetic pore solution. The synthetic pore solution composition is described in [36]. The pore solution composition is based on pore solution extraction from cement paste samples with w/c=0.42. The following table show the chemical composition of synthetic pore solution in 1 L of distilled water.

Table 3: Synthetic pore solution composition

<table>
<thead>
<tr>
<th>Components</th>
<th>Concentration (mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaOH</td>
<td>0.1</td>
</tr>
<tr>
<td>KOH</td>
<td>0.3</td>
</tr>
<tr>
<td>Ca(OH)$_2$</td>
<td>0.03</td>
</tr>
</tbody>
</table>

The hydrogel prepared were demolded from the glass molds after gelation and cut into circular pieces having 0.56 cm of diameter and then left to dry in the oven for 24 h. The dry mass of the hydrogel was measured using a high precision scale. A small mesh was placed inside a small container containing the pore solution and then the hydrogel was placed on top of the mesh. The mesh was constantly removed from the container and the extra pore solution was wiped and then the wet mass of hydrogel was measured. At the beginning, the mass was measured every 2 min for the first 10 min and then the mass was recorded every 10 min for 30 min and then every 20 min for the next hour. Finally, every
40 min a measurement was taken for 2 hours. The final mass was recorded after 24 hours and the swelling of the hydrogel was computed according to the following formula:

\[
Swelling\ Ratio = \frac{\text{Final weight} - \text{initial weight}}{\text{initial weight}} \times 100
\]  

(4)

2.7.3-Desorption of Hydrogel

The hydrogel prepared was demolded and submerged in synthetic pore solution until equilibrium is reached. The hydrogel is then cut into circular pieces and sandwiched between 2 dried cement paste cubes having the following dimensions (2.54 cm x 2.54 cm x 2.54 cm) (see Figure 13). A microscopic camera was set and focused on the sandwiched hydrogel and pictures were taken every 5 min for the first 30 min and then every 10 min for the next hour and then every 20 min for the next 1h 30 min. The last picture was taken after 24 h and the de-swelling curve was generated by analyzing the change of thickness of the hydrogel throughout time.

Figure 12: Image of the dry state of the hydrogel as well as the swollen state.

2.7.3-Desorption of Hydrogel
Figure 13: Desorption of hydrogel through time.
Chapter 3

Results and Discussion

3.1-Non-evaporable Water Content

The non-evaporable water content of the control cement paste and the cement paste with nutrients and the cement paste with bacteria is shown in Figure 14. Non-evaporable water content is used to evaluate the degree of hydration in the cement pastes. It is seen that the cement paste with bacteria showed higher degree of hydration than the control cement paste and the cement paste with nutrients at 4 days and 28 days. This increase in the degree of hydration could be due to the addition of nutrients and also the seeding effect of small bacteria size in the range of 1.5 µm. It is known that the addition of fine particles provides nucleation sites for hydration, thereby increasing hydration in cementitious materials [37].

![Figure 14: Non-evaporable water content results.](image-url)
3.2-Compression Test

The compressive strength of the control cement paste and the cement paste with nutrients and cement paste with bacteria are shown in Figure 15. It is seen that the compressive strength of the cement paste with bacteria is higher than that of the control cement paste and the cement paste with nutrients at early age. The increase in the compressive strength of the cement paste with bacteria at early age could be due to improved hydration of this cement paste compared to the control cement paste and the cement paste with nutrients, as realized from Figure 14. It should be noted that biomineralization of CaCO₃ could contribute to the improvement of compressive strength in the cement paste with bacteria. However, as discussed later, the amount of CaCO₃ precipitation was found to be low in the cement pastes studied here and therefore, its contribution to compressive strength is not expected to be significant. At a later age (28 days) the cement paste with bacteria exhibited a lower compressive strength than the control cement paste. The reason for this behavior is not clear and more investigations are needed to explain such behavior.
3.3-Electrical Resistivity Using EIS

The electrical resistivity measurement was used in order to get an insight into the microstructure of the cement paste. It is known that the electrical resistivity depends on the pore structure and denser is the structure the higher is the electrical resistivity. The measurements using EIS of the cement pastes are shown in Fig. 16. It is noticed that the cement paste with bacteria and the cement paste with nutrients showed higher electrical resistivity compared to the control cement paste at all ages studied here. The electrical resistivity of these two cement pastes is seen to be similar with the cement paste with bacteria having slightly higher electrical resistivity compared to the cement paste with nutrient at 28 days. The improved electrical resistivity of the cement paste with bacteria and the cement paste with nutrient is attributed to microstructure improvement, potentially due to increased hydration, as observed in Figure 14. It is also possible that the addition of
nutrients and bacteria could regulate hydration resulting in a denser microstructure, thereby improving electrical resistivity of the cement pastes. The precipitation of CaCO₃ could also contribute to increased electrical resistivity; however, as discussed later, the amount of CaCO₃ was found to be very small so that this is not expected to play a role in increased electrical resistivity of the cement paste with bacteria.

3.4-TGA Analysis

Fig. 17 and Fig. 18 show the mass percentage of calcium hydroxide and calcium carbonate, respectively, at 3 days and 14 days, calculated via TGA. The mass percentage of calcium hydroxide was determined by mass loss between 400°C and 500°C while the mass loss due to the decomposition of calcium carbonate was determined between 600°C and 800 °C [38],[8].

![Graph showing electrical resistivity results](image)
It is seen that Ca(OH)$_2$ of the control cement paste is higher than that of the cement paste with nutrient and the cement paste with bacteria. On the other hand, CaCO$_3$ of the cement paste with bacteria is seen to be higher than the control cement paste and the cement paste with nutrients as seen from Figure 18. Since the degree of hydration of the cement paste with bacteria and the cement paste with nutrient was higher than that of the control cement paste, it is unlikely that the reduction in Ca(OH)$_2$ of the cement paste with bacteria is due to delayed hydration. It is likely that the reduction in Ca(OH)$_2$ of the cement paste with bacteria could be due to biomineralization of CaCO$_3$, consuming Ca$^{2+}$ thereby reducing Ca(OH)$_2$ in the cement paste with bacteria. It is noted that the amount of CaCO$_3$ in the cement paste with bacteria was lower than expected and reported in the literature. One reason for this could be low viability of the bacteria in the microstructure environment of the cement paste lowering the CaCO$_3$ biomineralization efficiency of the bacteria.

![Figure 17: Calcium hydroxide content.](image-url)
3.5-Microscopic Examination

The SEM images of the control cement paste and the cement paste with bacteria at 14 days Fig 19. Unhydrated cement particles, hydration products and capillary pores are marked in this figure. It is noted that the microstructure of the cement paste with bacteria appeared to be denser than that of the control cement paste. This could explain higher electrical resistivity of the cement paste with bacteria than the control cement paste as observed in Figure 19.

Figure 18: Calcium carbonate content.
Figure 19: SEM images of (a) the control paste and (b) the cement paste with bacteria at 14 days of age.
3.6-Calcium Carbonate Morphology

3.6.1-SEM Imaging

The precipitation of calcium carbonate can lead to the formation of three anhydrous polymorphs: calcite, aragonite and vaterite, two hydrated forms and an amorphous phase [39]. The following pictures examined under SEM show the different shapes of crystals obtained when different calcium salts, different polymeric additives and different precipitation methods were added. The particle size was analyzed through ImageJ software. When the precipitation occurred without any additives, the crystals formed with all the three salts have a rhombohedral shape that characterizes calcite. Even though the crystals formed were calcite, the size of the crystals was slightly different when different salts were used. The use of calcium chloride resulted in crystals having an average particle size of 4 µm followed by calcium acetate with a 3.2 µm particle size then calcium lactate as the smallest crystals with a crystal size of 2.5 µm.

On the other hand, when Poly-acrylic acid (PAA) polymer solution was added to the calcium salts and sodium carbonate, the shape of the crystals changed and became spherical. The spherical shape characterizes vaterite. However, the particle size didn’t change when different salts were used and so all the particles have an average size of 1.5 µm. According to Amjad et al. [40] PAA was found to stabilize a less thermodynamically stable phase of CaCO$_3$ and capable of affecting the nucleation and the crystal growth stage of calcium carbonate. The SEM images showed a spherical shape that is a characteristic of vaterite and so vaterite is known for being the thermodynamically least stable phase of calcium carbonate and the results obtained are in accordance with the results found by Amjad et al. [40]. When Polyvinyl alcohol polymer (PVA) solution was added to calcium
salts and sodium carbonate solution, the crystals were found to be rhombohedral characterizing the formation of calcite. The average particle size was found to be 4 µm when calcium chloride was used, 3 µm when calcium lactate was used and 2.08 µm in the presence of calcium acetate. Finally, when precipitation was conducted through the biomineralization process, lamellar shaped crystals were observed in the presence of the calcium chloride and calcium lactate, while spherical shaped crystals were formed in the presence of calcium acetate. Lamellar shape and spherical shape crystals characterize the vaterite polymorph. From literature it was found that bio-precipitation in the presence of calcium chloride salt allowed the formation of rhombohedral crystals while the use of calcium acetate allowed the formation of lamellar shape crystals characteristics of vaterite. Finally, the use of calcium lactate led to the formation of spherical shape crystals another form of vaterite [13]. The results of the bio-precipitation experiment led to the formation of lamellar shape crystals in the presence of calcium chloride and calcium lactate and spherical crystals in the presence of calcium acetate. These crystals are characteristics of vaterite. This change in morphology could be attributed to the nutrient media or the bacterial strain used. According to Gorospe et al., [13], in the presence of calcium chloride salt calcite crystals were formed but in the presence of calcium acetate and calcium lactate vaterite crystals are formed. The difference of the crystals morphology could be attributed to the strain of the bacteria that exhibits different urease activities or the nutrient media that can contain some organic and inorganic component that can inhibit or alter the growth and therefore allow the formation of different calcium carbonate polymorphs. The crystals shape when different calcium salts are used are represented in Figures 20, 21 and 22.
Figure 20: SEM images showing the effect of calcium chloride on the morphology of the calcium carbonate obtained (a)-(c) from chemical precipitation with and without additives, and (d) from biomineralization.
Figure 21: SEM images showing the effect of calcium lactate on the morphology of the calcium carbonate obtained (a)-(c) from chemical precipitation with and without additives, and (d) from biomineralization.
(a) No additives
(b) PAA addition
(c) PVA addition
(d) Bio-precipitation

Figure 22: SEM images showing the effect of calcium acetate on the morphology of the calcium carbonate obtained (a)-(c) from chemical precipitation with and without additives, and (d) from biomineralization.
3.6.2-FTIR

Band assignment for FTIR of CaCO$_3$ is well established and these bands correspond to CO$_3$ out of plane bending mode ($v_2$) and doubly degenerated in-plane OCO deformation bending mode ($v_4$) [41]. The spectral region has been chosen between 600 cm$^{-1}$ and 1000 cm$^{-1}$ for qualitative analysis in order to emphasize on the peaks within this region. The calcite and the vaterite polymorphs are known to have characteristics absorption band in the following region. Vaterite $v_2$ at 849 cm$^{-1}$ and 877 cm$^{-1}$ and $v_4$ at 744 cm$^{-1}$ while for calcite $v_2$ is at 877 cm$^{-1}$ and $v_4$ at 712 cm$^{-1}$ [41]. Calcium carbonate precipitation was conducted first in the presence of calcium chloride. The results of the FTIR are represented in Fig.23. Peaks at 712 cm$^{-1}$ and 877 cm$^{-1}$ were observed when the chemical precipitation without any additives and the precipitation when PVA was conducted. The peaks present at these locations confirm the formation of calcite crystals. On the other hand, when a solution of PAA was added to calcium chloride and sodium carbonate solution, vaterite was formed. Also, vaterite was formed when calcium carbonate was precipitated through biomineralization. The FTIR graph shows peaks at 744 cm$^{-1}$ and 877 cm$^{-1}$ that are characteristics of the vaterite polymorph. These results are in accordance with the SEM images that showed rhombohedral crystals for calcite, spherical and lamellar shapes for vaterite.
Calcium carbonate precipitation using calcium acetate was characterized using FTIR and the results are represented in Fig. 24. Calcite was formed when the precipitation occurred without any additives and when PVA was added. The peaks in the FTIR graph were found at 712 cm\(^{-1}\) and 877 cm\(^{-1}\). These peaks correspond to calcite. On the other hand, the addition of PAA to the solution and when bio-precipitation was conducted, vaterite crystals was formed. This was confirmed by the FTIR characterization where peaks were found at 744 cm\(^{-1}\) and 877 cm\(^{-1}\), these peaks are the characteristics of vaterite crystals. These results are in accordance with the SEM images.

![FTIR spectra of calcium carbonate with calcium chloride](image)

Figure 23: FTIR spectra of calcium carbonate with calcium chloride.
Finally, the third precipitation was conducted using calcium lactate as the calcium source. The characterization was also done using FTIR. The peaks are shown in Fig. 25. Calcite was formed when the precipitation occurred without any additives and when PVA was added. The peaks in the FTIR graph were found at 712 cm$^{-1}$ and 877 cm$^{-1}$. The presence of these peaks confirms the formation of calcite crystals. The bio-precipitation allowed the formation of vaterite crystals. This was confirmed by the FTIR characterization where peaks were found at 744 cm$^{-1}$ and 877 cm$^{-1}$. However with the addition of a solution of PAA and in the presence of calcium lactate three peaks were observed on the graph: 712 cm$^{-1}$, 744 cm$^{-1}$ and 877 cm$^{-1}$, this indicates that a mixture of calcite and vaterite crystals.
were formed but the peak for vaterite was higher indicating that more vaterite was formed.

The characterization is in accordance with the SEM pictures.

Figure 25: FTIR spectra of calcium carbonate with calcium lactate.
### 3.7-Hydrogel Experiments

#### 3.7.1-Hydrogel Swelling

Hydrogels are hydrophilic polymers and ion exchangers that are able to absorb solvents in which they are submerged [42]. The absorption of the solvent causes the hydrogel to swell until equilibrium is reached. The hydrogel prepared are poly (acrylamide-co-acrylic acid hydrogels) and are pH sensitive [35]. pH sensitive hydrogels are known to either accept or release protons in response to appropriate pH and ionic strength changes in aqueous solution, therefore leading to a change in the network porosity due to the electrostatic repulsion [35].

The absorption of hydrogels in distilled water and synthetic pore solution with a pH value of 13.5 are shown in Figs 26 and 27, respectively. It is noticed that the absorption of hydrogels in distilled water is significantly higher than that in the synthetic pore solution. The reduction in hydrogel absorption in ionic solutions is due to the screening effect of...
counterions reducing the electrostatic repulsion between anionic polymeric networks in a hydrogels. It is seen that in distilled water hydrogels with encapsulation of bacteria or nutrients all showed a higher absorption compared to the control hydrogel. On the other hand, in synthetic pore solution, a reverse behavior is evident, where the control hydrogel exhibited the highest absorption among the hydrogels. The absorption of hydrogels is driven by the difference in the chemical potential of water inside and outside of hydrogels and is affected by the molecular structure of hydrogels, encapsulated materials, water content, and pH and ionic strength of solution. It is noted that the hydrogel with a high concentration of bacteria \((5\times10^6)\) showed the highest absorption compared to other hydrogels in distilled water. To provide an accurate explanation for the observed behavior of hydrogel absorption requires studying the effect of encapsulation of bacteria and nutrients on the molecular bonds in the hydrogel and this will be pursued in the future.

**Swelling of Hydrogel in Pore Solution**

![Swelling of Hydrogel in Pore Solution](image)

Figure 26: Swelling of hydrogels in synthetic pore solution.
work. The main purpose of the absorption test performed here was to elucidate the overall influence of bacteria encapsulation on the absorption behavior of hydrogels.

**3.7.2-Desorption of Hydrogel**

The results of desorption of the hydrogels are shown in Figure 28. The control hydrogel showed the least amount of desorption and desorption rate compared to the hydrogels with encapsulated bacteria and nutrients. It is seen that all hydrogels with bacteria and nutrients reached a similar final desorption although the initial desorption rate varied to some extent among these hydrogels. The primary driving force for desorption of hydrogels in contact with a cementitious material is the capillary effect exerted from the porous cementitious material on the hydrogels. Since hydrogels were in contact with a cementitious material with the same microstructure and relative humidity, the difference in the desorption of the hydrogels is attributed to a change in the diffusion rate of water within hydrogels as a result of encapsulating bacteria and nutrients. As mentioned previously, chemical analysis studies such as FTIR are needed to provide insight into the molecular structure of the hydrogels, which can be used to rationalize the observed desorption behavior of hydrogels in contact with cementitious materials. This will be further pursued in future work.
Deswelling of Hydrogel in Contact with Cement Paste

- Control
- Nutrients
- Bacteria 10e6 Cell/mL
- Bacteria 5X10e6 cell/mL
- Bacteria 10e6 cell/mL + Nutrients

Figure 27: Deswelling of hydrogels in contact with cement paste.
Chapter 4

Future Outlooks

The self-healing capability of microorganism induced mineralization of CaCO$_3$ in cementitious materials depends on the crack filling and crack binding ability of biomineralized CaCO$_3$ in a cementitious matrix. Crack binding is governed by the mechanical and chemical/biochemical interfacial binding strength between biomineralized CaCO$_3$ and cementitious materials. Currently, these fundamental interfacial mechanisms are not known and future work is required to elucidate these mechanisms in order to aid in the development of design guidelines for the self-healing cementitious infrastructure materials. In addition, further studies are needed to discover the chemical and mechanical interactions between hydrogels containing microorganisms and nutrients, and a cementitious material. Such studies permit understanding the factors determining the release kinetics as well as the physical and biochemical characteristics of resulting CaCO$_3$. 
Chapter 5

Conclusion

In conclusion:

- The addition of bacteria and nutrients increased the hydration of cement pastes compared to the control cement paste.

- Cement paste with bacteria and nutrients possessed a higher electrical resistivity than the control cement paste.

- For the microorganism, nutrients and concentrations used in this study, the amount of calcium carbonate precipitation quantified from TGA was lower than expected. Work is underway to obtain optimum concentrations of the bacteria and nutrients to increase biomineralization in cementitious materials.

- The calcium salt type and polymeric additives were shown to affect the morphology and chemical structure of chemically induced calcium carbonate crystals.

- It was found that for the microorganism and nutrients used in this study, vaterite was the primary phase of calcium carbonate.

- The incorporation of microorganism and nutrients into the hydrogel affected their swelling and de-swelling properties.
References


