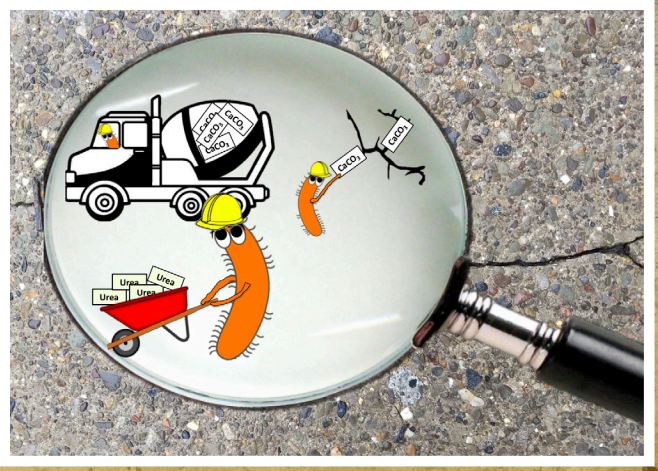
SUMMARY REPORT

Towards using Microbes for Sustainable Construction Materials: A Feasibility Study



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Impactful Resilient Infrastructure Science and Engineering

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7. Abstract: Reinforced Concrete (RC) structures are susceptible to damage from long-term exposure to chloride-based compounds (e.g., from marine environments or deicers) and/or repeated freeze/thaw cycles. To mitigate damage and degradation from environmental loading, an estimated $16 billion per year is spent on the rehabilitation of RC structures using cementitious patching materials and/or chemical treatments, which contribute to pollution and require repeated application and maintenance. To mitigate these detrimental environmental impacts, this study will evaluate the feasibility of increasing the durability, resiliency and sustainability of RC structures by using microbes to provide self-healing properties to prevent water and chloride ingress through structural and/or environmental cracking. State-of-the-art research has begun to explore microbial carbonate precipitation (MICP) for limestone, marble and, to a lesser extent, RC restoration. However, many challenges remain including: 1) finding non-pathogenic microbes capable of MICP; 2) developing methods to ensure microbial viability and even distribution throughout the material to be restored and; 3) creating and evaluating new RC formulations aimed at improving and or sustaining MICP. This research will focus on addressing these challenges, providing insight into the potential application of bio-restoration of RC, which will have far reaching applications for green building design and resilient and sustainable construction.	
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Towards using Microbes for Sustainable Construction Materials: A Feasibility Study

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The Impactful Resilient Infrastructure Science & Engineering consortium was established in the Department of Civil and Environmental Engineering in the Swanson School of Engineering at the University of Pittsburgh to address the challenges associated with aging transportation infrastructure. IRISE is addressing these challenges with a comprehensive approach that includes knowledge gathering, decision making, material durability and structural repair. It features a collaborative effort among the public agencies that own and operate the infrastructure, the private companies that design and build it and the academic community to develop creative solutions that can be implemented to meet the needs of its members. To learn more, visit: https://www.engineering.pitt.edu/irise/.

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Nomenclature

RC	Reinforced Concrete
MICP	Microbially Induced Carbonate Precipitation
XRD	X-ray diffraction spectrophotometer

1. Introduction

Background and Motivation

Figure 1: Photographs illustrating examples of cracked concrete.

Reinforced concrete (RC) is the most widely used construction material in the world [1]. Despite the strength, durability and economy of RC, it is susceptible to damage including corrosion, spalling and crack formation from long-term environmental exposure to chloride-based compounds (e.g., from marine environments or deicing treatment) and repeated freeze/thaw cycles as shown in Figure 1. This degradation causes microcracks, which result in the loss of structural integrity by diminishing RC's compressive strength and allowing water and other chemicals into the material [2]. Due to these issues, the lifespan of RC structures is dramatically reduced and is estimated to cost the US economy $21 billion dollars annually in repairs and improvements[2–4], with conventional concrete repair techniques (e.g., cementious materials, epoxies, and resins) costing up to $200 per cubic foot of RC[4]. Although there are many conventional cementitious patching techniques and chemical treatment options to repair cracks in RC structures, these methods contribute to environmental pollution (e.g., contributing to atmospheric emissions and hazardous runoff[5]), require repeated maintenance due to continued degradation and perform differently based on thermal conditions[6,7].

In nature, a variety of microbes utilize an array of different metabolic processes to produce carbonate precipitates (Table 1) which can form a cement-like material (typically calcium carbonate - $CaCO_3$[8]). Although a wide range of organisms can produce $CaCO_3$, the most widely studied mechanism is through the hydrolysis of urea by the enzyme, urease – a process used by ureolytic microbes[9]. In recent years, the control and use of microbes capable of microbially induced carbonate precipitation (MICP) has been explored for several rehabilitation applications including limestone, marble and, to a lesser extent, RC restoration[6,10,11]. However, despite previous initial success in identifying microbes capable of MICP, challenges have arisen with respect to their application in the human built environment: (1) identifying non-pathogenic microbes capable of MICP and (2) developing methods to ensure microbial viability and even distribution throughout the material to be restored. Furthermore, and perhaps most surprisingly, the mechanical properties of materials treated with MICP microbes and the impact of chemical / nutrients

formulation required for the development and maintenance of MICP microbes have not been characterized. The latter is of particular concern given that the leaching potential of biproduct produced from ureolysis, such as ammonium, which can cause health and environmental issues when present at high levels, has been largely ignored.

Problem Statement

Due to the short-term effectiveness, cost, and environmental hazards associated with conventional RC remediation, there is need for an innovative solution that reduces both the environmental and financial burden of the United States' aging structures. As an alternative to current repair techniques, microbially induced carbonate precipitation (MICP) has been studied for its potential as a biologically active binding agent. When a microbe is capable of MICP, it produces calcium carbonate from environmental calcium and bioavailable carbon, which can hypothetically act as an active mortar ingredient when applied to concrete cracks [8]. There are a wide range of microorganisms capable of MICP, and a variety of studies have begun to explore their application to repair structures (mainly ornamental stone) instead of the conventional materials. Although these studies have shown some success there are several deficiencies and limitations which must be overcome before these procedures could be applied to RC. For example MICP application to restore ornamental stone such as historic limestone structures has been popular due to conventional treatments exacerbating degradation and altering the aesthetic qualities of the stone [8,12]. However, RC biomortars have either been limited to surface-level calcium carbonate deposition applied over a traditional mortar, or using pure cultures of known MICP microorganisms as the biological binder [8,13–15]. These approaches result in the biomortar dying reasonably quickly either after the limited resources present in the mortar are exhausted, due to competition with the native RC microbiota or due to niche incompatibility (environment within the structure to be restored cannot sustain life), all of which raise the cost of using biomortar as a remediation option.

Herein we propose a study that will evaluate the feasibility of adding ureolytic microbes capable of MICP into RC in order to provide self-healing properties to prevent water and chloride ingress through structural and/or environmental cracking (Figure 2). This **bio-inspired regeneration of RC** provides a first-step towards developing a new RC design objective paradigm with an intentional **circular economy** focus. By increasing the service life whilst mitigating the detrimental environmental impacts associated with traditional

rehabilitation techniques this new approach will preserve the economic and environmental value of RC materials for as long as possible [16]. Since the long-term goal of this approach is very broad, we propose an initial study focusing on the rehabilitation of pre-cracked RC. More specifically, in order to determine the feasibility of the self-healing approach, we will isolate non-pathogenic microbes capable of MICP from existing RC, explore the development of new concrete formulations to promote MICP and develop methodology to deploy MICP capable microbes into existing cracks in RC.

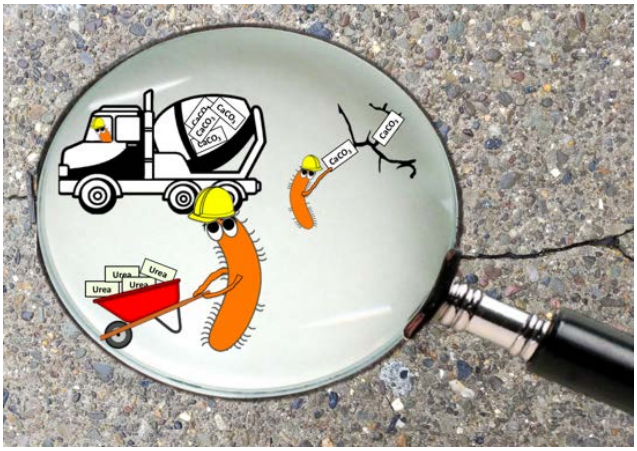

Figure 2: Graphical representation of MCP being used to seal cracks in concrete.

Research Plan

The overall objective of this project is to demonstrate the feasibility of using microbes to provide self-healing properties to prevent water and chloride ingress through structural and/or environmental cracking in RC structures. To meet this objective, the project team conducted four primary tasks:

(A) conduct a literature review to identify microbes which can be used for MICP in RC;
(B) isolate microbes that can be used for MICP that exist in RC in-situ;
(C) experimentally evaluate the mechanical properties of concrete mixture design which incorporate nutrients for MICP and;
(D) evaluate the self-healing and leaching properties of pre-cracked bench-scale concrete specimens treated externally with microbes identified in Tasks A and/or B.

2. Task A: Literature Review

RC is the most widely used construction material in the world due its small fabrication cost, high strength and stability, and ease of production[2]. Although these structures are fairly resilient to temperature changes and weathering conditions, they often degrade over time as a result of long-term weathering, chemical corrosion, and structural overloading[2]. This degradation causes microcracks, which result in loss of structural integrity by diminishing its compressive strength and allowing water and other chemicals into the

material, thus exacerbating the problem.[2] Microcracks can be attributed to significant infrastructure expense through intensive repair strategies, costing the United States up to $21 billion dollars annually[2,3, 4]. Conventional concrete repair techniques can cost up to $200 per meter of RC and commonly consist of cementious materials, epoxies, and resins[3]. While these materials may remediate the cracks temporarily, they use hazardous compounds that increase the material's overall environmental impact by contributing to atmospheric emissions and contaminating runoff[5].

2.1 RC as a widespread building material

In general terms, RC is traditional concrete that comprises of coarse and fine aggregates in cement that contains an embedded material that improves its compressive and tensile strength[17,18]. Steel is a common reinforcement material and is implemented as either rebar or as a mesh throughout the specimen[18]. In addition to cement, aggregate, and steel, additional chemicals called admixtures may be added to change some of the material characteristics of RC such as curing time or internal pore structure[19]. To produce RC, the desired constituents are combined in the presence of water resulting in a complex hydration reaction occurring in which the material is typically left to harden for up to 28 days[20]. A square foot of standard RC can cost up to $5.80, which is a desirable price for its implementation in construction projects[21]. However, this nominal immediate cost can significantly increase when cost assessments (e.g., repairs) over the materials whole lifetime are taken into consideration. In addition to economic constrains, cement and steel manufacturing are among the most environmentally harmful processes in the construction sector; accounting for 37.2% of manufacturing primary energy demand and nearly 50% of carbon dioxide emissions[22]. Overall, RC structures typically start to crack in as little as 10 years after curing, necessitating the expensive and toxic remediation efforts that increase the overall economic and environmental burdens of using RC[4,5,23].

2.2 Microbially induced carbonate precipitation

As an alternative to current repair techniques, microbially induced carbonate precipitation (MICP) has been studied for its potential as a biologically active binding agent. When a microbe is capable of MICP, it produces calcium carbonate from environmental calcium and bioavailable carbon, which can act as a mortar when applied to structural cracks by filling the available space with calcium carbonate crystals. This application of calcium carbonate crystals has been shown to slightly increase the strength of the once compromised material but typically provides structural integrity by preventing further

water ingress and hence slowing further crack formation. The MICP phenomenon occurs through a variety of metabolic pathways, including ureolysis, photosynthesis, sulfate reduction, nitrate reduction, and ammonification (Table 1)[24–26], however each pathway has varying pros and cons and hence suitability towards application for RC rehabilitation.
Across all of the metabolic pathways (Table 1), the degree of carbonate production is governed by environmental calcium concentrations, concentration of dissolved inorganic carbon, and pH.[25] In addition to calcium carbonate being a metabolic product of MICP, these microorganisms can also use their cell walls as nucleation sites for carbonate production to increase the rate.[27]

When determining the optimal metabolic pathway for microbes used in RC bioremediation, a variety of factors must be considered including the ability of the microbe to survive under anaerobic and basic conditions whilst growing quickly and producing enough calcium carbonate to make it a more sustainable and safe option than conventional techniques. The photosynthesis MICP pathway requires small environmental inputs and creates harmless metabolites, but is not a feasible pathway for concrete bioremediation due to its low rate of calcium carbonate precipitation within the cracks where sunlight cannot penetrate.[28] Ammonification and denitrification produce calcium carbonate at a rapid rate, but are not environmentally sustainable pathways because of the formation of basic nitrogenous byproducts, which can affect the environment in quantities as little as 1 ppm.[29–31] In addition, the microbes that precipitate calcium carbonate via denitrification that would be applicable for “healing” cracks in RC grow much slower than other potential MICP organisms, and ammonifying organisms cannot grow in the anaerobic conditions of the RC cracks.[6] Conversely from the ammonification and denitrification pathways, sulfate reduction and methane oxidation pathways precipitate calcium carbonate rapidly, but produce acidic sulfuric byproducts and can be pathogenic.[29,32]

Table 1: Comparison of MICP metabolic pathways. [3,8,27–30]

Metabolic Pathway	Simplified Reaction	Byproducts	Advantages	Disadvantages	Feasibility of RC Application
Photosynthesis	$HCO^-_3 + Ca^{2+} \rightarrow CaCO_3$	H_2O & O_2	No harmful byproducts	Light must penetrate deeply	Cannot survive in dark cracks
Ureolysis	$CO(NH_2)_2 + H_2O + Ca^{2+} \rightarrow CaCO_3$	NH_4^+	Rapid rate of $CaCO_3$ precipitation. Many non-pathogenic bacteria Aerobic and anaerobic	Byproducts can be toxic	Meets all parameters
Ammonification	Amino acids + O_2 + $Ca^{2+} \rightarrow CaCO_3$	NH_3	Many non-pathogenic bacteria	Byproducts can be toxic	Cannot survive in anaerobic RC matrix
Denitrification	Multiphase reaction, Final reaction: $CO_2 + OH^- + Ca^{2+} \rightarrow CaCO_3$	CO_2 & N_2	Facultative anaerobes	Byproducts can be toxic Slow growing organisms	Production is too slow to be feasible
Sulfate Reduction	$SO_4^{2-} + CH_2O + Ca^{2+} \rightarrow CaCO_3$	CO_2+HS^-	Can create Ca^{2+} by degrading parts of other organisms	Byproducts can be toxic Decrease pH	pH decrease is not conducive to RC HS^- is corrosive and odorous
Methane Oxidation	Anaerobic: $CH_4 + SO_4^{2-} + Ca^{2+} \rightarrow CaCO_3$ Aerobic $CH_4 + 2O_2 + Ca^2 \rightarrow CaCO_3$	H_2S	Aerobic and anaerobic	Byproducts can be toxic Decrease pH	H_2S is poisonous, flammable, and corrosive

2.3 Ureolysis as the optimal MICP pathway

In the context of RC remediation, microbial calcium carbonate synthesized via ureolysis is vastly favored in previous work due to the wide variety of microbes that undergo ureolysis, availability of necessary substrate, and rapid calcium carbonate precipitation[6,33]. Ureolytic organisms do not require oxygen in order to produce calcium carbonate, and therefore can function in aerobic and anaerobic conditions. Concrete provides a highly basic environment for microorganisms, which many ureolytic organisms are able to survive in[6,8]. Nonpathogenic bacteria such as *Sporosarcina pasteurii*, *Pseudomonas calcis*, and *Pseudomonas denitrificans are capable of MICP and* have been found in natural and built environments, which makes their application to RC favorable[6,8]. Most importantly, ureolytic microorganisms precipitate calcium carbonate readily under the conditions found in RC, which makes them ideal candidates for remediation use[6,33].

In order to precipitate calcium carbonate, ureolytic organisms contain a higher concentration of the enzyme urease that catalyzes the MICP reaction[8,34]. Urease has a strong affinity for calcium ions, so in a calcium-saturated environment, urease can cleave urea and form a bond with the resulting carbonate and calcium (Equations 1-6)[8,15,35]. The resulting ammonium from the reaction then increases the pH directly around the microbe, which enhances the conversion of carbon dioxide to carbonate ions in the vicinity and catalyzes the process further.

$$CO(NH_2)^2 + H_2O \rightarrow H_2COOH + NH_3 \quad (1)$$

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (2)$$

$$2NH_3 + 2H_2O \rightleftarrows 2NH_4^+ + 2OH^- \quad (3)$$

$$2OH^- + H_2CO_3 \rightleftarrows CO_3^{2-}\, 2H_2O \quad (4)$$

$$Ca^{2+} + cell \rightarrow cell - Ca^{2+} \quad (5)$$

$$cell - Ca^{2+} + CO_3^{2-} \rightarrow cell - CaCO_3 \quad (6)$$

Equations 1-6: Urease-catalyzed hydrolysis of urea to form calcium carbonate.

Ureolytic microbes need both urea and calcium sources in order to undergo the reaction describes in equations 1-6. Both of these nutrients must be applied along with the microbe as neither of these compounds pre-exist in concrete[36]. However, as urea is a well-defined substrate needed by these organisms, there are a variety of potential calcium sources that

they can utilize. Among these are the simple salt calcium chloride and more complex compounds such as calcium acetate, calcium nitrate, and calcium formate. Either type can be used as a calcium source in the MICP pathway, but there are advantages and disadvantages to each type. When using calcium chloride, the compound can readily dissociate and thus speeds up the overall reaction time during ureolysis, but the resulting chloride ion from the dissociation can cause further chemical damage of the cracked concrete[8]. The other complex compounds that can be used as a calcium source are more difficult for microbes to use, but don't cause the release of the corrosive chloride ion[8]. These variations in starting materials also impact the precipitated structure: calcium chlorite was found to make crystals with rhombohedral geometry, whereas calcium acetate made spherilitic crystals[37].

Another harmful compound involved in ureolysis that may cause more damage to cracked concrete is ammonium. For every mole of calcium carbonate that is created by urease, 2 moles of ammonium are released which contributes to the basic environment. In order to microbially remediate 1 m^2 of cracked concrete, the 10 g/L of urea necessary to facilitate MICP yields 4.7 g/L of nitrogen-containing compounds[8,37]. In context, this output is one-third of that of the daily nitrogen load of one person, so the large amounts of additional nitrogen output is a major concern for application of ureolytic MICP, hence the potential of ammonium leachate from rehabilitated RC must be assessed before application. Ammonium in high concentrations can volatilize into nitrogen oxide, which is a potent greenhouse gas and contributes to ozone depletion[37,38]. This metabolite could also be detrimental to the structural integrity of the concrete due to secondary reactions within the concrete matrix (e.g., formation of nitrogenous salts[8], or nitric acid by nitrifying bacteria[39]). While these nitrogenous salts and acids have the potential to impact the surrounded concrete, there is no data currently available on how these metabolites leach from rehabilitated RC or impact the strength of RC.

2.4 Ureolysis substrate considerations

Urea and calcium chloride are already used within the concrete industry as admixtures to alter some of the properties of RC during its fabrication. Calcium chloride is added to concrete mix as an accelerator to shorten setting times, and has been proven to improve short term strength in RC[40]. These benefits are only observed at concentrations lower than 2% due to ion corrosion of the internal rebar, so increasing the concentrations for the microbial feed source may not be feasible. Urea, on the other hand, can be added to

concrete mixes to lower the hydration and casting temperatures, and has shown to have no effect on the concrete's performance even at saturation conditions[41]. No studies have been conducted to the author's knowledge addressing the synergistic effects of calcium chloride and urea, so it is unclear as to the potential for the two chemicals to be added as a microbial feed stock without compromising the structural integrity of RC.

2.5 Current MICP applications

In order to apply microbes capable of MICP into RC to potentially seal/heal cracks there are numerous different methodologies which can be used (Table 2). Broadly speaking these techniques can be separated into two main categories: biodeposition and biocementation. Biodeposition describes MICP that forms a surface-level barrier of calcite that protects the structure underneath, whereas MICP classified as biocementation uses the precipitated calcite within the structure's matrix to increase adhesion of the internal components (Figure 2)[8,35].

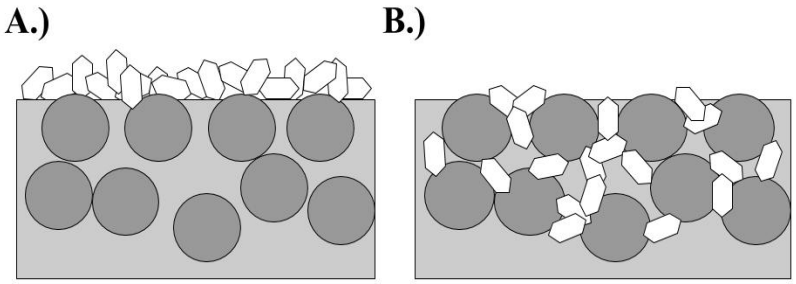

Figure 2: General categories of MICP application to concrete. Biodeposition **(A.)** results in a layer of calcium carbonate on the surface of the porous cement matrix, whereas biocementation **(B.)** adheres the cement matrix components together with calcium carbonate.

In terms of application biodeposition can be achieved relatively simply by using strategies such as spraying liquid bacterial culture or immersing the matrix in liquid bacterial culture, however has the disadvantage of only treating the material's exterior[8,42,43]. In contrast, biocementation can treat more than superficial cracks and can potentially increase the strength of RC, however, it is more difficult to implement due to difficulties in evenly mixing the microorganisms within the cementious slurry or maintaining a suitable environment for them to precipitate calcium carbonate[8,14,44].

In addition to categorizing these application methods as a surface or matrix treatment, the origin of the MICP organism being used can also be described as biostimulation or bioaugmentation[26,45]. Biostimulation techniques provide an environment that is conducive for calcium carbonate precipitation for the microbiota already present in the concrete. This technique does not introduce new bacteria to the structure, so the time and cost constraints of microbial culturing are not a factor[8]. However, the success of biostimulation relies on

the abundance of bacteria capable of MICP already being present in RC. Given the lack of knowledge pertaining to what if any viable microbes naturally reside within RC, bioaugmentation has been the approach typically investigated. Bioaugmentation is a techniques in which microorganisms with a desirable trait (e.g., MICP properties) are added to the matrix[26,45]. These microorganisms require culturing prior to application and need to be supplied with appropriate resources to allow them to grow in their new environment.

2.5.1 MICP-mediated biodeposition as a surface level sealant

Because of the purely superficial treatment biodeposition provides, it is more feasible for application for limestones than cementious materials such as RC. These remediation targets (e.g., ornamental stone) require a protective layer from erosion to be developed and not necessarily a proactive treatment for cracks as would be needed for RC. Furthermore, limestone and other stones used to construct statues and historic buildings are compatible with calcium carbonate, so initially MICP application was focused on their protection (Table 2). One of the first research groups to explore this concept led to a patented system of biologically active biodeposition and biocementation products to repair superficial cracking and seal these types of structures, called the Calcite Bioconcept[8]. These systems are implemented by spraying or brushing a liquid culture of MICP capable organisms onto the surface of the ornamental stone for a number of days until the calcin layer is established. The results of this surface treatment do not change the aesthetics of the stone and can be effective for years depending on the type of environment[8]. For the superficial biomortar produced by the Calcite Bioconcept, liquid culture with MICP capable organisms are mixed with a binding agent, then applied to small cracks in limestone objects, the resulting seal decreases water permeability and also aids in aesthetics. This type of in-situ remediation is typically used for projects with historic or sentimental value due to its low visual impact, lighter environmental footprint, and ease of application. Although traditional practices are much more cost effective, this is to the detriment of changing the visual attributes of these structures, as well as the high level of maintenance in re applications this method needs[3,8].

Table 2: Application methods of MICP-mediated remediation. Adapted from De Muynck et. al., 2010).

Biodeposition						
Method	**Advantages**	**Disadvantages**	**Ref.**	**Matrix Type**	**Microbe**	**Metabolic Pathway**
Spraying	• Easy application • Site-specific	• Superficial treatment • Requires frequent reapplications • Patented	46	Limestone	*B. cereus*	Ammonification
			47	Limestone	*Micrococcus Sp.* *Bacillus subtilis*	Ammonification Ureolysis
Brushing	• Applicable for pre-existing structures		47	Limestone	*Micrococcus Sp.* *Bacillus subtilis*	Ammonification Ureolysis
Immersing in liquid culture	• Even coverage • Conducive growth Conditions • Potential pore infiltration	• Not applicable for pre-existing structures • Requires large culture	3	Limestone	*Myxococcus xanthus*	Ammonification Ureolysis
			43	Limestone	*B. sphaericus*	Ureolysis
			47	Limestone	*Micrococcus Sp.* *Bacillus subtilis*	Ammonification Ureolysis
			42	Concrete	*B. sphaericus*	Ureolysis
			12	Limestone	Biostimulated native microbiota	n/a
Biocementation						
Biomortar	•RC compatible •Effective in-situ	• Requires application to cracks • Patented technology	46	Binder	*B. cereus*	Ammonification
Bacterial concrete	• Potential to increase RC strength • Changes RC microstructure	• No crack remediation	14	Concrete mix	*S. pasteurii*	Ureolysis
					Shewanella	Varied
Self-healing concrete	• Spontaneously heals cracks	• Difficult to keep bacteria alive • Difficulty distributing bacteria evenly	6		*S. pasteurii*	Ureolysis
			43		*B. sphaericus*	Ureolysis
			49		*B. pseudofirmus*	Denitrification

For smaller objects or projects not yet fully assembled into their final structures, biodeposition can also be utilized on a smaller scale by completely immersing the item of interest into liquid culture of MICP capable organisms[3,8,43]. This method insures even application of the calcite deposition in ideal environmental conditions, and may even promote the infiltration of microorganisms into the surface level pores to improve water

resistance. This method is however impractical due to the size limitations of the item being treated: the material must be fully submerged in order for the even application of calcite, so a large volume of the MICP culture must be grown and maintained. Further, the material must remain submerged until the calcin layer forms, which can be time-intensive. Most importantly for the application of in-situ remediation, it is impossible to submerge a pre-existing structure in order to achieve biodeposition[3,8,43].

2.5.2 MICP-mediated biocementation as a promising RC technique

While biodeposition is a beneficial process for the preservation of ornamental stones, it has very little relevance in remediating cracks in RC. Concrete has a higher resistance to environmental weathering, but is more prone to structurally significant cracks, which cannot be prevented with a biodeposited layer. In this case, biocementation treatments for RC focuses on expanding their serviceable lifetime by either improving their overall strength or mitigating the potential for the formation or increase of cracks, all while attempting to minimize the further environmental impact of RC (Table 2).

The most similar to traditional concrete remediation techniques for pre-existing structures, biomortar is a microorganism-enriched mortar that seals cracks like a traditional mortar but has the additional benefit of MICP crystal formation on the first few micrometers of the sealed crack. These crystals provide a stronger seal between the mortar and concrete by increasing the compatibility of the two materials and decreasing water permeability, but also function like a traditional remediation technique where the crack must first be identified and then treated directly[8]. A more efficient approach is necessary due to the difficulty of identifying remediation sites on a structure and the manpower required to monitor and retreat as needed.

Adding MICP capable microbes into the concrete prior to casting (i.e., as admixtures) is a way to circumvent the monitoring and remediation costs associated with traditional RC restoration. While these technologies are not applicable to pre-existing structures, they could be instrumental in increasing the lifetime of RC materials in the future and are thus paramount to study. Currently there are two main MICP admixture approaches which can be used: bacterial concrete and self-healing concrete. Bacterial concrete is RC that contains a small percentage of microorganisms within the mix that can change the RC's internal pore structure through MICP. The altered pore structure then provides the cured concrete

with more compressive and tensile strength, which makes it more resistant to cracking due to structural overloading as a result[14,42]. While this type of RC may withstand one type of stressor responsible for microcracks, it will require the same treatment that traditional RC does if cracks occur, even though infrequent.

The most promising novel MICP-mediated RC material is self-healing concrete, it should however be stressed that this is still a hypothetical system with early results coming from carefully controlled laboratory experiments. While similar in fabrication to bacterial concrete, self-healing concrete has the potential to heal cracks as they form, which would eliminate the need for screening or external maintenance[8,44,50]. In a self-healing RC system, a crack in the surface would allow water seepage into the internal RC matrix, but instead of allowing further ingress and potential rebar corrosion, the water would activate dormant MICP microorganisms, which would then begin producing calcium carbonate to heal the crack in its early stages[44,50]. For both bacterial concrete and self-healing concrete, a major challenge is to achieve an even distribution of the biological agent within the mix, or else the MICP properties will be unevenly applied through the material. Even more broadly, the MICP microorganisms chosen for this task must be able to withstand the hostile environment of curing, so it is likely that only spore-forming bacteria will be a feasible option[44]. These spore-forming bacteria often undergo ureolysis as their MICP metabolic pathway, so they live optimally in an anaerobic and alkophilic environment such as that found in RC, but urea and calcium need to be supplied to allow calcium carbonate production. For bacterial concrete, these nutrients can be added externally during curing[14]. Conversely, for self-healing concrete, the nutrients cannot be applied externally, therefore these compounds must be added to the mix so that the microorganisms embedded in the RC have access to the resources they need to survive and produce calcium carbonate. To date the exploration of different RC mix formulations optimized for sustaining MICP have not been explored – both from a microbial viability and RC mechanical perspective.

2.6 Considerations for MICP application in-situ

While there are a variety of MICP techniques with different uses, a suite of factors need to be considered to fully determine the feasibility of wide-scale application to RC. Some significant factors include effectiveness and reproducibility, cost, remediation lifetime, and environmental impact compared to conventional treatments. While implementation usually depends heavily on effectiveness and cost, some applications such as the remediation of historic limestone buildings mentioned previously are more concerned with other aspects

of the treatment such as material compatibility and treatment detection in the final product, which outweighs the slightly higher cost these materials have over traditional limestone treatments[3].

2.7. Efficacy of MICP technologies in RC remediation

The most important aspect of MICP based technologies is their ability to remediate RC. In all the studies to date, calcium carbonate was produced by these added organisms and had an effect on the targeted RC property, either as a surface layer to prevent water infiltration or an admixture to improve the RC. While promising, the results from these studies were attained using small bench scale experiments which don't comply with current ASTM standards, therefore it is unclear if these laboratory studies will retain their efficacy on a larger scale. As mentioned previously, many of these proposed techniques such as complete immersion into liquid culture are not applicable to large scale projects, or the application of sprays or mortars may not be achievable due to the remediation site on a structure. For bacterial concrete and self-healing concrete, there have been no field tests in scaled structures, and the results of these studies suggest that the larger scale will exacerbate many of the challenges found during laboratory experiments such as even mixing. Along with being effective at a meaningful scale these systems must also consistently undergo MICP under varying environmental conditions and be able to achieve the level of precipitation necessary for the treatment to be worthwhile. In laboratory experiments, many groups report consistent results, but these are not done in the environmental conditions necessary to provide information on the reproducibility in context.

2.8 Environmental impact of MICP technologies

One of the main goals for implementing MICP technologies into RC is to minimize the material's environmental impact. As mentioned, RC production can account for up to 10% of all carbon dioxide emissions and requires a large amount of energy[22,51]. In addition to these significant upfront amounts, the short functional lifetime of RC structures requires using a variety of environmentally harmful compounds to extend the structure's lifespan. These products are typically chemical resins or epoxies that emit volatile contaminants as well as produce runoff from the structure which contain these harmful compounds. While biodeposition and biocementation products may not decrease the production impact of RC, they can minimize additional environmental damage during the lifespan of the structure. For biodeposition techniques, the small layer of calcium carbonate created either over the

surface or superficially over a crack work similar to their chemical counterparts by reducing water and compound permeability, but do not contaminate the surrounding atmosphere and water table[8]. In the case of biocementation, the water and chemical resistance can be observed in the biomortar treatments, but the bacterial concrete does not require potentially harmful admixtures to create similar strength additions, and self-healing concrete negates the need for additional structural maintenance completely. In terms of structural upkeep alone, these biological treatments are superior to conventional systems.

When considering the implementation of bacterial and/or self-healing concrete in particular, there are additional factors to keep in mind. For example, to induce MICP either through naturally stimulating MICP organisms within RC (biostimulation) or adding organisms capable of MICP into RC (bioaugmentated), the supply of the appropriate nutrients needed to precipitate calcium carbonate must be considered. If bioaugmentation is utilized, the procedure radically changes the native microbiota of the RC, which could have unknown impacts of the physical properties of the RC as the significance of the native microbial population in RC has not been studied. In the case of biostimulation, the same problems mentioned earlier arise in the form of potential failure due to the endogenous microbial community not capable of MICP, slow precipitation when MICP organisms are present, and the need for maintenance.

Even when this protocol is feasible, the precipitate can only form at the concrete's surface due to lack of transport throughout the material. Therefore, adding MICP nutrients directly into RC mixes would maximize the potential advantages for using MICP. Besides the difficulty of evenly mixing the biologically active constituents throughout the concrete, the components themselves can carry significant environmental impacts. For example, the nitrogen source of ureolystic MICP is urea and the most commonly used calcium source is calcium chloride, both of which can contribute ammonium and chloride ions respectively, into the environment in the form of runoff which can cause eutrophication and react with other environmental components to form more dangerous compounds[8,3744,51]. One way to diminish the environmental impact of the urea used in these systems is to substitute synthesized urea with urea isolated from municipal waste in the context of a separated sewer system or via resource recovering using source separated urine streams[51]. This integration of infrastructure would minimize the energy consumption and cost of both the RC and wastewater treatment sectors, and aid in making bioconcrete a more attractive option for implementation. Even after self-healing concrete production, the ureolytic

metabolic reaction produces nearly half of the starting nitrogen concentration into ammonia, as ammonia can be corrosive to RC and chloride is a known RC curing accelerant their impacts on RC design and mechanics must be investigated. To date, no comprehensive studies have been conducted to assess the impact of ammonia or its secondary metabolites on both the structural integrity of RC or the environmental consequences of the ammonia load being introduced into the environment.

2.9 Effects of MICP technologies on RC lifetime

As discussed previously, traditional RC has a relatively short lifetime for the material's resource input[22,23]. When considering the applicability of the bacterial and self-healing concrete in particular, the functional lifetime and its total life cycle should be evaluated alongside the immediate environmental benefits. A longitudinal study on this topic is necessary before any conclusions can be drawn about the longevity of these materials. According to a life cycle assessment of bioconcrete in comparison to traditional concrete, bioconcrete's impact is half[51]. While higher initial capital input in required for bioconcrete, there is the opportunity for a closed-loop production stream where the bioconcrete at the end of its lifetime can be recycled into new aggregate for production, hence why its overall cost effectiveness is higher[52].

2.10 Cost analysis of MICP technologies

Economic feasibility is critical in the implementation of a novel material such as MICP concrete products. As with most novel technologies, the cost to produce biodeposition and biocementation products is slightly higher than traditional materials: for the company Calcite Bioconcept, it takes approximately $4 to treat a square foot of degraded limestone with a biodeposited treatment, which is significantly higher than $2.57/ft^2 for conventional water repelling treatments or $3.10/ft^2 for consolidation treatment[8]. Due to these cost disparages, it is unlikely that biodeposition treatments like those offered by Calcite Bioconcept will overtake the market standards. However, these treatments have found niches in the historic preservation sector where cost is less of a determining factor due to the material compatibility as mentioned earlier[3,8,43]. RC biomortar does not possess any niche qualities to improve its applicability: the production cost for a kilogram of biomortar binder alone is $1420.35 compared to conventional $0.67 binder[8]. Purely economically speaking, the most applicable bioremediation products discussed in this review in the context of RC would be the elimination of maintenance by self-healing concrete. Currently, it takes about $200 to remediate a square foot of cracked concrete, so the best way to

decrease costs would be to prevent the need to remediate cracks in the first place[4]. If these self-healing concrete products are applicable in-situ, the steep initial production cost may be mitigated by the minimal input throughout its lifetime. Furthermore, given the ever-increasing strict environmental pollution regulations, fines associated with contributions to eutrophication due to runoff from traditional RC maintenance and repair further highlight the economic benefit of biologically-inspired RC design. That being said, no longitudinal studies have been conducted at this time to determine whether this proposed economical trade-off actually occurs, so further work must be conducted.

2.11 Conclusions and future steps in the field of RC bioremediation

MICP technologies to bioremediate RC have a vast potential to improve the viable lifetime of RC while decreasing its environmental and economic impact. The body of literature discussing these technologies determined that ureolysis is the most effective metabolic pathway because of its rapid calcium carbonate precipitation, non-pathogenic species, and compatibility to the RC environment[8,44,53]. Using that pathway, biocementation technologies provide the most advantages in terms of increased strength in the case of bacterial concrete or decreased water and chemical permeability through cracks in the case of biomortar and self-healing concrete[14,44,53]. Overall, due to biomortar's economic drawbacks, self-healing concrete is the most attractive MICP technology because of its potential to decrease maintenance and increase the lifespan of RC, however, it is still in its testing phase[8,42].

Although, self-healing concrete shows promise at the bench-scale in carefully controlled laboratory conditions, longitudinal studies need to be conducted at pilot- and full-scale. To date, no such studies exist. In addition to these concept-driven experiments, self-healing concrete's lifetime and lifecycle analysis must be assessed to determine whether it is similar or superior to traditional concrete in its longevity, mechanics, cost and environmental impact. This assessment of environmental impact must also address the potential for ammonia leaching and the effects of other nitrogenous compounds reacted from ammonia on both the internal RC material and the surrounding environment, as well as the feasibility to utilize unconventional urea sources such as municipal wastewater to lower its environmental impact further. While MICP-mediated self-healing RC may still be in its infancy, it has great potential to contribute to a more sustainable future.

3. Task B: isolation of in-situ MICP microbes in concrete structures:

Summary: Microorganisms from a variety of RC samples were isolated and cultured on media that promotes MICP, colonies were chosen for isolation based on physical criteria previously described in the literature that are indicative of calcium carbonate production[54,55]. Overall, 24 potential MICP organisms were obtained. Compared to the best-known laboratory MICP organism (*Sporosarcina pasteurii)* the five most promising isolates based on physical characteristics (PA1, C1D, C2D, C3D and C1W) were taken forward for growth kinetic and calcium carbonate formation assessment. Four of the five best isolates were obtained from a fresh RC core taken from the PA I-70 deck, all of these isolates had faster growth and displayed a similar rate of calcium carbonate formation compared to *S. pasteurii*.

3.1 Materials and Methods

3.1.1 RC sample information

Reinforced concrete samples from a variety of structure types (e.g., sidewalks, highway bridge, steel reinforced structures) had previously been obtained by the Sachs group from Michigan, Pennsylvania, and Minnesota and were used to isolate native MICP microbes. In addition to these aged samples a fresh RC core from the I-70 bridge deck was provided by Jeremy Hughes (District 12, PA Department of Transportation).

3.1.2 Isolating and characterizing MICP organisms from RC specimens

Isolating potential MICP microorganisms from the RC specimens was accomplished in two ways: dry swabbing the RC sample with a sterile cotton swab or by submerging 2 g (finely ground) of the sample in 2 mL sterile phosphate buffer saline (PBS) solution. Collected swabs were smeared onto solid growth media containing nutrient broth, urea, and calcium chloride (NBUC) and 100 µL of the submerged PBS solution was plated using the spread plate method on solid NBUC growth media. All NBUC plates were incubated at 28 ºC for 7 days. NBUC media was made following the protocol outlined in Ghosh et al., 2019, where nutrient broth, urea,

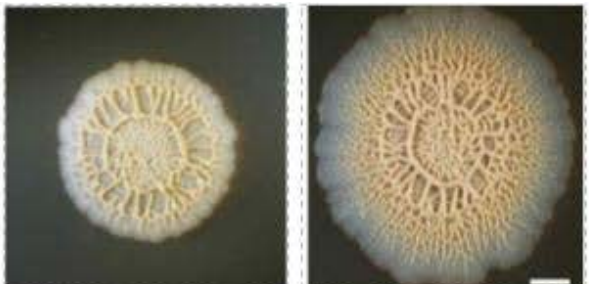

Figure 3: Physical characteristics of microbes capable of MICP. Adapted from Oppenheimer-Shaanan et. al, 2016.

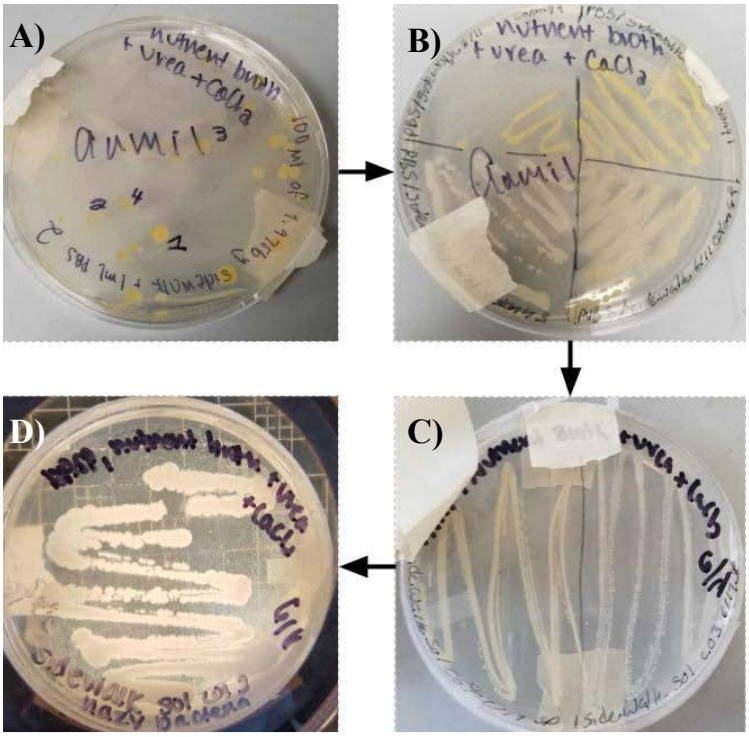

Figure 4: Isolation process of potential MICP organisms. A) All colonies grown from swabbing a RC sample. B) First round of purification of four colonies which display MICP characteristics taken from A. C) Purification of colonies from B. D) Pure culture of one of the colonies from C.

and calcium chloride are used to promote ureolytic MICP production [48]. *Sporosarcina pasteurii* (ATCC:11859) was used as a positive MICP control and was obtained from Dr. Koaru Ikuma from Iowa State University and both plate and PBS negative controls were ran for every sample.

After initial isolation, potential colonies capable of MICP were identified based on known MICP growth and appearance characteristics (Figure 3). The identified potential MICP organisms were then transferred using the streak plate method to fresh NBUC plates (Figure 4) and grown overnight at 28 ºC. Subsequent replating was performed until an axenic culture was obtained, which was determined using a Gram stain.

3.1.3 Growth kinetics

Once axenic cultures of the microbes isolated from RC specimens were obtained growth kinetic experiments were performed to determine their growth rate. Growth curves were established using the standard optical density procedure (600 nm for 24 h and plating serial dilutions of the culture at corresponding time points to the optical density readings). Generation rate (time required for the organism to double in abundance) was calculated using equations 7 and 8. All growth kinetic experiments were performed in triplicate with appropriate controls. Plates were parafilmed to reduce evaporation and allowed to grow overnight in a 28 ºC incubator before colony counting.

$$g = \frac{t}{n} \qquad 7$$

$$n = \frac{Log\ N - Log\ N0}{0.301} \quad (8)$$

3.1.4 Qualitative MICP performance analysis - agar column experiment

In order to assess the isolated microbes ability to form calcium carbonate, qualitative experiments were performed using agar columns following the procedure documented by [48]. Briefly, the environmental isolates were inoculated in 0.5% agar columns by creating a slit in the agar with a pipette tip, a single colony of each isolate was then introduced and incubated for 7 days at 28 ºC. Cultures were assessed by both visual inspection (looking for the appearance of crystalline structures) and calcium carbonate formation verification. Calcium carbonate formation was assessed visually and verified by melting the 7 day old agar columns and filtering them onto a 0.4 µm filter and analyzing the filtrate using X-ray diffraction spectrophotometer (XRD). *S. pasteurii* was used as the positive control and as a benchmark to compare the isolated MICP organisms to.

3.1.5 Confirming calcium carbonate production using XRD analysis

Microorganisms used in this study that showed MICP potential were grown in a 500 mL NBUC liquid culture for 7 days at 28 ºC. Grown cultures were pelleted by centrifugation at 8000g for 5 min and allowed to dry at room temperature. After drying, the pellet was ground into a powder and analyzed on a Bruker D8 powder X-ray diffraction spectrophotometer (XRD) with a Lynx Eye detector located in the University of Pittsburgh's Nanoscale Fabrication and Characterization Facility. Samples were run using an X-ray set to 40 kV and 40 mA from 3.5º-95º, with a locked coupled scantype at a scan speed of 0.4 sec/step and an increment of 0.04. Results from this instrument were analyzed for spectral similarity as well as through the analytical software EVA. Microbes grown in non NBUC media (not forming calcium carbonate) and pure calcium carbonate were run as controls.

3.2 Results & Discussion

Overall, 24 potential MICP microbes were isolated from the RC specimens aforementioned, 16 were Gram positive, five were Gram negative and three were unable to be assigned to a Gram designation (Table 3). MICP organisms are typically Gram positive due to the cell wall acting as a nucleation site for precipitate, therefore the five Gram positive isolates (PA1, C1D, C2D, C3D and C1W) which displayed MICP physical characteristics, had the fastest growth and performed the best in qualitative agar column procedure were taken forward for additional testing. Four of the five chosen isolates were

obtained from the freshly acquired RC core from a section of the PA I-70 bridge deck, and the other was sourced from a different sample of RC from Pennsylvania (Figure 5 and Table 3).

Table 3. Overview of the potential MICP microbes isolated from RC specimens. Note generation time was only determined for the five most promising microbes and potential to make calcium carbonate is based on visual observation.

Isolate ID	Specimen Origin	Isolation Method	Gram Stain	Form Calcium Carbonate	Generation time (minutes)
PA1	RC from PA	PBS soak	Positive	YES – confirmed by XRD	96.40
CID	I-70 Bridge, PA	Dry swab	Positive	YES – confirmed by XRD	181.60
C2D	I-70 Bridge, PA	Dry swab	Positive	YES – confirmed by XRD	148.35
C3D	I-70 Bridge, PA	Dry swab	Positive	YES – confirmed by XRD	482.57
C1W	I-70 Bridge, PA	PBS soak	Positive	YES – confirmed by XRD	26.44
S.pasteurii	Positive control	NA	Positive	YES – confirmed by XRD	133.02
SW 1	PA Sidewalk	PBS soak	Positive	YES –unconfirmed	NA
SW 2	PA Sidewalk	PBS soak	Positive	YES –unconfirmed	NA
SW3a	PA Sidewalk	PBS soak	Negative	No	NA
SW3b	PA Sidewalk	PBS soak	Positive	YES –unconfirmed	NA
SW3c	PA Sidewalk	PBS soak	Positive	YES –unconfirmed	NA
SW4	PA Sidewalk	PBS soak	Positive	YES –unconfirmed	NA
MN1	Un-reinforced concrete, MN	PBS soak	Positive	YES –unconfirmed	NA
MN2a	Un-reinforced concrete, MN	PBS soak	Positive	YES –unconfirmed	NA
MN2b	Un-reinforced concrete, MN	PBS soak	Positive	YES –unconfirmed	NA
MN3	Un-reinforced concrete, MN	PBS soak	Negative	No	NA
MI1	Un-reinforced concrete, MI	PBS soak	Negative	No	NA
PA2	RC from PA	PBS soak	Negative	No	NA
PA4	RC from PA	PBS soak	Neither	No	NA

C4D	I-70 Bridge, PA	Dry swab	Negative	No	NA
C2W	I-70 Bridge, PA	PBS soak	Positive	YES –unconfirmed	NA
C3W	I-70 Bridge, PA	PBS soak	Positive	YES –unconfirmed	NA
C4W	I-70 Bridge, PA	PBS soak	Neither	No	NA
C5W	I-70 Bridge, PA	PBS soak	Neither	No	NA

Growth kinetic experiments revealed that all five of the isolated presumptive MICP microbes grew faster than the gold standard laboratory microbe used in previous MICP studies (Table 3). In particular C1W, and PA1 grew ten and twelve times faster than *S. pasteurii*, respectively. The increased growth rate of these isolated organisms is a very beneficial characteristic as it potentially maximizes the calcium carbonate production rate and makes their future growth and application in biomortar economically viable (i.e., less

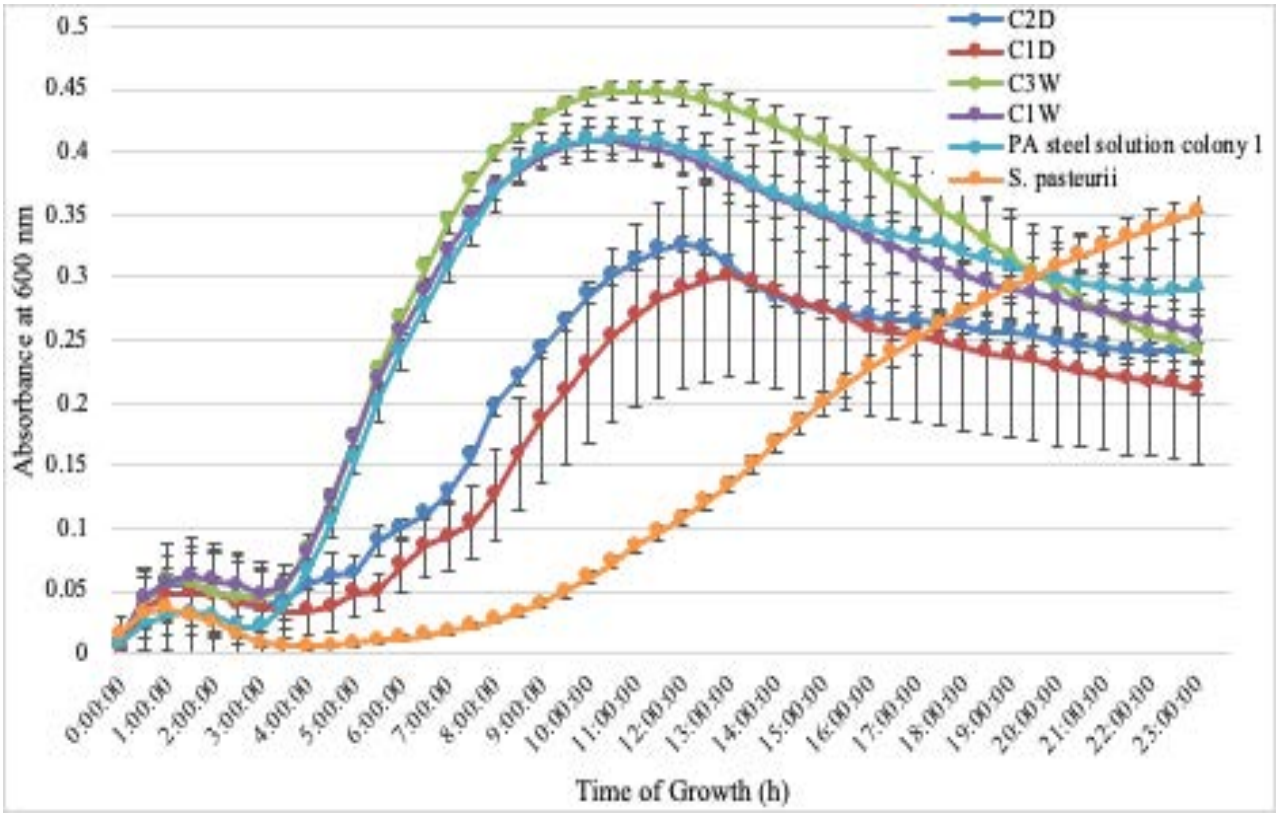

Figure 5: Growth kinetics of the top five RC-isolated MICP microorganisms. Points represent the average absorbance values and error bars represent the standard deviation based on nine replicas. Each curve represents all stages of microbial growth (lag, log, stationery and death). Note that decreases in absorbance values represent the death phase and are due to the resources within the batch reactor being consumed. Death phase was not observed for *S. pasteurri* due to its lower generation rate and hence slower rate on nutrient consumption.

time is required to grow them to achieve the desired density required for biomortar application).

In terms of calcium carbonate production, all five presumptive MICP microbes (C1D, C1W, C2D, C3W and PA1) were found by XRD analysis to have the same spectra as the known MICP microorganism *S. pasteurii* (a strong peak at 2Θ value 29, then duplets at 43, 48, 57, and 61 – Figure 6). It should however be noted that the background was higher in the presumptive MICP samples compared with the control, likely due to increased cellular debris which could be reduced by the introduction of additional washing steps during sample preparation. Sadly, due to COVID-19 restrictions the quantity of calcium carbonate produced by each organism could not be calculated, however qualitatively amounts appeared similar to the control.

To summarize, the five most promising MICP microbes isolated from RC samples were found to grow faster and produced calcium carbonate, similar to the gold standard microbe typically used (*S. pasteurii*). These results suggest that microorganisms isolated from pre-existing RC structures may be more desirable for biomortar applications due to their increased growth rate and confirmed MICP capabilities. In addition, these microbes unlike the typically used laboratory organism were isolated from RC specimens from real structures thus their likelihood of survival and successful application as a rehabilitation strategy is greater.

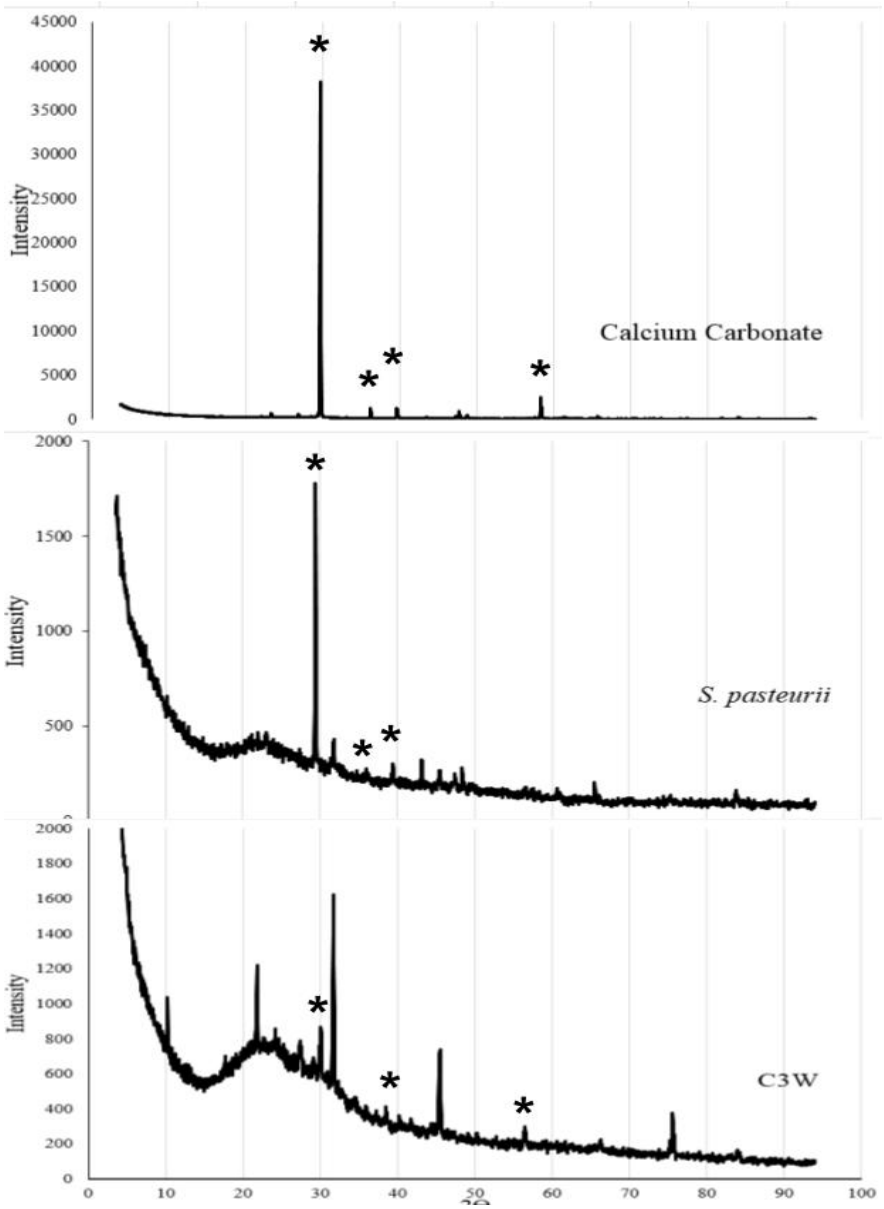

Figure 6: XRD spectral for calcium carbonate. Top panel shows pure calcium carbonate, middle panel shows calcium carbonate produced by *S. pasteurii* and the bottom panel shows calcium carbonate produced by one of the MICP organisms obtained from RC. Asterisks represent characteristic peaks for calcium carbonate that were used for identification.

4. Task C: evaluation of the mechanical properties of MICP inspired RC mix design

Summary: The purpose of this task is to demonstrate the feasibility of using microbes to provide self-healing properties to concrete. To this end, nutrients are needed for the microbes to engage in ureolysis to produce carbonate precipitation to fill the cracks or voids in the concrete. These nutrients are calcium chloride and urea. Therefore, in the mechanical evaluation, we need to demonstrate that the addition of calcium chloride and urea won't cause a negative impact on the mechanical properties of the concrete while providing the benefit of self-healing. The literature was investigated, and mortar testing was caried out in order to evaluate the effects of calcium and urea on the mechanical properties of the mix design. Five mixtures were evaluated: (1) control, (2) $CaCl_2$, (3) $CaCl_2$ + 1 g/L urea, (4) $CaCl_2$ + 5 g/L urea, and (5) $CaCl_2$ + 9 g/L urea. Compressive strength testing was carried out at 7, 14 and 28 days. Additionally, the effect of the nutrients on the hydration of the different mixtures was established by monitoring the temperature evolution via thermocouples. Finally, ammonia leaching tests were carried out to assess the leeching potential from the urea within the mixture.

4.1 Materials and Methods

As the two primary nutrients necessary to induce MICP are a chloride source, with calcium chloride being the most biologically available source and urea, the effects of these two constituents will be investigated first through an investigation of the literature. Then, mortar testing was conducted through various laboratory tests to examine the compressive strength and effect on the hydration.

4.1.1 Effect of Calcium Chloride on Portland Cement

Calcium chloride has been used as an accelerator (increases the hydration process or the speed at which Portland cement concrete hardens) for more than 100 years because of its low cost and availability[56]. Much of the research on the effect of calcium chloride on Portland cement was performed more than 50 years ago. In particular, Rapp (1935)[40] measured the setting time for 11 commercial types of cement with the addition of 0, 1, 1.5, and 2 percent of anhydrous calcium chloride by weight of cement and observed a negative correlation between the average setting time and amount of calcium chloride[40]. In addition, numerous studies have shown that the presence of calcium chloride greatly improves both

the early strengths of Portland cement mortar and the 1-year strengths (5-30% increase in strength)[14,57]. Although calcium chloride has been shown to improve the strength of concrete, this is only true when <4 % is added to the mix[14] as at concentrations >4% the presence of water, oxygen and chloride ions are in excess and can destroy the protective passive layer on the steel embedded in reinforced concrete; promoting corrosion[57]. Due to the corrosive potential of chloride, The American Concrete Institute[58] determined <1% water-soluble chloride ion should be presented to protect concrete from chloride corrosion. However, due to different suppliers and locations of materials it is very difficult to calculate the levels of chloride in concrete admixtures, therefore current industry guidelines state that <2% calcium chloride by weight of cement should be added for class C_0 exposure concrete[58].

4.1.2 Effect of Urea on Portland Cement

Urea could be used as an alternative deicer on concrete used for large scale infrastructure such as long-span bridges, motorway viaducts, and airport runways. In terms of urea addition, several studies have explored the structural impacts of urea addition to concrete[41,59,60]. For example, Sadegzadeh and Page, (1990) tested the mechanical properties of concrete cubes exposure to 5% and saturated urea solutions (nearly 50%) under conditions of alternate wetting and drying for 30 months and observed that the bulk mechanical properties of the concrete cubes were not significantly affected by the various pre-treatments and additives[41]. Likewise, Mwaiuwinga et al., (1997) found similar findings, including improved flowability, durability and shrinkage strain with increasing urea addition. In addition, Demirboga et al., (2014) measured the mechanical properties of concrete samples containing 6% urea (by weight of cement) cured in accordance with ASTM standard C192 in different deep freeze conditions (-5, -10, -15, -20 °C) for 7, 14 and 28 days and discovered that urea was an effective de-icing alternative up to -5 °C. Finally, from a corrosion perspective urea addition in concrete has not been found to cause corrosion to reinforcing steel or damage the concrete[41]. However, under chloride exposure conditions concrete containing urea in both 5% and 50% was found to be unable to produce a measurable reduction in the corrosion rate of the reinforced steel[41].

4.1.3 MICP inspired mortar specimen preparation

Standard 2-inch x 2-inch mortar cubes were mixed, cast and cured in conjunction with ASTM C305, C109, and C192[61–63]. A water to cementitious material (w/c) ratio of 0.45 was used along with a proportion of one-part cement to 2.75 parts sand. Type 1 Portland cement was employed, and the nutrients were introduced into the mix water from stock solutions of $CaCl_2$ and urea. The mixture formulations included a control which only used cement, sand, and water at the proportions outlined. The remaining four mixtures used 1% $CaCl_2$ by weight of cement and varying concentrations of urea. The urea concentrations in the four remaining mixtures were 0 g/L, 1 g/L, 5 g/L, and 9 g/L. These concentrations span the lower and upper concentrations acceptable in RC and required for growth of organisms capable of MICP.

4.1.4 Compressive strength testing of MICP inspired RC mixes

Compressive strength testing in accordance with ASTM C109 was carried out at 7, 14, and 28 days for each of the mixture formulations to examine adverse effects from the introduction of the $CaCl_2$ and urea nutrients. Finally, to examine any possible effects on the hydration that the nutrients would pose, the heat of hydration was indirectly measured by embedding a thermocouple within the fresh mortar to capture the time-temperature relationship for each of the five mixtures examined.

The heat evolved through cement hydration follows a curve similar to that outlined in Figure 7[64]. The hydration of cementitious materials in a concrete mixture results in a number of exothermic chemical reactions which liberate heat. The hydration process can be monitored by measuring the total liberated heat (via temperature changes) over time. A calorimetry test is traditionally used because it monitors heat of hydration with time. This testing can be time consuming and expensive, which is why it was decided to use thermocouples to monitor the temperature changes and investigate the effect of the nutrients on the hydration of the mortars.

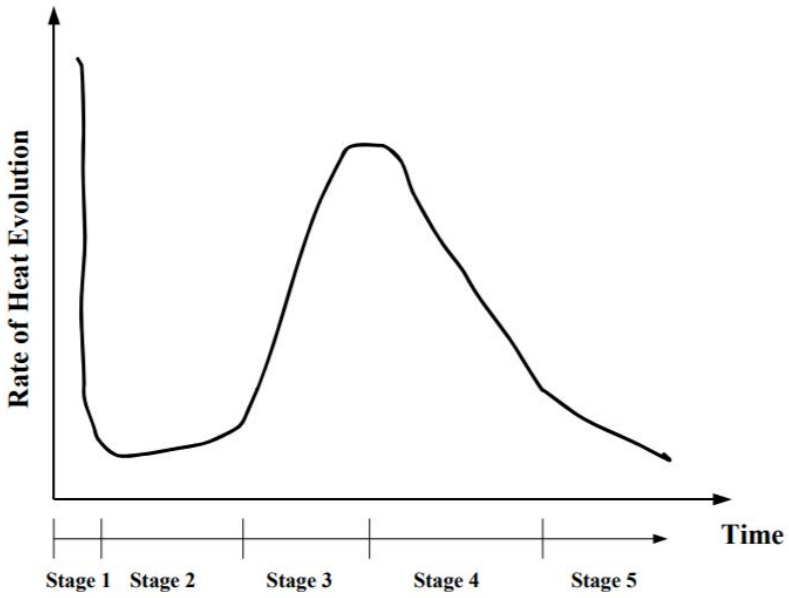

Figure 7. Cement Hydration Process (Wang et al., 2006)

The cement hydration process is typically divided into five stages. As soon as cement is mixed with water, a period of rapid heat evolution (stage 1) occurs and lasts about fifteen to thirty minutes. This stage is normally not captured by the calorimeter test due to its short reaction time. The heat evolution curves generally measured begin with the dormant period of cement hydration (stage 2). During the dormant period, cement hydration ceases, little heat is generated, and the concrete is flowable. This period generally lasts less than five hours. At the end of the dormant period, the significant hydration starts again during the acceleration period (stage 3). Concrete temperature increases rapidly during this period. As time increases, the rate of heat generation gradually slows (stage 4). Finally, cement hydration reaches the steady state (stage 5). Both stages 4 and 5 are known as the diffusion control phase[65].

For the specimens which were created in the lab, thermocouples were embedded in samples from the same mortar batches used to create compressive strength specimens. In order to compare and contrast the differences between the five established mixtures, the curves will

be evaluated to examine the visual difference between the curves, the time difference to the peak of the curve, as well as the area under the curves.

4.1.5 Ammonia leaching from MICP inspired RC mixes

The potential for leaching of by-products from urea hydrolysis in MICP inspired RC mixes was evaluated using 4-in diameter by 8-in high mortar cylinders that were air cured for 7 days using the following mixtures: the control, $CaCl_2$ + 1 g/L urea, and $CaCl_2$ + 9 g/L urea. The leeching test were performed in triplicate for each mixture and were conducted in accordance with JSCE-G 575-2005[66]. 4 L of distilled water was used as the leachate to immerse the specimen for 24 h and the concentration of ammonia in the leachate at day 1,2,3 and 4 was assessed using the Ammonia salicylate Method (Hach) in triplicate with appropriate controls.

4.2 Results & Discussion

The results of the 7-, 14-, and 28-day compressive strength testing are shown in Figure 8. Each of the values are the averages of three different specimens. Appendix A presents information for each of the specimens tested. To evaluate the differences between each mixture, hypothesis testing was performed to see if statistical differences exist. Tukey's range test is used to compare all possible pairs of means[67]. The null hypothesis is that the means of the two mixtures compared are equal while the alternative hypothesis is that the mean of one of the two mixtures differs from the other. From the analysis, there are no

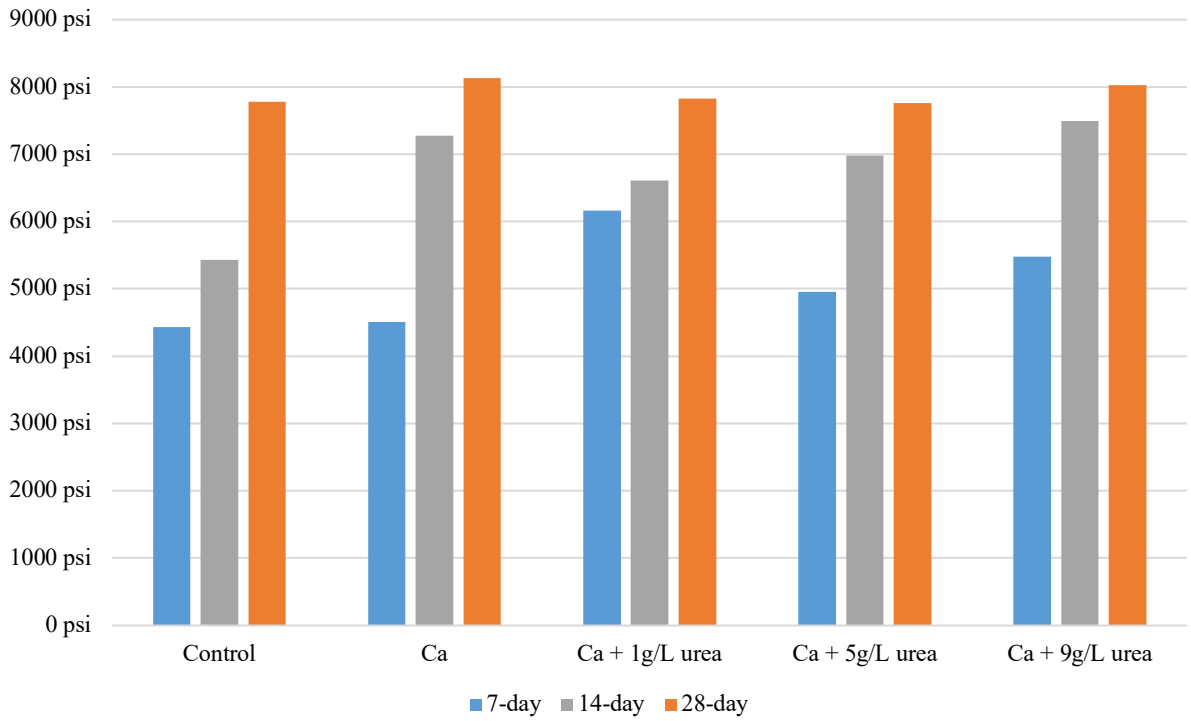

Figure 8. Compressive strength averages of different mixtures

statistical differences between any of the treatments at 28-days. At 14-days, the treatments with $CaCl_2$, $CaCl_2$ + 1 g/L urea, $CaCl_2$ + 5 g/L urea, and $CaCl_2$ + 9 g/L urea were found to be statistically higher than the control. At 7-days, $CaCl_2$ + 1 g/L urea was found to be statistically higher than the control and the treatments with $CaCl_2$, and $CaCl_2$ + 5 g/L urea. Additionally, $CaCl_2$ + 9 g/L urea was statistically higher than the control. From the results of the compressive strength testing, it is apparent that there is larger variability at 7-days and the variability dissipates by 28-days. Furthermore, these results suggest there is no detrimental effect on the compressive strength from the introduction of the nutrients for MICP. Indeed, it is also possible that the urea acts as a slight accelerant at early ages in addition to the calcium.

The hydration characteristics were observed by embedding thermocouples into mortar specimens. The temperature curves were all synced to the same time of water + cement. All specimens were stored in the same area to avoid any influences from external temperature fluctuations. Figure 8 presents the temperature vs. time plot for each of the mixtures and clearly shows that we captured the acceleration phase of hydration followed by deceleration and steady state conditions. It is also apparent that the curves have a similar

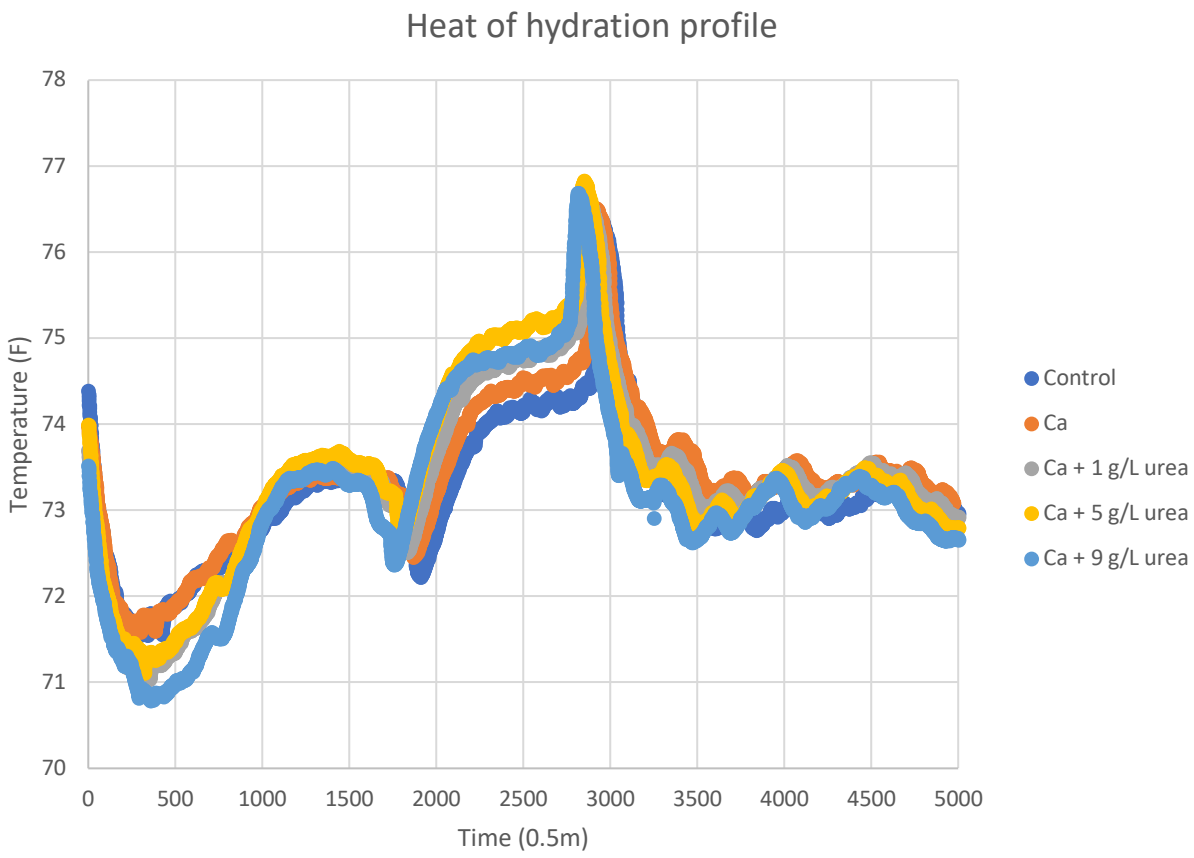

Figure 9. Time vs Temperature hydration plots of MICP inspired RC mixes

shape with a slight shift to the left compared to the control (Figure 9 and Table 4). The curve furthest right is the control plot indicating that it has the slowest hydration time which makes sense as it is the only mixture which does not contain calcium. The remaining four plots from furthest right to left are $CaCl_2$ + 0g/L urea, + 1 g/L urea, +5 g/L urea, +9 g/L urea (Figure 9). The observed time shift in hydration indicates that increasing urea concentration results in faster hydration. Additionally, the area under the curve was calculated to give a pseudo maturity value and it was found that each of the mixtures with calcium had a larger area under the curve than the control. Maturity is typically related to concrete strength in that the more time and higher temperature a sample has been subjected to, the higher the strength will be. This indicates that it is more likely that the compressive strength of the specimens with calcium will have a higher compressive strength at early ages compared to the control.

Table 4. Differences in hydration of the MICP nutrient addition RC formations compared to the control RC mix.

RC mix	Difference in time to peak temperature vs control (minutes)	Difference in area under hydration curve vs control (°F-min)
Ca	-19	483
Ca + 1 g/L urea	-35	629
Ca + 5 g/L urea	-52	214
Ca + 9 g/L urea	-67	527

The results from the leeching test are provided in Table 5 and show that both the low (1 g/L) and high (9 g/L) concentrations of urea resulted in essentially the same concentrations of ammonia at days 1-4 as control concrete specimen. This implies that leeching of ammonia should not be problematic at the concentrations of urea being used for MICP.

Table 5. Ammonia Leaching Test Results from MICP inspired RC mixes

Concrete specimen	Days after submerging in water	Average Leachate properties	
		pH	Ammonia concentration (NH3-N)
Control	1	11.43	0.02 mg/L
	2	11.50	0.01 mg/L
	3	11.20	0.01 mg/L
	4	11.26	0.00 mg/L
CaCl2 + 1 g/L urea	1	11.20	0.01 mg/L
	2	11.27	0.01 mg/L
	3	11.30	0.00 mg/L
	4	11.27	0.00 mg/L
CaCl2 + 9 g/L urea	1	11.40	0.02 mg/L
	2	11.49	0.02 mg/L
	3	11.25	0.02 mg/L
	4	11.23	0.01 mg/L

Overall, the results from this section demonstrate that the addition of nutrients required for MICP (urea and calcium chloride) did not have a significant effect on the compressive strength of RC. Although the hydration characteristics of the tested mixtures varied in terms of the location of the peak value of temperature and area under the curve compared

to control concrete specimens this is expected given calcium is known to accelerate the hydration process. Additionally, testing with the addition of calcium alone was shown to accelerate hydration also but to the same extent as when urea was also present, this suggests that urea also has some acceleration characteristics.

Given the lack of impact that calcium and urea addition had on the compressive strength alongside the limited ammonia release there should be little to no issue introducing the nutrients into the mixture to induce MICP. However, it is important to ensure that the levels of calcium added are kept below the recommended values from ACI so as not to significantly affect the rate of corrosion and the set of the mixture; as accelerated setting will adversely affect the placement of the concrete.

5. Task D: evaluate the self-healing and leaching properties of pre-cracked bench-scale concrete specimens treated externally with microbes identified

5.1 Summary

The environmental isolates and *S. pasteurii* characterized in Task B were used as the primary consolidating agent in a biologically-active mortar in bench-scale RC specimens. The mortar comprised of fine sand, a binder, urea and calcium chloride and MICP organisms in liquid culture. To determine the effectiveness of the biomortar, the RC specimens were dried out completely then submerged in water to determine the weight of water the block absorbed before and after biomortar application. If less water was absorbed after application, then the biomortar provided some protection from water ingress which is responsible for the corrosion of steel rebar in RC and a contributing factor to structure failure. When tested, the blocks treated with biomortar containing C1W (the fastest growing MICP microbe isolated from the I70 bridge deck) had a 37% reduction in water ingress compared to the same blocks prior to treatment and six times higher reduction than using the mortar alone. Although this result is promising the inability of the widely used positive control MICP organism (*S. pasteurii)* to reduce water ingress when applied as a biomortar indicates that more work is needed to evaluate the methodology to determine if these results are indicative of the biomortar composition or the testing method.

5.2 Materials and Methods

5.2.1 Preparation of RC Specimens

The specimens used in this study at the time of writing are 6-in by 6-in by 21-in concrete beams, obtained from several batches of three specimens each. All specimens were cast in accordance with ASTM C192[63] guidelines for laboratory concrete, cast as beams for use in ASTM C78[58] flexural strength testing, and were reinforced with fibers. All specimens were loaded to obtain an approximately 1mm flexural crack, running vertically in the direction of loading.

5.2.2 RC Mix Design

The concrete mix design was proportioned to meet requirements for both Class AAA-P bridge deck concrete, as specified in PennDOT Publication 408 (2016) section 704.1(b) Table A ("PennDOT Publication 408" 2020). The water-cement ratio used was 0.44. The cement used was standard Type I Portland cement, with no other cementitious materials.

Fine aggregate used was natural Type A sand with a calculated fineness modulus of 2.95, while the coarse aggregate used was crushed #57 limestone with a nominal maximum aggregate size of ¾-in. The final mix proportion of the concrete consisted of a water content of 11.55 lb/ft^3 (to account for water absorption by the aggregate), a cement content of approximately 24 lb/ft^3, fine aggregate content of 44.82 lb/ft^3, and a coarse aggregate content of 61.94 lb/ft^3. This leaves the mix ratio as 0.44:1:1.87:2.58. No other additives were included in the concrete mixture. Crack formation tends to occur quite rapidly in unreinforced concrete, making it difficult to obtain thin cracks without rupturing or spalling the specimen. As a result all specimens were cast with flexural reinforcement which consisted of Novomesh 950 polypropylene fibers during mixing, at a dosage of 5 lbs per cubic yard of concrete. In preliminary testing, steel rebar and mesh was used, however, it was more difficult to produce consistent cracking, therefore the polypropylene fibers were employed.

5.2.3 Cracking Procedure

Specimens were stored in a moisture curing room between demolding and cracking. Cracking was carried out at 7 days using a three-point loading apparatus as specified by ASTM C78[58]. The three-point loading apparatus ensured that flexural cracks and resulting failure would occur between the supports and point of load application in the region of constant moment. Loading was maintained at a constant rate and the specimens were carefully observed for cracking. For ease of transport and handling in further experiments, the ends of these specimens were sawed off, leaving 6-in cubic sections containing the flexural cracks. As the fiber-reinforced specimens provided more easily controlled cracking, reinforcing fibers were chosen over steel rebar.

5.2.4 Biomortar development and application

In order to minimize the environmental impact of crack remediation, a bioactive mortar consisting of MICP microorganisms (top 5 performing organisms from Task B), sand, and concrete binder was developed. Prior to preliminary testing of this biomortar, the concrete binder was assessed for its potential toxicity to the MICP isolates by growing *S. pasteurii* on NBUC plates made with 20% to 80% binder, then allowed to grow for 48 hours. All of the plates showed growth, so there were no concerns about the binder impacting the microorganism's viability in the biomortar. This mixture of sand, binder, and culture was initially tested in agar plates with a 5 mm wide slit cut out of the middle of the plate to

assess the consolidating effect of different mixtures of the constituents. The most consolidating of the combinations was then tested on surface fissures on a RC specimen to determine whether the ratios most successful in the agar plates were also the best in the RC matrix. The final biomortar was applied on triplicate cracked RC specimens using a sterile spatula.

5.2.5 Water ingress analysis

RC specimens with induced cracks were allowed to dry for 1 week, weighed, then submersed in tap water for 30 minutes. At the end of this period, the blocks were weighed again to determine the weight change when saturated with water. This protocol was repeated 7 days after the biomortar was applied to determine how water ingress potential had changed due to the crack treatment.

5.3 Results & Discussion

After numerous iterations of biomortar design, the final biomortar was achieved by creating a 3:8 mixture of 7-day old MICP culture and sterile sand to make a paste which was put in the lower half of the crack using a sterile spatula. The upper section of the crack was then filled with a slurry comprising of a 5:2:0.4 mixture of sterile sand, 7-day old culture of MICP microorganisms (Figure 10), and binder. The microbial dose in the biomortar was approximately 3.39 x 10^9 colony forming units /mL. This two-phase application provided the best visual seal in the RC cracked specimens likely due to the foundational paste acting as scaffolding to the slurry that had a greater proportion of microorganisms in it. Over the 7 days the biomortar was allowed to set, the consolidation was apparent on the biomortar's surface, which was likely a thin calcin layer created by the microorganisms (Figure 10).

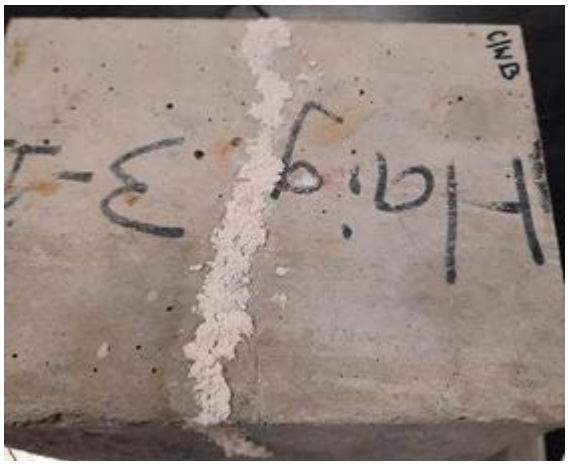

Figure 10. Photo showing a representative example of the biomortar after setting

The permeability results, however, did not reflect these visual observations. Out of the 5 environmental isolates and the conventionally used MICP organism *S. pasteurii,* only four

showed a reduction of water ingress after being submerged (Table 6). On average the four MICP isolated organisms reduced water ingress by 37.7%, however the positive control organism failed to reduce water ingress. This is unexpected due to *S. pasteurii* underperforming despite previous work highlighting its MICP properties and application to structural remediation[45,48,69]. One reason for the underperformance may have been due to a lack of time given to allow the biomortar to set. From an application perspective providing more time for the biomortar to set is not feasible, however, a thicker mortar could be produced which may help with initial binding and prevent washout (the likely reason for the observed results). In addition, the inconsistent findings could be attributed to a variety of problems with the experimental design and execution. For example, while the RC specimens themselves were cast using the same concrete protocol, there were inconsistencies in the reinforcement location and orientation, degree of cracking, and specimen age that could impact the porosity of the specimen outside of the treated crack. The experimental methodology of testing the biomortar's effectiveness may have also contributed to the unpredicted results. The test that was conducted allowed water to absorb into the entire block of concrete instead of exclusively the remediated section, resulting in weight differentials that may be more indicative of the matrix aging of the RC and not the biomortar effectiveness. When considering potential shortcomings in the biomortar itself, there is no way to guarantee that the microorganisms are evenly distributed within the mortar. This would result in areas within the remediated crack that have no consolidation and would allow water ingress. Additionally, no experiments were conducted to evaluate the viability of the microorganisms when added to the RC, so it is possible that the microorganisms desiccated when added into the crack. Future studies measuring adenosine triphosphate (energy) production on the surface of the remediated cracks will help provide evidence of viability and transmission electron microscopy of different sections of the mortar will help to determine how evenly distributed the microbes are.

Table 6. Permeability results of cracked RC specimen

Isolate ID	Average ± SD mass of water absorbed (kg)		Average ± SD difference in water absorbed (kg) [percentage water ingress reduction]	Water absorption reduction
	pre-mortar	post-mortar		
None – only chemical components of mortar	0.03 ± 0.01	0.04 ± 0.005	0.01 ± 0.007 [NA]	More water absorbed post-mortar
C1W	0.1 ± 0.005	0.04 ± 0.008	-0.07 ± 0.007 [60%]	Less water absorbed post-mortar
C1D	0.08 ± 0.006	0.05 ± 0.006	-0.03 ± 0 [37.5%]	Less water absorbed post-mortar
C2D	0.06 ± 0.006	0.09 ± 0.006	0.03 ± 0.007 [NA]	More water absorbed post-mortar
C3W	0.06 ± 0.006	0.04 ± 0	-0.02 ± 0.006 [33.3%]	Less water absorbed post-mortar
PA1	0.05 ± 0.006	0.04 ± 0.006	-0.01 ± 0.01 [20%]	Less water absorbed post-mortar
S. pasteurii (positive control)	0.03 ± 0	0.05 ± 0.003	0.02 ± 0.003 [NA]	More water absorbed post-mortar

6. Conclusion and Future Work

The results of this study suggest that isolating microorganisms capable of MICP from pre-existing RC structures is a viable option when developing sustainable remediation strategies. Microorganisms capable of growing on MICP-promoting media were isolated from all samples tested, and the representative few tested grew faster than *S. pasteurii,* but were less dense in culture. These same isolates also showed evidence of MICP when analyzed with XRD by presenting with the same spectral peaks as calcium carbonate. When implemented in a biomortar, consolidation occurred on the surface, but only water ingress reduction was achieved with four of the isolated organisms, with C1W achieving a 60% reduction.

Additional experiments need to be done to further evaluate the biomortar in RC specimens in Task D. This experiment should be replicated using RC specimens of the same age and containing the same reinforcement material. Additionally, viability testing during the biomortar setting period should be conducted to ensure that the microorganisms are still alive in the RC matrix. The methodology of these absorption tests should also be re-evaluated to better test the crack site on the RC alone and not the entire specimen for water intake to better exclusively determine the effects of the biomortar. Future work should also include remediation experiments using the different concrete mixes developed in Task C to determine if improved crack healing is achieved once all the essential nutrients required for MICP are present with the RC.

6.1 Estimated Cost Comparison

To assess the potential benefit of implementing this technology, it is necessary to compare the unit cost of MICP versus conventional remediation techniques. This is a difficult endeavor to make a consistent comparison taking into consideration the total overall cost considering labor materials, etc. First conventional techniques will be evaluated, and cost estimates provided to attempt the comparison.

The types of products to seal cracks are: epoxies, high molecular weight methacrylates (HMWM), urethanes, and water proofers[70]. HMWM products have good performance on depth penetration because of their low viscosity and they are an alternative for very narrow cracks (< 0.016 in.). HMWM are applied as a flood coat. For wider cracks (> 0.016 in.) an epoxy sealer is recommended, because of its higher bond strength. Epoxy sealer is applied to individual cracks[71,72]. For the epoxy resin or chemical grout injection, the procedure

involves two major steps. First, the crack is cleaned utilizing a high pressure wash. The next step will depend on which injection material is used. For the epoxy resin injection, the crack must be dry before injecting the resin. For the chemical grout injection, a wet surface is desirable as the chemical grout reacts with water, expands and fills the void of the crack while adhering to the concrete[73].

Since epoxies are recommended for wider cracks, they will be used for the comparison. Sources found cite an approximate unit cost of around $100 per linear foot for epoxy injection of structural cracks in concrete[74,75]. This process would include surface sealing of the cracks, installing injection ports, flushing the cracks with fresh water, injecting the cracks with a chemical grout, removing injection ports, and cleaning and patching the surface after the repair is complete. This includes the cost of labor, prep, etc. which is hard to gauge for MICP.

In order to make a more appropriate comparison, only the material cost will be evaluated. A concrete crack injector kit at Home Depot costs approximately $109 for 2 – 8.5 fl oz cartridges of material (https://www.homedepot.com/p/Sikadur-Concrete-Crack-Injection-Kit-432903/204076840#product-overview). It is worth noting that this cost also includes materials needed for injection (such as nozzles, injection ports, cartridges, etc.) and that it can most likely be obtained slightly cheaper at larger quantities, but provides a reasonable starting point for comparison. This equates to a cost of approximately $0.22/cm^3. Estimates from this research yield cost of materials for MICP of approximately $0.00032/cm^3. Therefore, large potential cost savings could be realized. However, significantly more research is needed to assess the ability of MICP to realize the same degree of crack sealing as epoxy. Additionally, the life cycle costs must be evaluated in terms of the expected life of the repairs.

6.2 Future work

Overall, the findings from this study suggest that there is great potential to use microbes capable of MICP to remediate cracks in pre-existing RC structures, however before full-scale implementation can be achieved further laboratory, pilot-scale and field-scale testing is needed. Below is a list of some of the major factors which need to be explored.

6.2.1 For application in pre-existing RC cracks

I. **Viability of microbes**: although we observed reductions in water ingress and the presence of calcium carbonate, future work needs to assess how long the microbes live and continue to perform MICP. This could be achieved by measuring the amount of adenosine triphosphate (ATP) produced within the crack. ATP is an intracellular energy storage molecule present in all forms of life and can be quantified rapidly in real-time via luminescence using handheld commercially available devices and kits. Although ATP measurement is routinely used to measure the efficacy of cleaning methods[76] to remove microbes in hospital it can be likewise used to assess growth.

II. **Environmental Pilot testing:** Assuming the MICP microbes remain viable for extended periods of time additional testing should be performed on concrete blocks which are left outside and exposed to the elements to assess their performance under non-laboratory conditions. This should include testing at various times of year to assess the impacts of differences in temperature, rainfall and salt exposure.

III. **Field testing:** If pilot testing is found to be successful, field testing should be performed on numerous existing RC structures using the same methodology as discussed in this report.

6.2.2 For application in new RC mixtures

I. **Testing of microbes in MICP inspired mortar designs:** Building on the findings from section 4.1.3, the MICP microbes isolated in this study should be applied into the three MICP inspired mixtures to assess their MICP capabilities and viability.

II. **Survival of MICP microbes during curing:** Assuming one of the MICP inspired mortar designs provides a viable environment for the MICP microbes it will be important to determine how to ensure the survival of these organisms during the curing process. This could involve exploring encapsulation methods and or inducing a sporing life stage (protective hibernation state).

III. **Even distribution of microbes within new mixtures:** Integral to the success of using MICP inspired mixtures to build new RC structures will be the assurance that microcracks formed anywhere in the structure will be “healed”. To ensure this, it is important that the MICP microbes will be evenly distributed throughout the RC structure or present at high-stress locations. Even distribution will likely be

challenging and will involve trying various approaches such as attaching the microbes to fibers.

IV. **Pilot and Field testing:** Following similar approaches as discuss above.

7. Scientific and educational impact

Scientific output

1) A. Shah, M. Stephen and SJ. Haig. Bio-concrete: exploring the use of microbes to improve the structural resilience and sustainability of reinforced concrete. Presented at the AEESP Conference, Arizona, May 2019. [Poster]
2) A. Shah, SJ. Haig, and M. Stephens. Exploring the potential of bioremediation to improve the structural resilience and sustainability of reinforced concrete. Presented at the Engineering Sustainability' 19 Conference, Pittsburgh, Pennsylvania, April 2019. [Poster]
3) C. Heckert, B. Wu, S. Sachs and SJ. Haig. Early detection and mitigaton of damage in concrete. Presented at the STRIVE pre-PhD summer research program conference, July 2019. [Oral]
4) S. Pitell, S. Sachs and SJ. Haig. Exploring the use of microbes to improve the structural resilience and sustainability of reinforced concrete. Presented at the American Society of Microbiology Conference, Online, June 2020. [Poster]
5) S. Pitell, S. Sachs and SJ. Haig. Microbially inspired self-healing concrete. To be presented at the Microbiology of the Bult Environment Gordon Research Conference, June 2021. [Poster]
6) S. Pitell, S. Sachs and SJ. Haig. Bio-concrete: harnessing the endogenous microbiota of reinforced concrete for crack remediation. *In preparation*: *Applied Environmental Microbiology Journal.*

Media attention

Article in the Pittsburgh Post-Gazette, July 2019
https://www.post-gazette.com/news/transportation/2019/07/15/Pitt-PennDOT-Pennsylvania-Turnpike-Allegheny-County-Golden-Triangle-Construction-Michael-Baker/stories/201907130009

Educational development

In addition to the named graduate students on the title page, Aamil Shah (sophomore undergraduate) contributed to Tasks A and B, Bin Wu (undergraduate – graduated Spring 2020) contributed to Tasks C and D and Christopher Heckert (REU student from UMBC) contributed to Task C.

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Appendix A

Group	No.	a1	a2	h	Area	Applied Load	Compressive Strength	Fracture Type
Control								
1	A	2.043 in.	2.024 in.	2.035 in.	4.14 in.^2	20910 lb	5053 psi	Cone
	B	2.042 in.	2.014 in.	2.038 in.	4.13 in.^2	17070 lb	4130 psi	Cone & Shear
	C	2.044 in.	2.042 in.	2.027 in.	4.14 in.^2	17030 lb	4112 psi	Cone & Shear
						Average	**4432 psi**	
+ CaCl2 Medium								
2	A	2.003 in.	1.997 in.	2.019 in.	4.04 in.^2	18300 lb	4532 psi	Cone
	B	2.006 in.	2.028 in.	2.035 in.	4.10 in.^2	17790 lb	4334 psi	Cone
	C	2.009 in.	2.000 in.	2.068 in.	4.15 in.^2	19360 lb	4670 psi	Cone
						Average	**4512 psi**	
+ CaCl2 Medium, + Urea Low								
3	A	2.024 in.	2.008 in.	2.047 in.	4.13 in.^2	26100 lb	6325 psi	Cone
	B	2.025 in.	2.035 in.	2.007 in.	4.07 in.^2	26500 lb	6504 psi	Cone
	C	2.021 in.	1.988 in.	2.024 in.	4.06 in.^2	22970 lb	5662 psi	Cone
						Average	**6164 psi**	
+ CaCl2 Medium, + Urea Medium								
4	A	2.049 in.	2.014 in.	2.032 in.	4.13 in.^2	21000 lb	5087 psi	Cone
	B	1.995 in.	2.006 in.	2.044 in.	4.09 in.^2	20090 lb	4913 psi	Cone
	C	2.009 in.	1.996 in.	2.034 in.	4.07 in.^2	19800 lb	4861 psi	Cone
						Average	**4954 psi**	
+ CaCl2 Medium, + Urea High								
5	A	2.010 in.	2.002 in.	2.054 in.	4.12 in.^2	23760 lb	5767 psi	Cone
	B	2.023 in.	2.002 in.	2.074 in.	4.17 in.^2	23450 lb	5618 psi	Cone
	C	2.032 in.	2.002 in.	2.043 in.	4.12 in.^2	20860 lb	5062 psi	Cone
						Average	**5482 lb**	

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