



WPI

A Study on Biologic Healing in Enzyme Modified Concrete

Presented By:

Emma Edwardson

Submitted to:

Professor Nima Rahbar and the Worcester Polytechnic Institute Civil and Environmental Engineering Department in partial fulfillment of the requirements for the Degree of Bachelor of Science
2019-2020

This report represents the work of a WPI undergraduate student submitted to the faculty as evidence of a degree requirement. WPI routinely publishes these reports on its web site without editorial or peer review.

For more information about the projects program at WPI, see

<http://www.wpi.edu/Academics/Projects>

Abstract

This project studied a biologically based concrete repair method to understand micro-characteristics and durability. Scanning electron microscopy (SEM) and rapid chloride permeability testing (RCPT) were used to study an enzyme modified (CA-Add) concrete and ordinary portland cement (OPC) concrete. Interfacial bonding of the repair to CA-Add substrate was visible in SEM. Permeability of CA-Add was lower than OPC. Results showed differences between the concrete mixes, however further research is recommended.

Acknowledgements

I would like to first thank my advisor, Professor Nima Rahbar. His encouragement and push for me to work on such a unique and innovative project has expanded my horizons and knowledge and allowed me to learn many new skills this year.

I would also like to thank Jessica Rosewitz for her invaluable help and guidance throughout the entire MQP process. Jessica guided me through all lab processes and helped me to become confident in my lab work. Her skills as a teacher and mentor were much appreciated during the project.

Lastly, I would like to thank Professor Aaron Sakulich. He provided me with informative background knowledge and advice during the proposal stage of the project.

Capstone Design Statement

To comply with the accreditation requirements established by the Accreditation Board for Engineering and Technology (ABET), the Department of Civil and Environmental Engineering at WPI requires all Major Qualifying Projects (MQP) to include a Capstone Design Experience. The Capstone Design Experience requires students to design a system, component, or process to meet desired needs. To fulfill this requirement, this MQP outlined and performed an experimental process to evaluate a method of biologically repairing concrete. The goal of the project was to address a lack of understanding of differences in the concrete matrices of normal OPC concrete and enzyme modified concrete. The project involved several design problems, including the design of unique molds as well as determining the best course of action for experimental set up and design. The MQP incorporated sustainability, environmental, and health and safety design constraints.

Sustainability

The project was framed in the context of sustainability. A large proportion of bridges and concrete structures in the United States need repair. Many concrete repair methods that are currently used are not long lasting and tend to debond from the concrete. The repair method studied in this project is a novel way of healing concrete, and it uses sustainable ingredients. Carbon dioxide is everywhere, and carbonic anhydrase is present in all living organisms, making them readily available for use.

Environmental

The enzyme repair method is environmentally friendly because it uses no harmful chemicals and involves biologically based healing in the concrete itself. The method of filling cracks uses water, carbon dioxide, tris, and carbonic anhydrase, none of which are harmful to the environment.

Health and Safety

None of the ingredients in the repair method are harmful to human health. Some methods of fixing damaged concrete, such as epoxies, can be harmful to human health. The repair method in this study is safe to produce and use.

Professional Licensure Statement

Engineering affects all aspects of life, and there is significant risk involved in engineering and design. Engineers must consider aspects such as health and safety, constructability, cost, and sustainability in their designs. Design and construction can have serious risks to public health safety, both during construction and after. As such, it is important for engineers to rigorously train and study in order to protect human life. A professional engineering license is a mark of dedication, hard work, and competence in the field of engineering. It is particularly important for a civil/structural engineer to pursue during their career because of the high risk associated with their designs.

In order to become a professional engineer, one must graduate from an engineering program approved by the state's licensure board. Next, one must pass the Fundamentals of Engineering exam, or FE, to become an engineer in training. A candidate for the Principles and Practice of Engineering exam, or PE, must have at least four years of professional experience before they can take the PE. Engineers must pass the PE in order to become a licensed engineer (How to get licensed, 2020).

Having a professional engineering license shows that an engineer is at the top of their profession, and that they are dedicated to safety, engineering ethics, and good design practice. It not only brings prestige, but also allows for career development and leadership in the workplace. All civil engineering projects need the approval of a professional engineer, so a professional license should be a goal for all civil engineers.

Table of Contents

Abstract	i
Acknowledgements	ii
Capstone Design Statement	iii
Professional Licensure Statement	iv
1.0 Introduction	1
2.0 Background	2
2.1 Climate Change and Concrete.....	2
2.2 An Overview of Concrete and Concrete Chemistry.....	2
2.2.1 Concrete Hydration	3
2.2.2 Concrete Degradation.....	4
2.2.3 Concrete Repair	6
2.3 Innovative Concrete Repair.....	7
2.3.1 A History of Bacterial Concrete	7
2.3.2 Urease and Carbonic Anhydrase	9
2.3.3 Biologically Induced Healing and Carbonic Anhydrase	10
2.4 Summary	11
3.0 Project Methods	12
3.1 Sample Preparation for SEM.....	12
3.2 Preparation of the Calcium Carbonate Repair.....	14
3.3 Rapid Chloride Permeability Testing.....	15
4.0 Experimental Results and Discussion	19
4.1 SEM Imaging	19
4.2 Rapid Chloride Permeability.....	22
5.0 Conclusions and Recommendations	26
6.0 References	28
Appendix A: SEM Image Gallery	31
Appendix B: RCPT Results	43

List of Figures

Figure 1: The process of concrete hydration.....	3
Figure 2: Structurally deficient bridges in the U.S.....	5
Figure 3: Epoxy crack repair.....	6
Figure 4: Mechanism of healing in concrete.....	8
Figure 5: Carbonic anhydrase reaction.....	10
Figure 6: Silicone mold for SEM samples.....	13
Figure 7: Four part enzyme mixture for topically applied repairs.....	14
Figure 8: A repaired and non-repaired SEM sample comparison.....	15
Figure 9: Rapid chloride permeability test setup.....	17
Figure 10: SEM images of the concrete-repair interface.....	19
Figure 11: SEM image of a single calcium carbonate crystal.....	20
Figure 12: SEM image of the concrete repair interface showing interfacial growth.....	21
Figure 13: SEM image of CA-Add and control surfaces.....	22
Figure 14: Graphic representation of RCPT results.....	25

List of Tables

Table 1: RCPT mix design.....	16
Table 2: ASTM C1202 chloride ion penetrability classifications.....	23
Table 3: Cumulative results for RCPT.....	24

1.0 Introduction

Concrete has been used as a building material since the days of the Roman Empire, although its uses have evolved since. It can be formed into almost any shape, is fire-resistant, inexpensive, and has a high compressive strength (Monaghan, 2017). The strength of concrete can be further increased by using steel reinforcing bars to prevent tensile cracking. Although concrete is a versatile material, it does have a number of flaws, namely its susceptibility to cracking. Cracks are often caused by environmental conditions, such as freeze thaw damage or corrosion by carbon dioxide and chloride. Environmental conditions, such as temperature, humidity, and weather patterns have been changing due to climate change, and it has affected concrete structures in a negative way.

Climate change is a growing concern, it has already been observed that the global average land surface air temperature has risen by 1.53 °C (IPCC, 2019). Although climate change is directly affecting the durability of concrete, the production and construction of concrete structures further contributes to climate change. Concrete is produced in high volumes all over the world because it is needed for new construction projects as well as repair and maintenance in older structures. There have been numerous studies in the past years that have addressed the need for concrete to be a more sustainable and environmentally friendly material. Biologic and self-healing concrete has become a promising solution. Bacteria, and the enzymes they produce, have the ability to fill cracks and reduce the permeability of damaged concrete by precipitating calcium carbonate. It has become a novel and environmentally friendly possibility for concrete repair.

This study focuses on a unique method of healing concrete using the enzyme carbonic anhydrase. The method is new, and the micro-characteristics of the repair and its durability are not well understood. In order to gain a deeper understanding of how the concrete matrix changes with the addition of carbonic anhydrase, scanning electron microscopy and rapid chloride permeability testing was used. The final outcomes of the project produced detailed visuals of the repair-concrete interface, and a quantitative comparison between normal concrete and concrete containing carbonic anhydrase.

2.0 Background

This chapter provides a detailed background on concrete and its many uses in the modern world. The section also discusses the benefits and drawbacks of concrete as a building material. Although widely used, concrete has several weaknesses when exposed to continual loading and unfavorable environmental conditions. There are a multitude of solutions currently used in industry to repair concrete, but they are often not long lasting. The chapter concludes with a discussion on innovative concrete repairs, and why those repairs can help make infrastructure sustainable and resistant to climate change.

2.1 Climate Change and Concrete

Concrete is one of the most used building materials in the world, and therefore needs to be produced in high volumes. In 2016, concrete production generated about eight percent of the total global carbon dioxide emissions, and 90% of that total is due to cement production (Rodgers, 2018). As a material, concrete has a relatively low amount of embodied carbon dioxide, but it is the sheer amount produced every day that creates such a high level of emissions (Barcelo et al., 2014). Concrete production and use is not sustainable, particularly with rising concerns about climate change. Wang et al. determined that the rising temperatures will most likely increase corrosion rates in concrete, with a fifteen percent increase in corrosion if there is a two degree increase in global temperature (2011). The high carbon footprint of concrete combined with the need for long lasting and inexpensive repairs are challenges that must be overcome in the future.

Climate change is already affecting concrete structures due to changes in temperature, humidity, and extreme weather events. Two major methods of deterioration in concrete, carbonation and chloride-induced corrosion are influenced strongly by temperature and humidity. Wang et. al. states that higher temperatures increase carbonation, and there is evidence that chloride-induced corrosion is accelerated by carbonation (Wang et al., 2010). Carbonation is directly caused by atmospheric carbon dioxide, and the likelihood of carbonation induced corrosion has grown more likely as global carbon dioxide levels increase. Furthermore, increased temperature elevates the likelihood of both carbonation and chloride-induced corrosion in concrete structures (Wang et al., 2011). The effects of climate change on concrete infrastructure will only get worse in the future. There is a need for innovative solutions that can address issues with concrete durability and sustainability.

2.2 An Overview of Concrete and Concrete Chemistry

Concrete is one of the most versatile and well known building materials in the world. It is also relatively easy to make. At its most basic, concrete is made up of cement, water, and aggregate. Aggregate, both fine and coarse, is inexpensive, easy to come by, and makes up seventy to eighty percent of concrete volume (Scientific Principles, 1995). Cement production on the other hand requires a slightly more involved process. Cement is formed during a high temperature process “during which minerals like clay, iron-ore, sand and limestone are partially melted and recombined at up to 2,700F in large kilns” (Monaghan, 2017, p.96). The resulting material is called clinker.

The clinker is ground up and combined with gypsum in order to regulate the setting time and create typical portland cement (Scientific Principles, 1995). Although concrete only has three main ingredients, cement, aggregate, and water, at a deeper level the minerals and hydration processes of concrete are far more complicated.

2.2.1 Concrete Hydration

Water is one of the main ingredients in concrete, and it is vital for workability, hardening, and it greatly influences material properties. Too much water in a mix can detract from strength due to unfilled voids in the concrete. Too little water and the concrete will not be workable and there will not be enough free water for concrete to gain maximum strength (Monaghan, 2017). There are numerous compounds found in cement powder responsible for the strength of the concrete, but the main ingredients are tricalcium silicate, dicalcium silicate, tricalcium aluminate, tetracalcium aluminoferrite, and gypsum (Monaghan, 2017). The most important minerals are tricalcium silicate and dicalcium silicate.

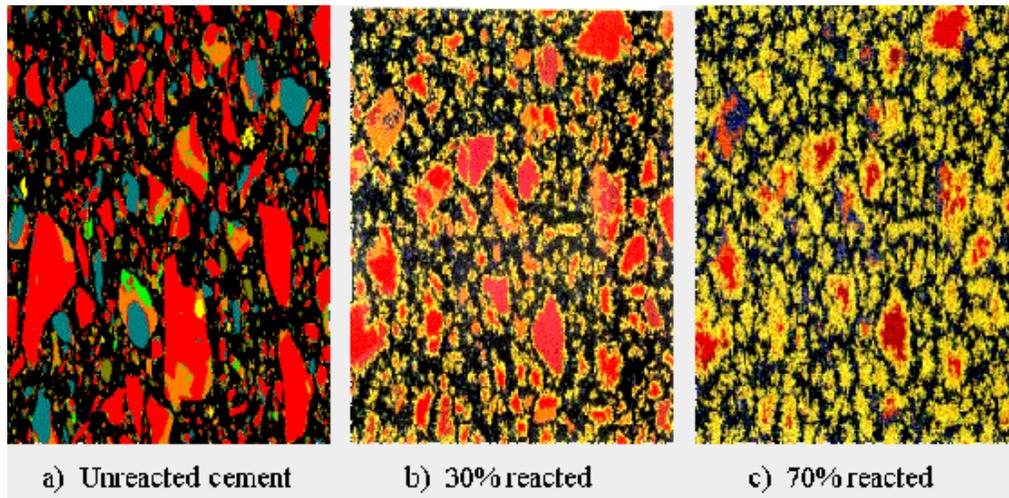


Figure 1: The process of concrete hydration. Water is represented by black, alite is represented by red, belite is represented by blue, and the yellow is C-S-H gel. From left to right, the amount of C-S-H increases and the water filled pores decrease as the hydration process continues, adding to the strength of the concrete (Thomas & Jennings, 2018).

Tricalcium silicate, also called alite, is the most common mineral in portland cement. It is illustrated in Figure 1 in red. Alite is responsible for the initial strength of the hardened concrete. Alite is highly reactive, and it quickly releases calcium and hydroxide ions as well as a significant amount of heat. The reaction causes calcium silicate hydrate gel, or CSH, to begin forming. CSH is illustrated in Figure 1 in yellow and is responsible for binding the particles together. Although CSH is not strong itself, it grows thicker over time, making it harder for the alite to react with water (Monaghan, 2017). Dicalcium silicate, or belite, reacts over a longer period and is responsible for increasing the concrete strength in the long run. It is illustrated in blue in Figure 1.

Belite reacts in a similar fashion to alite, but in a slower manner. It also produces CSH gel and contributes to the build-up of concrete strength over time (Scientific Principles, 1995). Both alite and belite produce calcium hydroxide, which helps to close pores and resist shrinkage (Monaghan, 2017). Calcium, which is in both alite and belite, is an integral part of concrete hardening and it is one of the most vital ingredients in portland cement. Calcium is responsible for binding the concrete matrix together and giving it strength in the long run.

2.2.2 Concrete Degradation

While concrete is a relatively cheap and versatile material, it does have a number of drawbacks, one being its susceptibility to cracking. The degradation of concrete structures is inevitable over time, but cracking increases the permeability of the concrete and makes a structure more vulnerable to corrosion of steel reinforcement by water, air, or harmful chemicals (Muhammad et al., 2016). There are a number of ways for concrete structures to deteriorate, many of which are influenced by environmental conditions.

Carbonation is one of the most significant threats to concrete, and is caused by the ingress of atmospheric carbon dioxide. It is influenced by temperature and humidity as well, with maximum carbonation occurring at fifty to seventy percent humidity (Šavija & Luković, 2016). The carbon dioxide reduces alkalinity of the concrete and makes the steel vulnerable to corrosion through depassivation. Carbonation also causes shrinkage, worsening existing cracks. During carbonation, carbon dioxide reacts with calcium hydroxide crystals and CSH gel to produce calcium carbonate. The process reduces the pH of the concrete and destroys the passive layer, leading to an increased threat of corrosion (Wang et al., 2010). Concrete is more susceptible to carbonation over time as the concrete is continually exposed to atmospheric carbon dioxide. There are also “changes in porosity, (micro)mechanical properties and appearance of cracks” that result from carbonation (Šavija & Luković, 2016, p. 286). On the other hand, carbonation can sometimes have positive effects on the properties of cement paste and concrete. It has been observed that strength, both compressive and tensile, can be increased by carbonation because the cement paste becomes stiffer. Another beneficial use of the carbonation process is accelerated carbonation curing. Fresh concrete is exposed to a high concentration of carbon dioxide which results in a rapid and high early age strength gain. Calcium hydroxide is converted to calcium carbonate, resulting in a high amount of calcite (Šavija & Luković, 2016). Overall, exposure to carbon dioxide can have positive effects for concrete in the short term but negative effects over time.

Chloride-induced corrosion can also pose a significant threat to structures, particularly in marine environments. Cracks in concrete allow chloride ions to permeate the structure, which then leads to corrosion of the steel reinforcement. Chloride-induced corrosion is strongly influenced by the concrete cover depth over the reinforcement. A greater cover depth typically leads to less corrosion (Wang et al., 2010). The products from corrosion have more volume than the original steel, leading to significant internal stresses that cause cracking and a loss of steel cross-sectional area (Li et al., 2017). Both carbonation and chloride corrosion can result in structural deterioration and even failure of a structure. Other processes that cause deterioration include sulphate attack in

areas that have acidic sulfate soils, reactions between alkalis and the aggregate that cause expansion, and freeze thaw cycles that cause expansion of water in concrete pores (Wang et al., 2010). While concrete is a strong building material, it has weaknesses that need to be addressed throughout the service lifetime of a structure.

A case study involving surveys of industry experts by Gardner et al. found that the most common problem experienced with concrete structures is cracking and poor workmanship leading to cracking. Cracking was reported by almost ninety percent of survey respondents. Freeze thaw damage was reported by about fifty percent of respondents, while carbonation and chloride induced damage was reported by about thirty percent. Additionally, the study reported that bridges, particularly joints, bearings, and the deck were the most vulnerable to damage and therefore needed the most repairs (Gardner et al., 2018).

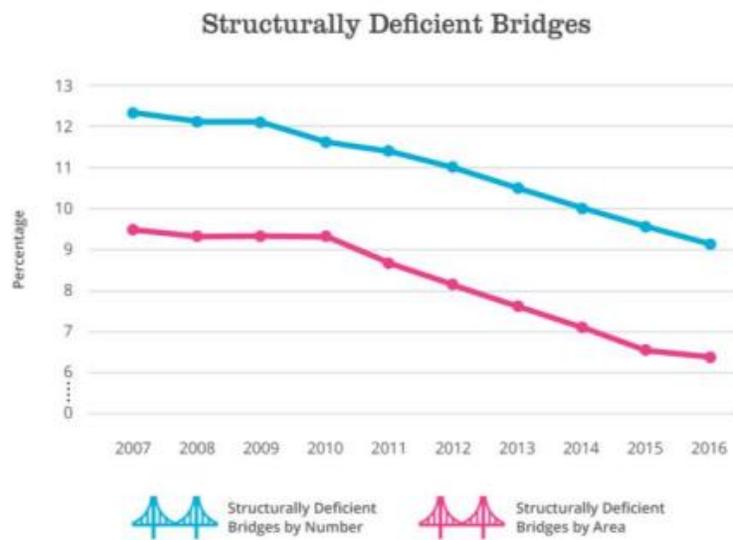


Figure 2: Structurally deficient bridges in the U.S. Although the number of structurally deficient bridges in the U.S. has decreased, there is still a large number of bridges needing repair. (American Society of Civil Engineers, 2017).

Concrete repairs have significant economic impacts due materials, manpower, and the cost of service disruption to the public. Combined with the sheer number of concrete structures that need repair, there is a significant amount of money that needs to be invested into maintenance and repair of concrete. The American Society of Civil Engineers, or ASCE, has studied the need for infrastructure investment in the past as well. Every four years, ASCE releases a report card detailing the quality of infrastructure across the United States. The current infrastructure grade is a D plus. Figure 2 shows the percent of structurally deficient bridges in the US. The report card states that 9.1 percent of bridges in the U.S. are structurally deficient, and an estimated \$123 billion is needed to update and repair the bridges (American Society of Civil Engineers, 2017). The National Academy of Engineering, or NAE, has identified fourteen engineering challenges that must be addressed in the 21st century, and one of them is to restore and improve urban

infrastructure. The NAE states that solutions must consider sustainability, environmental and energy-use considerations, and aesthetic elements (National Academy of Engineering,) n.d.). Overall, there is a tremendous need for solutions that address repair and maintenance of concrete structures, especially as they continue to age and deteriorate.

2.2.3 Concrete Repair

According to a 2015 concrete repair guide prepared by the U.S. Department of the Interior Bureau of Reclamation, there are four main types of concrete repair. The first kind is sealers and coatings that repair small cracks and protect from surface damage. Sealers often have to be reapplied every other year in order to maintain protection. Some sealers dry rapidly and have a strong odor, making them a challenge to apply to structures (Monaghan, 2017). An example of a sealing repair is shown in Figure 3. The next type of repair is a thin repair. They provide a non-permanent repair for cracks that are not deep or large. Thin repairs can sometimes cause more damage if the repair debonds from the concrete due to freeze thaw damage, or if the new and old concrete create an alkali-silica reaction. On the other hand, thick repairs are used for large cracks, and are typically the most cost effective and successful repairs. Lastly, there are crack and water leak repairs, which typically involve extra reinforcement placement or an injection of resin (von Fay, 2015). Although there are numerous ways to repair concrete structures, the repairs are not always effective over time.



Figure 3: Epoxy crack repair. These types of repair are not always long lasting and often have to be reapplied every few years (von Fay, 2015).

A case study by Grantham looked at 230 case histories of repairs of different concrete structures and different repair methods around Europe. The most common issue encountered in the structures was corrosion of steel reinforcement. In the end, the report found that only fifty percent of repairs to concrete were successful. In fact, twenty five percent of repairs failed outright (Grantham, 2011). The repairs were intended to last for the rest of the service life for the structure, but rarely lasted that long.

Another report prepared by the U.S. Department of the Interior Bureau of Reclamation found that most concrete repairs last only five to seven years. It is impossible to perfectly match the properties of a fully cured concrete specimen to a new repair. As a result, the repairs have a tendency to debond, allowing harmful contaminants to once again invade the concrete. Furthermore, many of the repairs had a high initial bond strength, but that is not always an accurate measure of bond durability. Structures experience variable loading throughout their lifetime due to environmental and internal conditions, leading to bond deterioration over time (Vaysburd, Bissonnette, & von Fay, 2014). Thin and thick repairs, also called patching repairs, can involve patching of concrete using either a cementitious material or a polymer based material. Grantham found that about fifty percent of patching repairs are successful, with polymer based materials being slightly more effective than cementitious repairs. The failures were most likely due to debonding of the repair and the concrete, although it was found that a combination of patch repair and a sealer was more successful than a patch repair alone (Grantham, 2011). Some other limitations of traditional concrete repair are sensitivity to moisture and heat, poor weather resistance, and degradation of repair due to differing thermal expansion coefficients (Seifan, Samani, & Berenjian, 2016). Overall, there are numerous repair methods used in industry today, but they are not always effective and long lasting.

2.3 Innovative Concrete Repair

The need for innovative concrete repair methods and innovative cementitious materials has given rise to some unique solutions. In order to make concrete more sustainable, mixes often use recycled ingredients. Fly ash, for example, can form a cementitious material when combined with water that can substitute up to thirty percent of cement powder. Fly ash can also increase workability of concrete, leading to less water used in a mix (Monaghan, 2017). The process makes fly ash, an otherwise harmful byproduct of coal fired power plants, a useful and cheap alternative to ordinary portland cement. Another development in concrete repair has been the evolution of bacterially induced healing in concrete.

2.3.1 A History of Bacterial Concrete

Self-healing in concrete is not a new phenomenon, and has been observed in many concrete specimens. The different mechanisms of autogenous healing are shown in Figure 4. Autogenous healing can occur when unhydrated cement particles are hydrated, or by carbonation of dissolved calcium hydroxide (Seifan et al., 2016). It is a natural process, but it depends on the amount of water and unhydrated cement particles that are present. Autogenous healing is not always an option for healing cracks in concrete because it is dependent on internal conditions and it cannot be regulated. A more promising method of healing concrete is through biomineralization. Some bacteria are able to precipitate minerals such as carbonates and silicates. Calcium carbonate, one of the common minerals in concrete, can be precipitated by bacteria as long as there is a source of calcium (Seifan et al., 2016). In many cases, the calcium carbonate is produced through urea

hydrolysis, using the enzyme urease, but it can also be produced using the enzyme carbonic anhydrase.

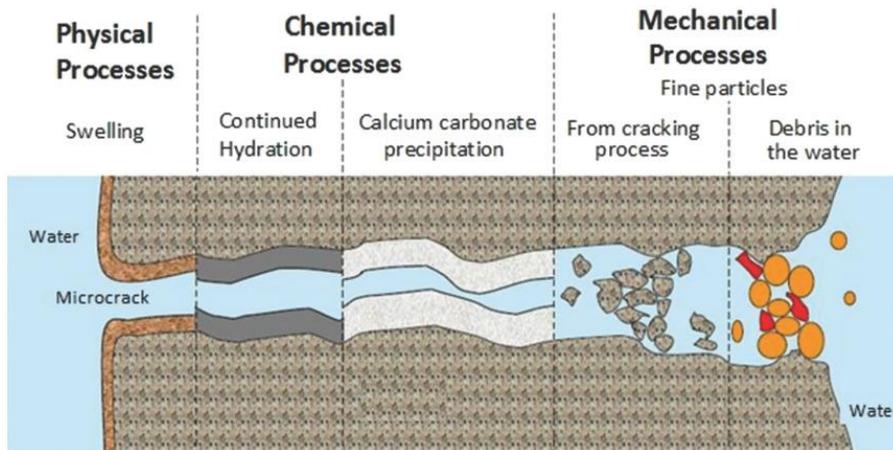


Figure 4: Mechanisms of healing in concrete. Chemical processes are the most common autogenous healing methods. Continued hydration is common in concrete. Carbonation can lead to the precipitation of calcium carbonate, stiffening the cement and filling small cracks. Mechanical and physical processes are less common than chemical (De Belie et al., 2018).

Henk Jonkers has been a pioneer in the field of bacterial concrete. His research evolved from studying bacterial calcification in different environments to the use of bacteria to heal cracks in concrete. One of the first articles Jonkers published on bacterial concrete was in 2010. The article begins by framing the bacterial concrete in the context of sustainability. Jonkers states that in previous studies, bacteria or derived ureolytic enzymes were applied externally to cracks. One of the drawbacks of the ureolytic enzymes is that the reaction can produce ammonium ions that lead to excessive nitrogen loading in the concrete. Jonkers tested the possibility of integrating bacteria into the concrete matrix, which ultimately led to numerous developments in the field of self-healing concrete (Jonkers et al., 2010). Jonkers determined that the next challenge was to find bacteria that could efficiently precipitate calcium carbonate in a harsh environment and effectively lay dormant in concrete until needed. Jonkers also discussed the need to quantify the behavior of self-healing and measure efficiency of crack healing.

Jonkers continued by studying different methods of encapsulating bacteria in concrete. A second study, published in 2011, investigated the possibility of encapsulating bacteria in expanded clay particles. The method showed promising results, particularly in wet environments, but Jonkers recognized that “the long term (years) durability and cost efficiency” of the bacterial concrete must be studied “before practical application can be considered” (Wiktor & Jonkers, 2011), p. 769). Furthermore, Jonkers has continued to frame a significant amount of his research in the context of sustainability. In a conference proceeding from 2017, Jonkers discusses a research program entitled *Bio-Based Geo & Civil Engineering for a Sustainable Society*. One of the objectives is to develop environmentally friendly biologically based materials, such as bacterial concrete (Jonkers,

2017). There are a number of programs, but all the research has a focus on reducing the carbon footprint of civil and geotechnical engineering projects.

Most recently, Jonkers has published a review on the damage management potential of self-healing concrete. The study discusses natural autogenous healing in concrete, which is only sufficient for healing small cracks. Cementitious additives such as fly ash or blast furnace slag can also promote increased autogenous healing in concrete. The review gives a history of all non-biological healing methods before discussing bacterial healing in concrete. The article discusses calcium carbonate formation by bacteria, and why it is effective at healing cracks in concrete. Calcium carbonate is compatible with the concrete matrix, has a strong bonding capacity, and results in densification of the concrete by filling pores and decreasing permeability (De Belie et al., 2018). Many bacteria strains have been used in studies on bacterial concrete, but most use the enzyme urease to facilitate calcium carbonate production. Jonkers recognizes that while bacterial self-healing is effective and environmentally friendly, there are a number of drawbacks that must still be investigated or overcome. The hydrolysis of urea produces ammonia as well as calcium carbonate, which could potentially corrode the steel reinforcement. Furthermore, the mechanisms of self-healing must be studied in un-ideal conditions outside of the laboratory in order to better understand how they work in the real world (De Belie et al., 2018). Overall, bacterial healing in concrete is an exciting field with many developments in the past years, but there are still many challenges and tests to be performed before it can be used commercially. The mechanisms behind self-healing need to be further studied, particularly the enzymes involved in the precipitation of calcium carbonate in concrete.

2.3.2 Urease and Carbonic Anhydrase

There are two main enzymes that are involved in bio-mineralization: urease and carbonic anhydrase. In most studies on bacterial self-healing in concrete, the enzyme urease has been researched. Another enzyme, carbonic anhydrase (CA), is also related to calcium carbonate production. Urease precipitates calcium carbonate by the hydrolysis of urea, and reactions can occur if there is enough calcium. Urea hydrolysis can produce a significant amount of calcium carbonate in a short time, but it also produces ammonia that can sometimes be harmful, as discussed earlier (Achal & Pan, 2011). On the other hand, carbonic anhydrase in the context of bio-mineralization in concrete has been studied far less than urease. CA acts when carbon dioxide and water react to form bicarbonate. CA facilitates the reaction of bicarbonate and calcium to form calcium carbonate (Rahbar et al., 2019). Figure 6 illustrates the bio-mineralization process. It is a quick process, and it has the potential to increase healing in concrete structures.

In most literature on self-healing concrete, carbonic anhydrase is rarely or never mentioned. CA is present in all organisms and is involved in all processes that involve carbon dioxide or bicarbonate (Achal & Pan, 2011). It is also associated with bio-mineralization, although it is only recently that CA has been studied in relation to bio-mineralization. Although there was comparatively less literature on CA, two studies by Qian et al. and Alshalif et al. researched the potential of carbonic anhydrase to sequester carbon dioxide in concrete and precipitate calcium

carbonate. Both studies were framed in the context of sustainability, and how the enzyme can be used to mitigate the effect of concrete repair on climate change. Qian et al. placed CA producing bacteria on concrete walls in order to absorb carbon dioxide and repair micro-defects through the precipitation of calcium carbonate. The concrete surface was treated with nutrients for the bacteria. The solution restored some strength to the structure, but there was a low bond strength between the surface and the deposited minerals (Qian et al., 2016). The study shows that carbonic anhydrase is environmentally friendly and has the potential to make concrete structures more durable and sustainable.

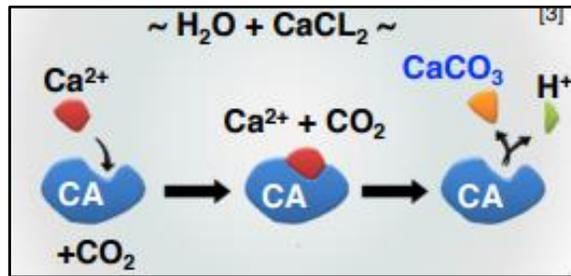


Figure 5: Carbonic anhydrase reaction. CA facilitates the conversion of carbon dioxide to calcium carbonate, as long as there is a sufficient amount of calcium present (Rahbar et al., 2018).

The other study, by Alshalif et al., looked at both urease and carbonic anhydrase and their potential to sequester carbon dioxide into concrete. Different strains of bacteria were studied, but the authors found that CA facilitated the production of calcium carbonate in all strains. The study showed that both enzymes can effectively sequester carbon dioxide, but the authors predicted that CA “has the most important role in the sequestration process of carbon dioxide” (Alshalif et al., 2018, p.8). Overall, most literature on carbonic anhydrase has focused more on the carbon dioxide sequestering potential of the enzyme, and not its capacity to heal cracks in concrete. Carbonic anhydrase is productive, readily available, and environmentally friendly, making it a viable option for healing defects in concrete.

2.3.3 Biologically Induced Healing and Carbonic Anhydrase

Recently, carbonic anhydrase has been studied as a repair method without the use of bacteria. Rahbar et al. developed a carbonic anhydrase solution that is saturated with carbon dioxide gas in order to precipitate calcium carbonate. The solution is typically applied on cracks in concrete specimens in order to facilitate healing. The repair is strong, fast acting, consumes carbon dioxide, and does not pose any risk to human health. The repair has also shown great promise in returning strength and reducing permeability (2018). Carbonic anhydrase is readily available for use, making the repair economic as well. Although a new method of healing concrete, the CA solution has a high potential for commercial use. Before that, more studies should be done to understand the material properties of the repair, particularly the bond between the repair and the concrete matrix. The study proposed in this report will involve an in-depth study of the repair-concrete interface of the enzyme repair and its durability.

2.4 Summary

The innovative repair methods discussed above have the potential to make infrastructure more sustainable and environmentally friendly. The United States is already behind on infrastructure management and repair, and climate change will only make matters worse. Wang et al. states that one of the main components “in climate adaptation is the...capability of the system to maintain its functionality and integrity” under the external stresses that come with a changing climate (2011, p.49). In recent years there has been several developments in the field of sustainable infrastructure, namely in biologically induced healing in concrete. The use of bacteria to heal flaws in concrete is well-studied and successful, but biologically mediated repair using enzymes has the potential to be just as effective.

3.0 Project Methods

This chapter provides an overview of the methods that were used to complete the project. The goal of the project was to study the durability of the enzyme modified concrete using scanning electron microscopy (SEM) and rapid chloride permeability testing (RCPT). SEM was used to produce detailed visuals of the interface between the calcium carbonate repair and the concrete. It was also used to look at the differences in the concrete matrix between a control sample and a sample that contained carbonic anhydrase. Previously, the micro-characteristics of the enzyme modified concrete was not well understood. The SEM graphics allowed for a qualitative analysis of the enzyme modified concrete and led to a better understanding of the strength and durability of the enzyme modified concrete.

RCPT was the second test performed in the study. Testing followed the procedure outlined by ASTM standard C1202. The test measures the resistivity of the concrete by using 60 volts of electricity to push chloride ions into the concrete (ASTM, 2019). Although it does not accurately predict field conditions, the test is widely accepted by the concrete industry because it is non-destructive and gives a good prediction of the permeability of concrete. The test can have large variability in results, even in samples that are very similar (Jacobs & Malpas, 2019). Although the test can be inaccurate for measuring field conditions, it was constructive in determining if there is a significant difference between the resistivity of normal OPC concrete and the enzyme modified concrete.

3.1 Sample Preparation for SEM

The samples used in the scanning electron microscope were small cylinders, 25mm in diameter and 5mm in height. The dimensions were chosen because they are the maximum height and width that can be used in the Phenom G1 Scanning Electron Microscope. In order to create the samples, a reverse mold was created using Solidworks and printed using rapid prototyping. A few iterations were printed until the final design was chosen. Ultimately, it was found that having three cylinders in one mold was the easiest to demold the silicone from the plastic. After printing, the mold was placed on a vibrating table and filled with silicone. The mold was left on the vibrating table for 10 minutes. It was then covered with plastic wrap and left to set for 24 hours. After 24 hours, the silicone mold was removed and hot-glued to a small sheet of clean acrylic. The mold was placed atop a piece of acrylic in order to create a smooth top on the sample. A surface with flat topography is easier to observe with SEM, and the acrylic provided a smooth finish on the cylinders. The final mold is shown in Figure 6.



Figure 6: The silicon mold used to make concrete samples with a small v-shaped notch. Samples were used for SEM. Three cylinders were put in each mold for efficiency and ease in demolding.

A small v-shaped notch was added in the middle of the cylinder on the top face. The notch was added in order to create a clear boundary between the concrete and the CA repair. A clear boundary with a smooth surface allowed for an easy comparison between the CA repair and the concrete substrate under SEM. Two types of samples were studied under the scanning electron microscope. The first type was a control sample with ordinary portland cement and water, and the notch left as is. The second type CA-Add, had the same proportions of cement and water but with carbonic anhydrase added. The notch was also filled with the calcium carbonate repair. A sample with the notch filled with the calcium carbonate repair is shown next to a non-repaired sample in Figure 8.

All samples mixed for SEM had a 0.4 water to cement ratio. The ratio was chosen because it was found to be the most workable when making the small disks. The control sample contained 50g of cement powder and 20g of water. The CA samples contained the same amount of cement powder and water, but also contained 10 μL of 100 μM carbonic anhydrase. For the samples containing CA, the cement powder was first weighed out on a bench scale and added to a small beaker. Next, 10g of water was measured out in a separate beaker. The carbonic anhydrase was then added to the beaker of water using a pipette to rinse out the micro-centrifuge tube three times. The tube containing the enzyme is rinsed three times to make sure all the enzyme is out of the container and in the mix. Once the enzyme is in the beaker, the rest of the 20g of water was measured out and added to the cement powder. The cement was mixed until the texture was smooth with no lumps. The acrylic and silicone mold was placed on a vibrating table and the three disks were filled with cement. The top was smoothed over and the mold was left on the vibrating table for 10 to 15 minutes. The vibrating table was turned off and the mold was wrapped in plastic wrap and left in a flat, dry space for 24 hours. After 24 hours the disks were demolded left in a moist curing room.

3.2 Preparation of the calcium carbonate repair

In order to fill the v-shaped notch in the CA-2 samples for SEM, the calcium carbonate repair was prepared after the samples cured for 7 days in a moist curing room. On day 7, the samples were taken out of the curing room and the top surfaces were covered with scotch tape. Scotch tape was chosen because it kept the surface of the sample smooth but did not impede the repair solution from filling the notch. After taping, the samples were placed in separate 80 mL beakers.

In a large graduated cylinder, 800 mL of deionized water was measured out. A 1000 L Erlenmeyer flask was placed atop a stir plate beneath a fume hood. Half of the deionized water added to the flask. A small bench scale was used to measure 400 mL of $2M\text{CaCl}_2$, or 88.792g. The scale was also used to measure 400 mL of 0.1M tris, or 4.844g. The calcium chloride and tris were added to the flask using a funnel, using a transfer pipette to rinse the measuring dishes. The stir plate was turned on high in order to let the calcium chloride and tris dissolve completely. Once the mixture turned clear, carbon dioxide gas was added. The gas was added to the mixture using a carbon dioxide canister with a hose and nozzle attached. The gas was turned on to a low bubble in the mixture. The last ingredient for the calcium carbonate repair is the enzyme itself. 20 μL of 100 μM carbonic anhydrase was added to the mixture, using a pipette to rinse out the microcentrifuge tubes three times in order to get all the enzyme in the mixture. The four-part mixture is shown in Figure 7.

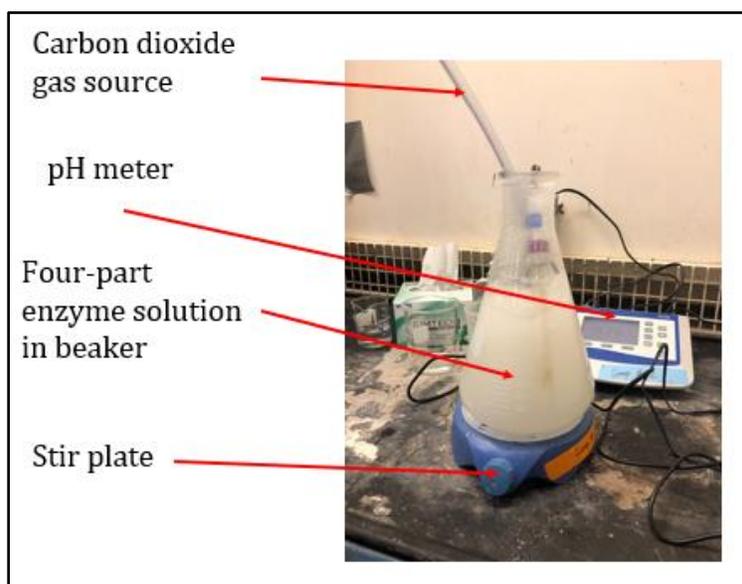


Figure 7: The four-part enzyme mixture for topically applied repairs. The four ingredients are water, calcium chloride, carbon dioxide, and carbonic anhydrase, with tris added to stabilize the pH. A pH meter was used to monitor the pH as it fell to a neutral level. The stir plate kept the mixture homogeneous.

Once the enzyme is added the pH of the mixture jumps to about 12. The tris is added in order to stabilize the pH, but it should be monitored with pH strips or a pH meter. Once the pH

falls to 7, the carbon dioxide gas was turned off. After turning off the gas, the flask was removed from the fume hood. Six 50 mL centrifuge tubes were filled with 45 mL of the enzyme mixture. The tubes were placed in a centrifuge for 9 minutes at 5000 rpm. The centrifuge concentrates most of the calcium carbonate at the bottom of the tube. The supernatant on top was poured into a large beaker, leaving about 5-10 mL of supernatant mixed with the concentrated repair. The tube was capped and shaken for 5 seconds and the concentrate was poured into a separate, smaller beaker. The process was repeated for all six centrifuge tubes. The centrifuge tubes were filled again with the mixture from the flask and placed in the centrifuge. The entire process was repeated until the mixture from the flask was entirely depleted. Once the mixture was separated into supernatant and concentrate, about 35 mL of the supernatant was poured over each sample. The samples were covered with parafilm, labeled, and left to sit for 48 hours. The final repaired sample and a non-repaired sample are shown side by side in Figure 8.

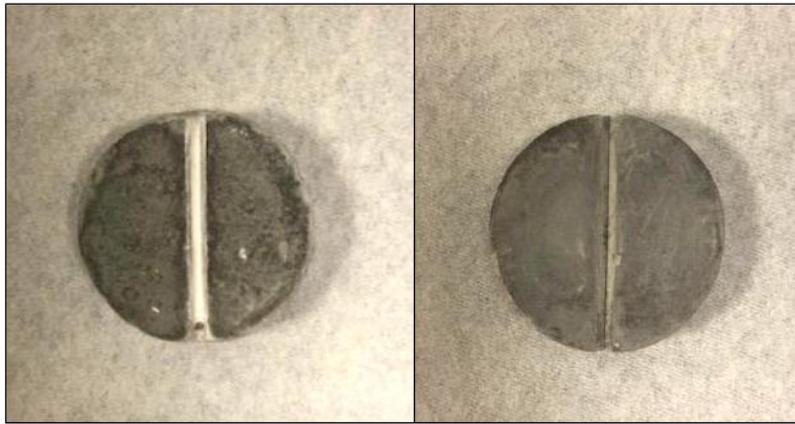


Figure 8: On the left is a repaired sample containing carbonic anhydrase in the concrete mixture (CA-Add). On the right is a control sample containing only water and OPC (Con). The two samples were among those compared using SEM.

After 48 hours, the supernatant was removed from the 80 mL beakers using an auto pipette and put in a separate beaker. The pH of the supernatant is between 6 and 7 and was disposed of down the drain. The samples were placed in a desiccator so they could be dried for use in the scanning electron microscope. The samples were in the desiccator for three days. Each day a vacuum pump was run for one hour. After day three, the samples were placed in a storage desiccator.

3.3 Rapid Chloride Permeability Testing

Two different types of samples were prepared for rapid chloride permeability testing: a control sample and a sample containing carbonic anhydrase (CA-Add). The exact mix design is shown in Table 1. The mixes are 51.4% coarse aggregate by weight. A large amount of coarse aggregate was used in order to increase the resistivity of the concrete during testing. The coarse and fine aggregate used in the mixes was saturated surface dry. While pouring, the concrete was

tamped 20 to 25 times every two inches in order to leave as few air voids as possible. The mix ratio filled two 4 inch by 8 inch cylinders. The concrete cylinders were removed from the mold after 24 hours and left in a moist curing room at ambient temperature. Two batches of samples were tested. The first batch cured for about two weeks, and the second batch of testing occurred around 11 weeks.

Table 1: RCPT mix design. All ratios are the same except for the addition of carbonic anhydrase in the CA-add mix. Both mixes are 51.4% coarse aggregate by weight.

	Control	CA-Add
Cement (g)	1377.5	1377.5
Coarse Aggregate (g)	3970.5	3970.5
Fine Aggregate (g)	1983.5	1983.5
Water (g)	394	394
Enzyme (μ L)	N/A	5

The rapid chloride permeability test followed the procedure outlined in ASTM standard C1202. Before testing, all 4 inch by 8 inch cylinders were cut into 50 mm disks. A water cut with an industrial 18” diamond blade (Highland Park Lapidary Co., Whitinsville, MA) was used to cut the cylinders. The top 50 mm disk was discarded, and the middle 50 mm disk was used from each cylinder. The diamond blade left a slight lip on the edge of the disks. In order to protect the screen of the RCPT set-up, the raised edge was carefully chipped off with a chisel and hammer.

Two days before testing, all solutions needed for the test were prepared. The needed solutions are degassed water, sodium chloride, and sodium hydroxide. For the degassed water, two large flasks were filled with deionized water and capped with a tin foil hat. The flasks were placed on a hot plate and left to boil for one hour to completely degas the water. After one hour, the flasks were removed from the hot plate and left overnight to cool.

Two solutions are needed for RCPT: 3N sodium hydroxide and 3% sodium chloride. The 3% sodium chloride was prepared first. A 1000 mL flask was filled with 250 mL of deionized water and placed on a stir plate with a small stir bar in the bottom of the flask. Using a bench scale, 15g of sodium chloride was measured and added to the flask. Another 235 mL of deionized water was added, and the stir plate was turned on high. The flask was covered with a tin foil hat and left to mix until the solution turned clear. Once the solution was clear, the stir plate was turned off and the solution was left to sit overnight. The 3N sodium hydroxide solution was prepared next. Another 1000 mL flask was placed on a stir plate with a small stir bar. Using a bench scale, 60g of sodium hydroxide was measured and added to the flask. Next, 500 mL of deionized water was added. The flask was capped with a tin foil hat and the stir plate was turned on high. Once the solution turned clear the stir plate was turned off. The reaction of sodium hydroxide and water is exothermic, so the solution was left to cool overnight.

On the day before testing all samples sat in a vacuum chamber for 3 hours. The sample was placed in the bottom of a small vacuum chamber, and the rubber gasket around the chamber was brushed with silicone oil. The chamber was closed, and a vacuum pump was turned on for 3 hours. After 3 hours, degassed water was added to the vacuum and the sample sat for 1 hour in the water vacuum. After 1 hour, one of the nozzles in the chamber was opened and the sample was left to sit and return to ambient pressure for 18 hours. The actual test was performed using the Proove'it system by Germann Instruments. testing apparatus and ran for 6 hours, according to the ASTM standard. The test set up is shown in Figure 9.

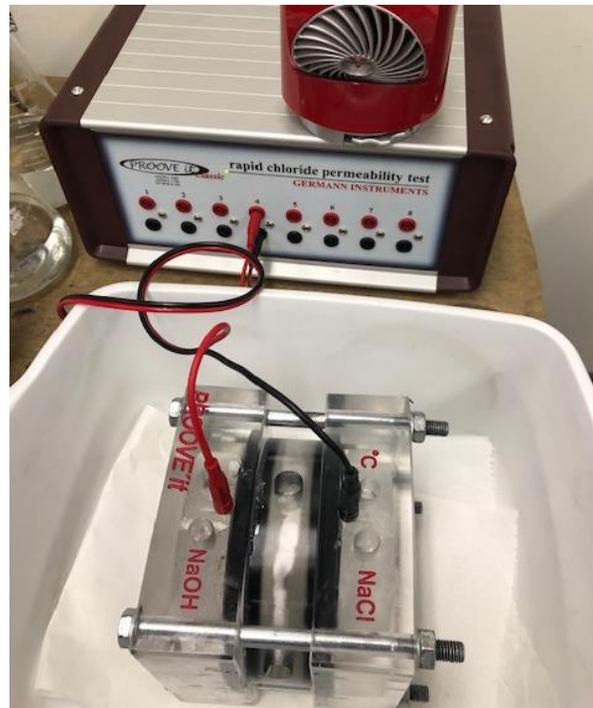


Figure 9: The RCPT test set up using the Proove'it system by Germann Instruments. One side of the apparatus contains 3% Sodium Chloride, and the other side contains 3N Sodium hydroxide. The instrument provides 60V of electricity to push chloride ions into the concrete over 6 hours.

In order to prevent leaks and create a strong seal in the test set-up, the ASTM standard was modified slightly, and the following procedure was used. First, the four bolts were placed in one of the voltage cells. Silicone oil was brushed onto one side of one of the rubber gaskets. The side with the silicone oil was placed on the metal of the voltage cell with the bolts. Then, Dow Corning high vacuum grease was applied around one edge of the concrete sample. The greased side of the sample was then pushed into the rubber gasket. More vacuum grease was added around the other edge of the sample. Next, the acrylic middle piece was placed around the sample. The second rubber gasket was placed around the top of the sample. Silicone oil was carefully brushed on top of the rubber gasket and the second voltage cell was placed on top with the metal touching the silicone oil. Next, a washer and nut were placed on each bolt. The assembly was tightened in a star pattern until the acrylic parts meshed together. The assembly tightness was checked by filling up

each voltage cell with degassed water. If any leaks were detected, more vacuum grease was added to the outside of the assembly. If no leaks were detected, the cells were emptied of water and filled with their respective solutions. Next, the assembly wires were connected to the machine, making sure not to cross the red and black wires. The temperature probe was added and placed in the cell with sodium chloride. The test generates a significant amount of heat, so a small fan was placed near the assembly to aid in cooling. The actual test was then started and ran for 6 hours according to the ASTM C1202 standard.

After 6 hours, the test set up was taken apart and cleaned. After disconnecting the wires, the solutions were emptied from the cells and disposed of in a hazardous waste container. The bolts were loosened in a star pattern and each assembly part was wiped off with paper towels before being placed in a tub of warm water and dish soap. Because vacuum grease is hard to remove, it is important to wipe off as much grease as possible before placing the part in water. Each part was scrubbed for 2-4 minutes and rinsed before being placed in a separate container to dry. The concrete samples were discarded after the test.

4.0 Experimental Results and Discussion

SEM was used to qualitatively assess the strength and durability of the enzyme modified concrete, while RCPT was used to quantitatively compare normal OPC concrete to the enzyme modified concrete. The images and results gathered show that there are differences in durability and makeup of the different concrete mixes.

4.1 SEM Imaging

Control and enzyme modified samples were examined using a Phenom G1 scanning electron microscope. The complete collection of images is shown in Appendix A. Figure 10 shows the interface between the CA-Add concrete substrate and the repair. The crystalline structures are precipitated calcium carbonate from the enzyme repair method. Although the crystal growth is asymmetrical, the size of the crystals is even. The crystals all appear to be in focus in the images, which suggests that the trough is evenly filled. The interface between the concrete and the crystals is clearly distinguished, and there appears to be direct bonding between the concrete and the crystals. The ridged contours in the concrete substrate are from the 3D printed mold.

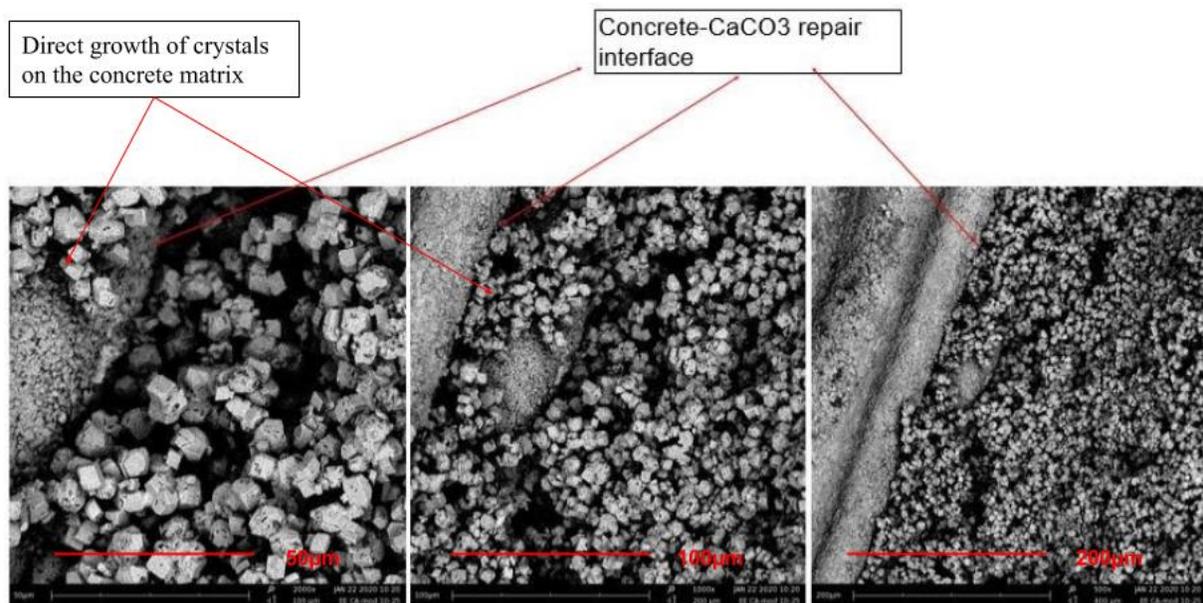


Figure 10: Images of the interface between the repair and the concrete. From left to right the magnification is 2000x, 1000x, and 500x. The crystals are precipitated calcium carbonate. The image shows that there are several gaps in the repair matrix, but the trough is evenly filled.

Figure 11 shows a precipitated calcium carbonate crystal at full magnification (20000x). The surface topography is rough, and the crystal contains several cracks, but there is good bonding between neighboring crystals. Not all the calcium carbonate crystals are cracked, but the distribution appears to be random.

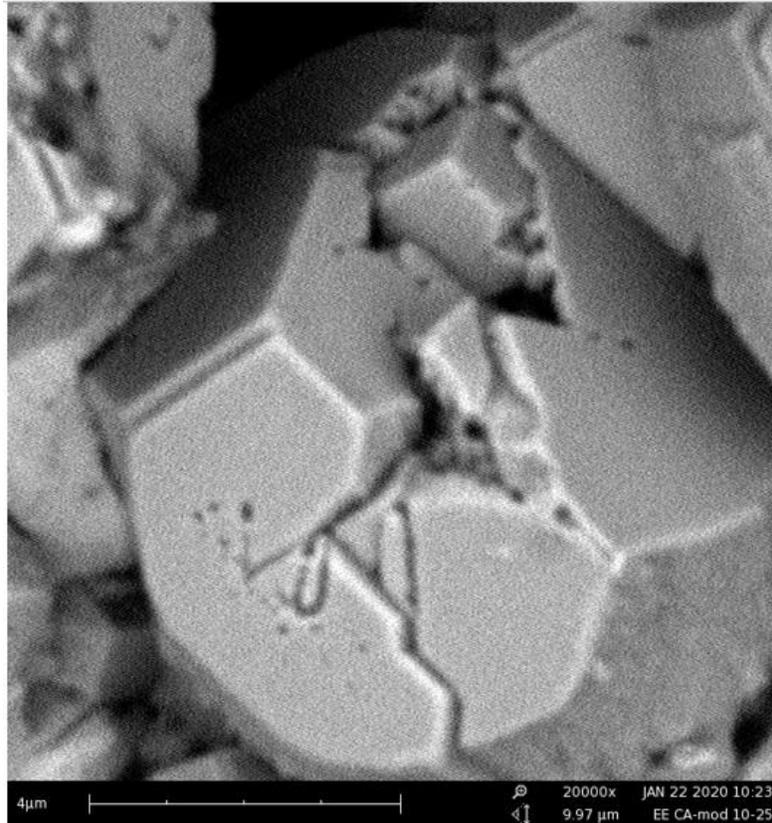


Figure 11: An SEM graphic of a calcium carbonate crystal at 20000x. The crystal has several imperfections and cracks, but it appears to be well bonded to other crystals.

There also appears to be interfacial growth of calcium carbonate crystals in the concrete matrix near the boundary of the repair. Figure 12 shows direct interfacial growth of calcium carbonate crystals in the CA-Add concrete. Figures 10 and 12 show the variable crystal adherence onto a CA-Add substrate; each is from identical but separate samples prepared side-by-side in the laboratory. While Figure 10 shows direct crystal growth on the concrete, the crystals in Figure 12 appear to sit in a glue-like substance and grow into the substrate. This suggests that the CA-Add substrate is promoting an advanced bond between the calcium carbonate repair material and the cement matrix, but not in all specimens. The interfacial growth suggests that the addition of carbonic anhydrase to the concrete mix changes the properties of the bond to the repair. However, the variable appearance suggests that further work is needed to obtain consistent repair characteristics.

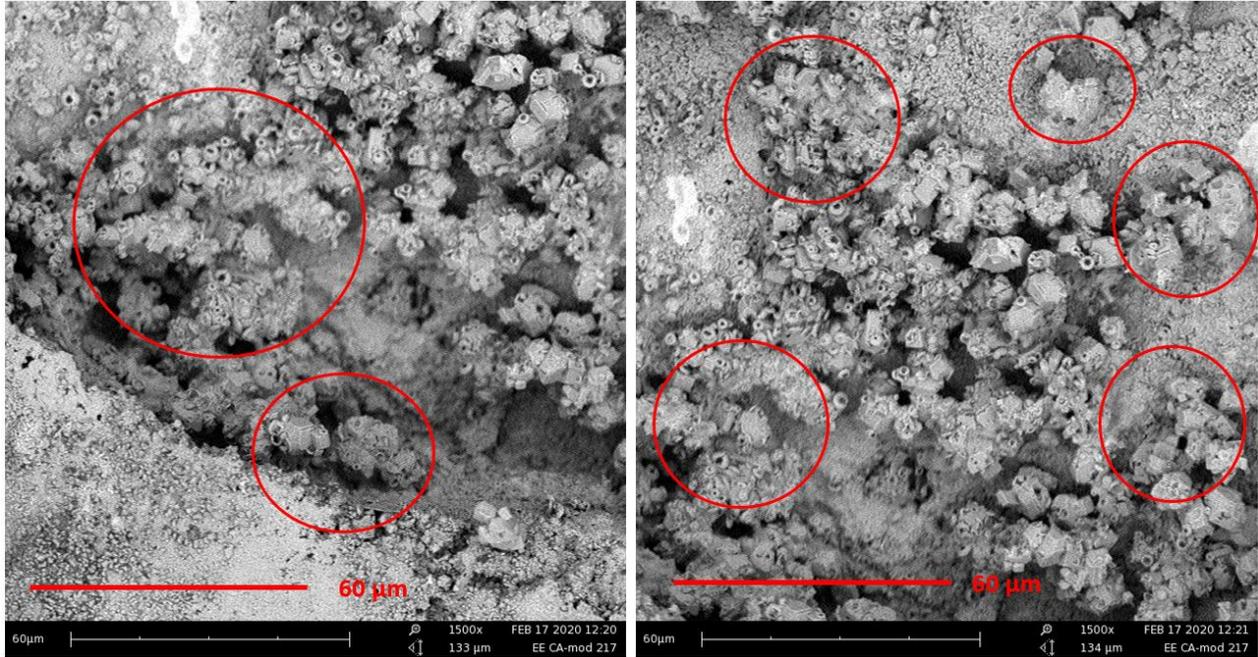


Figure 12: SEM graphic of the repair-concrete interface. The red circles show areas of direct interfacial growth of calcium carbonate crystals onto the concrete matrix. The area pictured is at the edge of the calcium carbonate filled notch. The magnification of the image is 1500x.

The surfaces of the control and CA-Add samples were also examined. Away from the notched area, the topographies of the samples were visually similar. There was no crystal growth on the CA-Add samples away from the filled notch. The two samples are compared side-by-side at 5000x magnification in Figure 13.

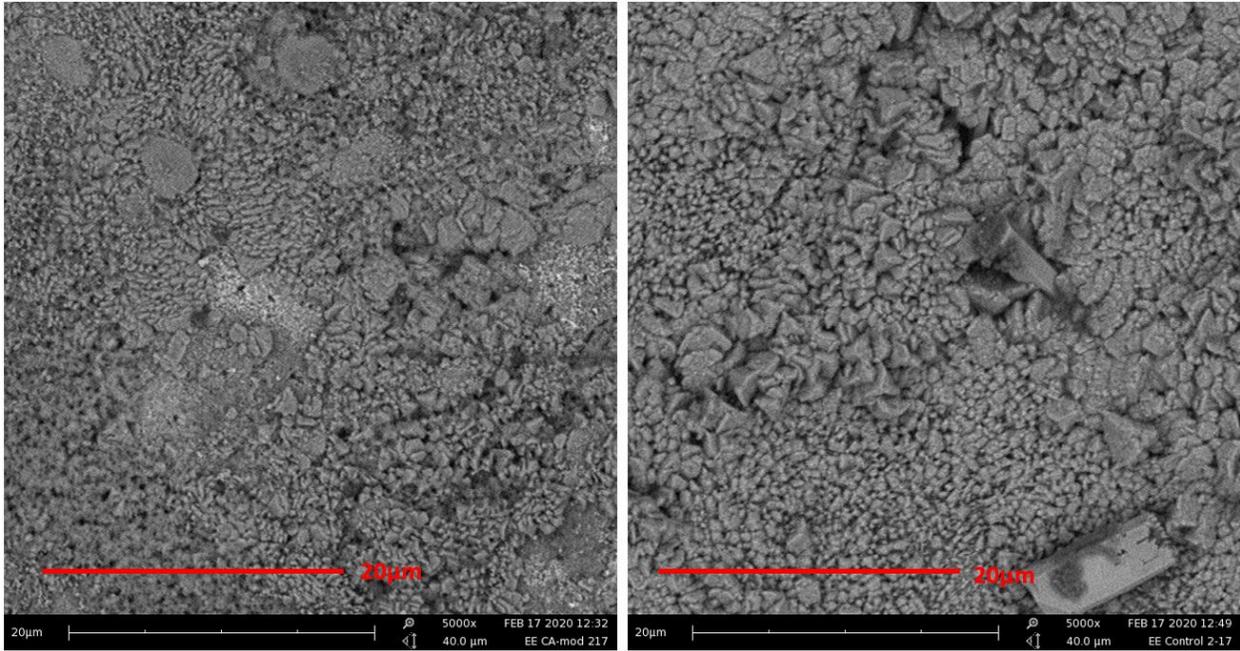


Figure 13: An SEM graphic of the surfaces of CA-Add and control samples respectively. The topographies of the different samples are similar in roughness and homogeneity. The magnification of the image is 5000x.

4.2 Rapid Chloride Permeability

RCPT was determined to be the most appropriate method for comparing the durability of the enzyme modified concrete to normal OPC concrete. Although RCPT can have a large amount of variation in results and is not always accurate for predicting durability in real environmental conditions, it demonstrated that there is a difference in the resistance of the CA-Add mix to the control mix. The overall results shown in Table 3 and Figure 14 show that the concrete mix containing carbonic anhydrase has a greater resistance to chloride ion penetration. The permeability class is determined according to ASTM standard C1202. The exact classifications are outlined below in Table 2.

Table 2: According to ASTM C1202, samples passing a charge greater than 4,000 coulombs has high permeability. Samples passing between 2,000 and 4,000 coulombs have moderate permeability. Samples passing between 1,000 and 2,000 coulombs have low permeability, and samples passing less than 1,000 coulombs have low to negligible permeability.

Charge Passed (coulombs)	Chloride Penetrability Ion
>4,000	High
2,000-4,000	Moderate
1,000-2,000	Low
100-1,000	Very Low
<100	Negligible

The cumulative results for RCPT are shown below in Table 3. Each sample type has two averages, one for a two-week curing time and one for an 11-week curing time. All samples had the same mix proportions and were prepared the same way.

Table 3: Chloride ion penetrability and charge passed in control and CA-mod samples. The average charge passed for samples cured at two weeks is eleven percent lower for CA-mod samples than control samples. At two months, the average for CA-mod is 39 percent lower than the average for control.

Sample	Charge Passed (Coulombs)	Perm. Class	Days Cured	Average (Coulombs)
Control	4320	High	15	4302
	5341	High	15	
	3244	Mod	23	
	3509	Mod	77	4085
	5085	High	80	
	3581	Mod	82	
CA-Mod	3643	Mod	14	3868
	3156	Mod	14	
	4806	High	14	
	2707	Mod	76	2760
	2833	Mod	76	
	2740	Mod	76	

The RCPT results are represented graphically in Figure 14. The two linear trend lines are between the averages of the two types of samples at two weeks of curing and two months of curing.

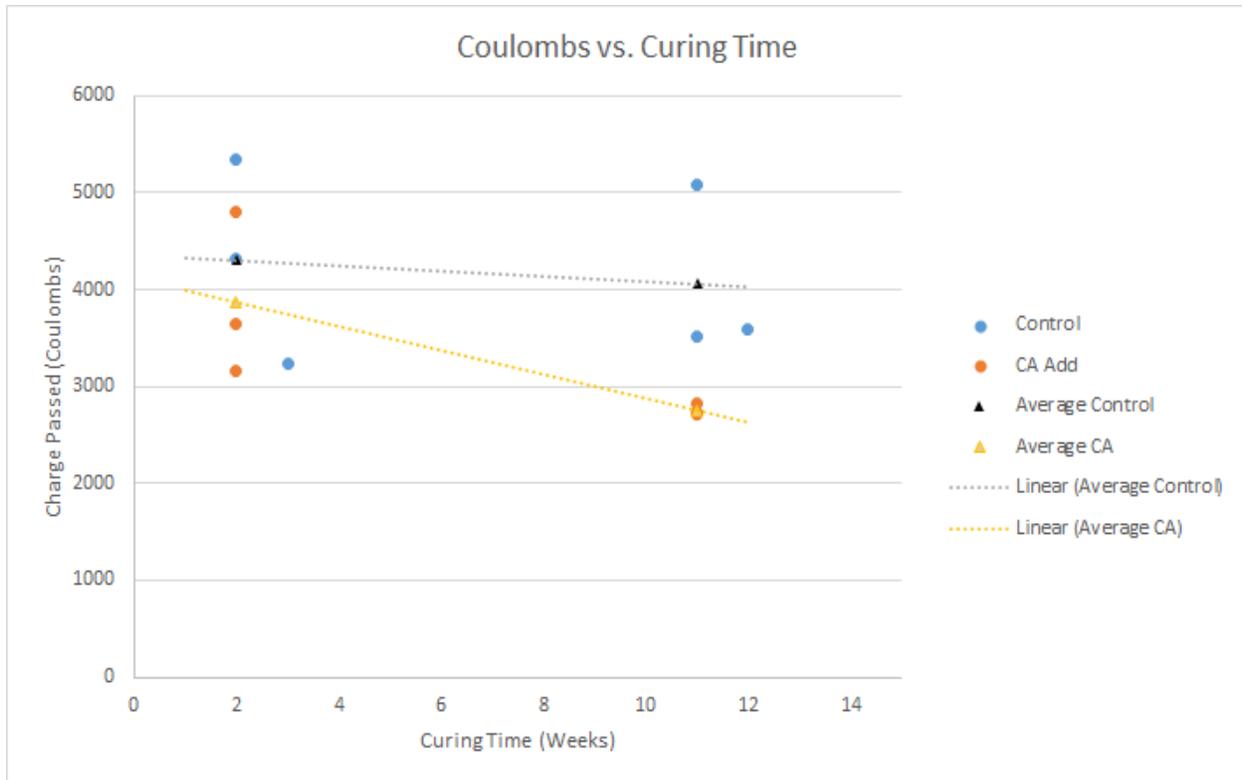


Figure 14: Graphic representation of RCPT results. The permeability of control samples decreased at 36.2 coulombs per week, while the permeability of CA-Add samples decreased at 184.7 coulombs per week.

Although the CA-Add average was lower than the control average at both ages, the results were not statistically significant for either age. The p-value of a student's t-test for the two-week age was 0.61. The result was not unexpected, because the averages of the 2-week test groups are different only by 217 coulombs. Furthermore, the data set is not extensive, which may contribute to the high p-value. The p-value of a student's t-test for the 11-week age was 0.13. Both t-test values suggest that the means are similar, and the data is not statistically significant. It is possible the t-test values are so high because the number of samples in each group is only 3. However, the reduction in the p-value from 0.61 to 0.13 over 2 to 11 weeks, respectively, shows promise that with more data points and refined methods, a more statistically significant outcome could be obtained.

Although the data showed no statistical significance, there was a greater decline in permeability and lower averages overall in the CA-Add mix. The result was achieved with only the addition of 5 μL of 100 μM carbonic anhydrase. The number of samples tested during the project was mostly limited by time during the study. It is recommended to further study the CA-Add mix by conducting more rapid chloride permeability tests in the future at different ages of curing.

5.0 Conclusions and Recommendations

This study was concerned with the micro-characteristics of the enzyme modified concrete as well as its durability. The SEM images suggest that although there are some gaps in the repair matrix, the trough it evenly filled and there is good bonding between neighboring crystals. All samples prepared for SEM imaging followed strict laboratory procedures. All samples were cast on a smooth acrylic surface to create flat surfaces. The notch that was filled with the repair had to be small, only 1 mm deep. In order to create such precision, a small printer nozzle was used to create the mold. All samples contained the same mix proportions and were prepared the same way. The samples that had the notch filled with the enzyme repair were carefully taped so the repair would only fill the notch and not affect the concrete surface. Even with the strict procedures, there were many differences observed in crystal growth. Some crystals appear to have grown on top of the concrete, while other crystals have grown into the concrete matrix and created a stronger bond. Furthermore, some precipitated crystals have cracks and chips, while others do not. Further study is needed to understand why there is such variation in crystal growth, even in the same sample.

Furthermore, the enzyme repair method itself used few ingredients, but took a large amount of time to repair the small notches. A strict laboratory procedure was followed, but the procedure does not seem viable on a large scale. The procedure also does not seem viable for large applications, since it is topically applied and needs a large amount of solution to effectively fill a crack or notch. Although the repair is not commercially viable in its current state, it could be an ecologically sensitive alternative to concrete repair. Carbonic anhydrase is biodegradable and poses no risk to human health. Furthermore, the repair consumes carbon dioxide and could potentially sequester it from the environment. The repair is still in early stages of development, and with more study it could have the potential to be a successful alternative in concrete repair.

The RCPT results determined that the average permeability of samples tested at 11 weeks was 39% lower for enzyme modified samples than control samples. Although the results are promising, they were of no statistical significance based on a student's t-test. All samples prepared for RCPT followed strict laboratory procedures. The mix design was the same for all samples, with a large proportion of coarse aggregate in order to increase the resistivity of the samples. The samples were cut with a diamond saw to create smooth surfaces, and the ASTM 1202 test procedure was modified to create a better seal with vacuum grease on the test setup. Even with strict methods and practice, there was still large variation in results. This is likely because RCPT is not a consistently repeatable test. According to the ASTM C1202 standard, results from the same batch of concrete can differ by up to 34% (ASTM, 2019). Conducting more RCPT testing on normal and enzyme modified concrete would give more data and hopefully results that are similar to the ones found in this study. The number of samples tested was limited by time and manpower during the study, but more data would increase the likelihood of finding statistically significant results. The enzyme modified concrete could also be studied with a less variable testing method, which would increase confidence in the results.

In the future, energy dispersive X-ray analysis (EDX) could be conducted to study the elemental makeup of the enzyme modified concrete. EDX would aid in understanding the differences between normal OPC concrete and CA modified concrete by determining if there is more calcium present in the enzyme modified samples, or if it is similar to OPC concrete in terms of minerals present. The presence of more calcium in the enzyme modified concrete would suggest that there is more calcium carbonate due to the addition of the enzyme. Calcium carbonate makes the concrete matrix denser and less permeable by filling pores within the concrete. EDX would aid in understanding the differences between OPC concrete and enzyme modified concrete.

Additionally, strength testing, both compressive and tensile, would determine if the repair method returns strength to damaged specimens. Testing the enzyme modified concrete on a larger scale could be a next step in studying the repair method. It would also give insight on the quantitative bond strength of the repair. Another investigation could be conducted to see if differing amounts of carbonic anhydrase in the mix design affects the durability or strength of the concrete.

Overall, the enzyme repair method is a new and innovative way of healing cracks in concrete and strengthening the concrete itself. Biologic concrete, namely the use of bacteria to heal concrete, has been studied increasingly often in the past. Most studies focus on the use of the enzyme urease to precipitate calcium carbonate. One drawback of urease is that it creates ammonia during hydrolysis. The ammonia can corrode any steel reinforcing that is present. Carbonic anhydrase does not create ammonia or any other harmful byproducts. The use of carbonic anhydrase in biologic concrete is relatively new and there are many unknowns to be investigated. There is still much to be understood about the enzyme repair, but the current results are promising and there are many paths to explore in the future.

6.0 References

- Achal, V., & Pan, X. (2011). Characterization of urease and carbonic anhydrase producing bacteria and their role in calcite precipitation. *Current Microbiology*, 62(3), 894-902. doi:10.1007/s00284-010-9801-4
- Alshalif, A. F., Irwan, J. M., Othman, N., Al-Gheethi, A., Hassan, A., & Nasser, I. M. (2018). Potential of carbonic anhydrase and urease bacteria for sequestration of CO₂ into aerated concrete. *MATEC Web Conf.*, 250 Retrieved from <https://doi.org/10.1051/mateconf/201825003004>
- American Society of Civil Engineers. (2017). America's infrastructure grade. Retrieved from <https://www.infrastructurereportcard.org/>
- ASTM International. (2019). *C1202-19 Standard Test Method for Electrical Indication of Concrete's Ability to Resist Chloride Ion Penetration*. Retrieved from <https://doi.org/10.1520/C1202-19>
- Barcelo, L., Kline, J., Walenta, G., & Gartner, E. (2014). Cement and carbon emissions. *Materials and Structures*, 47(6), 1055-1065. doi:10.1617/s11527-013-0114-5
- De Belie, N., Gruyaert, E., Al-Tabbaa, A., Antonaci, P., Baera, C., Bajare, D., . . . Jonkers, H. M. (2018). A review of self-healing concrete for damage management of structures. *Advanced Materials Interfaces*, 5(17), 1800074. doi:10.1002/admi.201800074
- Gardner, D., Lark, R., Jefferson, T., & Davies, R. (2018). *A survey on problems encountered in current concrete construction and the potential benefits of self-healing cementitious materials* doi://doi.org/10.1016/j.cscm.2018.02.002
- Grantham, M. (2011). *Concrete repair : A practical guide*. London ;: Routledge.
- How to get licensed. (2020). Retrieved from <https://www.nspe.org/resources/licensure/how-get-licensed>
- IPCC. (2019). *Climate change and land*. (). Retrieved from https://www.ipcc.ch/site/assets/uploads/2019/08/Edited-SPM_Approved_Microsite_FINAL.pdf
- Jonkers, H. M. (2017). *Toward bio-based geo- & civil engineering for a sustainable society* doi://doi.org/10.1016/j.proeng.2017.01.323
- Jonkers, H. M., Thijssen, A., Muyzer, G., Copuroglu, O., & Schlangen, E. (2010). *Application of bacteria as self-healing agent for the development of sustainable concrete* doi://doi.org/10.1016/j.ecoleng.2008.12.036
- Li, W., Xu, C., Ho, S. C. M., Wang, B., & Song, G. (2017). Monitoring concrete deterioration due

- to reinforcement corrosion by integrating acoustic emission and FBG strain measurements. *Sensors (Basel, Switzerland)*, 17(3), 657. doi:10.3390/s17030657
- Monaghan, G. (2017, Concrete chemistry and protective coatings.22, 96+. Retrieved from https://link.gale.com/apps/doc/A499719485/ITOF?u=mlin_c_worpoly&sid=ITOF&xid=228ce811
- Muhammad, N. Z., Shafaghat, A., Keyvanfar, A., Abd. Majid, M. Z., Ghoshal, S. K., Mohammadyan Yasouj, S. E., . . . McCaffer, R. (2016). *Tests and methods of evaluating the self-healing efficiency of concrete: A review* doi://doi.org/10.1016/j.conbuildmat.2016.03.017
- National Academy of Engineering.Restore and improve urban infrastructure. Retrieved from <http://www.engineeringchallenges.org/challenges/infrastructure.aspx>
- Qian, C., Ren, L., Xue, B., & Cao, T. (2016). Bio-mineralization on cement-based materials consuming CO2 from atmosphere. *Construction and Building Materials*, 106, 126-132. doi:10.1016/j.conbuildmat.2015.10.105
- Rahbar, N., Rosewitz, J., & Scarlata, S. (2019). *Naturally motivated concrete healing*. Unpublished manuscript. Retrieved from https://nam03.safelinks.protection.outlook.com/?url=https%3A%2F%2Fdrive.google.com%2Ffile%2Fd%2F1yOWQRBE0DuAhOYoxYgEhfsj5Rzcxv3qZ%2Fview%3Fusp%3Ddrive_web&data=02%7C01%7Cejedwardson%40wpi.edu%7C515aa7a7bdf41d8c7f808d7273a171c%7C589c76f5ca1541f9884b55ec15a0672a%7C0%7C0%7C637021002763212753&sdata=8HrX%2FXonJlfzvn9s6D9TqekOL4RdZF1ZMwVtt6mTd6Y%3D&reserved=0
- Rodgers, L. (2018, Dec 17,). Climate change: The massive CO2 emitter you may not know about. *BBC News* Retrieved from <https://www.bbc.com/news/science-environment-46455844>
- Šavija, B., & Luković, M. (2016). *Carbonation of cement paste: Understanding, challenges, and opportunities* doi://doi.org/10.1016/j.conbuildmat.2016.04.138
- Scientific principles. (1995). Retrieved from <http://matse1.matse.illinois.edu/concrete/concrete.html>
- Seifan, M., Samani, A. K., & Berenjian, A. (2016). Bioconcrete: Next generation of self-healing concrete. *Applied Microbiology and Biotechnology*, 100(6), 2591-2602. doi:10.1007/s00253-016-7316-z
- Thomas, J., & Jennings, H. (2018). The science of concrete. Retrieved from <http://iti.northwestern.edu/cement/index.html>
- Vaysburd, A., Bissonnette, B., & von Fay, K. (2014). *Compatibility issues in design and implementation of concrete repairs and overlays*. (). Denver: Retrieved from https://www.usbr.gov/research/projects/download_product.cfm?id=1416

von Fay, K. F. (2015). *Guide to concrete repair* (2nd ed.) U.S. Department of the Interior Bureau of Reclamation. Retrieved from https://www.usbr.gov/tsc/techreferences/mands/mands-pdfs/Guide2ConcreteRepair2015_Final.pdf

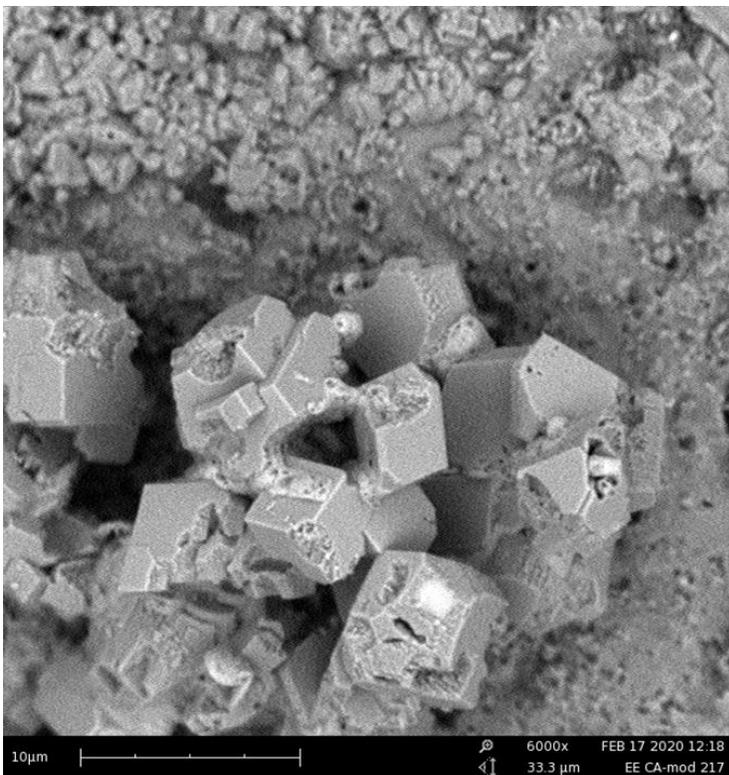
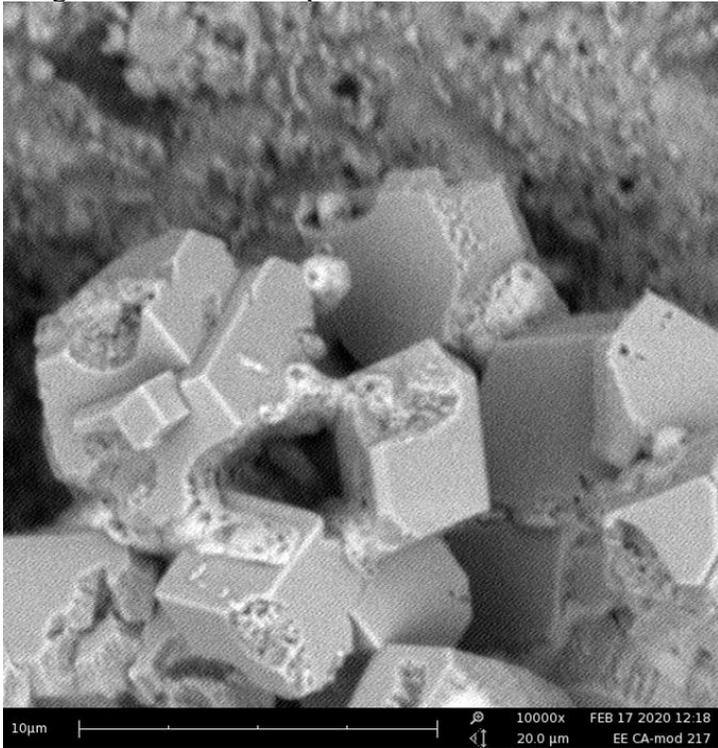
Wang, X., Nguyen, M., Stewart, M., Syme, M., & Leitch, A. (2011). *Analysis of climate change impacts on the deterioration of concrete infrastructure part 3: Case studies of concrete deterioration and adaptation. synthesis report* doi:<https://doi.org/10.4225/08/584ee966eac70>

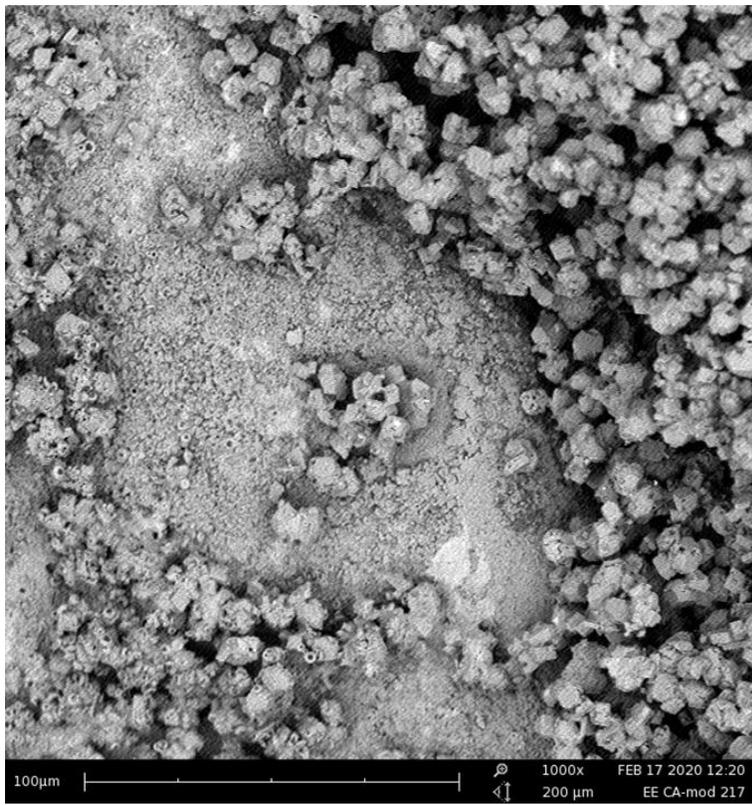
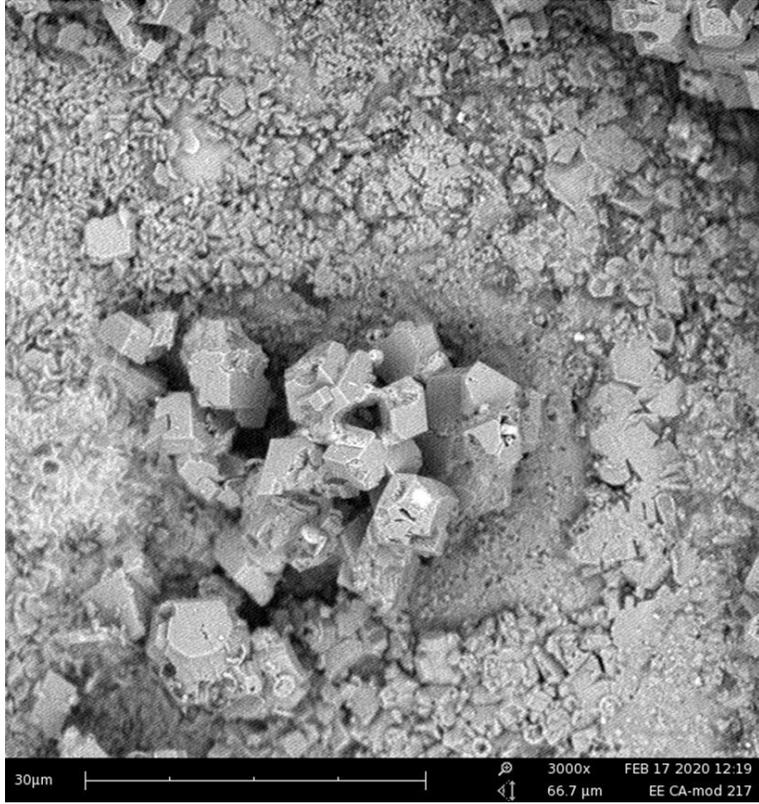
Wang, X., Nguyen, M., Stewart, M., Syme, M., & Leitch, A. (2010). *Analysis of climate change impacts on the deterioration of concrete infrastructure – part 1: Mechanisms, practices, modelling and simulations – A review* doi:10.13140/RG.2.1.3984.5521

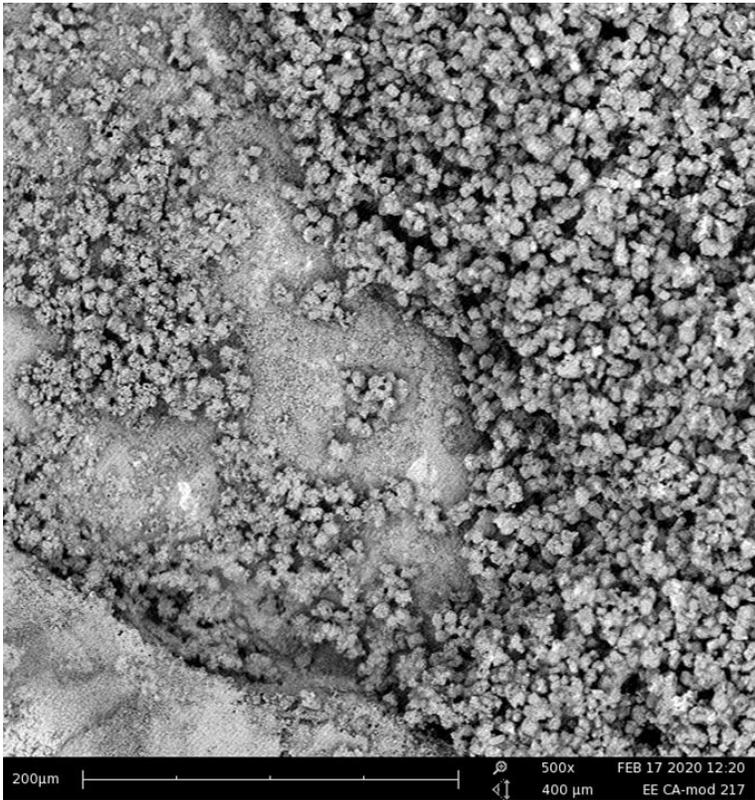
Wiktor, V., & Jonkers, H. M. (2011). *Quantification of crack-healing in novel bacteria-based self-healing concrete* doi://doi.org/10.1016/j.cemconcomp.2011.03.012

Appendix A: SEM Image Gallery

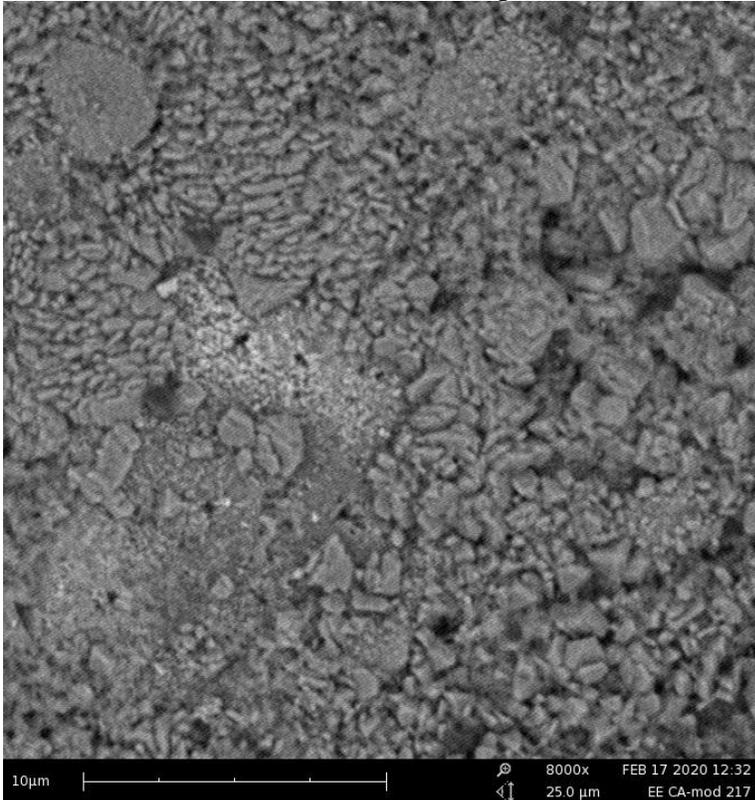
Images of CA-Add samples with the calcium carbonate repair:

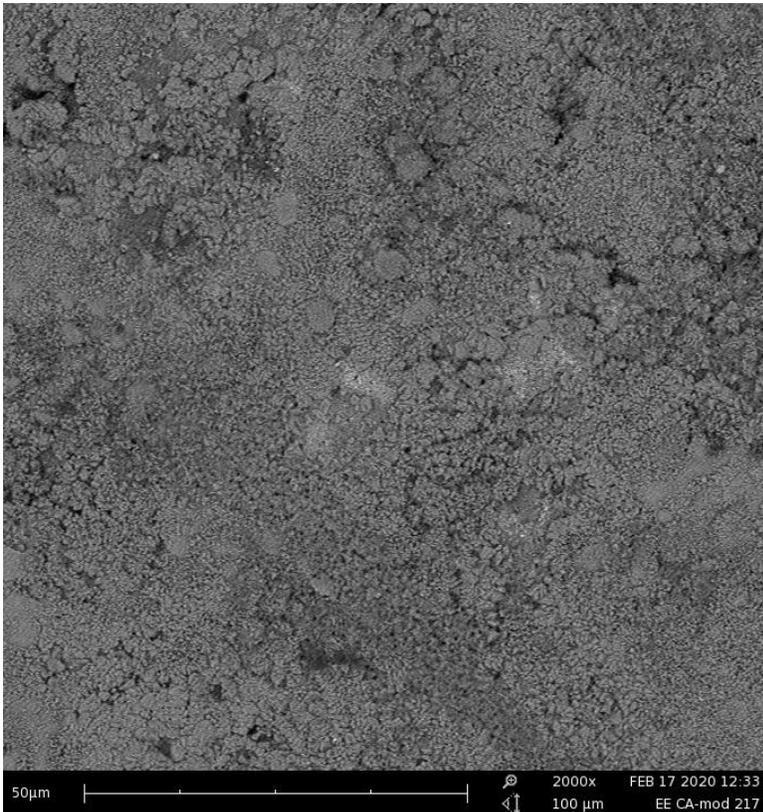
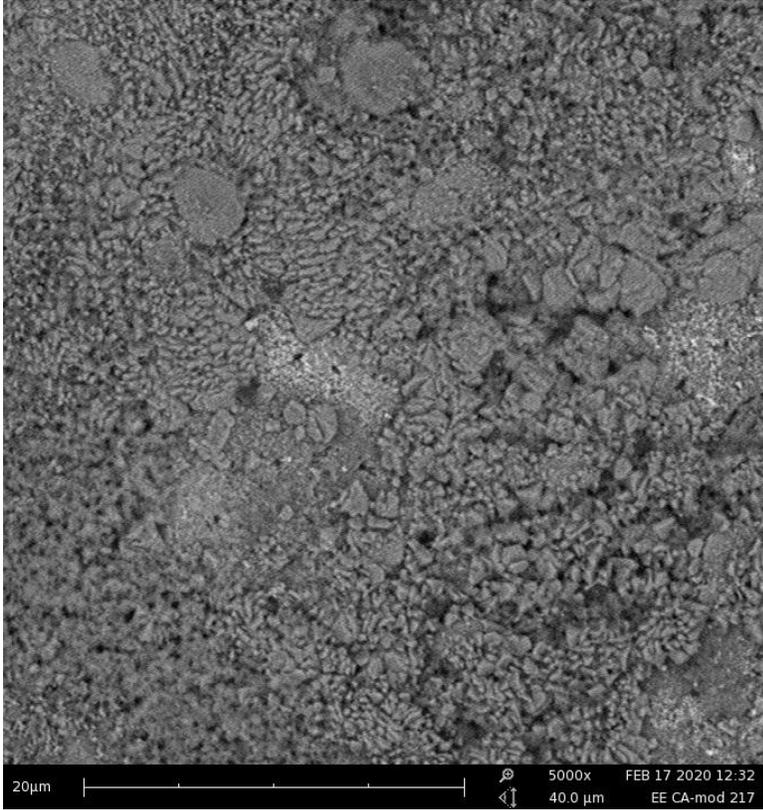




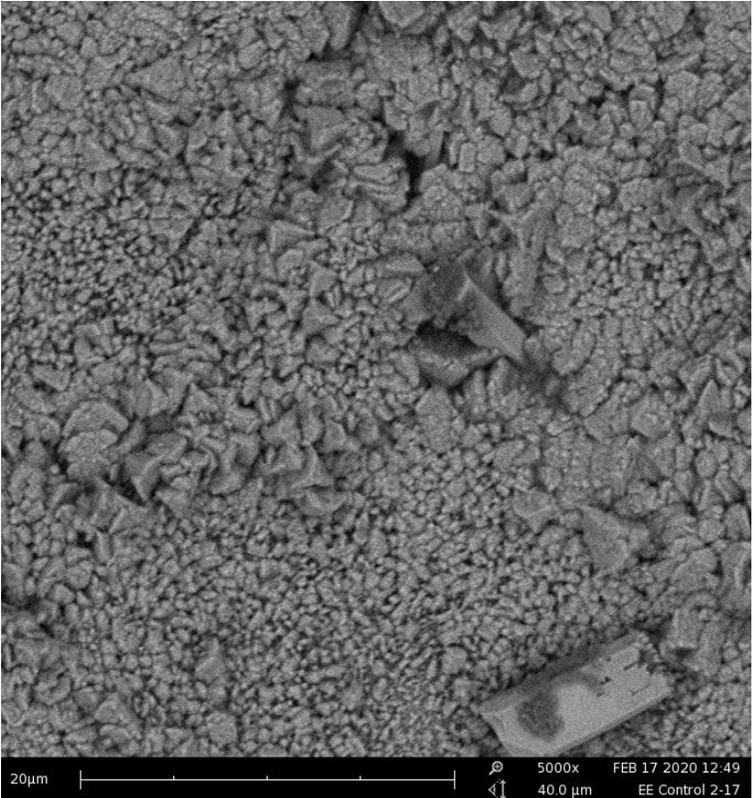
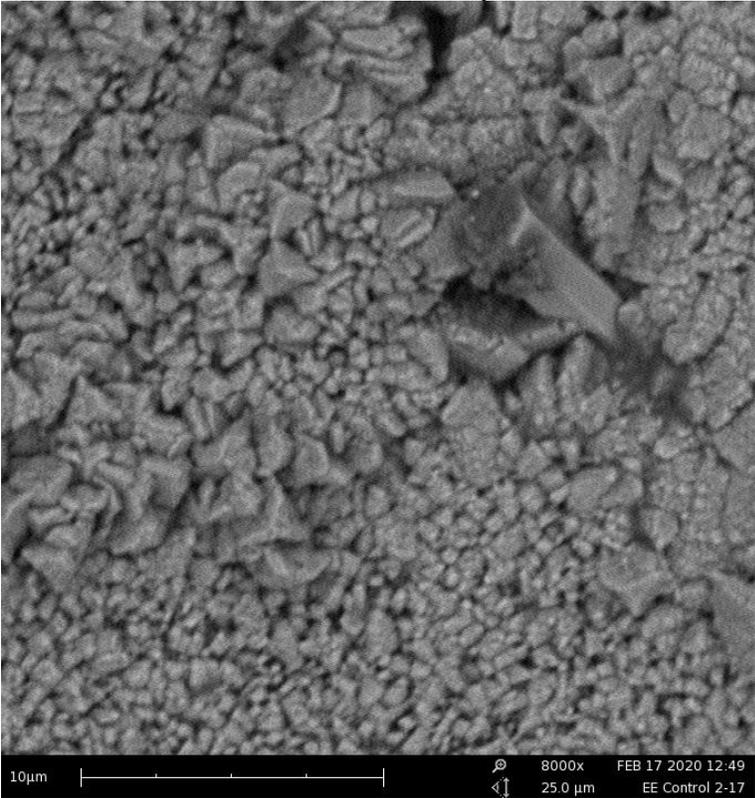


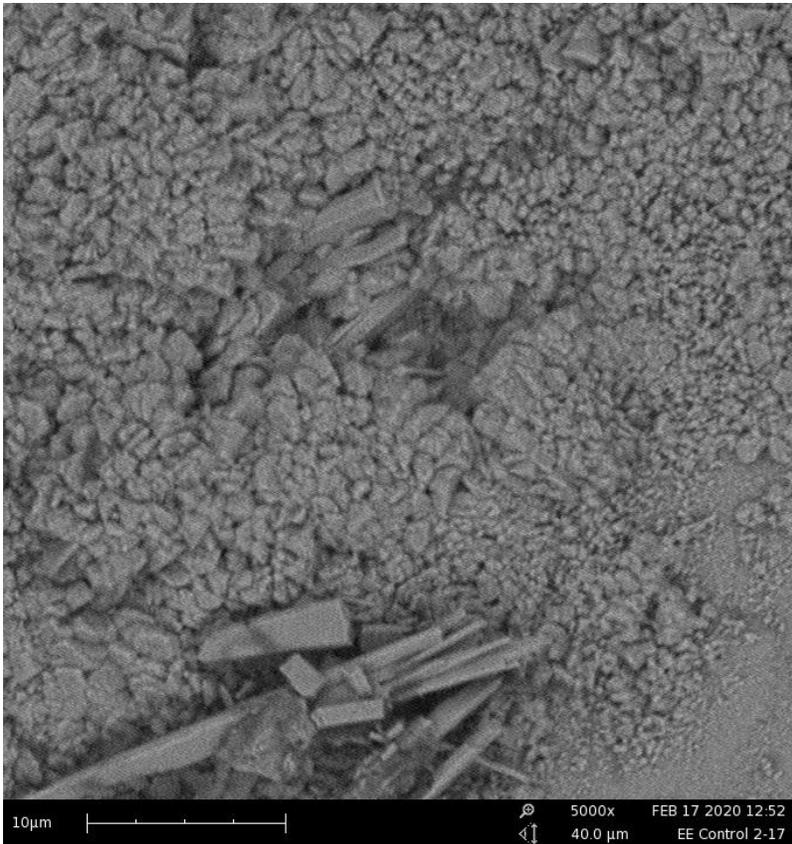
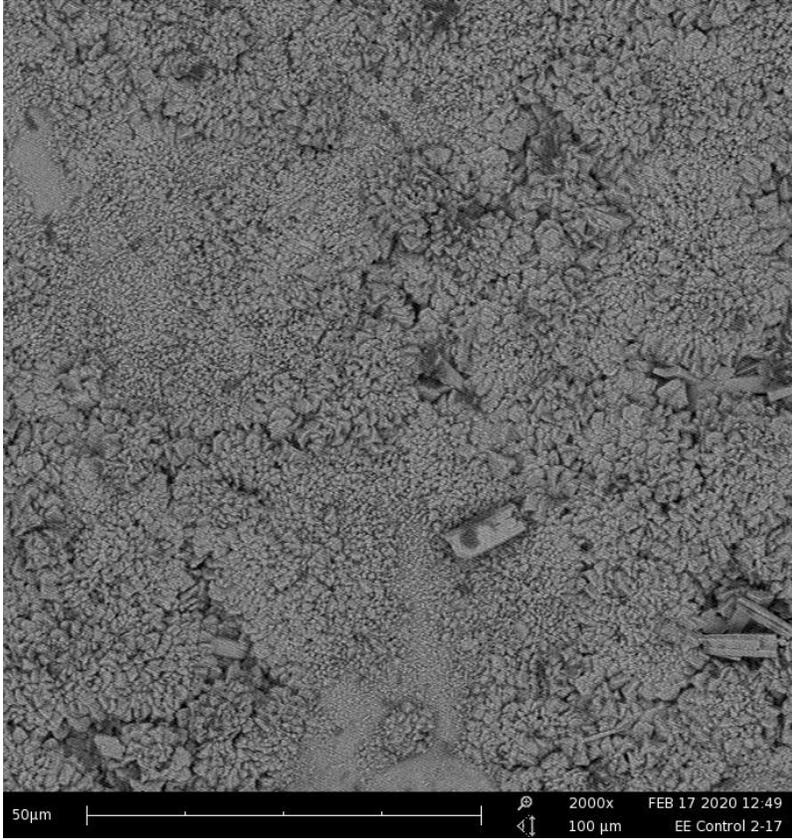
Surface characteristics of CA Add samples:



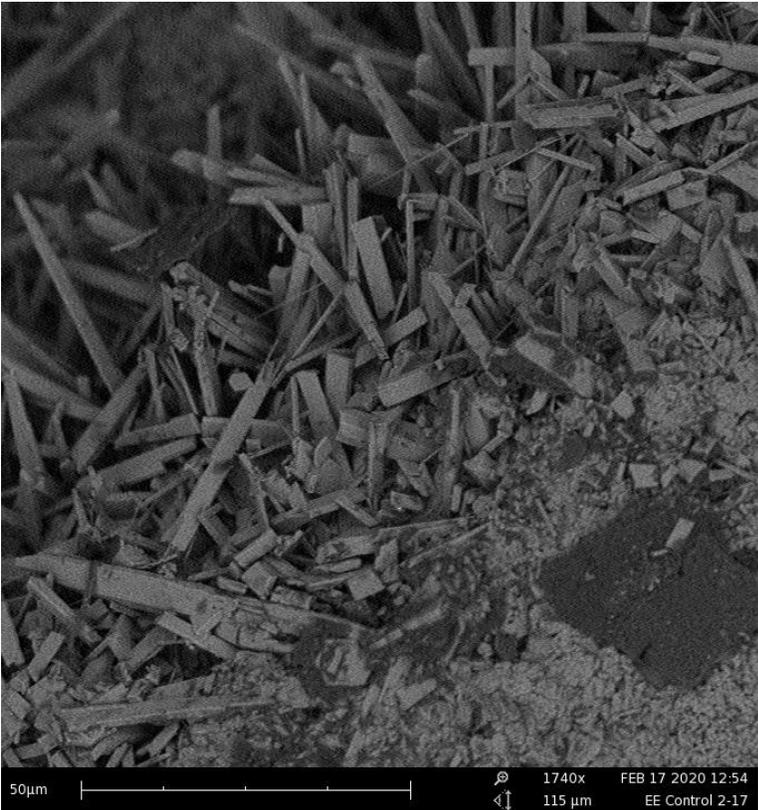
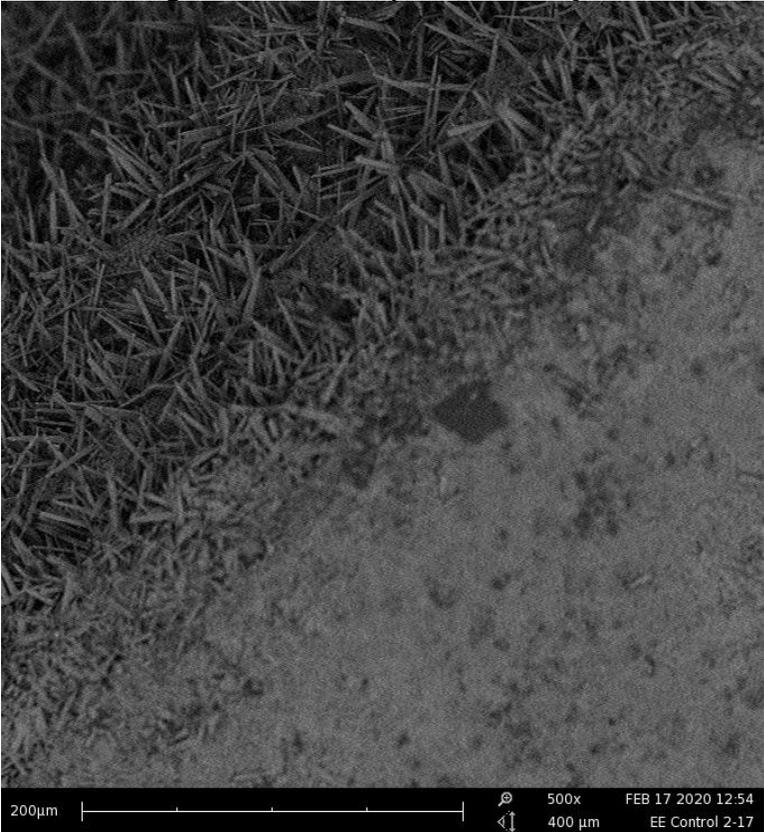


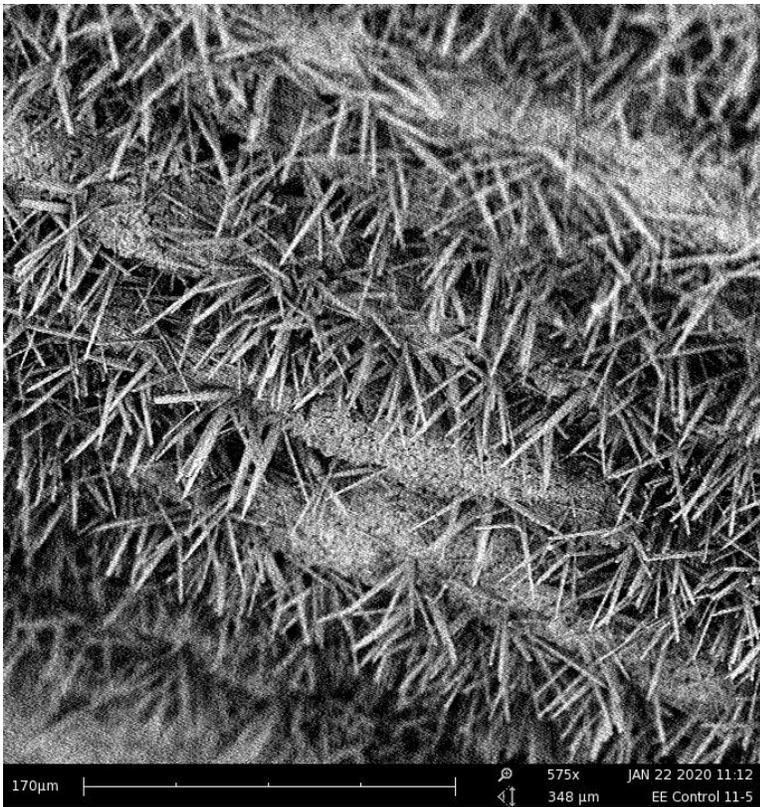
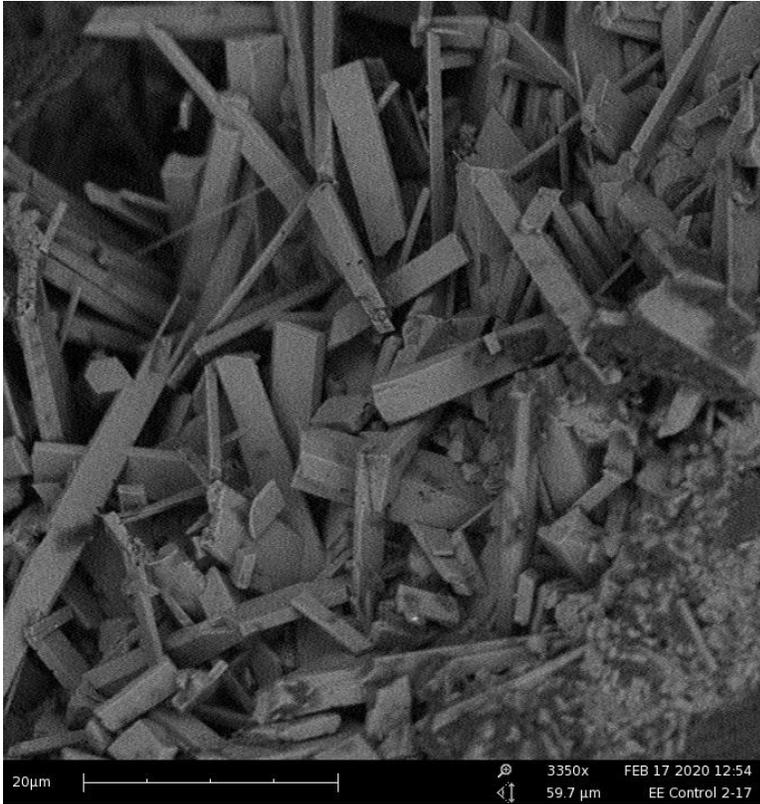
Surface characteristics of control samples:





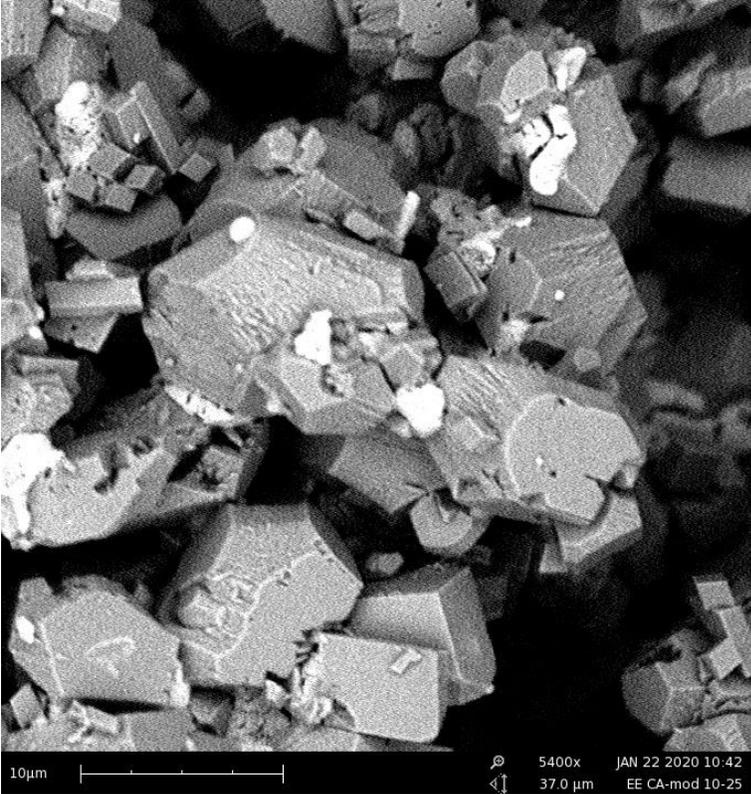
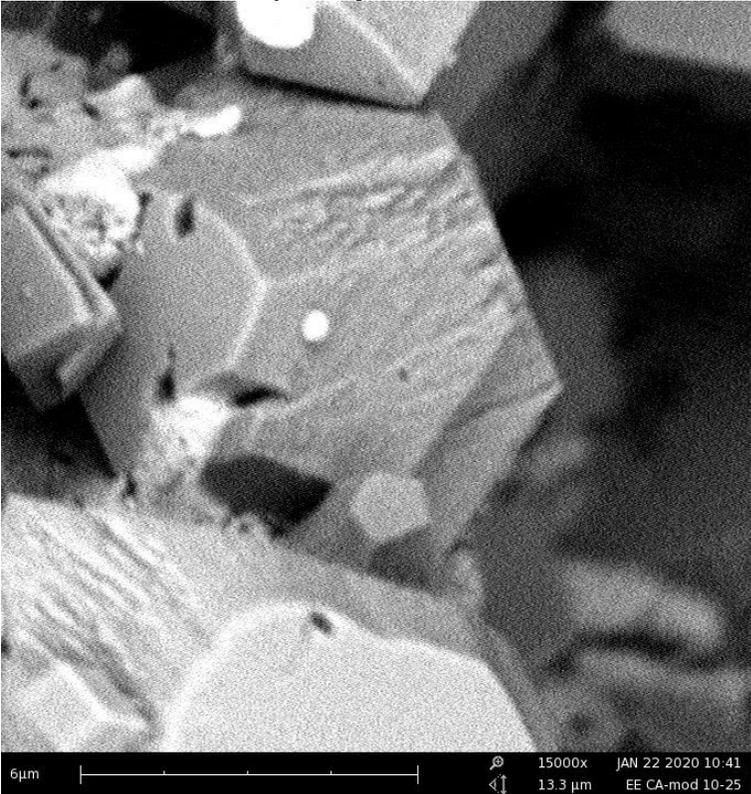
Unfilled trough of control samples (with unhydrated cement crystals):

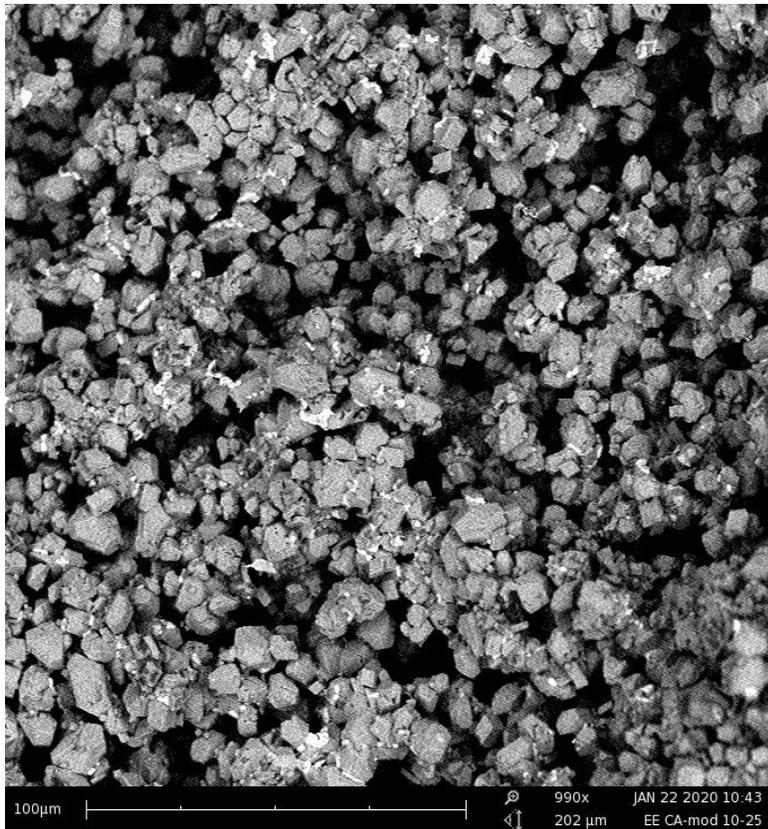
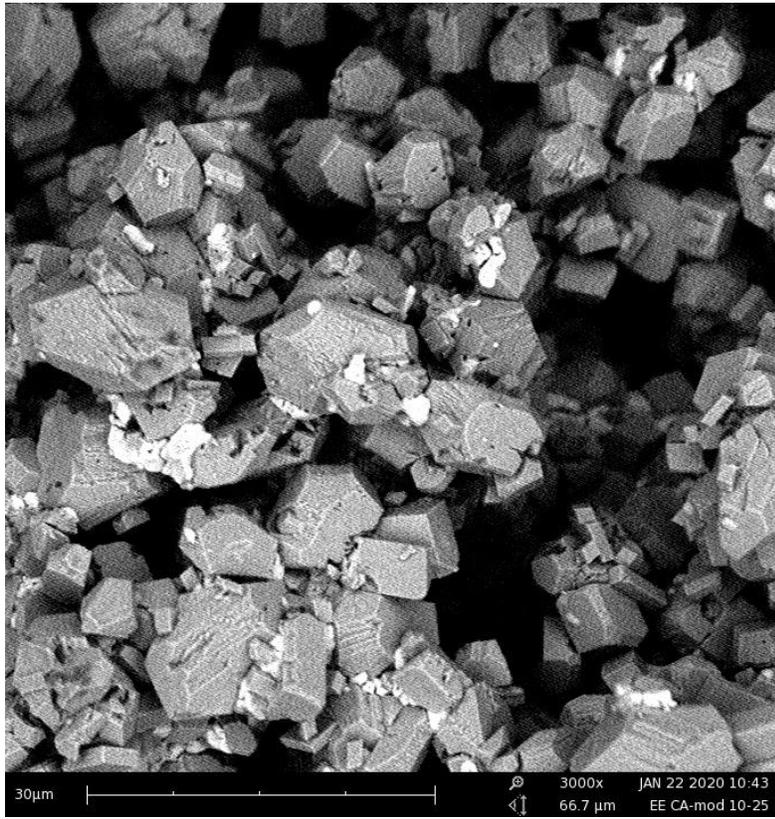


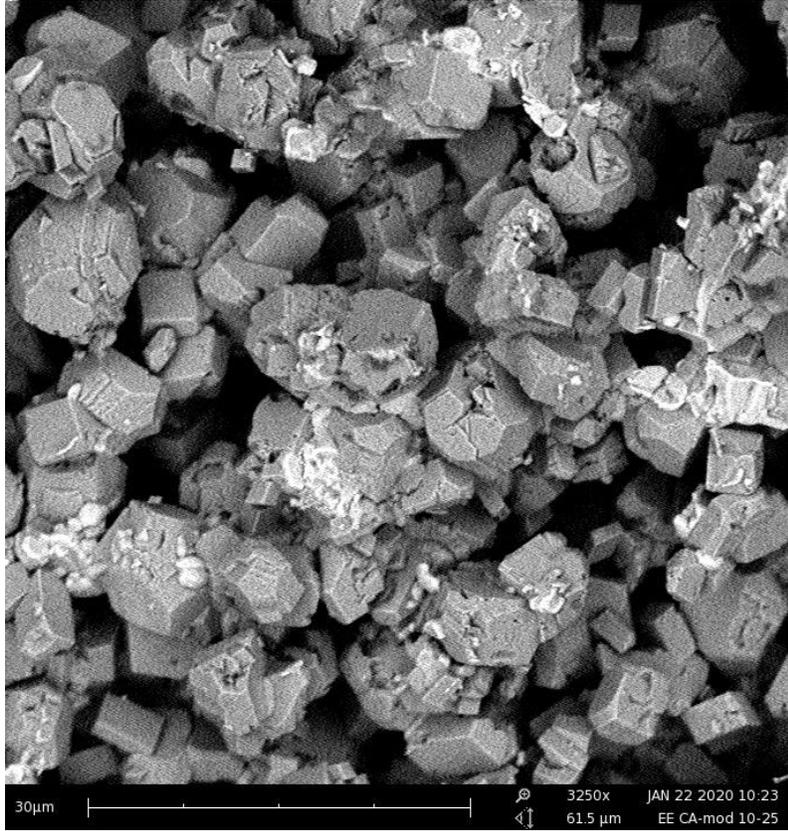




Calcium carbonate repair crystals:







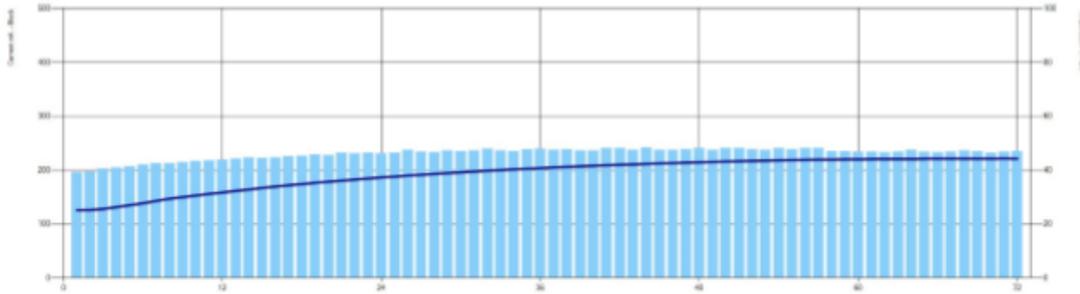
Appendix B: RCPT Results

Control 1



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 4320.24 coulomb
 Penetrability Class: High
 Instrument Number: 181624
 Channel Number: 5
 Report Saved: Thursday, November 21, 2019 2:44 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: FIN
 System ID: I0065
 Test ID: ejedwardson



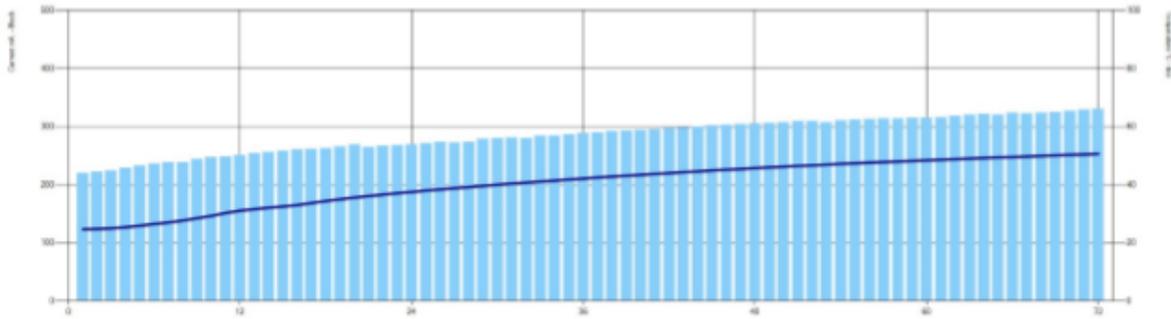
Time	°C	mA									
00:05	25	195.4	01:35	35	228.6	03:05	41	237.2	04:35	44	238.6
00:10	25	197.1	01:40	36	227.6	03:10	41	238.4	04:40	44	241.0
00:15	26	202.8	01:45	36	231.4	03:15	41	236.6	04:45	44	240.6
00:20	26	204.7	01:50	37	230.5	03:20	42	236.1	04:50	44	235.4
00:25	27	207.2	01:55	37	231.5	03:25	42	240.8	04:55	44	235.4
00:30	28	210.6	02:00	37	230.3	03:30	42	240.6	05:00	44	234.6
00:35	29	212.3	02:05	38	232.2	03:35	42	237.3	05:05	44	234.3
00:40	29	212.3	02:10	38	237.1	03:40	42	241.4	05:10	44	233.2
00:45	30	214.4	02:15	38	234.0	03:45	43	237.2	05:15	44	234.6
00:50	31	216.2	02:20	39	232.6	03:50	43	237.9	05:20	44	237.1
00:55	31	217.9	02:25	39	236.3	03:55	43	238.7	05:25	44	233.6
01:00	32	218.4	02:30	39	235.6	04:00	43	240.2	05:30	44	233.3
01:05	32	220.9	02:35	40	235.9	04:05	43	237.8	05:35	44	233.9
01:10	33	222.8	02:40	40	239.1	04:10	43	241.1	05:40	44	236.3
01:15	33	222.1	02:45	40	236.7	04:15	43	240.5	05:45	44	235.7
01:20	34	223.5	02:50	40	235.5	04:20	43	238.5	05:50	44	232.2
01:25	34	225.6	02:55	41	238.6	04:25	44	237.6	05:55	44	234.1
01:30	35	226.7	03:00	41	239.9	04:30	44	240.3	06:00	44	234.8

Control 2



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 5341.08 coulomb
 Penetrability Class: High
 Instrument Number: 181624
 Channel Number: 6
 Report Saved: Friday, November 22, 2019 2:50 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: FIN
 System ID: I0066
 Test ID: ejedwardson



Time	°C	mA									
00:05	25	220.0	01:35	35	265.9	03:05	43	290.3	04:35	47	311.8
00:10	25	221.7	01:40	36	269.1	03:10	43	291.7	04:40	48	312.7
00:15	25	224.8	01:45	36	264.5	03:15	43	293.2	04:45	48	314.0
00:20	25	229.0	01:50	37	266.9	03:20	43	294.3	04:50	48	314.2
00:25	26	232.5	01:55	37	267.8	03:25	44	295.6	04:55	48	314.5
00:30	27	236.1	02:00	38	268.8	03:30	44	296.9	05:00	48	315.3
00:35	27	238.7	02:05	38	271.7	03:35	44	298.2	05:05	49	316.4
00:40	28	238.9	02:10	38	273.5	03:40	45	299.7	05:10	49	317.8
00:45	29	244.3	02:15	39	272.3	03:45	45	302.1	05:15	49	320.7
00:50	29	247.3	02:20	39	273.2	03:50	45	303.3	05:20	49	321.3
00:55	30	248.8	02:25	40	278.5	03:55	45	303.9	05:25	49	320.8
01:00	31	250.9	02:30	40	280.1	04:00	46	304.7	05:30	50	323.1
01:05	32	253.2	02:35	40	280.8	04:05	46	306.0	05:35	50	323.0
01:10	32	256.0	02:40	41	279.7	04:10	46	307.4	05:40	50	323.6
01:15	33	258.4	02:45	41	284.6	04:15	47	309.3	05:45	50	324.8
01:20	33	259.8	02:50	41	284.1	04:20	47	308.9	05:50	50	326.7
01:25	34	261.0	02:55	42	286.2	04:25	47	306.9	05:55	50	329.0
01:30	34	262.6	03:00	42	289.2	04:30	47	311.0	06:00	51	329.8

Control 3



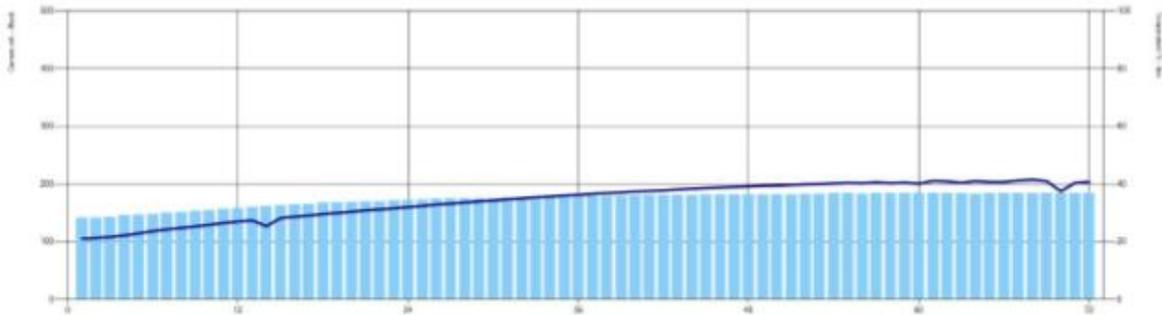
Test-company
Testing street 45
CompagnyCity
Some Country

Your own logo,
size=20x10mm



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 3244.14 coulomb
 Penetrability Class: Moderate
 Instrument Number: 181624
 Channel Number: 8
 Report Saved: Tuesday, December 3, 2019 2:26 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: FIN
 System ID: 10068
 Test ID: ejedwardson



Time	°C	mA									
00:05	21	140.8	01:35	30	167.5	03:05	37	179.1	04:35	40	183.5
00:10	21	140.8	01:40	30	168.4	03:10	37	179.4	04:40	40	183.3
00:15	22	142.3	01:45	31	169.7	03:15	37	179.6	04:45	41	183.5
00:20	22	145.5	01:50	31	170.2	03:20	37	180.1	04:50	40	183.5
00:25	23	146.8	01:55	32	171.5	03:25	38	180.2	04:55	41	183.5
00:30	24	147.9	02:00	32	172.4	03:30	38	180.5	05:00	40	183.5
00:35	24	150.3	02:05	32	173.3	03:35	38	180.7	05:05	41	183.6
00:40	25	151.5	02:10	33	173.7	03:40	38	181.1	05:10	41	183.7
00:45	25	153.7	02:15	33	174.8	03:45	39	181.4	05:15	40	183.5
00:50	26	154.9	02:20	34	174.7	03:50	39	181.6	05:20	41	183.3
00:55	27	156.5	02:25	34	175.1	03:55	39	181.7	05:25	41	183.4
01:00	27	157.9	02:30	34	175.5	04:00	39	181.7	05:30	41	183.6
01:05	27	159.5	02:35	35	176.6	04:05	39	182.2	05:35	41	183.6
01:10	25	160.9	02:40	35	176.8	04:10	40	182.1	05:40	42	183.6
01:15	28	162.9	02:45	35	176.9	04:15	40	182.1	05:45	41	183.6
01:20	29	163.8	02:50	36	177.8	04:20	40	182.5	05:50	38	183.7
01:25	29	165.6	02:55	36	178.1	04:25	40	183.0	05:55	40	183.6
01:30	30	167.0	03:00	36	178.7	04:30	40	183.4	06:00	41	183.4

Control 4



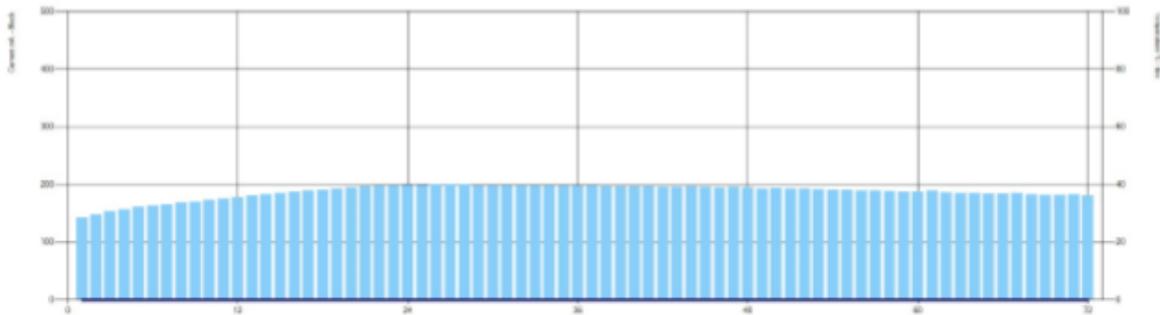
Test-compagny
Testing street 45
CompagnyCity
Some Country

Your own logo,
size=20x80mm



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 3509.26 coulomb
 Penetrability Class: Moderate
 Instrument Number: 181624
 Channel Number: 4
 Report Saved: Wednesday, January 22, 2020 2:53 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: FIN
 System ID: I0072
 Test ID: ejedwardson



Time	°C	mA									
00:05	0	142.1	01:35	0	193.1	03:05	0	198.3	04:35	0	190.6
00:10	0	147.8	01:40	0	194.8	03:10	0	197.0	04:40	0	189.8
00:15	0	152.9	01:45	0	196.6	03:15	0	197.0	04:45	0	189.8
00:20	0	156.7	01:50	0	197.9	03:20	0	196.6	04:50	0	188.5
00:25	0	160.6	01:55	0	198.3	03:25	0	196.5	04:55	0	187.5
00:30	0	162.8	02:00	0	199.3	03:30	0	196.1	05:00	0	187.6
00:35	0	165.4	02:05	0	199.5	03:35	0	196.4	05:05	0	188.9
00:40	0	168.2	02:10	0	200.5	03:40	0	196.7	05:10	0	186.5
00:45	0	170.2	02:15	0	199.9	03:45	0	196.2	05:15	0	185.2
00:50	0	172.6	02:20	0	200.3	03:50	0	195.3	05:20	0	185.0
00:55	0	174.8	02:25	0	198.2	03:55	0	195.4	05:25	0	184.1
01:00	0	177.2	02:30	0	198.0	04:00	0	194.5	05:30	0	184.3
01:05	0	180.5	02:35	0	198.4	04:05	0	192.7	05:35	0	184.9
01:10	0	183.0	02:40	0	198.0	04:10	0	194.2	05:40	0	182.9
01:15	0	184.9	02:45	0	198.2	04:15	0	192.7	05:45	0	182.1
01:20	0	186.9	02:50	0	198.3	04:20	0	192.8	05:50	0	181.5
01:25	0	188.8	02:55	0	197.7	04:25	0	191.4	05:55	0	183.2
01:30	0	190.7	03:00	0	198.1	04:30	0	190.4	06:00	0	181.0

Control 5



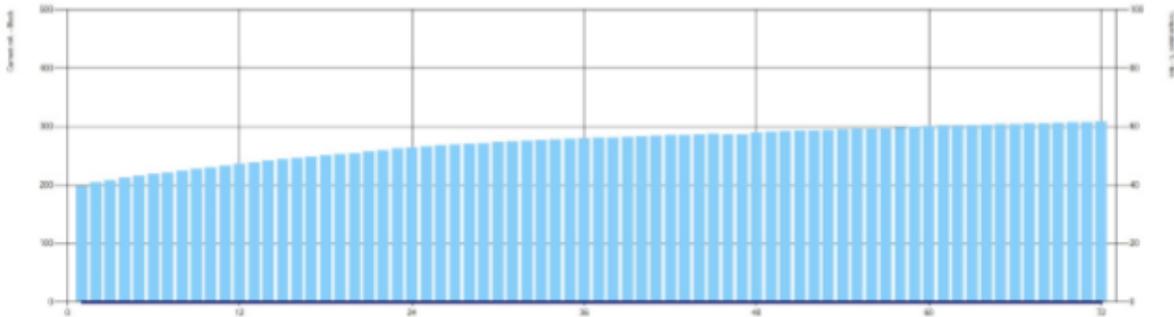
Test-compagny
Testing street 45
CompagnyCity
Some Country

Your own logo.
size=20x80px



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 5085.61 coulomb
 Penetrability Class: High
 Instrument Number: 181624
 Channel Number: 5
 Report Saved: Monday, January 27, 2020 2:34 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: _FIN
 System ID: 10073
 Test ID: ejedwardson



Time	°C	mA									
00:05	0	198.0	01:35	0	252.6	03:05	0	280.9	04:35	0	295.8
00:10	0	204.3	01:40	0	254.2	03:10	0	281.4	04:40	0	296.4
00:15	0	207.7	01:45	0	256.9	03:15	0	282.3	04:45	0	296.6
00:20	0	211.9	01:50	0	259.6	03:20	0	283.4	04:50	0	298.5
00:25	0	215.0	01:55	0	262.0	03:25	0	284.2	04:55	0	299.8
00:30	0	218.3	02:00	0	263.9	03:30	0	285.1	05:00	0	300.4
00:35	0	220.6	02:05	0	265.7	03:35	0	285.9	05:05	0	301.5
00:40	0	224.1	02:10	0	267.4	03:40	0	286.6	05:10	0	301.9
00:45	0	227.4	02:15	0	268.9	03:45	0	287.1	05:15	0	302.3
00:50	0	229.9	02:20	0	270.2	03:50	0	286.4	05:20	0	302.7
00:55	0	232.8	02:25	0	271.4	03:55	0	286.7	05:25	0	303.6
01:00	0	235.8	02:30	0	273.1	04:00	0	289.6	05:30	0	304.1
01:05	0	238.6	02:35	0	274.4	04:05	0	290.8	05:35	0	304.9
01:10	0	241.4	02:40	0	275.4	04:10	0	291.6	05:40	0	305.4
01:15	0	243.8	02:45	0	276.7	04:15	0	292.5	05:45	0	306.3
01:20	0	246.3	02:50	0	277.6	04:20	0	292.8	05:50	0	306.8
01:25	0	248.4	02:55	0	279.1	04:25	0	293.8	05:55	0	307.4
01:30	0	250.9	03:00	0	279.9	04:30	0	294.7	06:00	0	308.2

Control 6



Test-company
Testing street 45
CompagnyCity
Some Country

Your own logo.
size=20x80mm



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 3581.83 coulomb
 Penetrability Class: Moderate
 Instrument Number: 181624
 Channel Number: 6
 Report Saved: Wednesday, January 29, 2020 2:48 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: FIN
 System ID: 10074
 Test ID: ejedwardson



Time	°C	mA									
00:05	0	164.9	01:35	0	190.7	03:05	0	199.9	04:35	0	194.8
00:10	0	166.8	01:40	0	192.0	03:10	0	199.3	04:40	0	194.1
00:15	0	167.3	01:45	0	193.3	03:15	0	199.2	04:45	0	194.5
00:20	0	169.7	01:50	0	194.0	03:20	0	198.2	04:50	0	193.8
00:25	0	170.6	01:55	0	195.2	03:25	0	198.6	04:55	0	193.0
00:30	0	172.4	02:00	0	196.1	03:30	0	198.1	05:00	0	193.0
00:35	0	173.7	02:05	0	196.9	03:35	0	198.8	05:05	0	192.2
00:40	0	175.2	02:10	0	198.2	03:40	0	197.6	05:10	0	192.5
00:45	0	176.9	02:15	0	199.4	03:45	0	197.3	05:15	0	192.5
00:50	0	179.0	02:20	0	199.9	03:50	0	196.9	05:20	0	192.3
00:55	0	180.4	02:25	0	200.0	03:55	0	196.7	05:25	0	192.4
01:00	0	181.5	02:30	0	200.4	04:00	0	196.3	05:30	0	191.3
01:05	0	183.3	02:35	0	200.8	04:05	0	196.5	05:35	0	190.8
01:10	0	184.6	02:40	0	200.4	04:10	0	195.9	05:40	0	190.7
01:15	0	185.9	02:45	0	200.6	04:15	0	195.5	05:45	0	190.1
01:20	0	187.4	02:50	0	200.8	04:20	0	195.2	05:50	0	190.1
01:25	0	188.4	02:55	0	200.5	04:25	0	194.8	05:55	0	189.5
01:30	0	189.8	03:00	0	199.7	04:30	0	195.0	06:00	0	189.3

CA-Add 1



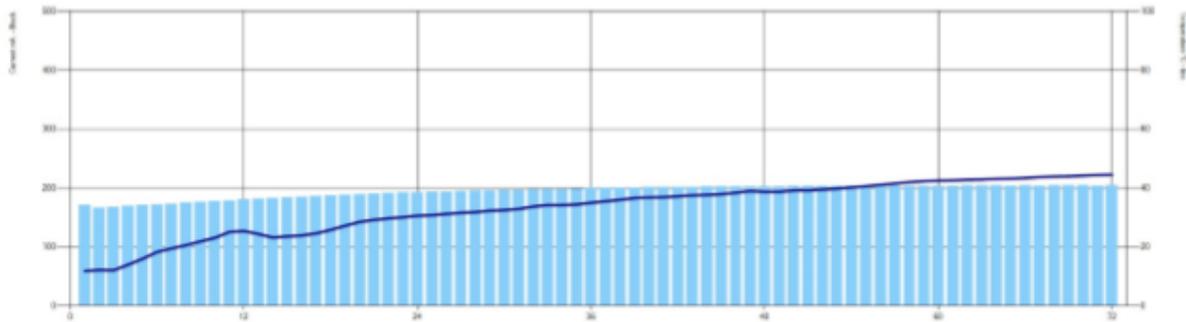
Test-compagny
Testing street 45
CompagnyCity
Some Country

Your own logo.
size=20x80mm



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 3643.41 coulomb
 Penetrability Class: Moderate
 Instrument Number: 181624
 Channel Number: 1
 Report Saved: Wednesday, December 4, 2019 1:43 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: FIN
 System ID: 10069
 Test ID: ejedwardson



Time	°C	mA									
00:05	12	171.2	01:35	27	188.4	03:05	36	200.2	04:35	41	203.4
00:10	12	166.3	01:40	29	189.6	03:10	36	200.5	04:40	41	203.5
00:15	12	167.6	01:45	29	190.4	03:15	37	200.7	04:45	42	203.4
00:20	14	169.3	01:50	30	191.3	03:20	37	201.2	04:50	42	203.8
00:25	16	170.5	01:55	30	192.3	03:25	37	201.5	04:55	42	203.7
00:30	18	171.9	02:00	31	192.8	03:30	37	201.6	05:00	43	203.8
00:35	20	173.2	02:05	31	193.7	03:35	38	201.8	05:05	43	203.7
00:40	21	174.6	02:10	31	194.1	03:40	38	202.0	05:10	43	204.1
00:45	22	176.2	02:15	32	194.8	03:45	38	202.0	05:15	43	204.1
00:50	23	177.3	02:20	32	195.4	03:50	38	202.4	05:20	43	204.1
00:55	25	178.9	02:25	32	196.2	03:55	39	202.5	05:25	43	204.0
01:00	25	180.2	02:30	33	196.8	04:00	39	202.7	05:30	43	204.1
01:05	24	181.6	02:35	33	197.4	04:05	39	202.9	05:35	44	204.0
01:10	23	183.0	02:40	34	197.7	04:10	39	203.1	05:40	44	204.1
01:15	24	184.2	02:45	34	197.9	04:15	39	203.1	05:45	44	204.1
01:20	24	185.3	02:50	34	198.4	04:20	40	203.2	05:50	44	204.3
01:25	25	186.4	02:55	34	199.0	04:25	40	203.3	05:55	44	203.8
01:30	26	187.5	03:00	35	199.7	04:30	40	203.7	06:00	45	204.7

CA-Add 2



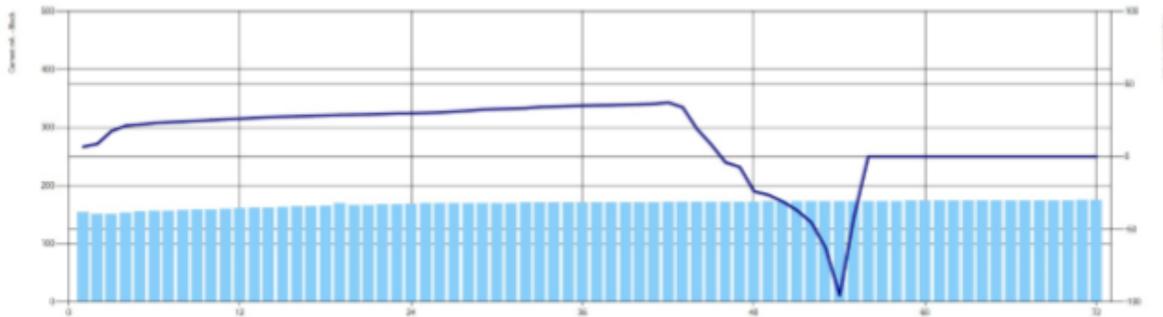
Test-compagny
Testing street 45
CompagnyCity
Some Country

Your own logo,
size=20x80mm



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 3156.44 coulomb
 Penetrability Class: Moderate
 Instrument Number: 181624
 Channel Number: 2
 Report Saved: Thursday, December 5, 2019 1:40 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: FIN
 System ID: 10070
 Test ID: ejedwardson



Time	°C	mA	Time	°C	mA	Time	°C	mA	Time	°C	mA
00:05	7	154.8	01:35	29	169.3	03:05	35	170.6	04:35	-41	173.2
00:10	9	151.2	01:40	29	166.4	03:10	36	170.9	04:40	0	173.3
00:15	18	151.2	01:45	29	166.8	03:15	36	171.1	04:45	0	173.3
00:20	21	152.8	01:50	29	167.5	03:20	36	171.1	04:50	0	173.4
00:25	22	155.1	01:55	30	167.9	03:25	36	171.3	04:55	0	173.6
00:30	23	156.3	02:00	30	168.4	03:30	37	171.6	05:00	0	173.7
00:35	24	157.1	02:05	30	169.2	03:35	34	171.8	05:05	0	173.8
00:40	24	157.9	02:10	31	169.4	03:40	19	172.0	05:10	0	173.9
00:45	25	158.8	02:15	31	169.5	03:45	8	172.0	05:15	0	173.9
00:50	25	159.2	02:20	32	169.4	03:50	-4	172.2	05:20	0	174.0
00:55	26	160.1	02:25	32	169.9	03:55	-7	172.3	05:25	0	174.0
01:00	26	161.0	02:30	33	169.9	04:00	-24	172.4	05:30	0	174.2
01:05	27	161.5	02:35	33	169.9	04:05	-26	172.6	05:35	0	174.3
01:10	27	162.1	02:40	33	170.3	04:10	-31	172.6	05:40	0	174.3
01:15	28	163.1	02:45	34	170.3	04:15	-37	172.8	05:45	0	174.4
01:20	28	163.8	02:50	35	170.4	04:20	-45	172.9	05:50	0	174.4
01:25	28	164.6	02:55	35	170.4	04:25	-62	173.0	05:55	0	174.6
01:30	28	165.4	03:00	35	170.7	04:30	-95	173.1	06:00	0	174.6

CA-Add 3



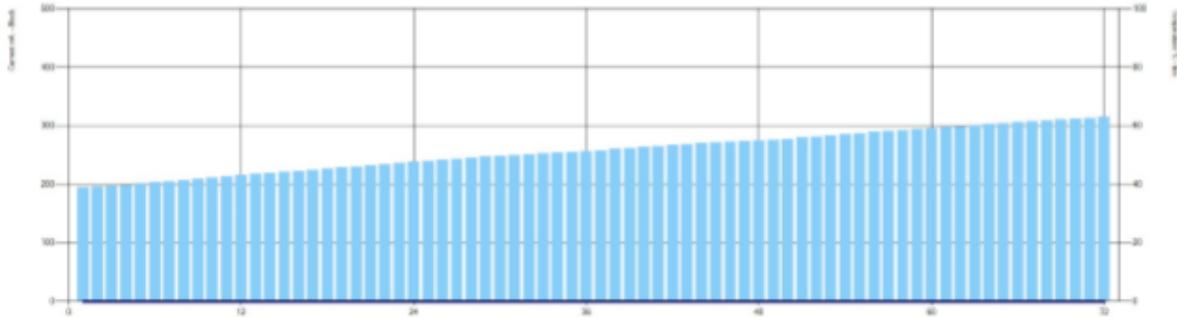
Test-compagny
Testing street 45
CompagnyCity
Some Country

Your own logo
size=20x80mm



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 4806.32 coulomb
 Penetrability Class: High
 Instrument Number: 181624
 Channel Number: 3
 Report Saved: Tuesday, December 10, 2019 4:15 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: _FIN
 System ID: T0071
 Test ID: ejedwardson



Time	°C	mA									
00:05	0	194.7	01:35	0	228.4	03:05	0	257.3	04:35	0	286.8
00:10	0	196.0	01:40	0	230.2	03:10	0	259.8	04:40	0	289.3
00:15	0	197.2	01:45	0	232.3	03:15	0	261.2	04:45	0	290.7
00:20	0	198.5	01:50	0	234.1	03:20	0	263.3	04:50	0	292.1
00:25	0	201.0	01:55	0	236.1	03:25	0	264.8	04:55	0	293.7
00:30	0	203.2	02:00	0	238.0	03:30	0	266.8	05:00	0	295.6
00:35	0	204.9	02:05	0	239.6	03:35	0	268.4	05:05	0	297.2
00:40	0	207.2	02:10	0	241.2	03:40	0	270.0	05:10	0	299.0
00:45	0	209.3	02:15	0	242.8	03:45	0	270.7	05:15	0	300.8
00:50	0	211.3	02:20	0	244.6	03:50	0	272.1	05:20	0	302.5
00:55	0	213.4	02:25	0	246.9	03:55	0	273.3	05:25	0	304.2
01:00	0	215.3	02:30	0	248.0	04:00	0	274.2	05:30	0	305.8
01:05	0	217.3	02:35	0	249.6	04:05	0	275.3	05:35	0	307.1
01:10	0	218.9	02:40	0	250.8	04:10	0	277.1	05:40	0	308.5
01:15	0	220.6	02:45	0	252.2	04:15	0	279.7	05:45	0	310.4
01:20	0	222.3	02:50	0	253.8	04:20	0	281.5	05:50	0	312.1
01:25	0	224.5	02:55	0	255.0	04:25	0	283.3	05:55	0	313.1
01:30	0	226.1	03:00	0	256.0	04:30	0	285.3	06:00	0	314.4

CA-Add 4



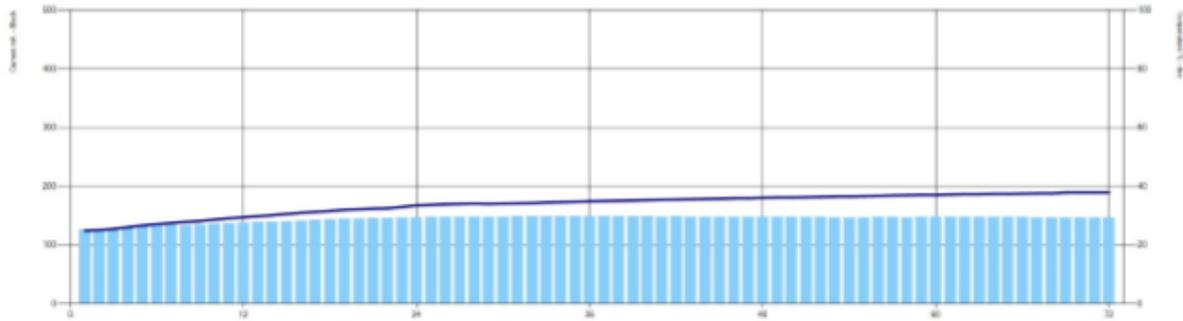
Test-company
Testing street 45
CompagnyCity
Some Country

Your own logo.
Size=20x80mm



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 2706.9 coulomb
 Penetrability Class: Moderate
 Instrument Number: 181624
 Channel Number: 7
 Report Saved: Tuesday, February 4, 2020 2:29 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: _FIN
 System ID: 10075
 Test ID: ejedwardson



Time	°C	mA									
00:05	25	125.5	01:35	32	144.2	03:05	35	148.6	04:35	37	147.2
00:10	25	127.0	01:40	32	144.8	03:10	35	148.4	04:40	37	147.3
00:15	26	128.4	01:45	32	145.7	03:15	35	148.5	04:45	37	147.4
00:20	26	130.0	01:50	33	146.0	03:20	35	148.4	04:50	37	147.2
00:25	27	131.2	01:55	33	146.8	03:25	35	148.3	04:55	37	147.3
00:30	27	132.1	02:00	34	147.1	03:30	36	148.4	05:00	37	147.3
00:35	28	133.1	02:05	34	147.8	03:35	36	148.2	05:05	37	147.3
00:40	28	134.1	02:10	34	147.6	03:40	36	148.2	05:10	37	147.3
00:45	28	134.8	02:15	34	147.8	03:45	36	148.3	05:15	37	147.3
00:50	29	135.8	02:20	34	147.9	03:50	36	148.2	05:20	37	147.3
00:55	29	136.8	02:25	34	148.1	03:55	36	148.1	05:25	37	147.4
01:00	30	137.8	02:30	34	148.3	04:00	36	148.0	05:30	38	147.3
01:05	30	138.7	02:35	34	148.7	04:05	36	148.1	05:35	38	147.2
01:10	30	139.6	02:40	34	148.7	04:10	36	147.9	05:40	38	147.1
01:15	31	140.7	02:45	35	148.5	04:15	36	147.5	05:45	38	147.0
01:20	31	141.4	02:50	35	148.7	04:20	36	147.5	05:50	38	147.0
01:25	31	142.5	02:55	35	148.5	04:25	37	147.2	05:55	38	147.2
01:30	32	143.3	03:00	35	148.6	04:30	37	147.0	06:00	38	147.1

CA-Add 5



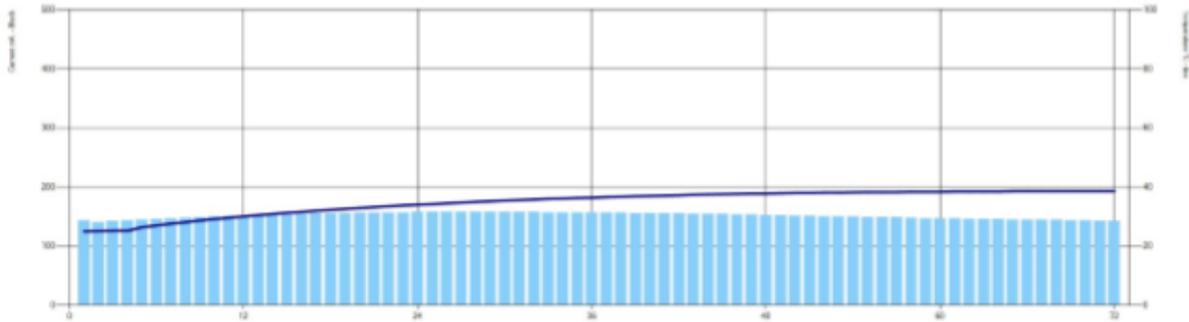
Test-compagny
Testing street 45
CompagnyCity
Some Country

Your own logo
size=20x80px



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 2833.21 coulomb
 Penetrability Class: Moderate
 Instrument Number: 181624
 Channel Number: 8
 Report Saved: Wednesday, February 5, 2020 3:13 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: FIN
 System ID: I0076
 Test ID: ejedwardson



Time	°C	mA									
00:05	25	143.9	01:35	33	155.8	03:05	37	156.3	04:35	38	149.1
00:10	25	140.4	01:40	33	156.3	03:10	37	156.2	04:40	38	148.7
00:15	25	142.0	01:45	33	156.6	03:15	37	155.9	04:45	38	148.6
00:20	25	143.6	01:50	34	156.7	03:20	37	155.7	04:50	38	148.2
00:25	26	144.8	01:55	34	157.0	03:25	37	155.5	04:55	38	147.1
00:30	27	145.6	02:00	34	157.2	03:30	37	155.2	05:00	38	146.6
00:35	28	146.5	02:05	34	157.6	03:35	37	154.6	05:05	39	146.3
00:40	28	147.7	02:10	35	157.6	03:40	38	154.5	05:10	39	145.9
00:45	29	148.6	02:15	35	157.3	03:45	38	154.4	05:15	39	145.6
00:50	29	149.7	02:20	35	157.3	03:50	38	153.8	05:20	39	145.3
00:55	30	150.6	02:25	35	157.7	03:55	38	153.0	05:25	39	144.8
01:00	30	151.1	02:30	35	157.4	04:00	38	152.2	05:30	39	144.5
01:05	31	152.1	02:35	36	157.5	04:05	38	151.8	05:35	39	144.1
01:10	31	152.8	02:40	36	157.4	04:10	38	151.4	05:40	39	144.4
01:15	31	153.5	02:45	36	157.1	04:15	38	150.9	05:45	39	143.5
01:20	32	154.4	02:50	36	156.7	04:20	38	150.3	05:50	39	143.2
01:25	32	155.2	02:55	36	156.8	04:25	38	149.8	05:55	39	142.8
01:30	32	155.5	03:00	36	156.6	04:30	38	149.7	06:00	39	142.5

CA-Add 6



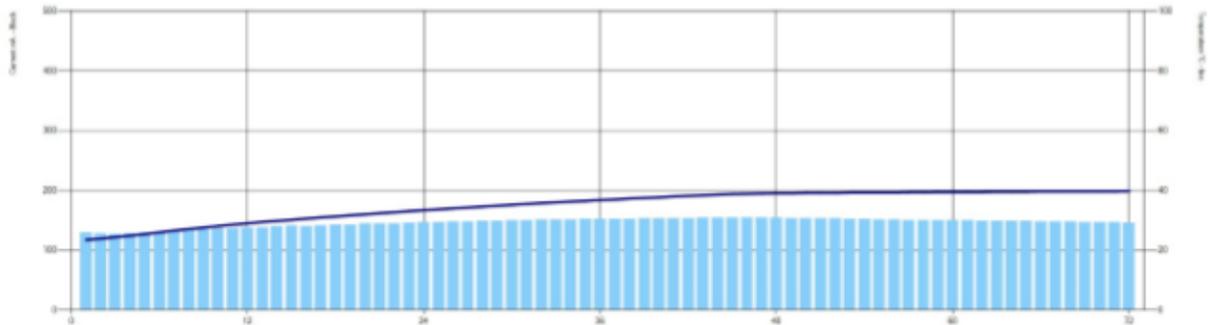
Test-compagny
Testing street 45
CompagnyCity
Some Country

Your own logo,
size=20x80px



Test report

Voltage Used: 60 V
 Testing Time: 6:00 hours
 Charge Passed (Adjusted): 2740.9 coulomb
 Penetrability Class: Moderate
 Instrument Number: 181624
 Channel Number: 1
 Report Saved: Thursday, February 6, 2020 3:27 PM
 Testing By: Emma Edwardson
 Reference:
 Sample Diameter: 102 mm
 Sample Length: 50 mm
 Instrument Status: FIN
 System ID: 10077
 Test ID: ejedwardson



Time	°C	mA									
00:05	24	128.9	01:35	32	142.9	03:05	37	152.6	04:35	39	151.5
00:10	24	127.9	01:40	32	144.5	03:10	37	152.6	04:40	39	151.1
00:15	24	127.6	01:45	32	144.1	03:15	38	153.2	04:45	39	150.5
00:20	25	128.7	01:50	33	145.0	03:20	38	153.8	04:50	39	150.2
00:25	25	129.7	01:55	33	145.7	03:25	38	153.2	04:55	40	150.2
00:30	26	130.5	02:00	33	146.2	03:30	38	153.6	05:00	40	149.5
00:35	27	131.4	02:05	34	146.9	03:35	38	153.9	05:05	40	149.6
00:40	27	133.4	02:10	34	147.3	03:40	39	154.1	05:10	40	149.3
00:45	28	135.9	02:15	34	148.3	03:45	39	154.0	05:15	40	148.8
00:50	28	135.6	02:20	35	148.9	03:50	39	154.7	05:20	40	149.2
00:55	29	137.4	02:25	35	149.2	03:55	39	154.2	05:25	40	148.5
01:00	29	137.9	02:30	35	149.6	04:00	39	154.1	05:30	40	148.0
01:05	30	138.5	02:35	36	150.2	04:05	39	153.5	05:35	40	147.9
01:10	30	139.9	02:40	36	150.8	04:10	39	153.3	05:40	40	147.7
01:15	30	140.8	02:45	36	151.0	04:15	39	153.0	05:45	40	147.0
01:20	31	140.4	02:50	36	151.1	04:20	39	152.9	05:50	40	146.9
01:25	31	141.5	02:55	37	151.7	04:25	39	152.1	05:55	40	146.7
01:30	31	142.1	03:00	37	151.8	04:30	39	151.9	06:00	40	145.7