DEVELOPMENT OF TWO-PHASE BIOLOGICAL SELF-HEALING AGENTS FOR CEMENT-BASED MORTAR

A Thesis

by

Mustafa Mert Tezer

Submitted to the

Graduate School of Engineering
In Partial Fulfillment of the Requirements for
the Degree of

Master of Science

in the

Department of Civil Engineering

Özyeğin University

January 2020

DEVELOPMENT OF TWO-PHASE BIOLOGICAL SELF-HEALING AGENTS FOR CEMENT-BASED MORTAR

Approved by:

Asst. Prof. Dr. Zeynep Başaran Bundur, Advisor,
Department of Civil Engineering
Özyeğin University

Prof. Dr. Safiye Feyza Çinicioğlu
Department of Civil Engineering
Özyeğin University

Assoc. Prof. Dr. Nilüfer Özyurt Zihnioğlu
Department of Civil Engineering
Boğaziçi University

Date Approved: 13 January 2020

I dedicate this thesis to my parents,

Ayşegül and Hicabi Tezer.

ABSTRACT

Factors affecting durability of concrete structures are generally associated with each other. Due to its brittle nature, concrete can crack under stress and these cracks can decrease the service life of concrete structures. Therefore, it is crucial to find alternative methods to detect and recover microcracks, then to repair them before they were developed into wider cracks. Recent research in the field of concrete materials suggested that it might be possible to develop a smart cement-based material that is capable of remediate cracks by triggering biogenic calcium carbonate ($CaCO_3$). The mineral precipitation was obtained by leveraging the metabolic activity of microorganisms to provide microbial induced calcium carbonate precipitation (MICP). The research done on possible use of MICP in cement-based systems has showed promising results and the studies suggest this process could be a useful approach for remediation of cracks on the surface of concrete.

The goal of this study was to design a *2-phase biological self-healing agent for cement-based materials*. For this specific goal vegetative *Sporosarcina pasteurii (S. pasteurii)* cells were immobilized on the selected natural minerals or light weight aggregate (LWA). Herein, the bacterial cells were immobilized on diatomaceous earth, bentonite, sepiolite and pumice, to remediate flexural cracks on mortar in early ages (14 and 28 days after mixing). To obtain the 2-phase bio additive, half of the minerals were saturated with a nutrient medium consisting of urea, corn-steep liqueur (CSL) and calcium acetate and the cells with immobilized to the other half without nutrients. Screening of the healing process was done with ultrasonic pulse velocity (UPV) testing and stereomicroscopy. Additional evaluations were conducted with water absorption test and viability

assessment. Precipitated formations were further examined with scanning electron microscopy (SEM) analysis. With this approach, the cracks on mortar surface were sealed with the biogenic precipitate and the water absorption capacity of the *so-called* self-healed mortar was decreased compared to its counterpart cracked mortar samples. Calcite was found to be the dominant precipitate in the remediated cracks. At last, the bacterial cells immobilized on various protection barriers were viable and functional for extended time periods.

ÖZETÇE

Beton dayanıklılığını etkileyen faktörler kendi içlerinde bir etkileşim içerisindedir. Stres altında çatlak oluşumu, beton yapılarda hizmet ömrünü kısaltan kaçınılmaz bir sorundur. Beton yapılarda oluşan kılcal çatlakların hızlı bir şekilde tespit edilmesi ve ilerleme kaydedip büyük ölçekli çatlaklara dönüşmeden onarılması oldukça önemlidir. Son yıllarda yapılan araştırmalar kendiliğinden iyileşen çimento-esaslı malzemeler ile kılcal çatlakların onarılmasının mümkün olduğunu göstermiştir. Bu amaçla kullanılabilecek en yenilikçi yöntemlerden biri biyomineralizasyondur. Bu reaksiyonda mikroorganizmaların metabolik aktivitelerin sonucu ürün olarak kalsiyum karbonat ($CaCO_3$) oluşur ve oluşan $CaCO_3$ çökeltisinin çatlakları doldurması ile kendiliğinden iyileşme elde edilir. Bu sistemde mineral çökelmesi, bakterilerin metaboik aktiviteleri kullanılarak tetiklenmektedir. Bu sistemin çimento bazlı yapılarda çatlak kapanması amacı ile kullanılableceği, yapılan çalışmalar sonucunda gelecek vaat eden bir yöntem olarak öne çıkmıştır. Bu çalışmanın hedefi iki bileşenli bir biyolojik katkı maddesinin geliştirilmesiydi. Bu hedef doğrultusunda, *Sporasarcina pasteurii* bakterisinin doğal mineraller olan diyatomlu toprak, bentonit, lületaşı ve ponza üzerine sabitlenmesiyle erken yaşta oluşturlan çatlakların (14 ve 28 gün) onarılması incelenmiştir. İki fazlı bir biyo-onarım malzemesi elde etmek için hücreler ilk olarak 10^8 CFU/mL konsantrasyon ile sterilize edilmiş fosfat çözeltisi (PBS) içerisine eklendi. Kullanılan minerallerin yarısı bu solüsyon içinde en az 24 saat tutuldu. Minerallerin diğer yarısı ise Üre-MMS (Mısır maserasyon sıvısı) ve kalsiyum asetat içeren sulu besi yerine 24 saat boyunca batırıldı. Onarım etkinliği stereo mikroskop ve ultrason dalga takibi ile incelendi. Ek olarak kirişlerin su emme kapasitesi ve sonrasında hücre canlılığı test edildi. Çatlak içerisindeki çökeltilerin yapısı taramalı elektron mikroskopu (SEM) ile incelendi. Bu yöntem ile bakteri içeren örneklerde çatlak kapanması ve su emme kapasitlerinde azalma gözlemlendi. Ultrason dalga takibi sonuçları biyo-onarım malzemesi ile hazırlanan örneklerde, kontrol örneklerine oranla artış gösterdi. Çökelti yapısı

mikroskop incelemeli sonucunda kalsit yoğunluğu gösterdi. Kullanılan minerallerin, hücreleri beton içerisinde uzun periyotlar için canlı tutabileceği kanıtlandı.

ACKNOWLEDGEMENT

I want to express my gratitude for my advisor, Dr Zeynep Basaran Bundur for her mentorship and patient guidance. Her contribution to my academic development was invaluable. I will always be grateful for her support and advice. I would like to acknowledge the support of the Scientific and Technical Research Council of Turkey (TUBITAK). The conducted research was made possible with the funding of TUBITAK 1001 Grant-118M327.

Special thanks to the jury members of this thesis, Prof. Nilüfer Özyurt Zihnioğlu and Prof. Safiye Feyza Çinicioğlu for their attention to my study and the valuable suggestions.

I am thankful to my laboratory partner Ayca Gisu Güldoğan for her precious friendship and support through my study. I am also thankful to my roommate Onur Cem Aygın for his support and understanding. I want to acknowledge my good friends Gökhan Tekbaş, Berkant Bayar and Burak Talha Kılıç for their friendship. I am thankful to former M.Sc. students Ali Amirii and Mahzad Azima for their guidance and friendship.

I would like to appreciate my beloved Kübra Yılmaz for her infinite love and support.

The last and most importantly, I am grateful to my parents who dedicated their lives to me. Thank you for teaching me how to love, live and to be ambitious.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

Introduction

Despite its negative perception in the society, concrete is still the most used building material in the construction sector. In facts, it is still the most-used man-made material in the earth. Concrete serves a lot of advantages such as easy casting, high compressive strength, good fire-resistance, and low cost and these pros make it the prominent material, especially for large volume construction. From the early 19^{th} century to present, it has an exponential growth in production and investment basis [1]. Throughout the last decade, build environments in most of the cities were renovated for more equitable and sustainable cities. This also substantial increased the demand for cement-based materials, particularly concrete.

Concrete is a composite material containing Portland cement, water, aggregates, pozzolans and various chemicals. As the concrete production increases the demand for cement production also increases. Environmental impacts on cement production raises a great concern in the society. While cement manufacturing requires significant energy inputs, it also generates a vast amount of waste material. Cement manufacturing accounts for 8% of the total carbon dioxide (CO_2) emission worldwide [2]. Alongside with CO_2, different pollutants such as nitrogen oxide and sulphur dioxide are also released into the air [3].

For the last decade, the number research studies to mitigate the negative environmental impact of cement production significantly increase. These studies involve use of alternative binders, capture and store the CO_2 gas and improve the durability of concrete structures to reduce demand for cement production. Durability of concrete is directly related to its permeability. Even though a concrete mix designed to be low permeable, cracks in concrete can increase the

permeability of the material. Depending on the design quality and service environment it can perform under different loads such as compressive, tensile and shear but at the end cracking is inevitable. These cracks might be caused by external and thermal stresses, poor curing, rough environments etc.[4]. Also, plastic shrinkage and expansive reactions are possible causes[5].

A part of these deformations can be repaired by maintenance. Conventional methods could be listed such as epoxy injection, routing and sealing, grouting or polymer impregnation etc. Obtaining a successful repair requires three steps. First, detection of the cracks and resolving the causes, afterwards assessing the degree of damage caused by crack and at last deciding on a suitable repair method. However, even the external repair costs, need of extra workforce and time is covered, these methods might still be insufficient. Microcracks are generally hard to detect and they are mainly formed at early ages of concrete. These types of cracks are known to not affect the strength of the matrix. However, they increase the permeability of the matrix and disrupt the structural integrity [6]. Thus, they create pathways for hazardous chemicals such as Cl^- and SO_4^{-2} and allow excessive water penetration which can lead to corroded rebars [7]. With the corrosion of the rebars, additional internal stresses are created which could eventually lead to new cracks. Microcracks could also propagate to a point where they also threaten the strength of the matrix. These problems demand a solution where the detection is not prominent, and the method should be applicable with the narrow-sized cracks. In addition, maintenance interference comes with additional costs and the deformation requires to be noticed in the first place. An investigation conducted by The American Society of Civil Engineers estimated the total maintenance costs as $2.2 trillion for the U.S. and $2 trillion for Asia between 2016 and 2021[8]. When combined with environmental effects and high maintenance costs, a need for a sustainable solution arises.

Recently in literature, there is an increasing number of studies conducted on the *so-called* self-healing phenomenon which could be an efficient solution to the narrow-sized cracks[9]. The

idea of self-healing is mainly built around the Damage Management Approach (DMA) which is initially an alternative concept for Damage Prevention Approach (DPA). Main distinction between the two approaches is DPA would always show a neutral or positive rate of damage formation where DMA could possess a negative rate[10]. Self-healing is generally summarized under 2 main topics as autogenous and autonomous self-healing.

Autogenous healing primarily depends on the infinite hydration process of the cement and carbonation of calcium hydroxide ($Ca(OH)_2$)[11]. Also, hydrated cement paste could swell and remediate the crack and free particles around the matrix could clog inside the crack zone[12]. This intrinsic behaviour peaks interests due to its possibility of an effortless contribution. However, the phenomenon only works on very narrow cracks and needs long time periods where suitable amount of water is available. Overtime, various methods were proposed in order to boost the autogenous healing efficiency. These methods were mainly crack restriction, polymer modified concrete, usage of different absorbents to supply water and the addition of the crystalline admixtures in order to promote the un-hydrated cement to form crystalline product [15,16]. Despite the efforts, in order to reach a complete remediation, environmental conditions should be perfect fit and the maximum width of a crack should not exceed 200μm which is a very restricting list of needs [13,17]. To obtain a more efficient and controllable mechanism, autonomous healing approach was proposed. Autonomous healing concept is the total of engineered methods mainly relying on the encapsulation of different healing agents or incorporation of bacteria [13].

Capsule mechanism depends on the disintegration of the carrier at the time of cracking. After the crack propagation, dismembered capsule releases the agent inside the crack zone. Thus, healing is achieved. Method can be identified as one-component or two-components according to the core mechanisms behaviour. First type needs a healing agent to react with water and air or the curing agent spread on the concrete hence the reaction product fills the crack. For the

two-component method, healing and curing agents are incorporated into two different capsules. With the propagated crack, released agents mix and reaction products fill the cracks [14]. However, it is a challenging method because distribution of the capsules are random and heterogeneous[16]. Also, there is a possibility that the mechanical force applied onto the capsules at the mixing process could dismember the carrier before the desired instance[17].

The idea of using bacterial cells as self-healing agents is built on a mechanism called *biomineralization*, aka MICP, which first came into light in the early 2000s [18]. Biomineralization is a mechanism where different kinds of minerals are precipitated by the metabolic activity of a microorganism[19]. Further discussion made on the mineral nucleation, subsequent crystal formation and the growth stimulated by a microorganism would be similar to an abiotic case [20]. It is known that the most advantageous autogenic healing type is through the formation of calcium carbonate ($CaCO_3$) by the dissolved calcium hydroxide ($Ca(OH)_2$) [21]. Aim of integrating the biomineralization concept into the concrete is increasing the $CaCO_3$ precipitation through urea hydrolysis and an additional calcium source[22]. In theory, gram positive microorganisms can decompose urea into ammonia and carbonates through hydrolysis. Their negative surface charge attracts [Ca^{+2}] ions, if they present, and $CaCO_3$ precipitation nucleate on bacterial cells[9]. Throughout literature, there are different methods of bacteria incorporation to remediate cracks in cement-based materials. In 2001, injection of a mixture consisting of *Bacillus pasteurii*, urea and calcium chloride ($CaCl_2$) into the manually generated cracks showed that the calcite precipitation can be enhanced with the use of a microorganism and effectively remediate a crack[18]. However, ideally a more optimized way compared to the injection, where an outside intervention was made, could increase the usability of the method. This way of thinking encouraged the researchers in order to focus on the methods where the direct incorporation of the bacteria could be possible. However, the challenging environment constituted by cement was not an ideal case for using a bacterail cells. Highly alkaline nature

of cement based matrix would decrease the viable bacterial cells where the mineral-forming ability is directly related with the decreasing cell concetration [20,25]. Additionally, density of the cementitious matrix increases as the hydration continues reducing the pore space, which would supposedly host the bacteria[23]. As an initial attempt to overcome such challanges, the researchers studied incorporation of spores raher than the active cells and as a result, this approach prolonged the viability up to 4 months inside the matrix[24]. However, endospores are rather protein based structures which could dissolve in high pH. The studies showed that the compressive strength of concrete was decreased by time[25]. Thus, researchers proposed various encapsulation methods which used for non-biogenic healing agents as a solution. The aim for this methodology was to protect the microorganisms from the in-situ effects of cement and also to guarantee a prolonged healing mechanism regardless of the cell state. Several attemts were made using fabricated capsules such as polyurethane, silica gel and hydrogels [28,29]. In these studies, selected capsulation materials were also acted as fillers alongside the precipitated calcite. Studies showed sufficient results where the precipitation performance was not affected by the capsulation , on the contrary viability of the microorganisms was positively effected. Wiktor et al. [22] proposed the usage of porous expanded clay particles as a possible carrier of a two-component bio-agent consisting of bacteria and calcium lactate. Oxygen consumption measurements showed a prolonged behaviour at the bacteria activity. Additionally, the application was also evaluated as a possible protection against the corrosion of the rebars by the means of oxygen consumption inside the matrix. Various research showed the usage of the immobilization method would be beneficial to enhance the self-sealing ability and improve the microorganism viability. However, the methodologies proposed were mostly synthetic and requires additional processes requiring experienced workers. Thus, it is crucial to develop a simpler, economically feasible and sustainable protection system to improve the robustness of the bacterial cells against the restrictive environment. As possible barriers, some

natural minerals such as diatomaceous earth, zeolite, metakaolin and expanded perlite were proposed[30-32]. However, when the number of porous minerals are considired, it is clear that there is still a need for additional studies on the mineral usage and alternative methadologies.

1.2 Goal and Objectives

In literature, various research on the self-healing and the adaptation of biomineralization is present but the viability of the bacteria is still dependent on the new studies. Protective barrier and/or capsulation methods are promising approaches. However, current state of the phenomenon is insufficient in order to supply a material which is cheap, easy to find and compatible with bacteria. *The goal of this study was to design a 2-phase biological self-healing agent for cement-based materials. For this specific goal the bacterial cells were immobilized on the selected natural mineral or light weight aggregate (LWA).* Diatomaceous earth, pumice, bentonite and sepiolite were selected due to their porous structure and was evaluated as possible protective barriers. Self-healing ability of designed 2-phase biological additive was evaluated through stereomicroscopy, ultrasonic pulse velocity (UPV), water absorption test and scanning electron microscopy (SEM). At last, the viability of bacterial cells was correlated with the efficiency of crack healing for different immobilization barriers.

To achieve the abovementioned goal, the specific objectives of the study can be listed as follows:

1- Characterization of protection barriers,
2- Development of 2-phase biological self-healing agent by immobilizing the cells on natural minerals and LWA,
3- Qualitative analysis of crack healing through visual inspection, SEM and UPV,
4- Quantifying the self-healing through change in water absorption,
5- Correlating crack-healing with bacterial viability.

Chapter II

Literature Review

An alternative methodology to repair crack is concrete is self-healing or self-sealing of cracks. Self-healing in cement-based materials is a very broad topic where the list of possible integration methodologies is extensive. A RILEM report named and categorize this self-healing behavior as autogenous and autonomous self-healing. Terminology was also explained for concrete as the remediation of a crack with time[31].

2.1 Autogenous self-healing

Generally, self-healing methodologies include starting pathway like in the bio-systems where the living organisms can heal small body damage by themselves without any other intervention. As so, concrete also has a potential of intrinsic self-healing, which is named autogenous healing. Autogenous healing concept mainly relies on the continuous hydration process of the cement; dissolution and carbonation of calcium hydroxide [13,14,34]. Different approaches were proposed to trigger autogenous healing in concrete. These approaches mainly focussed on crack restriction, polymer modified concrete, usage of different absorbents to supply water and the addition of the crystalline admixtures in order to promote the un-hydrated cement to form crystalline product[15,16].

However, the behavior is dependent on various environmental and compositional factors and it is an uncontrollable mechanism which makes it unreliable. This unpredictable nature of the autogenous self-healing led researchers to focus on new methods where engineered enhancements could be integrated to the concrete. These methods were mainly defined as autonomous self-healing. The potential of the intrinsic self-healing of the concrete peaked the

interests due to the advantages that it could give to a structure. An effortless repair system might be a very cost efficient and an easy to adapt system. Even though the autogenous self-healing behavior is not exactly an ideal system, it was a very promising and advantageous method to abandon.

2.2 Autonomous self-healing

To achieve faster and controllable healing, researchers focus on incorporating additional self-healing agents to the mix. Promoting the self-healing behavior with various engineered modifications was became a popular topic around 1990's. In general, studies focused on systems which constituted by self-healing agents carried through encapsulate member. The encapsulate member, impregnated by the healing agent, is incorporated into the matrix. After the cracking, the encapsulation breaks and releases the agent inside the crack zone. Method can be identified as single-component or two-components according to the core mechanisms behaviour. For single-component agents, upon cracking the healing agent directly reacts either with water (or air) and the reaction product fills the crack or the curing agent by itself fill the cracks [14]. For the two-component systems, healing agents and initiators are incorporated into two different capsules. With the crack propagation, the agents are released to crack and reaction products remediate the crack[14].

Capsules are the initial tools that carry and protect the agents inside a matrix. Ideally, when a crack starts to propagate it will, theoretically, crack the a capsule, releasing the agent into the cracked zone [33]. There are different conditions that should be considered to achieve an efficient self-healing with 2-component autonomous self-healing. The encapsulation (protective) material should be selected such that it should resist the forces during the mixing and later hardening process of concrete. As an example, glass capsule usage as a carrier shows very poor results when the mixing forces encountered [34]. As a solution, studies conducted

where the glass tubes were placed inside the matrix rather than the inclusion at the mixing stage[35]. Relatedly, it should be resistant to high alkalinity of cement paste. Even though, these conditions lead to use of a strong capsulation barriers, the material should not be very strong so it could be broken and release the healing agent when a crack hits to capsule.[36] Urea formaldehyde (UF), double-walled polyurethane/urea-formaldehyde (PU/UF) and melamine-formaldehyde are some of the materials that are used for encapsulation [40–42]. These materials are classified as thermosetting polymers and selected due to their high strength and resistance to highly alkaline environments. As an alternative, paraffin which is a low strength thermoplastic material can be used[14]. An important advantage of this method is the bonding strength between the capsule and the matrix compared to other thermosetting polymers. If the bonding between the capsule and the matrix is weak and the capsule is stiff enough to resist, there is a possibility that the propagation will continue through between them. This would leave the capsule intact thus, the agent to be non-released. Interfacial transition zone (ITZ) between the capsule and the matrix should be investigated in order to understand this mechanism[36]. However, selecting a material that could develop a more powerful bond would possibly eliminate this problem. Other materials such as light weight aggregates (LWA) were also used but it can be classified as a carrier rather than a capsule [40].

Various materials were proposed in literature as possible healing agents. While selecting a healing agent, there are some crucial points to consider. Agent should not have a high viscosity in order to flow through the crack zone. Additionally, their reaction time is a key point. Agent should provide sufficient reaction time for a complete curing process to take place[13].

Epoxy resins were proposed as a possible healing agent. It is a class of polymers which is also an adhesive agent[39,44]. In a study, Dong et.al. [42] used epoxy resin as healing agent, butyl glycidyl ether (BGE) as thinner agent and MC120D as harden agent. Urea-formaldehyde was

used as the shell material. A significant strength gain and a decrease in chloride penetration was observed as a result[42].

Another adhesive agent, cyanoacrylates, was used as a healing agent. It is a family of adhesives where they contain strength and fast-reactant properties. It is also called as the superglue (ethyl cyanoacrylate) and it is a favourable one compared to epoxy resin due to its low viscosity and single-agent nature [43]. Different forms of isocyanate can be seen in literature such as methyl cyanoacrylate and ethyl cyanoacrylate [46-48]. Agent even though the agent is highly reactive in moist environment, time frame between the exposure and reaction rate is sufficient for self-healing to occur [46]. Agent is generally transported to the zone by the means of glass hollow fibers, microcapsules or vascular systems [46,47]. It shows a very rapid and accurate strength regain of a samples pre-cracking strength. Cyanoacrylate showed superior results in both stiffness recovery and permanent crack closure compared to samples prepared with two part epoxy resin and silicon based adhesive agent, as the means of self-repairing, stiffness and frequency of cracking[47]. Another alternative material as a self-healing agent is methyl methacrylate. Multiple loading cycles was applied in order to understand the sealing behaviour and its continuity. Between the first and second loading specimen containing the agent showed no strength loss where control specimen had 33% decrease. Also, it was stated that, specimen containing agent showed a different crack pattern instead of the re-opening of the previous crack zone. Improved permeability was another beneficial aspect of the agent usage[48].

Sodium silicate, a low viscosity material was also used as a healing agent in various studies. Alghamri et. al. [40] attempted the impregnation of light weight aggregates with sodium silicate as a potential healing agent. Performance was evaluated in terms of visual inspection and reduction in water absorption. Crack remediation was obtained at 28 days in concrete samples were sodium silicate was encapsulated in LWAs as self-healing agents. Crack widths around

0.14 mm was recovered. In addition, there was a 50% reduction in the sorptivity index compared to control samples where no crack healing was obtained[40].

2.3 Bacterial concrete

The search of a more sustainable and stable method as an alternative self-healing system, recent studies in the literature showed that it might be possible to develop a bio-based self-healing system where bacterial cells are being used to remediate cracks via the microorganism's metabolic activities [15,52,53]. In this particular case, the process is called as MICP aka biomineralization.

Biomineralization is a complex bio-chemical reaction chain where the mineral precipitation takes place as a by-product of interactions between the metabolic activities of the microorganisms[22]. Mineral precipitation could be triggered by different reactions such as urea hydrolysis, denitrification and by a charged surface area [21,22].

MICP through urea hydrolysis is one of the most commonly used approach for cement-based materials due to its controllable nature and high calcium carbonate output. Suitable strains that possesses urease enzyme can act as a catalyser promoting urea ($CO(NH_2)_2$) hydrolysis to ammonium [NH_4^+] and carbonate [CO_3^{2-}] , and increase the pH of the environment. If free calcium ions [Ca^{2+}] are present in the environment, they might be attracted by the negatively charged surface of the cells and trigger crystal nucleation on the cell. Calcium carbonate crystals start to nucleate on the cell wall and encapsulate the microorganisms. Due to the complexity of the reactions, there is more than one factor influencing microbially induced calcium carbonate precipitation, such as calcium ion concentration, the concentration of dissolved inorganic carbon, pH of the environment and availability of nucleation sites for crystal growth [10,24].

Overall urea hydrolysis reactions are as follows:

$$CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3 \quad (1)$$

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

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+ \quad (3)$$

$$HCO_3^- \leftrightarrow CO_3^{2-} + H^+ \quad (4)$$

$$CaCO_3 \leftrightarrow Ca^{2+} + CO_3^{2-} \quad (5)$$

Efficiency of MICP depends on the type of the strain, availability of sites that microorganisms can attach to, concentration of cells and the accessibility of nutrient sources. To keep microorganisms active in a metabolic state, carbon and nitrogen source are required. Urea is the main nitrogen source to keep the microorganisms viable and trigger equations from 1 to 5 Deposition sites for the mineral precipitation are also supplied by the bacteria where it does not even have to be alive[52]. Yeast extract is commonly used as a carbon source; in addition to providing a source of carbon, yeast extract has many amino acids and vitamins required for survival of bacteria[53]. An alternative for yeast extract is corn steep liquor (CSL), which is the by-product obtained from corn-industry[54].

2.3.1 Morphology of the precipitate

There are six known polymorphs of calcium carbonate. Vaterite, calcite and aragonite are the anhydrous polymorphs of the calcium carbonate where monohydrocalcite and ikaite are the hydrated crystal phases [56,57]. Additionally, amorphous calcium carbonate (ACC) formations were also observed as the initial phase of calcite formation[57]. Calcite and aragonite are more stable than vaterite and monohydrocalcite while ikaite and amorphous calcium carbonate (ACC) are rarely observed. Calcite and vaterite, rarely aragonite, are mostly observed in MICP applications[18,50]. The morphology of $CaCO_3$ crystals is more critical in self- healing

applications such that bonding between precipitate and the substrate should be durable and strong. Regarding to this aspect, more stable aragonite and calcite are more favourable than vaterite in self-healing applications.

2.3.2 Use of MICP for crack remediation in mortar

In the early 2000s, Ramachandran et. al. attempted to remediate the microcracks on the cement where they filled the manually cut crack zones with sand and *Bacillus pasteurii*[18]. Afterwards, specimens were cured in a solution consisting of urea-$CaCl_2$ for 28 days. A comparison was made between the control samples (without bacteria) and samples containing biomass in terms of stiffness values. Significant increase was observed on the bacterial beams compared to control ones[18]. However, it was seen that since the cut depths increase the performance decreases which can be directly correlated to the restriction of the bacteria from the required environments for MICP to take place.

A system that the bacteria is supposedly injected or incorporated by an outside intervention is not ideally align with the term self-healing. This ideology led the studies to focus on a system where the direct incorporation of the bacterial cells into the concrete could be possible. While it would be the optimal method for increasing the performance of the cementitious matrix, it was also a very difficult attempt due to the extreme conditions that would be projected on the microorganisms. There are several reasons for this. First of all, highly alkaline nature of cement is a serious factor which would decrease the viable bacterial cells where the mineral-forming ability is directly related with the cell concetration.[18], [22] Additionally, density of the cementitious matrix increases as the hydration continues. Thus, the pores inside it, which would supposedly host the bacteria, will get tighter to a point where the bacteria viability is not possible.[23]

Due to the above mentioned conditions, the main challenge of the application is to find a microorganism that can tolerate highly alkaline conditions of cement paste, can survive the mixing process, and can remain viable with limited access to nutrients[58] In this scope, Jonkers et. al. attempted to incorporate endospores into the matrix with the mixing water instead of viable cells and as a result bacterial cells were remained active for 4 months[24]. However, an attempt where vegetative *S. pasteurii* cells were added to the mix in active stage, it was observed that the cells could survive in mortar up to 11 months[59]. However, limited viability and lack of O_2 decreased the performance of $CaCO_3$ yield through all crack the depth. Instead, the precipitation was found to be limited to the crack mouth in microscale cracks[60].These findings support that, it is very important to consider the quantity of viable bacteria since it is possible to detect viable cells but as their count decrease the performance of the mechanism also decreases.

An alternative method which ideally could increase the bacterial viability for prolonged periods was encapsulation. Theoretically, a suitable carrier can protect the cells from the harsh environment and increase the mineral precipitation by keeping the cell quantity stable. The encapsulation methods consist of embedding the cells in a protective covering, e.g. inorganic lightweight porous aggregates (LWAs)[22], polymeric membrane [11,62], microcapsules [62], hydrogels [63] and natural minerals [31,65].

2.3.3 Protective barrier methodologies

Tittelboom et. al. used *Bacillus sphaericus* as the bacterial strain and silica gel as the protective barrier. Performance was compared to the unprotected bacteria in terms of water absorption, UPV and visual inspection[6]. Samples cured for more than 1 year where the visual crack remediation was observed in the specimens with protective barriers where the direct incorporation of the bacteria did not yield in calcium carbonate precipitation. Additionally,

protective barrier usage increased both UPV and water absorption performances when compared to samples prepared with unprotected cells[6]. A similar approach adopted by Wang et. al. where the silica gel and polyurethane were impregnated by *Bacillus sphaericus*[26]. Polyurethane showed superior results compared to silica gel in terms of water permeability where the coefficient, k, was 10^{-10}-10^{-11} m/s and 10^{-7}-10^{-9} m/s respectively[26].

Melamine based microcapsules were impregnated with *Bacillus sphaericus* spores where the self-healing efficiency was evaluated through water permeability and visual inspection[62]. After 8 weeks of curing through wet and dry cycles, 48%-80% healing ratio was observed in specimens with bio-microcapsules compared to control samples (18%-50%). Additionally, the decrease in the water permeability was ten times lower than the control specimens[62]. Hydrogel encapsulation was also attempted with a similar approach where the maximum remediated crack width was 0.5 mm where this value was 0.97 mm when microcapsules were used[63,64].

A comprehensive study was conducted by Erşan et. al. where diatomaceous earth, metakaolin, expanded clay, granular activated carbon, zeolite and air entrainment were evaluated as potential protective barriers[29]. However, the evaluation was made on the compressive strength and setting times where calcium carbonate precipitation and related scopes were not investigated. Diatomaceous earth and metakaolin resolved in a decrease in initial and final setting where other protective barriers did not show an apparent change. This behaviour was attributed to the addition of the fine particle minerals which might increase the reaction rate through an increase in surface area in contact with water[29]. Metakaolin, expanded clay and granular activated carbon showed positive or neutral effect on compressive strength where remaining minerals resolved in a decrease[29].

Wiktor et. al. proposed the impregnation of porous expanded clay particles with *Bacillus alkalinitrilicus* in order to compose a two-component self-healing agent[22]. Maximum

remediated crack width was 0.46 mm after a 100 days period of curing in water. Oxygen consumption profile measurements were also conducted in order to evaluate bacterial activity at prolonged periods. As a result, significant oxygen consumption was monitored in bacteria incorporated samples after months of casting where the consumption was insignificant in specimens without bacteria[22].

In 2017, impregnation of expanded perlite with nutrients and *Bacillus pseudofirmus* strain[30]. 4.1×10^{9} spores, 0.3 g calcium acetate and 0.03 g of yeast extract was used per gram of expanded perlite. Performance was evaluated through visual inspection and initial surface absorption of water. Full closure was observed in water cured samples at 165 days when the impregnated perlite was incorporated to the mortar. Additionally, bacteria-nutrient containing samples had 47% less absorption compared to control samples[30].

A natural mineral, which also evaluated for this study, diatomaceous earth was used as a potential carrier[28]. Performance of the system was evaluated through ureolytic activity, visual inspection and capillary water absorption. Crack widths varied between 0.15-0.17 mm where full closure was obtained when specimens were cured in nutrient medium (urea-calcium source) for 40 days. Additionally, a serious increase was observed in ureolytic activity of DE immobilized bacteria compared to the free cells where the cement mimicked environment was constituted. A decrease water absorption of 50-70% was seen when bacteria incorporated and control specimens compared[28].

This study introduces three new potential protective barriers being as bentonite, sepiolite and pumice on top of diatomaceous earth. Usage of the mentioned three barriers were not evaluated in literature by the means of self-healing through MICP.

Chapter III

Materials and Methods

3.1. Bacteria Selection and Growth

For this study, *Leibniz Institute- German Collection of Microorganisms and Cell Cultures: S. pasteurii (DSMZ 33)* strain was selected due to its high ureolytic activity and resistance to highly alkaline environments and nutrient depletion. Microorganism was also known as *Bacillus pasteurii*[65]. *S. pasteurii* is a gram-positive bacterial with a negative surface charge, thus it becomes a very ideal strain that can hydrolyse urea and trigger MICP in presence of calcium source.

Cells were incubated in a nutrient medium consisting of tris base (0.13M), corn steep liquor (CSL) (10 g), sodium acetate (10g) and urea (20 g) per liter of distilled (DI) water (DI). This solution is further referenced as Urea-CSL-Sodium Acetate (UCSLS) throughout the text. Twelve grams agar was added to per liter of solution when solid medium was needed. pH value was fixed at 9.

The nutrient medium was sterilized at 121°C for 45 minutes with an autoclave (HIRAYAMA HV 25-L, Japan). Afterwards, the solution was cooled the room temperature and the cells were added into the medium. Then the culture was incubated aerobically in sterilized liquid medium with shaking conditions (at 175 rpm) at 30°C using an incubator (IKA KS 4000, Germany). Aliquots were collected from the incubated bacterial culture periodically for viable plate counts. Evaluation was conducted by placing the droplets onto agar-petri dishes and their incubation at 30 °C. Colony forming units (CFU) were counted after 24 hours of plating. A correlation of bacterial growth (CFU/mL) vs. time was obtained. Bacterial growth curve was plotted as colony

forming units (CFU/mL) vs. time which can be seen in Figure 3.1. This was used for further determination of cell concentration in the study. The *S. pasteurii* inoculum for mortar mixes was grown from freezer-stock in 300-mL batches until the stationary phase (10^9 CFU/mL) was reached. Agar-petri plates were also used in order to reproduce the bacteria as an alternative to freezer-stock.

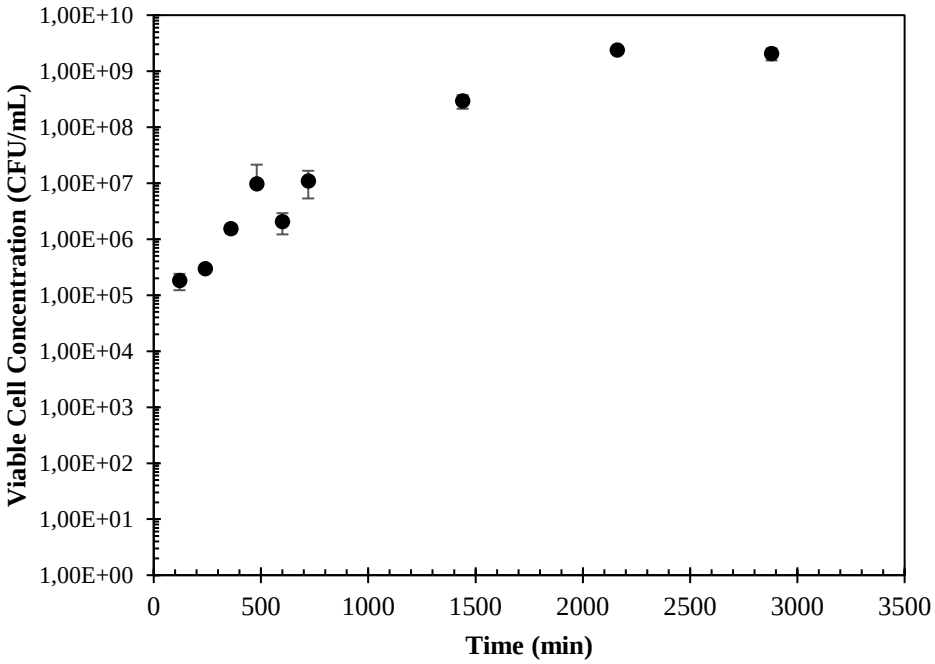


Figure 3. 1 *Representative growth profile for S. pasteurii (DSMZ 33) in UCSLS medium. Data points average of triplicates of samples and error bars represent the one standard deviation.*

As seen from Figure 3.1, the stationary phase of the cells was determined as 10^9 CFU/mL and this concentration was reached between 24-36 hours. Thus, the cells were incubated at least for 24 hours to obtain the desired concentration.

After the incubation, the cells were collected from the culture by centrifuging at 8000g for 10 min. The cells were washed twice by a sterilized phosphate buffered solution (PBS) and stored at 4°C until the immobilization.

3.2 Cement, aggregates and protective barriers

For this study, Ordinary Portland Cement (OPC) CEM I 42.5 R was used with an average particle size of 23.2 μm (Table 3.1). A standard sand compatible with the BS EN 196 was used as aggregate in the mortar mixes.

The goal of this study was to design a 2-phase biological self-healing agent for cement-based materials. For this specific purpose the bacterial cells were immobilized on diatomaceous earth (DE), pumice (PUM), bentonite (BT) and sepiolite (SEP), respectively. These minerals were selected due to their porous structure. DE, BT and SEP were used in fine powder form and PUM were rather used as fine LWA with a maximum particle size of 2.36 mm.

To define an immobilizing procedure the absorption capacity of the minerals and LWA were determined. In particular, the immobilization procedure was achieved by submerging the barriers to aqueous medium for 24 hours, thus so-called absorption capacity of the minerals could be specified as *24-hour absorption capacity*. This was determined by submerging the oven dry barriers in water for 24 hours and calculating the total absorbed water within the specified time frame. A similar procedure was also done for 48 hours and since the total absorbed water for the protection barriers did not show a significant change (except BT), *24-hour absorption capacity* was used though out the study. The *24-hour absorption capacity* of the minerals and LWA are summarized in Table 3.1.

Following the absorption capacity, a particle size analysis was conducted with a Mastersizer 2000 particle size analyzer with a Hydro MU 2000 (Malvern, Worcestershire, United Kingdom) wet dispersion unit. DE and BT were acquired in powder form from the supplier. However, SEP was not rather in the powder form. Thus, to obtain limit particle size, the mineral was sieved through a 150μm sieve and passing powder content was used in the experiments. Figure 3.2 summarized the particle size distribution of cement and minerals used in the study. In addition, pumice was used as fine LWA rather than powder form. Thus, the PSD was determined to ASTM C136 standard and the particle size used in the mixes was limited to those passing from 2.36mm sieve and retain on the 1.18mm sieve[66].

Table 3. 1 Characteristics of the cement, minerals and pumice.

Sample	*Particle size range*	*Averagee partice size*	*Absorption Capacity*
Cement	5-90 μm	23.2 μm	-
Bentonite (BE)	0.375-52.6 μm	22,1 μm	300%
Diatomaceous earth(DE)	0,375-90 μm	19.2μm	110%
Sepiolite (SEP)	0.375-864 μm	277,8μm	80%
Pumice (PUM)	0.3-2.5 mm	-	45%

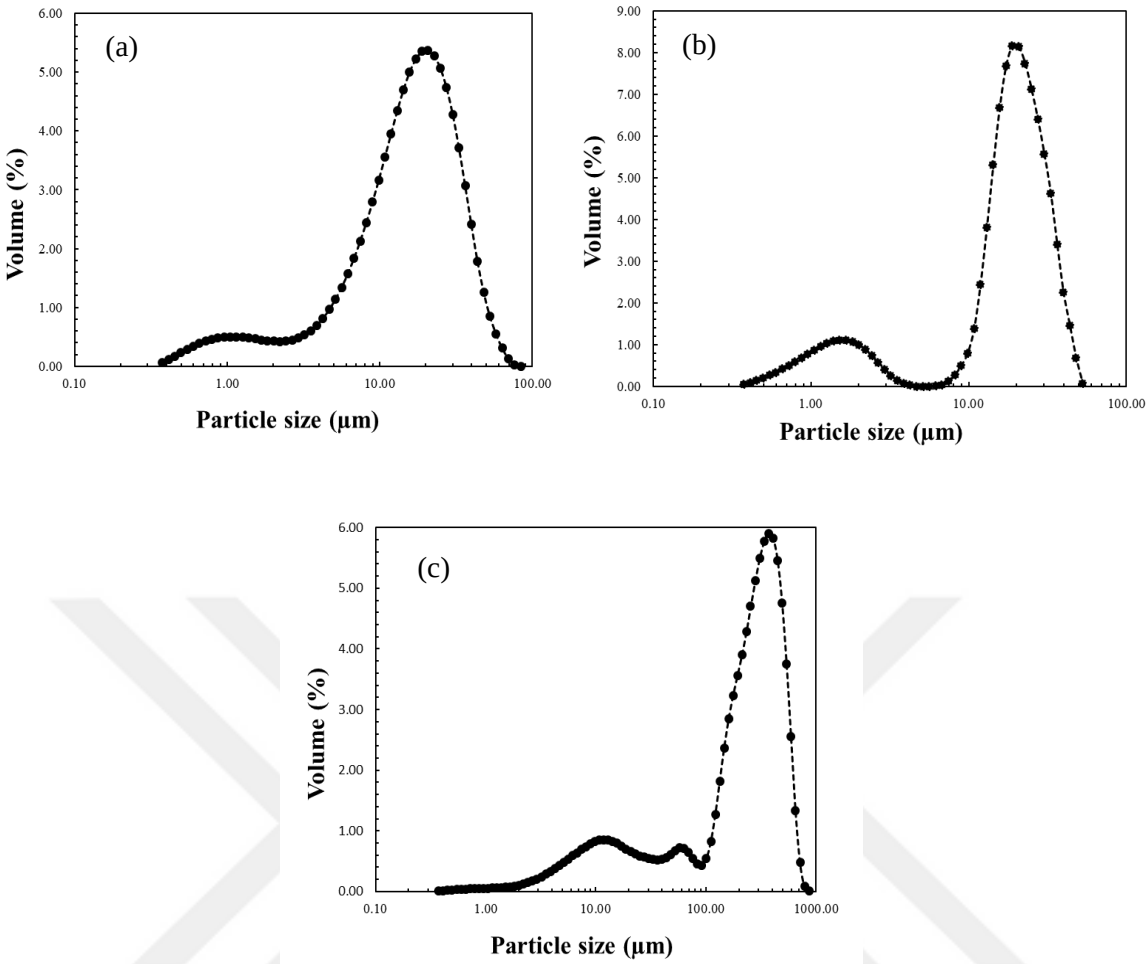
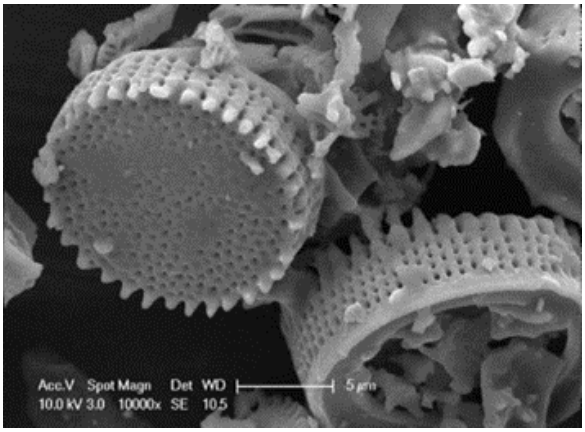
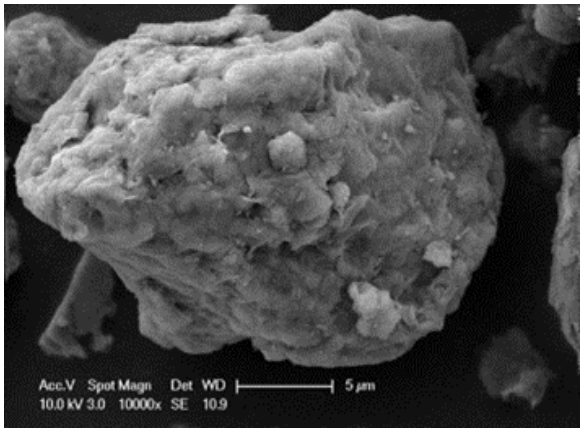


Figure 3. 2 Particle size distributions of (a) diatomaceous earth, (b) bentonite and (c) sepiolite.

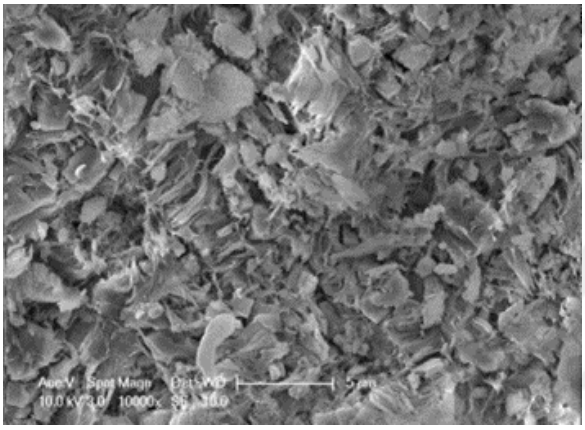
Additionally, morphology of the protection barriers was evaluated through SEM analysis. The samples were gold-coated prior to imaging with a FEI-Philips XL30 Environmental Scanning Electron Microscope with Field Emission Gun (FEG) (Boğaziçi University, Istanbul). The accelerating voltage was kept at 10 kV while the working distance was held at 10 ± 1 mm at various magnifications. Figure 3.3 shows the SEM images of protections barriers before the immobilization process.



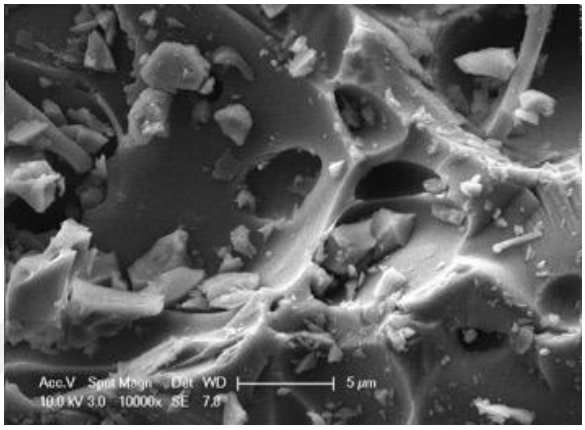
(a)



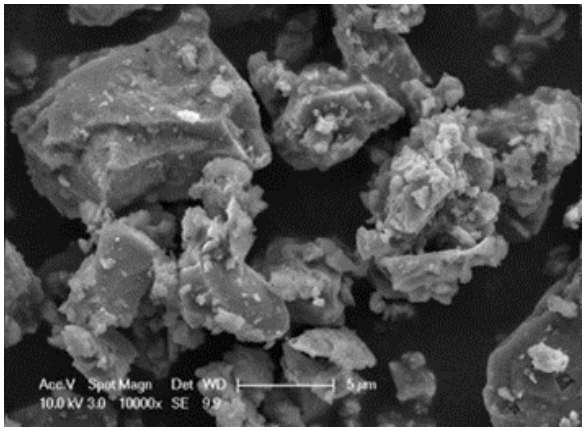
(b)



(c)



(d)



(e)

Figure 3. 3 *Initial SEM images of the (a) diatomaceous earth, (b) bentonite, (c) sepiolite, (d) pumice and (e) CEM I 42.5 R cement.*

3.3 Immobilization procedure

As mentioned, the aim of this study was to design a 2-phase biological agent to trigger self-healing in cement-based materials. To design the 2-phase biological self-healing agent, *S. pasteurii* cells were grown and collected as mentioned in Section 3.1. Then, these cells were immobilized on barriers characterized in Section 3.2.

The immobilization was simply done by (1) resuspending the collected bacterial cells either in sterilized PBS (2) submerging the protection barriers (minerals and pumice) in the bacterial solution or UCSLC medium (for 2 phase samples) for 24 hours under shaking conditions (175 rpm at 30°C). (3) impregnated minerals were removed from the incubator and any remaining solution was filtered through MN615 A Grade I filter paper (4) the obtained saturated minerals were partially dried in oven (at 40°C) until SSD condition was achieved.

Two different aqueous media were used for immobilization procedure. First, the collected cells were resuspended in PBS and all the protection barriers were submerged in this solution. These set of samples were rather used as control mixes. The 2-phase biological additive was obtained by submerging the half of the protection barriers to cell-PBS solution and the other half to the nutrient medium including of urea (20g/L), CSL (10g/L) and calcium acetate 10g/L (UCSLC). Compositions is further referenced as Mineral-Bac and Mineral-2P, respectively. A flow chart was presented at Figure 3.4 in order to clarify the immobilization procedure.

For immobilization process 2 g of vegetative *S. pasteurii* cells were immobilized on 22.5 g of DE, BT or SEP. However, since the particle size of pumice was larger, 6 g of cells were immobilized on 67.5 g of pumice. Proportioning for immobilization procedure was summarized in Table 3.2.

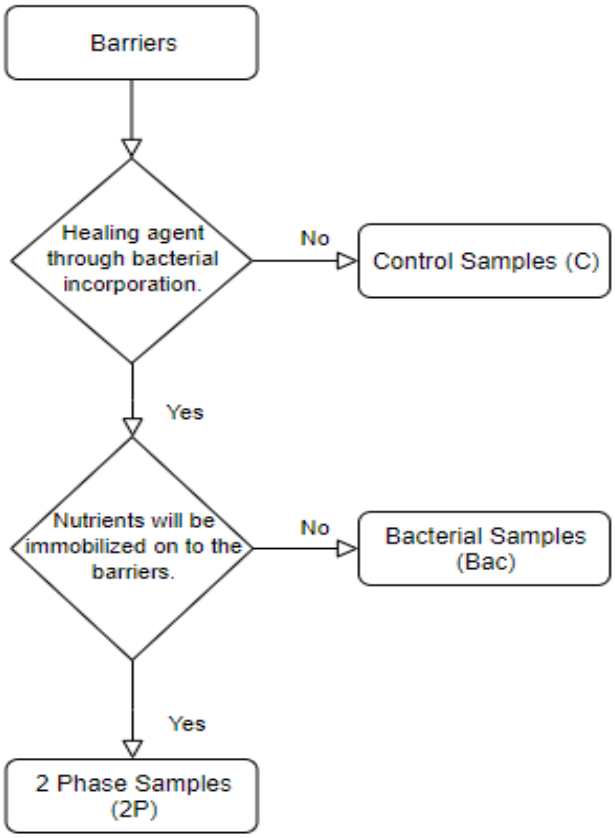
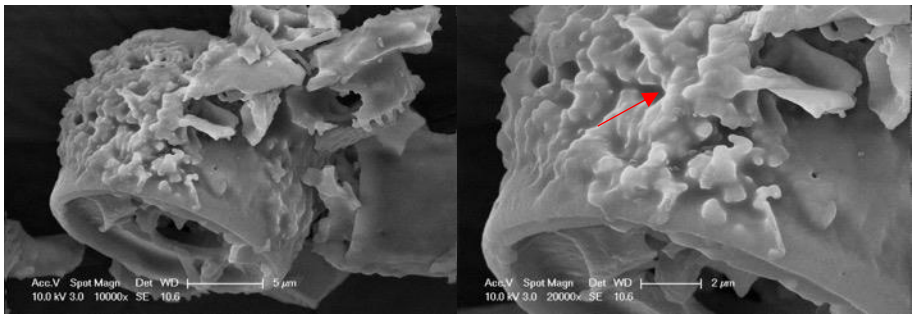


Figure 3. 4 Representative flow chart for the immobilization procedure.

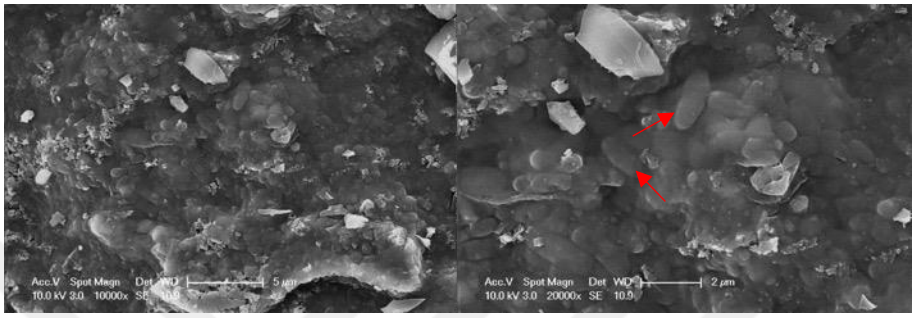
Table 3. 2 Proportioning for the immobilization process.

Additive Composition	Bacteria (g)	Mineral (g)	PBS (ml)	UCSLC (ml)
DE-Bac	2	22.5	87.5	-
PUM-Bac	6	67.5	262.5	-
BT-Bac	2	22.5	87.5	-
SEP-Bac	2	22.5	87.5	-
DE-2P	1	22.5	45	42.5
PUM-2P	3	67.5	135	127.5
BT-2P	1	22.5	45	42.5
SEP-2P	1	22.5	45	42.5

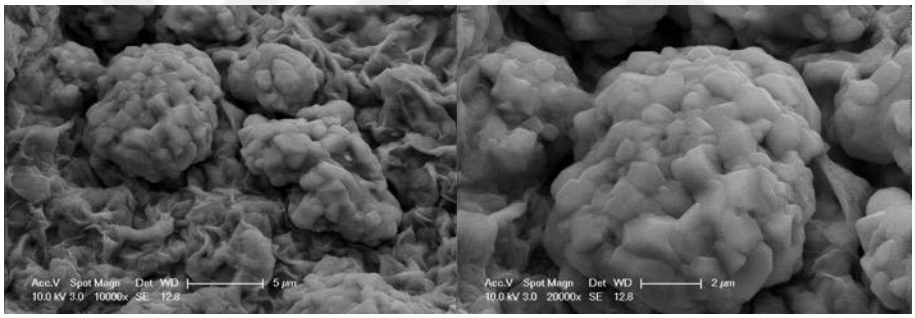
A SEM analysis was also conducted to validate immobilization procedure (Figure 3.5). Immobilized bacteria were visually observed in diatomaceous earth and pumice. Pores on the DE particles were filled with bacteria mass. Bacteria was also spotted on pumice particles where the surface of the particles were penetrated by the bacteria. However, there were no clear evidence of bacterial presence in the bentonite and sepiolite samples. This might be a result of the pore sizes difference between DE and PUM; BT and SEP particles. Since bentonite and sepiolite has high absorption capacities and complex morphologies, bacteria might be fixed inside the minerals. Additionally, inspected samples are a very small part of the total portion. This might resolve in a challenge to observe bacteria on the minerals.



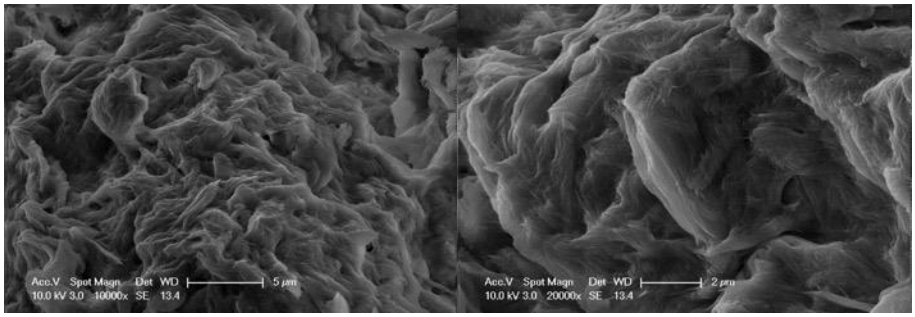
(a)



(b)



(c)



(d)

Figure 3. 5 *SEM images of the (a) diatomaceous earth, (b) pumice, (c) bentonite and (d) sepiolite after immobilization.*

3.4. Sample preparation, crack formation and curing

Mortar samples with OPC, standard sand and protection barriers were prepared to evaluate the self-healing ability of the 2- phase biological additive. Water to cement (w/c) and sand to cement ratios were 0.45 and 3, respectively. To provide flexural resistance and crack bridging affect during crack initiation, 12-mm micro synthetic fibers were added to mortar (6 g/m^3 of mortar). While the mineral protection barriers were used at 5% addition by the cement weight, 5% of the standard sand was replaced when pumice was used as protection barrier.

Since all of these minerals, LWA and fibers affect the workability of the mix, the mini slump flow of the mixes was adjusted with a polycarboxylate ether (PCE)-based superplasticizer (BASF). The workability of the mixes were evaluated according to ASTM C1437-15 Standard Test Method for Flow of Hydraulic Cement Mortar and ASTM C230-14 Standard Specification for Flow Table for Use in Tests of Hydraulic Cement [38,39]. The base plate and the conical mold were lubricated prior to the test for preventing the adhesion. Four readings were taken with a standard caliper. The flow percentage of the mortars was calculated by Eq. (3.1).

$$Flow\ percentage, \% = \left(\frac{A}{d_{base}}\right) \times 100 \qquad (3.1)$$

in *A* being the average of four reading minus the base diameter of the cone.

Used minerals and LWA, effected the flow of the mixes. In order the minimize this, iterations were made by changing the superplasticizer amount until the flow of the mortars were ±10% of the control neat mortar. The workability of pastes was adjusted using PCE superplasticizer.

Average flow values were calculated from triplicates of samples from 5 batches (15 samples in total). A detailed table including the prepared samples proportioning and their average flow values can be seen in Table 3.3.

Table 3. 3 Mix design of the beams and their flow.

Sample	Cement (g)	Sand (g)	Mineral (g)	Superplasticizer (g)	Flow (%)
Control	450	1350	-	3	21 ± 0.4
DE-C	450	1350	22.5	4.5	30 ± 0.4
BT-C	450	1350	22.5	4.5	12 ± 0.4
SEP-C	450	1350	22.5	4.5	15 ± 0.4
PUM-C	450	1282.5	67.5	4.5	22 ± 0.4
DE-Bac	450	1350	22.5	3	36 ± 0.4
BT-Bac	450	1350	22.5	3	24 ± 0.4
SEP-Bac	450	1350	22.5	3	20 ± 0.3
PUM-Bac	450	1282.5	67.5	3	25 ± 0.4
DE-2P	450	1350	22.5	1.5	34 ± 0.4
BT-2P	450	1350	22.5	1.5	33 ± 0.3
SEP-2P	450	1350	22.5	1.5	22 ± 0.3
PUM-2P	450	1282.5	67.5	1.5	32 ± 0.4

Mortar samples were prepared by ASTM C305-14 Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars [69]. Then, the prepared mortar samples were cast into 40 x 40 x 160 mm molds and kept in humid environment at 21°C. After 24 hours, beams were demolded and the samples were further submerged in tap water (at 21°C) for further curing until the testing. The tap water, used for curing, was refreshed weekly.

At 14 and 28 days after mixing, the samples were removed from the curing environment and wiped with a towel to remove excess water. Cracks were formed by flexural loading using a servo hydraulic displacement-controlled device (0.05 mm/sec). The samples were unloaded once the crack was formed visually, which corresponded to 50 ± 3% of the ultimate flexural strength of samples. Upon unloading, the remaining average crack width ranged from 0.3 mm to 0.5 mm. A set (3 samples) of samples were not cracked as negative controls for further analysis.

Once the cracks were formed a set of cracked samples were cured in tap water and another set was cured in nutrient medium (UCSLC). Triplicates of samples were submerged into the curing

media. To prevent contamination UCSLC medium was autoclaved prior to submersion and refreshed, periodically. Curing process was carried out by submerging the samples into curing solutions for 2-days and then, subsequently leaving them at ambient conditions for 2 days. This wet and dry cycle was applied until at least 90% crack sealing was observed. Table 3.4 summarizes the quantity of the samples, curing environment and the cracking periods. A total of 195 samples were casted and evaluated for analysis.

Table 3. 4 Representative table of sample quantity, curing environments and crack periods.

Sample	Not Cracked	Cracked at 14 Days		Cracked at 28 Days	
	Water Cured	Water Cured	UCSLC Cured	Water Cured	UCSLC Cured
Control	3	3	3	3	3
Mineral / LWA-C	3	3	3	3	3
Mineral / LWA-Bac	3	3	3	3	3
Mineral / LWA-2P	3	3	3	3	3

3.5 Evaluation of Self-healing

Most important part of this project is to evaluate and quantify the crack remediation ability of the developed biological self-healing agent. In order to fully understand the effects, various evaluation methods were used. This section will briefly explain the relevance of the methods to the sealing performance evaluation and clarify their methodologies. Used methods were stereomicroscopy, UPV, water absorption, SEM analysis and cell viability.

3.5.1 Stereomicroscopy evaluation

For the optical inspection of samples, Nikon SMZ745T Stereomicroscope and Clemex Vision Lite software were used. In order to evaluate the sealing, samples were evaluated every 7 days until the crack closure was observed. Samples were taken out from their specific curing solution a day ago and let to air dry. The basic work principle of the measurement was depended on the

software. In the setup stage, every pixel's dimensions were assigned through a micro-scale rod. This allowed the measurement of the crack width's and the closure percentages weekly. Images were taken at 6.7x and 50x scales which corresponds to 7000 μm and 1000 μm respectively. Lighting conditions were adjusted manually. However, when needed auto exposure was used.

3.5.2 Ultrasonic pulse velocity (UPV)

In order to evaluate the crack closure performance, UPV test was conducted according to ASTM C597-16 Standard Test Method for Pulse Velocity Through Concrete [70]. The test was simply conducted by measuring the transverse time of longitudinal stress waves generated by electro-acoustical transducer. Transverse time is affected by the cracks and voids located inside the concrete.

Measurements were taken weekly in order to evaluate the healing performance until the complete closure was seen. Prior to the test, transducers and the sides of the beams were carefully wiped in order to obtain a smooth contact zone. Also, calibration check was conducted before each test with a calibration rod. Test was conducted while the samples were at air-dry condition. Grease was applied to the transducer faces as a coupling agent in order to eliminate air at the contact surfaces. Measurements were taken by simply pressing the transmitting and receiving transducers to the two sides of the beam where they were located opposite to each other. Transducers were connected to the pulse generator and the resonant frequency was adjusted as 20 kHz.

Afterwards, pulse velocity was calculated according to the following formula:

$$V = L/T \quad (3.2)$$

Where V is pulse velocity in m/s, L is the beam length in meters and T is the time in seconds.

3.5.3 Water absorption

Water absorption capacity of the beams were evaluated with respect to RILEM 25 PEM II-6 [71]. After the sealing of the samples were observed, curing stage was ended and the samples were further inspected on their absorption ability.

Beams were removed from their assigned curing environment and put on an oven at 40°C. The samples were dried until the mass change was within the range of ±0.1% in the periodic measurements. When a stationary mass change achieved, samples were partially covered with paraffin in order to restrain the water penetration. Only 40x40 mm area around the sealed or non-sealed crack zone was left uncovered. Also, the opposite side of the crack surface was not covered with paraffin in order to allow water flow. Dry weight (W_d) of the samples were measured before the submersion. Afterwards, beams were submerged into a water bath where submersion depth was 2 mm and only the uncoated surface was in contact with water. Specimens were periodically removed at 15 and 30 min;1,2,3,8,24,48 and 120 hours from the water bath in order to measure the wet mass (W_w). Water droplets on the samples were carefully wiped with a towel without disturbing the precipitates sealing the crack. After the measurement, specimens put back into the water immediately.

Water absorption coefficient (k) was calculated with the following formula:

$$k(t^{0.5}) = Q/A \quad (3.3)$$

Where;

k (kg/ (m^2.$s^{0.5}$)): water absorption coefficient,

t (seconds): time,

Q (kg): absorbed water mass (W_w - W_d),

A (m^2): submerged surface area.

3.5.4 SEM analysis

After the water absorption test, specimens were collected from the sealed crack zones with a hammer. Samples were obtained from crack surfaces. Then, the collected samples were further split into the pieces to a dimension smaller than 1 cm^3. Morphology of the specimens were inspected with a FEI-Philips XL30 Environmental Scanning Electron Microscope (ESEM) with Field Emission Gun (FEG) to identify the presence of bacterial cells and to evaluate the precipitate morphology. The accelerating voltage was kept at 10 kV while the working distance was held at 10 ± 1 mm at various magnifications.

3.5.5 Viability of the cells

A part of the samples obtained from the cross section (Section 3.5.4) were used to determine the bacterial viability. First, the collected samples were powdered into very small pieces with a sterilized pestle and mortar. Then, approximately 20±1 g of this powdered samples put in the 50 ml sterile centrifuge tubes. Then, the pre-prepared and autoclaved UCSLS medium was injected into the tubes. Approximately, 25±5 ml nutrient medium was used for every tube. Tubes were then left to the incubator in shaking conditions at 30℃ for one hour. Afterwards, tubes were taken out of the incubator and submerged into to water in an ultrasonic bath. Sonication was applied for 20 minutes at 30℃ in order to separate the bacteria from the matrix.

It should be noted that, sonication is not a wide-spread method to separate bacteria from different carriers. There are several studies present that discusses this method as a possible practical method. The effects of the sonication on the bacteria is dependent on various factors such as exposure duration, temperature, bacteria type and the tube material [72]. However, it is clear that the sonication is an effective method in order to remove the bacterial cells from the carrier mortar.

After the sonication, supernatant liquid was taken out with a sterile syringe and suspended into a 275 mL of new UCSLS growth medium and incubated in shaking conditions at 30℃ for 24 hours. Special care was taken in order to not incorporate any matrix sediment while the suspension process. For the bacteria count, viable plate count method was used where 1 ml aliquots were taken from the growth medium and 10-µL drops of each dilution were inoculated on agar-petri dishes [73]. Plates placed on the incubator for 24 hours than the viable cell count was carried out. Samples were taken as duplicates in order to eliminate errors and to obtain a standard deviation.

Chapter IV

Results and Discussion

In general, visual crack closure was observed in all samples including bacterial cells cured in UCSLC medium, regardless of the protective barrier. In contrast, no visual crack closure was observed in negative control samples without any bacterial cells. Throughout the evaluation 2 different curing methodology (water and UCSLC medium) for 14 day and 28 day cracked samples. This chapter includes a brief explanation of results for each different protective barrier and a cumulative discussion on their performance as self-healing agents.

Analysis were conducted to evaluate the self-healing in cement-based mortar samples including the negative control samples without any bacterial cells and samples containing bacterial cells (*Bac* and *2P* samples in Table 3.3). Since the visual crack healing was almost the same for each set, one representative image from triplicates of samples were presented in the text. Control sample images were presented at the Appendix A. However, rest of the data was presented on triplicates of samples.

4.1. Diatomaceous Earth (DE) as a protective barrier:

4.1.1 Visual crack healing evaluation:

Tables 4.1 to 4.4 shows the representative images of cracked DE-Bac and DE-2P samples before and after crack healing. A set of samples from each series were cracked after 14 days of mixing (*Table 4.1 and Table 4.3*) and another set was cracked at 28-days (*Table 4.2 and Table 4.4*). Average crack size for these samples were recorded as 0.32 ± 0.03 mm.

Crack sealing was observed in all samples cured in UCSLC medium regardless of if the cells were added to the mix with (DE-2P) or without nutrients (DE-Bac). Similar trend was observed at both 14 and 28-day cracked samples. Visually there was not any difference in the crack sealing of the bacterial cells at different ages. Mineral precipitation was observed in all through the crack. For 2-phase samples, a complete closure was observed in cracks having a size of 0.28 mm and 0.38 mm for 14 and 28-days, respectively.

Interestingly, partial crystal formation was observed in DE-2P 28 -day cracked samples cured in water. Precipitate formation was very limited at the 14-day cracked samples compared to those in 28-day cracked samples. Beams had more apparent formations especially located near the crack walls. This can be explained with the heterogenous distribution of the bacteria. It is known that the MICP would not be possible at the water cured samples simply because there was no urea supplied to the bacteria to evoke their ureolytic activity. However, intrinsic calcite precipitation due to hydration can actualize without urea and due to the negative surface charge of the bacteria, calcite precipitates may deposit near the crack walls[20].

Another aspect was the observation of the surface voids closure. Pores on the beam surface were filled with white precipitates when the bacteria immobilized samples were cured in UCSLC medium. However, this behaviour not observed at water cured samples. *In short, precipitation was only observed when a certain amount of nutrients existed in the environment.*

Another point that should be noted is the duration of healing process. Crystal precipitation was initiated after 7 days of UCSLC curing and 80% crack sealing was achieved almost at 3 weeks. Full crack closure was observed latest at 28-days.

At last, the colour of the precipitate was different in *DE-Bac* samples. While a yellowish precipitate was observed in *DE-Bac* samples, a while crystal was seen in *DE-2P* samples. To understand the possible difference in crystal morphology, further SEM evaluation was conducted (*see* Section 4.1.4).

Table 4. 1 DE-Bac beams cracked at 14 days.

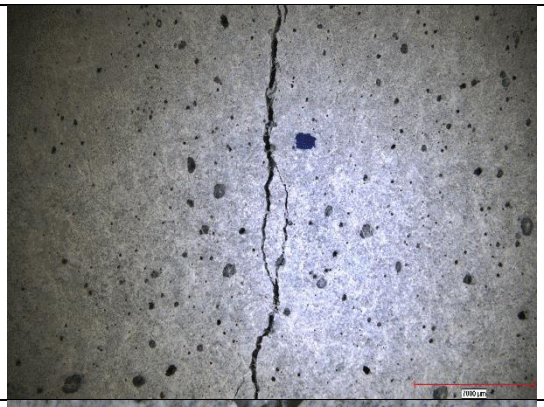
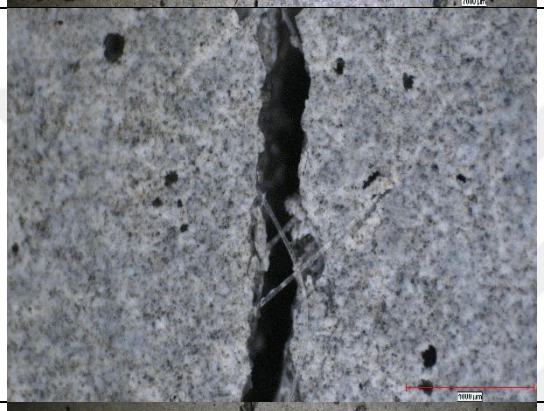
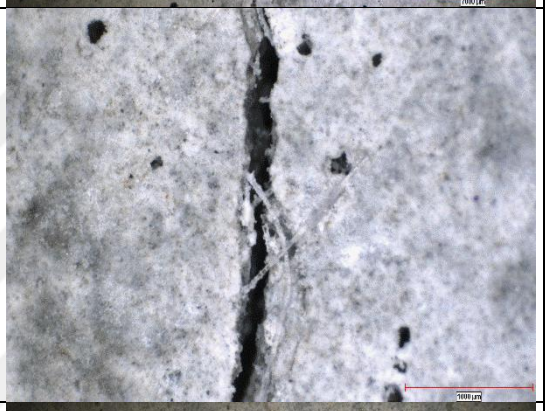
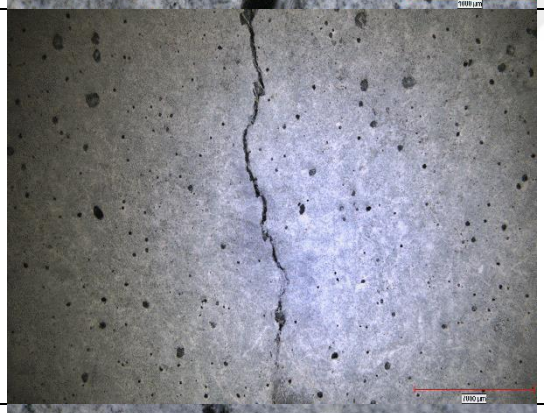
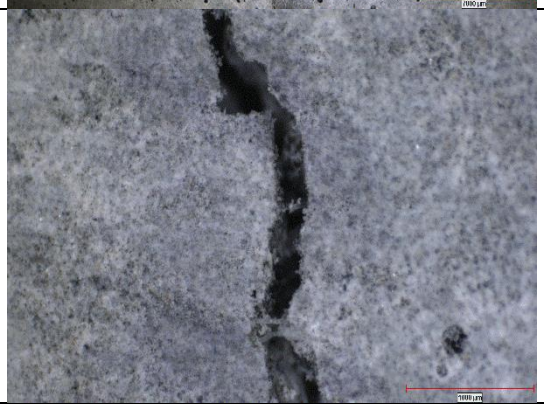
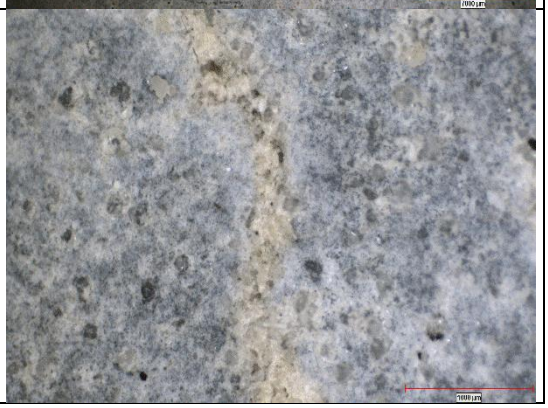
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 2 DE-Bac beams cracked at 28 days.

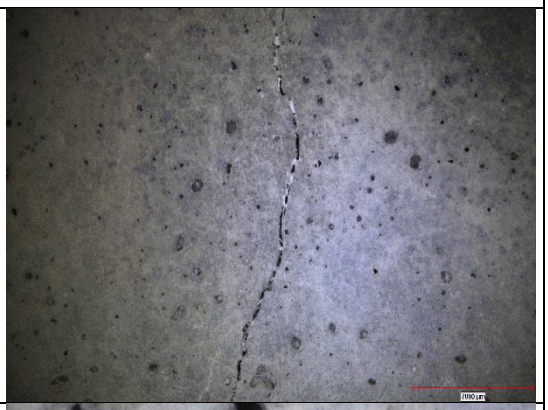
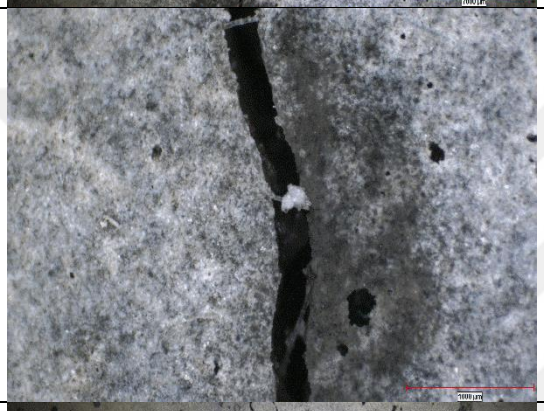
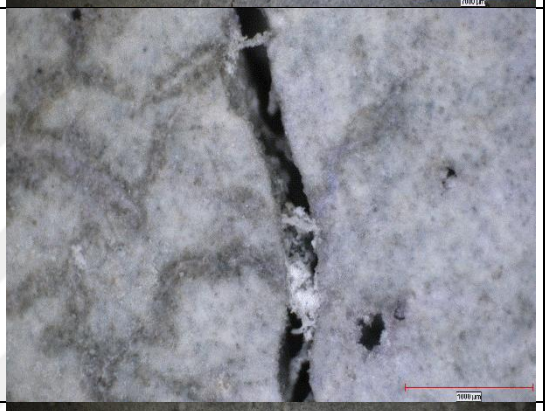
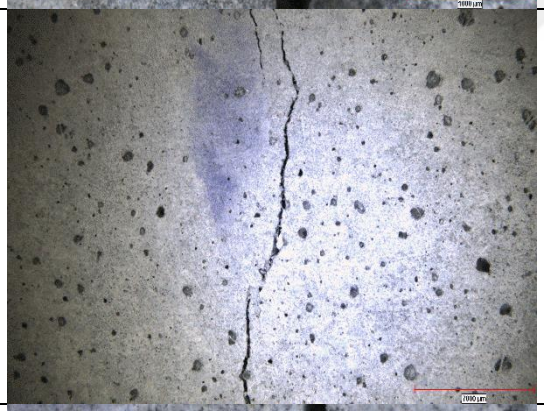
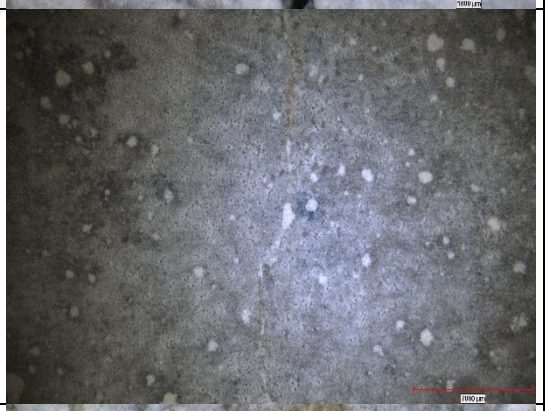
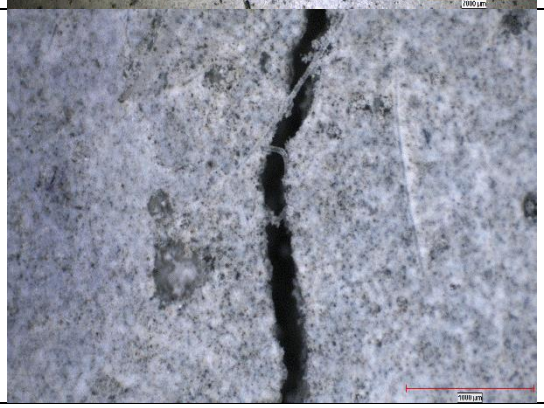
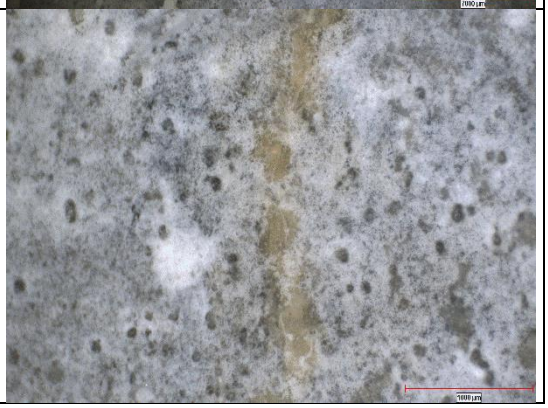
28 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 3 DE-2 Phase beams cracked at 14 days.

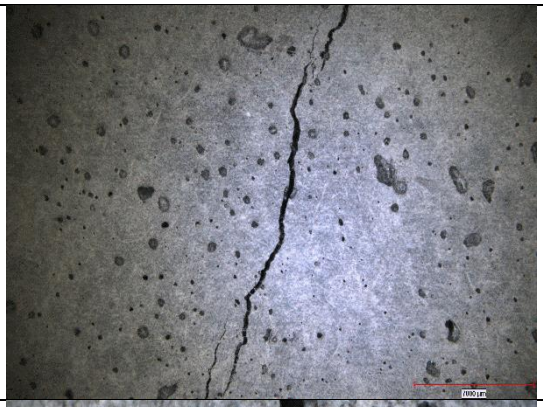
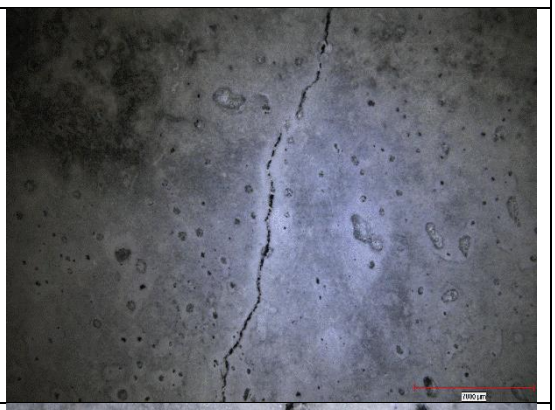
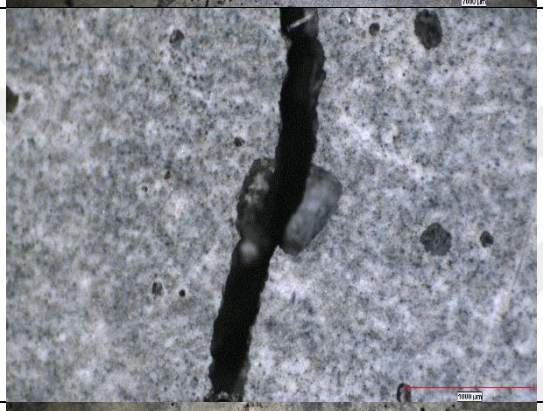
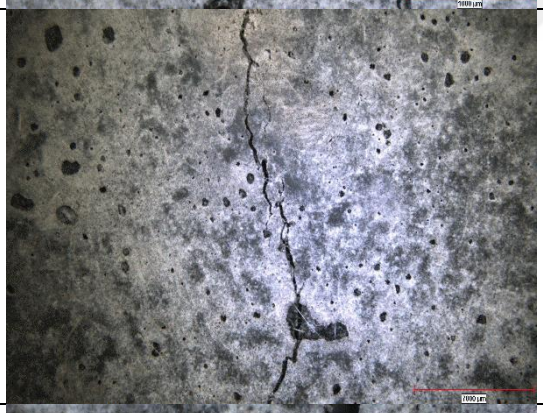
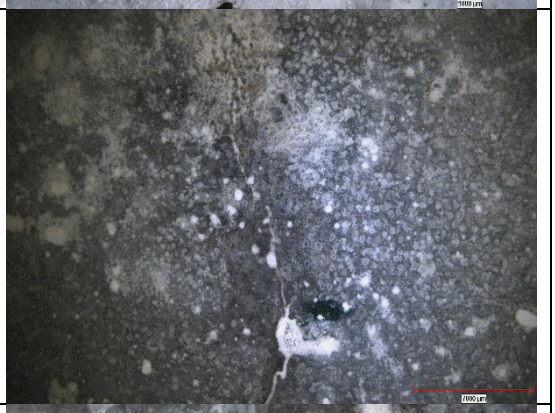
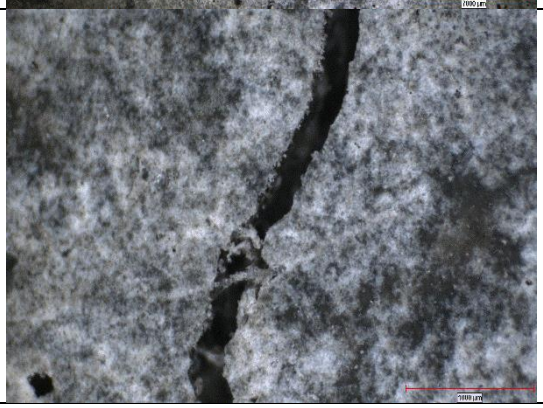
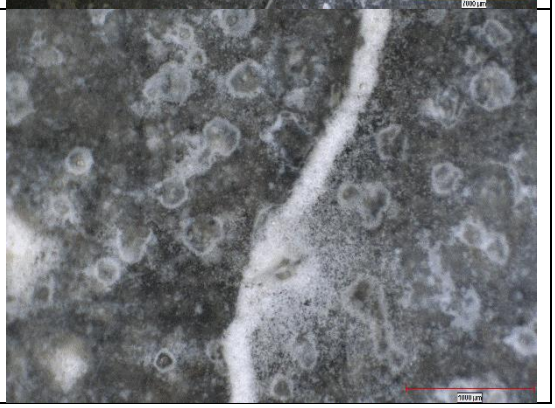
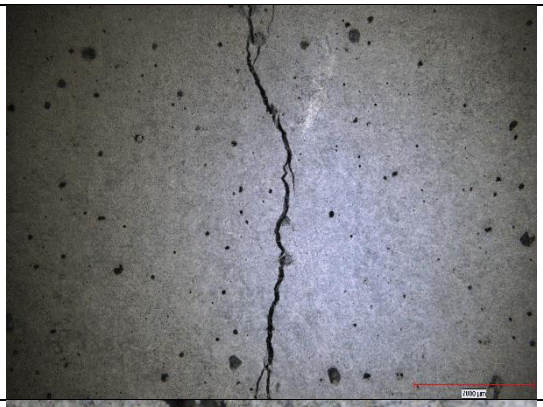
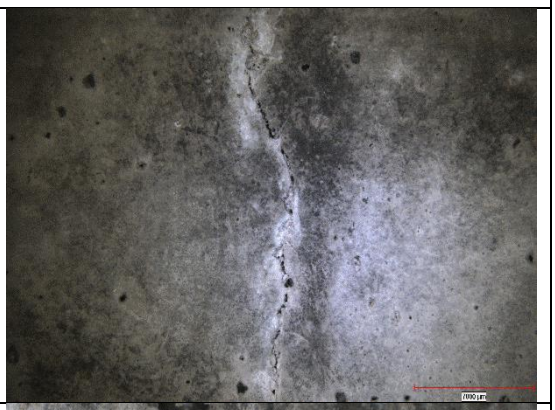
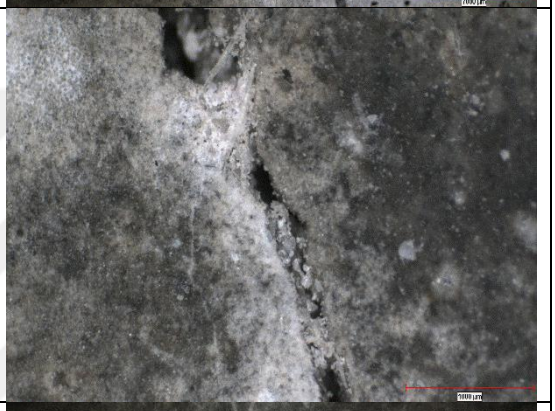
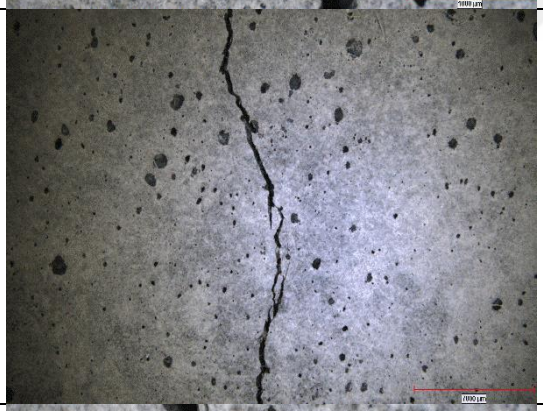
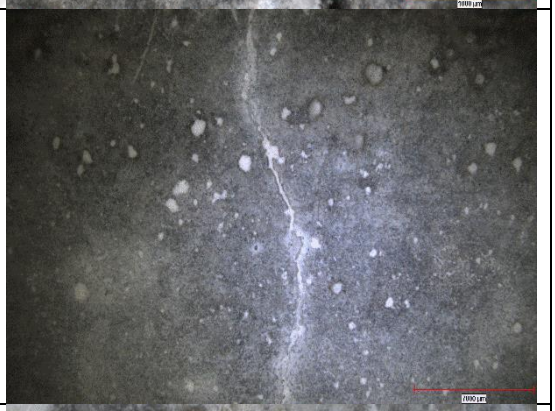
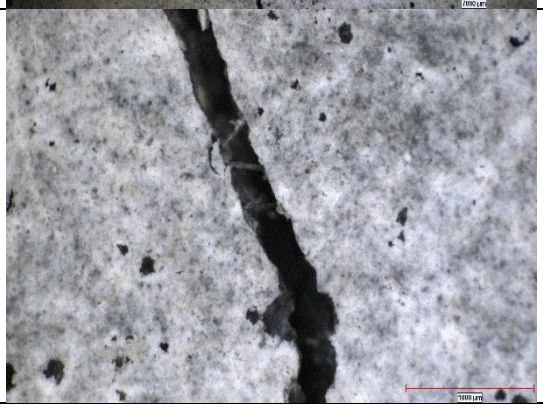
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 4 DE-2 Phase beams cracked at 28 days.

28 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

4.1.2 UPV analysis for detecting crack healing:

UPV test was conducted as a semi-quantitative method to evaluate the crack closure. This test enabled to evaluate the possible crack closure not only on the crack mouth but rather through the depth of the crack. Similarly, test was conducted on samples with and without any bacterial cells (*DE-C, DE-Bac and DE-2P*). The samples were evaluated by measuring the transverse time of the stress waves with an ultrasonic pulse velocity equipment. The waves would propagate faster in a denser environment. Thus, crack closure or the remediation of internal pores could be further evaluated with this test. Figure 4.1 and Figure 4.2 show the UPV readings obtained through 28 days of evaluation and change in the UPV between before and after healing for samples cracked at 14 and 28 days after mixing.

Results of the DE-Bac samples were coherent with the visual inspection where a higher increase was recorded for the samples with crack closure. The difference was interesting between the 14 and 28 day cracked samples. Samples cracked at 14 days showed a superior increase in velocity compared to 28-day ones. This was interpreted as the decrease in bacterial activity in 28 day cracked samples caused by longer isolation time of the bacteria. This claim is supported by the behaviour of DE-2P samples where 28 day cracked samples showed higher velocity compared to 14-day samples. Immobilization of the nutrients alongside with the bacteria might increase their activity at prolonged periods.

The UPV results obtained were in line with the visual inspection except the DE-2P 14-day cracked samples cured in UCSLC medium. Abovementioned set showed a very similar increase in velocity compared to DE-C samples cured in UCSLC medium. This was an indication that even the sealing was observed at the crack mouth, internal crack healing might not be attained. Additionally, this behaviour was not observed at 28-day cracked samples with the same composition. Further investigations such as water absorption and SEM analysis would clarify

the deviation in this data set. This was also the case for DE-2P water cured samples where for 14-day cracked samples DE-C attained a higher velocity. Similarly, to the 14-day cracked samples, DE-2P 28-day cracked beams had a higher velocity compared to DE-C samples. In fact, the highest strength change was recorded in DE-Bac samples cured in UCSLC medium. Such that upon crack initiation the velocity dropped to 3.8 km/h and upon 14 days of treatment it was increased to 4.1. km/h. This might be attributed to filling of cracks with the precipitate. In addition, the relatively higher change in UPV for 14 day cracked DE-Bac samples compared to its counterpart DE-2P samples could be attributed to the amount of the cells present in the mix. DE-Bac samples contain 1g of bacterial cells per kg of mortar whereas DE-2P contain 0.5g of bacterial cells per kg of mortar. The higher amount of bacterial cell dosage might increase the amount of precipitation in the cracks. This relative difference was less pronounced in 28-day cracked samples, which might be related to possible decrease in viability of cells in DE-Bac samples since the cells do not have any access to nutrients prior to crack initiation.

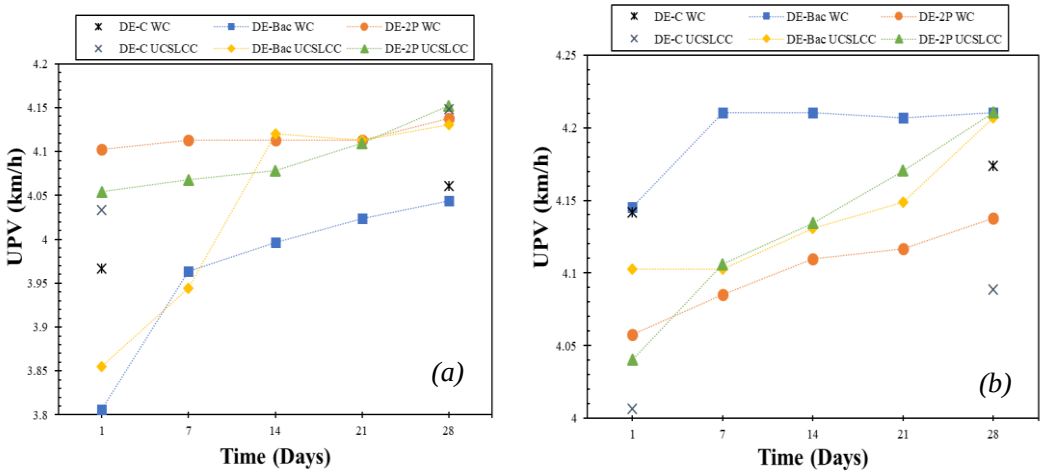


Figure 4. 1 *UPV recordings DE containing samples for 28 days (a) samples cracked at 14-day after mixing (a) samples cracked at 28-day after mixing. DE: Diatomaceous earth; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing.*

Another point that should be mentioned is the UPV values obtained in samples. Even though the absolute change in DE-2P samples were lower compared to DE-C and DE-Bac samples, particularly for samples cracked at 14 days after mixing, the UPV readings obtained in these samples were relatively higher compared to rest of the samples. Throughout the literature, generally it is agreed that very high velocities (> 4570 m/s) indicates very good concrete quality, while very low velocity ranges (< 3050 m/s) are indicative of poor concrete quality [74]. In addition, period changes in velocity might indicates the possible change in the quality of the concrete [74]. Thus, the increase in velocity is also an indicator of an improvement in quality of the material. Even though the relative change obtained through 28 days was lower, the velocity recorded in DE-2P was already high which is an indicative of a high quality.

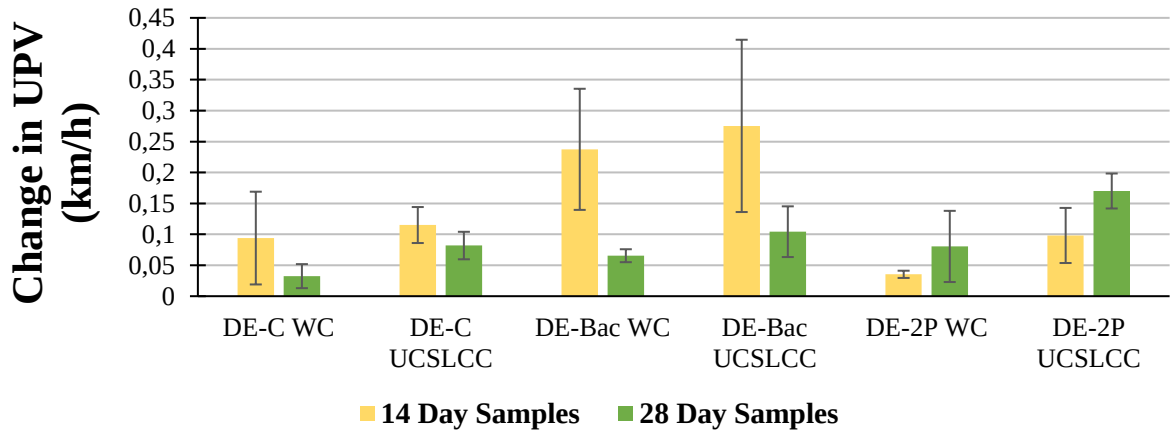


Figure 4. 2 *UPV change of the DE containing samples at the end of curing stage. DE: Diatomaceous earth; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing. Error Bars represent the standard deviation obtained from triplicates of samples and columns represent the average of 3 samples.*

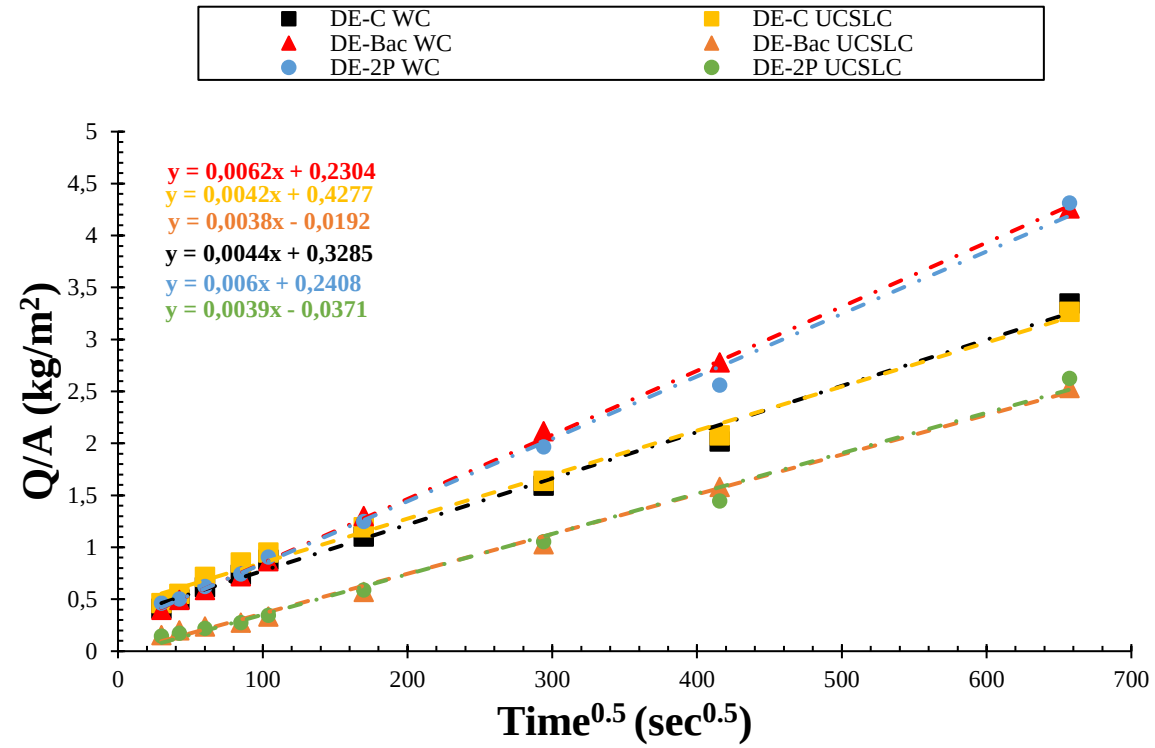
4.1.3 Water absorption:

A water absorption test was conducted to quantify the degree of crack closure in terms of water penetration. Based on visual inspection crack mouth and the pores on DE-Bac and DE-2P samples were sealed with a white precipitate when the samples were cured in UCSLC medium. In addition, UPV test revealed that the sealing might only occur on the crack mouth rather than through-out the crack. This might also be expected since *S. pasteurii* cells are aerobic and would also need oxygen to be metabolically active. Thus, it would be more accurate to quantify the crack healing in terms of water tightness upon crack sealing. Herein, water tightness was correlated with water absorption. To determine the water absorption coefficient, the water absorption capacity of healed samples was plotted as a function, of time and the slope of this graph shows the water absorption coefficient (k) value. The water absorption coefficient, k, was calculated by determining the slope of the trendlines.

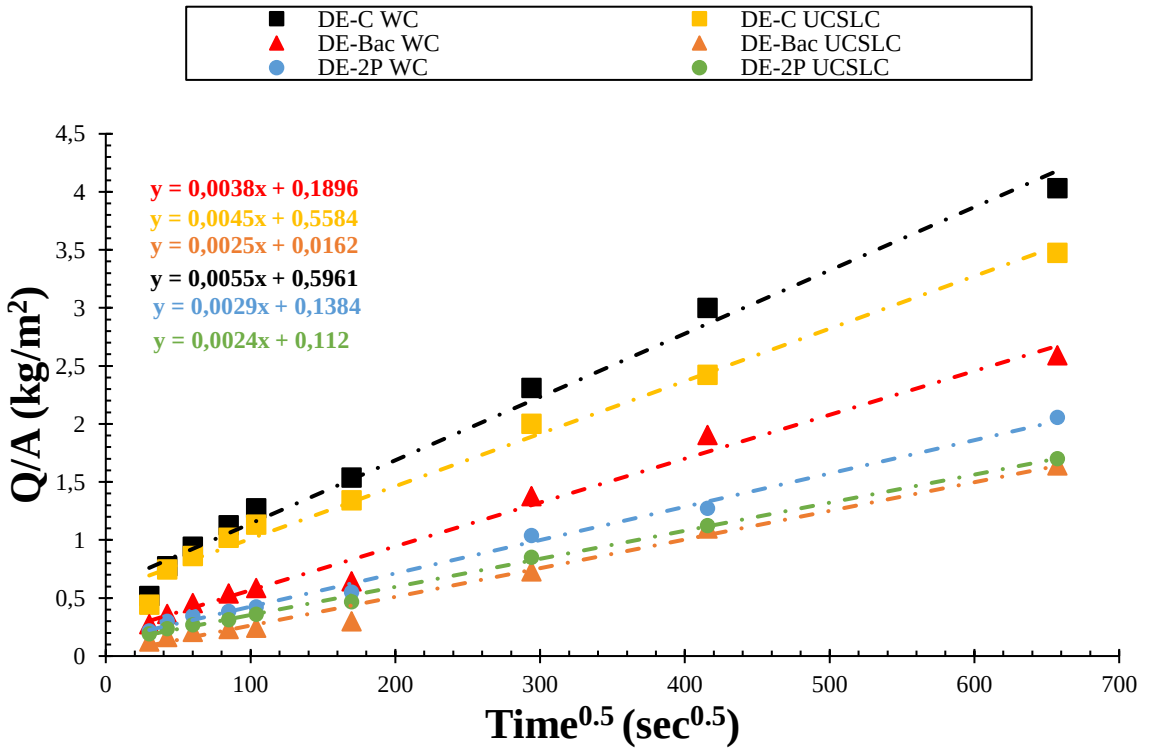
Figure 4.3 presents the water absorption capacity of *DE-C*, *DE-Bac* and *DE-2P* samples as a function of time. The data points represent the average values obtained from triplicates of samples. For 28 day cracked samples (*see* Figure 4.3 (b)), the water absorption coefficient, k, calculated from the slope of the graphs was in line with the visual inspection such that control samples without the bacterial cells have the highest k values and DE-Bac and DE-2P samples cured in UCSL medium have the lowest. There was a 45% decrease in water absorption coefficient in these samples compared to DE-C sample cured in UCSL medium. Relatedly, there was a considerable improvement in k value (almost 50 %) in 28 day cracked DE-2P sample cured in water compared to its counterpart control sample DE-C cured in water. This is slightly inconsistent with visual inspection result where there was only a partial precipitate observed around the crack mouth. However, there was a slight increase in UPV in 28 day cracked DE-2P sample cured in water. This might indicate that the healing process in this

sample is internal rather than the crack edge. Further SEM analysis through the crack surface might reveal the discussion on this set.

The change in absorption coefficient was lower in 14 day cracked samples compared to the changes observed in 28 day cracked samples. Such that, the k coefficient was lower in 14 day cracked DE-Bac and DE-2P samples cured in UCSLC medium compared to their counterpart control sample, the change in k value was limited to only 10%. Even though the precipitates fully sealed cracks and pores, this did not affect the water tightness of the sample. In fact, this might be understood with the SEM and viability results obtained from this particular set of samples but also could be a simple experimental error. Generally, calcite was known to be a denser and a more stable polymorph of $CaCO_3$, thus might lead to a better improvement in water absorption. While the other polymorphs like vaterite and aragonite are meta-stable which might result with a variation in water absorption [55]. Further evaluation has to be done to understand the mechanism of crack sealing and water tightness.



(a)



(b)

Figure 4. 3 *Mass of absorbed water per m^2 for (a) 14 day (b) 28-day diatomaceous earth samples. DE: Diatomaceous earth; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing. Data points represent the average of samples.*

4.1.4 Precipitate characterization:

SEM evaluations were conducted on samples that self-healing was observed. Images of crack surfaces obtained from 14-day old *DE-Bac* and *DE-2P* samples can be seen in Figure 4.4.

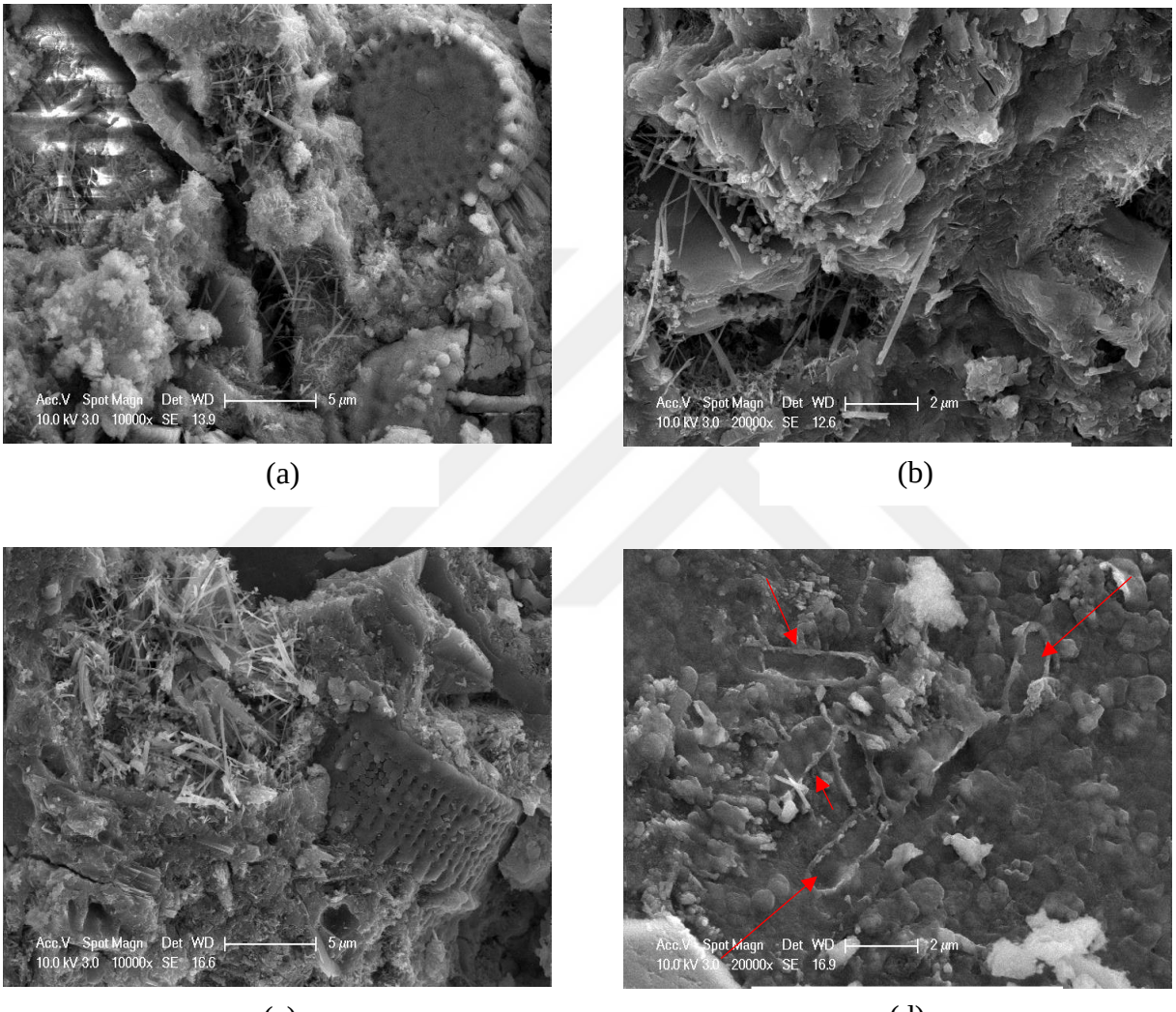


Figure 4. 4 *SEM images of the 14-day cracked samples cured in UCSLC medium (a) and (b) DE-Bac samples, (c) and (d) DE-2P samples with red arrows highlighting the bacterial presence*

As seen from Figure 4.4 (a) to (c) similar crystals morphologies were observed in both DE-Bac and DE-2P samples. Vast amounts of amorphous calcium carbonate (ACC) formation were observed. However, ACC formations on the needle shaped crystals were increased the chance

of this needle shaped crystals might be aragonite [57]. Further EDAX or XRD analysis have to be done to fully comprehend the exact nature of the crystals. In addition, calcite crystals were also observed through the analysis. These results showed that *S. pasteurii* cells immobilized on DE was successfully triggered MICP and resulted with different polymorphs of $CaCO_3$. Another note that should be mentioned that evidence of bacterial cells was also observed (*see* Figure 4.4 (d)), particularly for DE-2P sample cured in UCSLC medium. Rod shape indications, having an approximate size of 2 μm, proves that the immobilization was achieved and the cells could be found in cracks.

4.2. Bentonite (BT) as a protective barrier:

4.2.1 Visual crack healing evaluation:

Tables 4.5 to 4.8 present the stereomicroscopy images of *BT-Bac* and *BT-2P* either cured in water or UCSLC nutrient medium. A similar trend was also observed when bacterial cells were immobilized on bentonite in terms of visual crack sealing. Such that full crack remediation was observed in *BT-Bac* and *BT-2P* samples cured in UCSLC regardless of their age. Average crack size for these samples were recorded as 0.30 ± 0.02 mm. In contrast there was not any crack sealing observed in control samples without any bacterial cells (BT-C cured in water and UCSLC medium).

A particular note on BT samples is the rate of crack sealing. The cracks were sealed faster than that of in samples prepared with DE. While full crack sealing in *DE-Bac* and *DE-2P* samples could only be achieved in 28 days, this duration was 14 to 21 days in *BT-Bac* and *BT-2P* samples. This might be attributed to the autogenous healing effect of bentonite by itself. Bentonite is a clay that could also be used as a self-healing agent in cement-based materials[75]. Suleiman et al. [75] showed that partial crack healing was observed in concrete samples

containing bentonite in 1 year. Thus, incorporation of bentonite with another self-healing agent, such like bacterial cells, significantly improved the rate of crack remediation compared to the cases where these 2 agents used separately [75]. This might be related to the limited access of bacterial cells to nutrients.

Another different trend observed in BT samples was crack closure was observed in *BT-Bac* and *BT-2P* samples cured in water. However, the sample ages were rather different, such that crack closure was observed in 14 day cracked *BT-Bac* and 28 day cracked *BT-2P* samples cured in water. Curing rate of these samples were also different, 28 day cracked *BT-2P* sample was cured in 21 days while 14 day cracked *BT-Bac* sample cured in 28 days.

The colour of the precipitates was clear white, except for 14 day cracked *Bac-2P* sample cured in UCSLC medium. This might again be related to the different crystal morphology, but it could also be simply due to the colour of the curing medium.

Table 4. 5 BT-Bac beams cracked at 14 days.

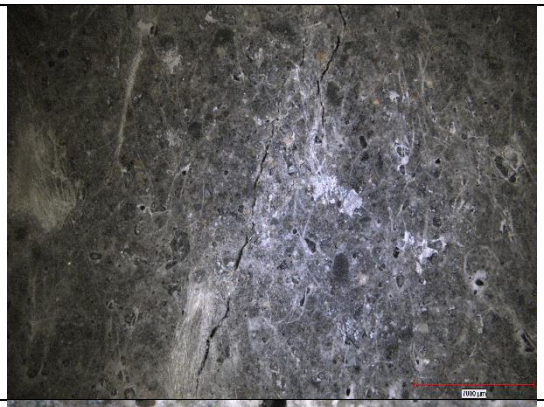
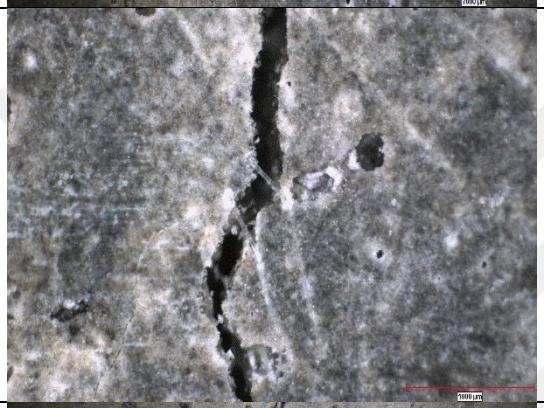
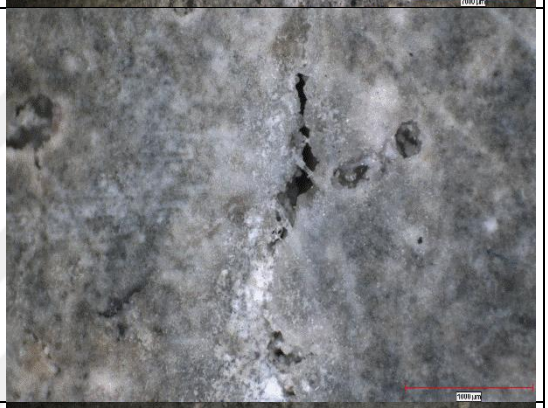
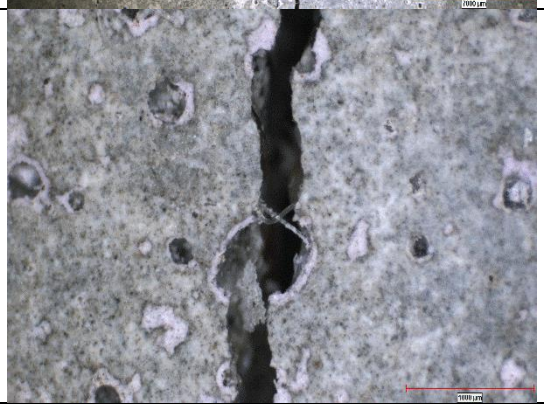
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 6 BT-Bac beams cracked at 28 days.

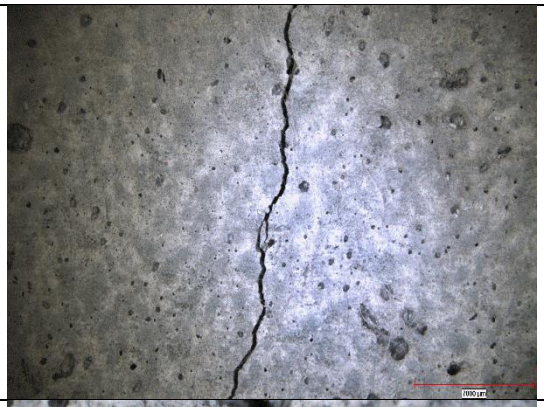
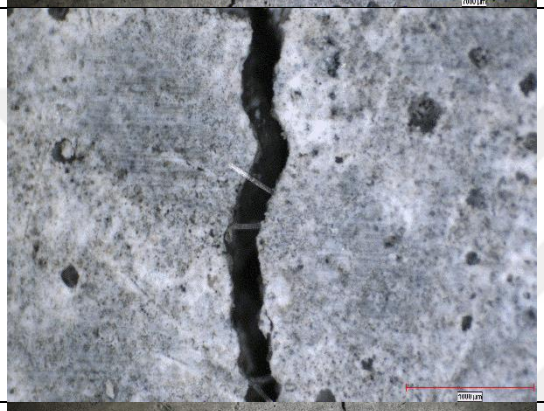
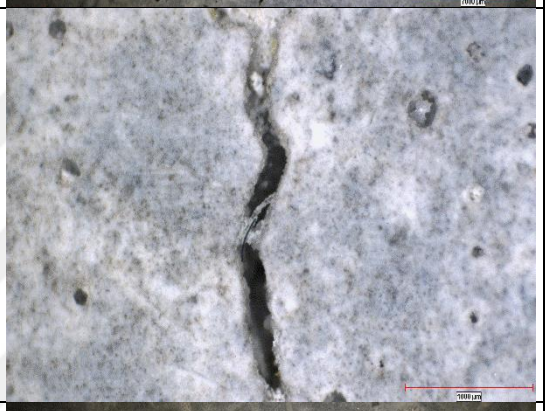
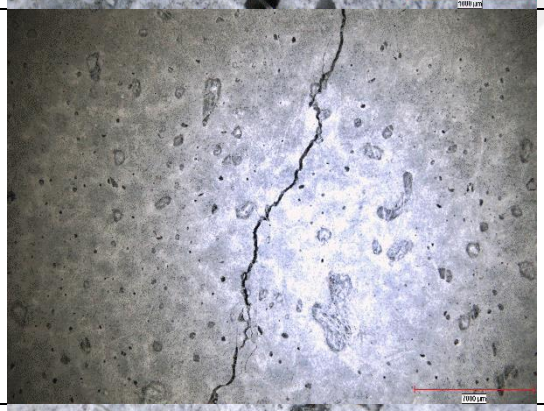
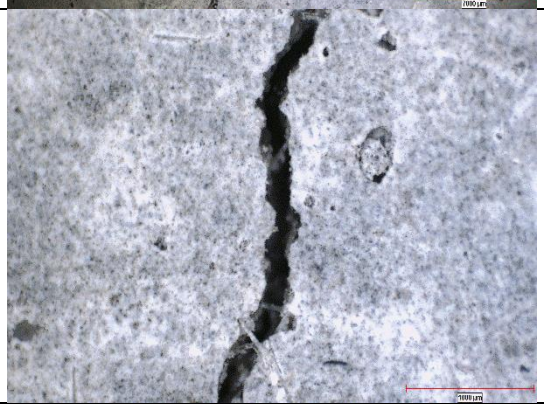
28 Day	Initial Crack	14th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 7 BT-2P beams cracked at 14 days.

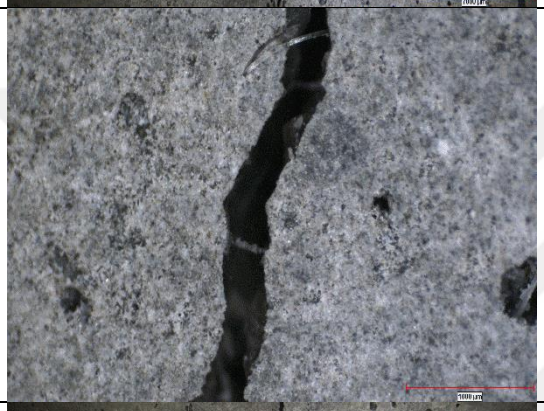
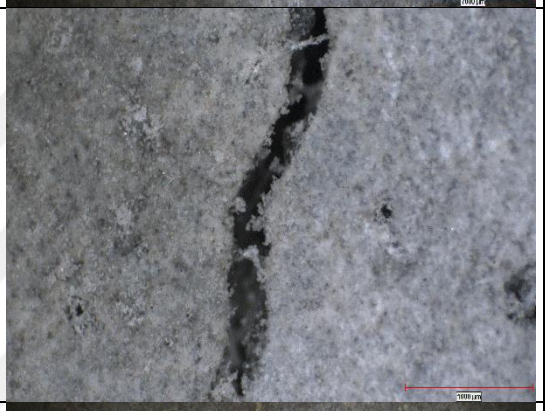
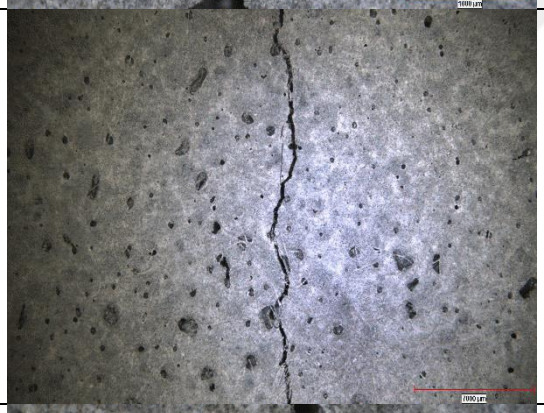
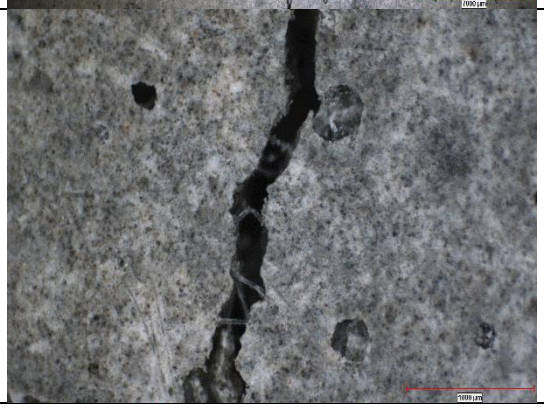
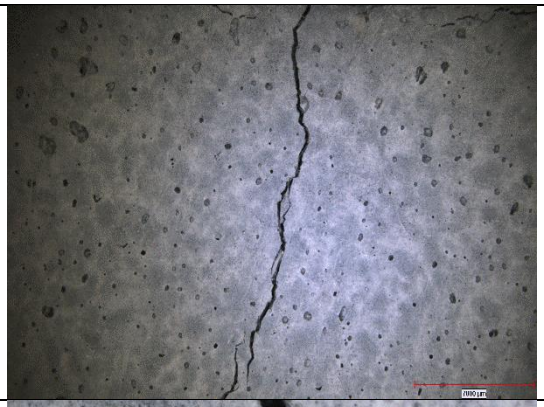
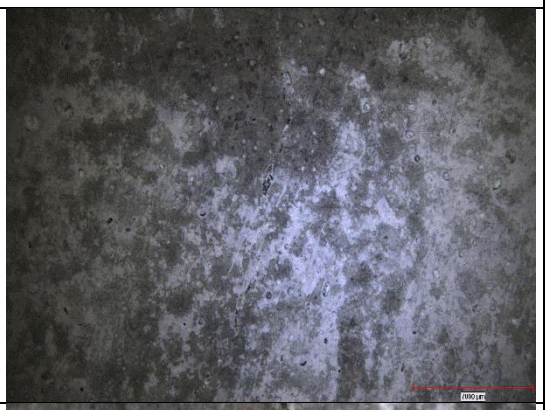
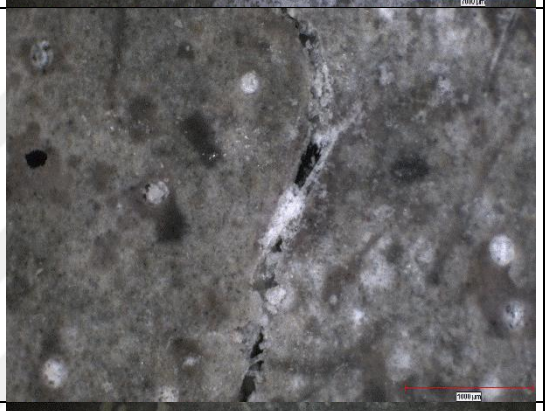
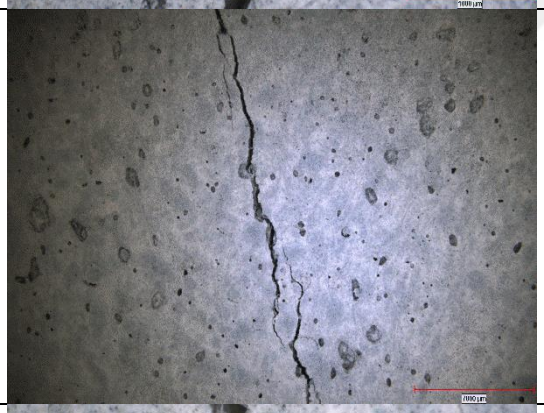
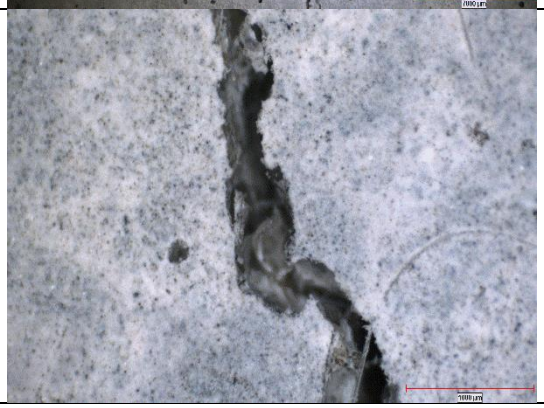
14 Day	Initial Crack	21st Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 8 BT-2P beams cracked at 28 days.

28 Day	Initial Crack	21st Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

4.2.2. UPV Analysis:

Closure efficiency was examined for *BT-C*, *BT-Bac* and *BT-2P* samples by the means of transverse time of waves. Figure 4.5 and 4.6 summarize the test results for UPV readings for 28 consecutive days and velocity change between the first and last day of curing.

The highest change in UPV was observed in 14 day cracked *BT-Bac* beams cured in UCSLC medium among all the samples containing BT. Difference was 0.18 km/h increase after the curing. This was attributed to longer curing period of the mentioned specimens. Additionally, BT-Bac 28 day cracked samples were cured for 14 days in UCSLC medium were full crack closure was achieved. However, this did not affect the velocity change in a negative way.

One particular thing about samples containing bentonite is that there was a considerable change in UPV velocity in BT-C samples even there was not any crack healing observed. As it was mentioned before, bentonite has a high absorption capacity and that could swell in presence of moisture. This might interfere with the accuracy of the readings. Such that, bentonite can swell during curing without sealing the cracks, but it might result with a change in UPV reading.

Similar to samples containing DE, BT-2P samples also showed a relatively weaker performance compared to the BT-Bac samples. This performance was attributed to the amount of the bacterial cells incorporated in the mix. As mentioned before *2P* samples have 0.5 g of cells per kg of mortar, while *Bac* samples have 1 g per kg of mortar. In addition, the velocity readings obtained in BT-2P samples were relatively higher compared to its counterpart BT-Bac samples, particularly at samples cracked after 14 days from mixing. This might also be an indication of a better quality of concrete and self-healing efficiency.

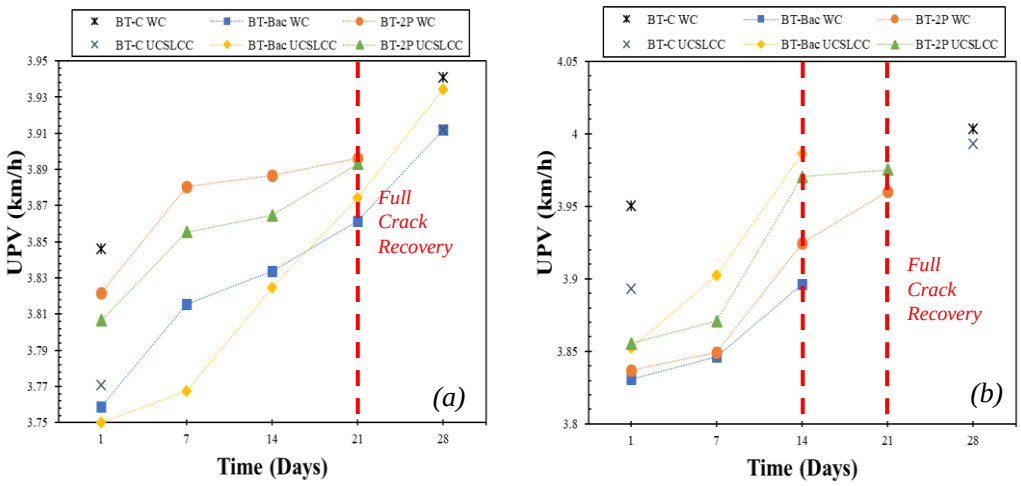


Figure 4. 5 *UPV recordings bentonite containing samples for 28 days (a) samples cracked at 14-day after mixing (a) samples cracked at 28-day after mixing. BT: Bentonite; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing.*

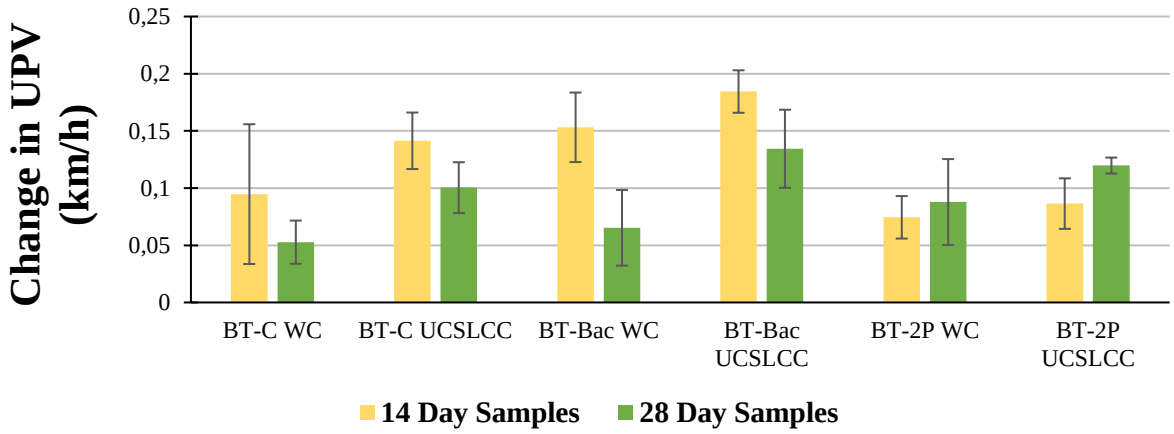


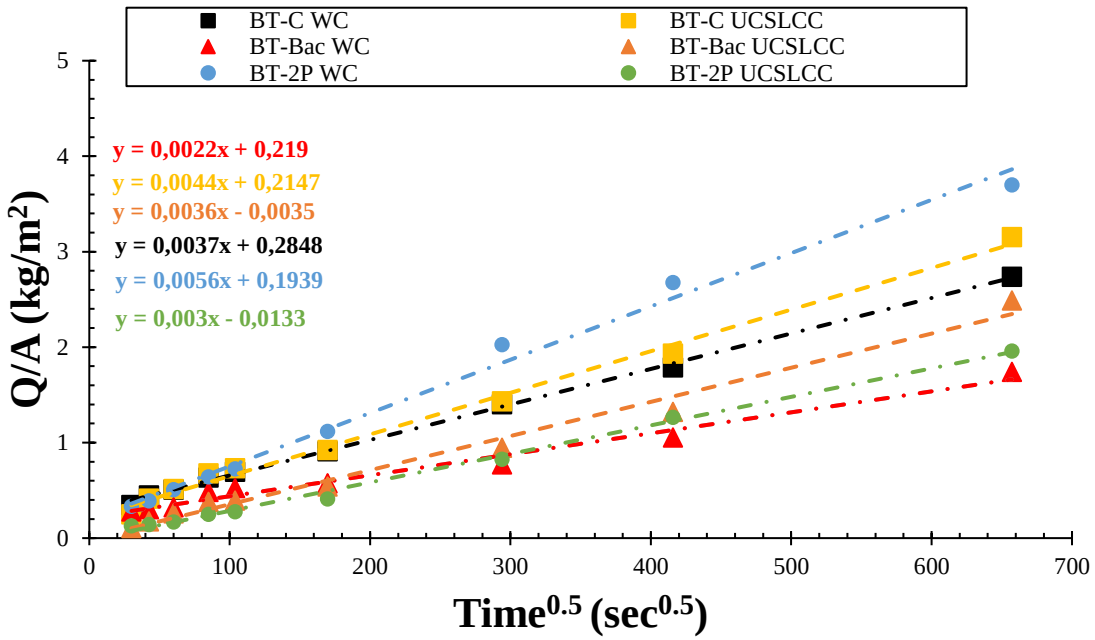
Figure 4. 6 *UPV change of the Bentonite containing samples at the end of curing stage. BT: Bentonite; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing. Error Bars represent the standard deviation obtained from triplicates of samples and columns represent the average of 3 samples.*

4.2.3 Water absorption:

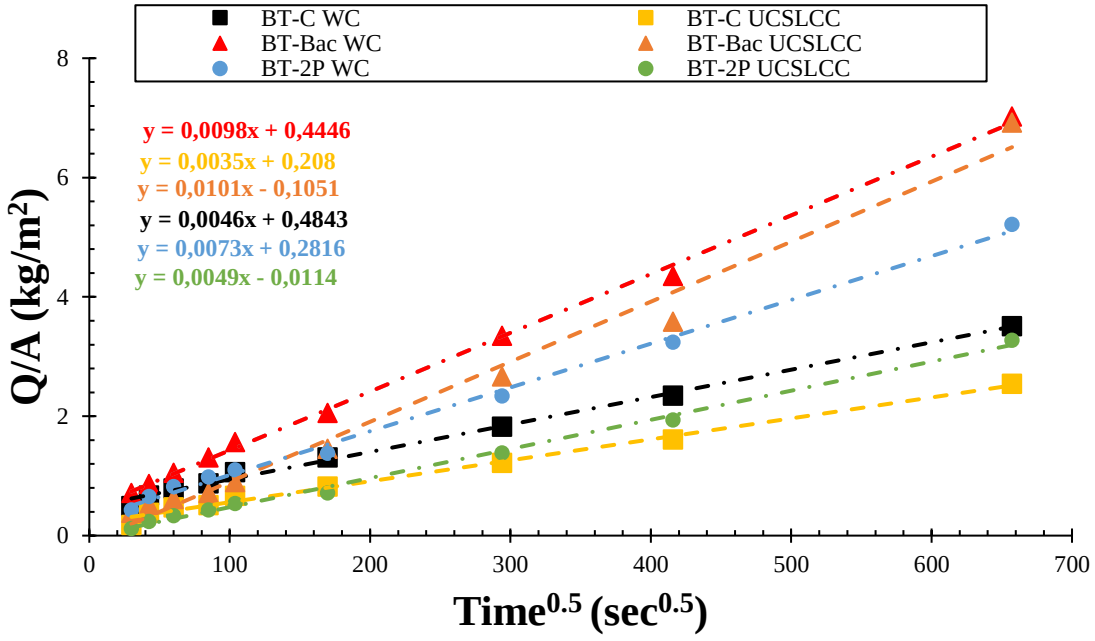
The visual evaluation on self-healing showed that the most efficient crack closure was achieved in BT-Bac and BT-2P samples cured in UCSLC medium. Data obtained from UPV also indicate that this healing might also be applied through the depth of the crack. To quantify the change in self-healing efficiency a water absorption test was conducted.

Figure 4.7 presents the water absorption capacity of the BT-C, BT-Bac and BT-2P samples cracked at 14 and 28 days after mixing. The data points show average of triplicates of samples and the slope of the trendline indicates the water absorption coefficient based on RILEM PEM II (*see* Section 3.5.3). BT-Bac samples cracked at 14 days showed an opposite behaviour to the visual inspection and UPV evaluation. Water cured BT-Bac samples cracked at 14 days showed partial crack closure where UCSLC cured samples showed complete remediation. This was also coherent with the UPV test where samples cured in UCSLC medium had better increase compared to water cured samples. Surprisingly, water absorption coefficient of the 14-day cracked BT-Bac samples cured in water was 40% lower than the BT-Bac samples cured in UCSLC medium. Since other evaluations show an opposite behaviour this might be better understood with SEM inspection if it was an experimental error or a difference caused by precipitate type. BT-Bac and BT-2P samples cured in UCSLC showed a coherent behaviour with other inspections in terms of the decrease in k when compared to BT-C samples cured in UCSLC medium. Highest absorption coefficient, k value, was detected at BT-2P water cured samples. This was again an unexpected behaviour where the UPV results showed an increase in velocity compared to BT-C water cured samples. One sound explanation for this is the inconsistent trend in water absorption of samples containing bentonite. This could be again related to high absorption capacity of the mineral leading to an experimental error due to the use of paraffin. Even though, the absorption capacity of bentonite was recorded as 300% for 24 hours, extended saturation might lead to a higher degree of absorption capacity. Paraffin covers

the sample as a very thin layer which was peeled off on some parts of the samples, particularly samples containing bentonite. This might be due to gradual expansion of bentonite under moisture.



(a)



(b)

Figure 4. 7 *Mass of absorbed water per m^2 for (a) 14 day (b) 28-day bentonite samples. BT: Bentonite; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing. Data points represent the average of samples.*

4.2.4 Precipitate characterization:

The self-healing mechanisms occurred in samples containing bentonite was more complex compared to that of in samples containing DE. Even though the data obtained from stereomicroscopy showed that the cracks were sealed, the water absorption data was not in line with the findings. Figure 4.8 show the SEM images obtained from the crack surface area in BT-Bac and BT-2P samples. SEM analysis showed that both amorphous calcite crystals, vaterite and calcite precipitated within the cracks. In addition, there were rod-shaped indications of bacterial cells (showed with red arrows). SEM images revealed that the bacterial cells were immobilized in bentonite and triggered MICP when cracks were induced. The bacterial cells observed was in line with the expected bacteria dimensions which was approximately 2 μm length.

Furthermore, results were also interpreted by correlating the possible precipitate identities and the curing time. It was seen the calcite precipitation was much more profound in more aged samples.

The spherical crystals at Figure 4.8 (b) might be a direct result of the calcium source (calcium acetate) that was used for this study since it is proven that the morphology of the crystals could be constituted by the calcium source type [76].

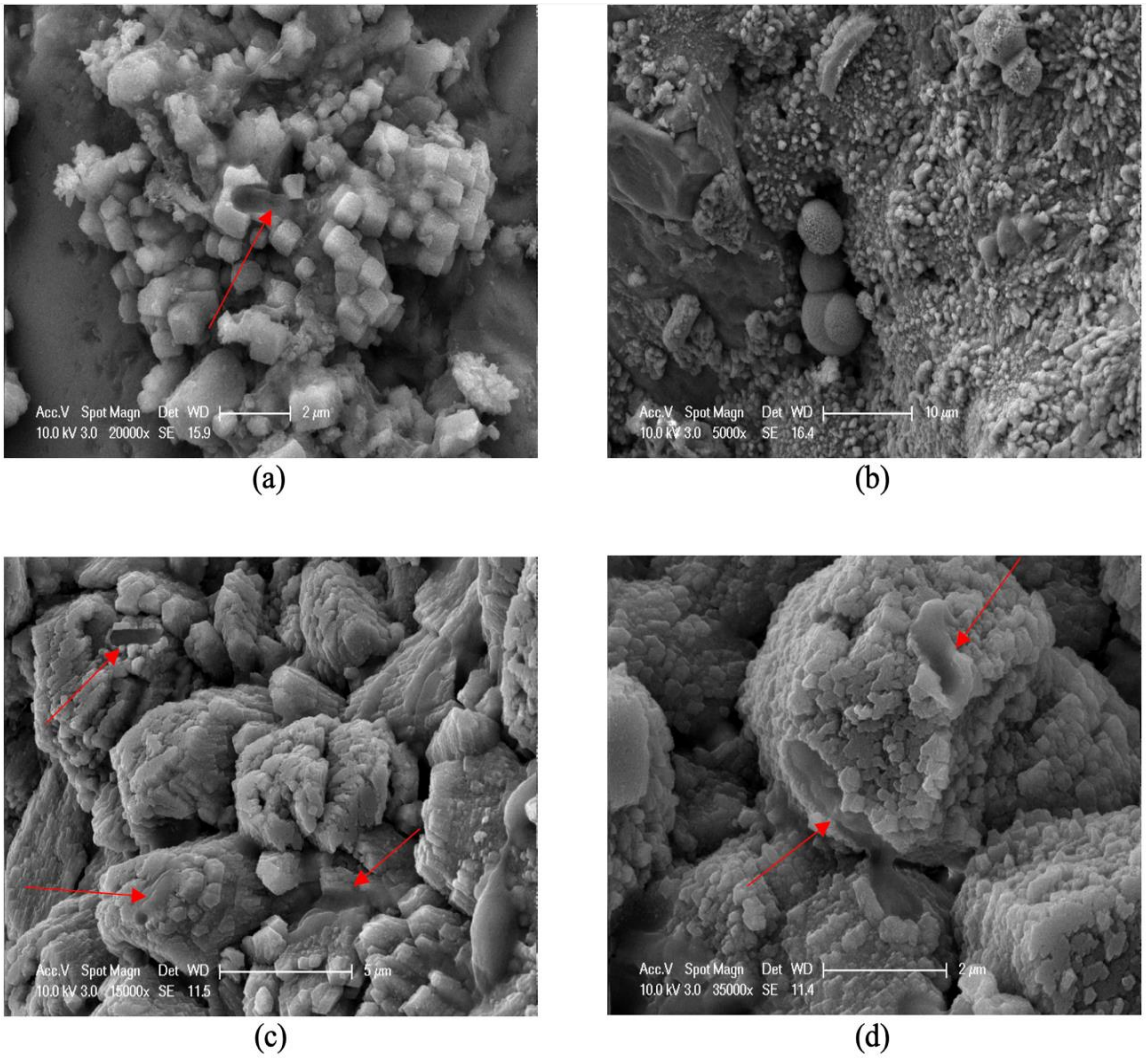


Figure 4. 8 *SEM images of the crack surfaces in BT- Bac samples cured in UCSLC medium (a) and (b) BT-Bac samples cracked at 14-day , (c) and (d) BT-Bac samples cracked at 28-day . Red arrows highlighting the indications of possible presence of S.pasteurii cells.*

4.3. Sepiolite (SEP) as a protective barrier:

4.3.1. Visual crack evaluation:

Tables 4.9 to 4.12 present the stereomicroscopy images of *Sep-Bac* and *Sep-2P* either cured in water or UCSLC nutrient medium. Similar to what was observed in the previous 2 protection barriers, full crack sealing was observed in *Sep-Bac* and *Sep-2P* samples cured in UCSLC medium, regardless of their age. In contrast there was not any healing observed in *Sep-Bac* and *Sep-2P* samples cured in water. As expected, there was no precipitation observed in negative control samples without any bacterial cells. Crack remediation was achieved in crack width ranges from 0.30 to 0. 50 mm. In addition, there were precipitation observed not only within the cracks but also in the surface pores. The colour of the precipitates was also white.

Table 4. 9 Sep-Bac samples cracked at 14 days.

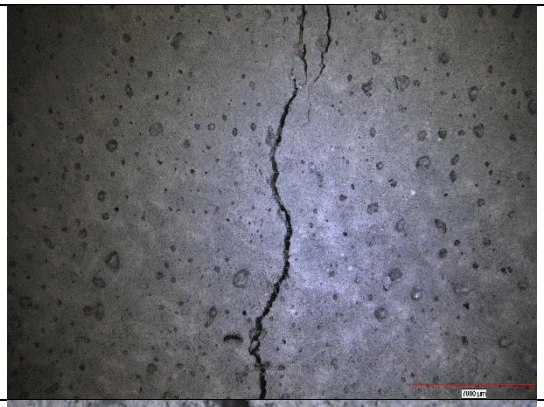
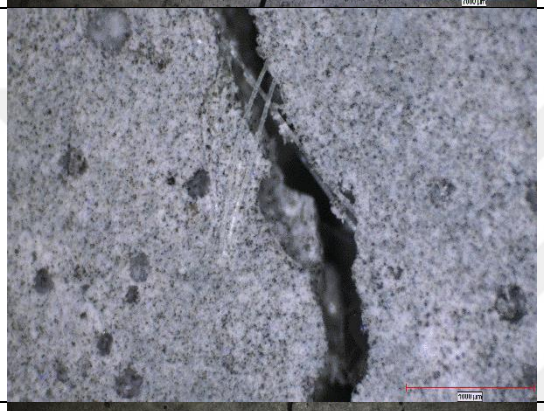
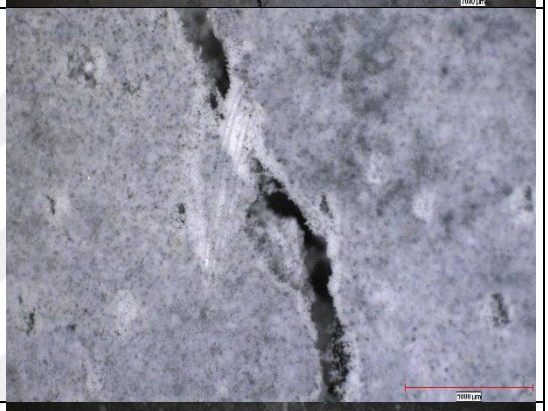
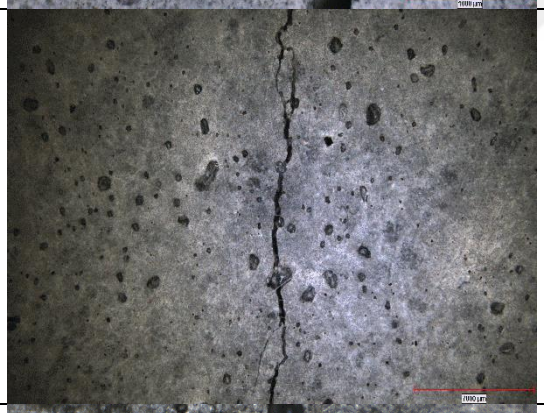
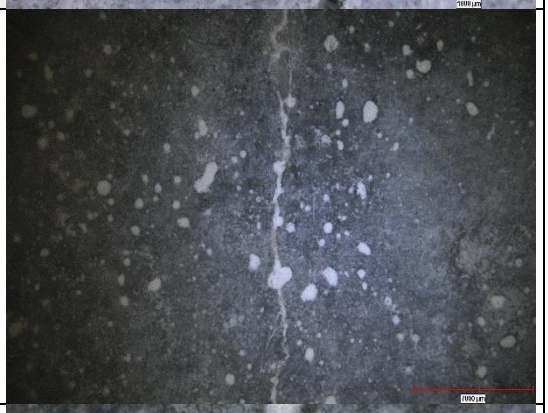
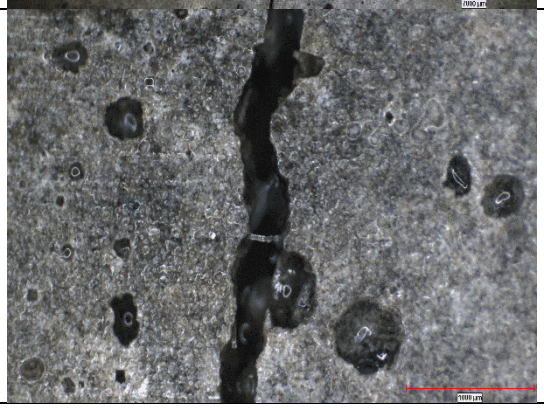
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 10 Sep-Bac samples cracked at 28 days.

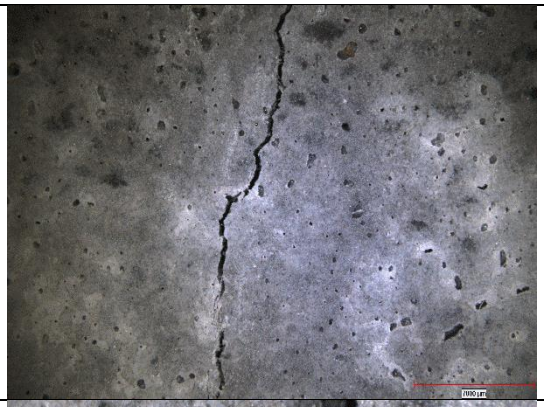
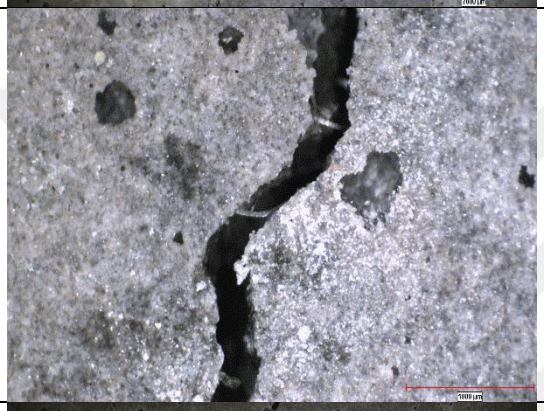
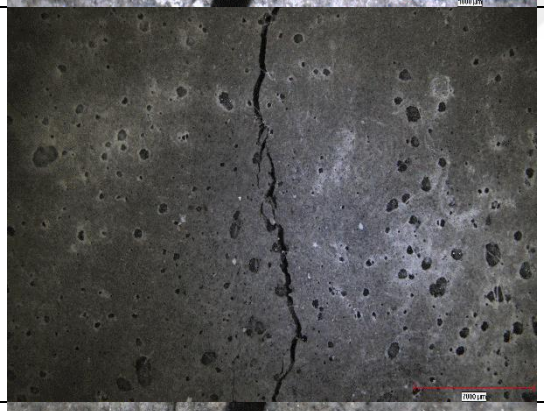
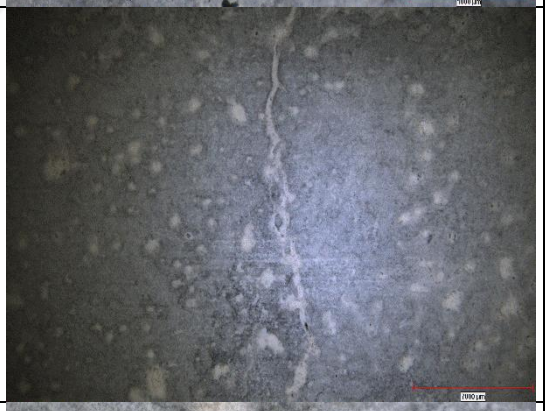
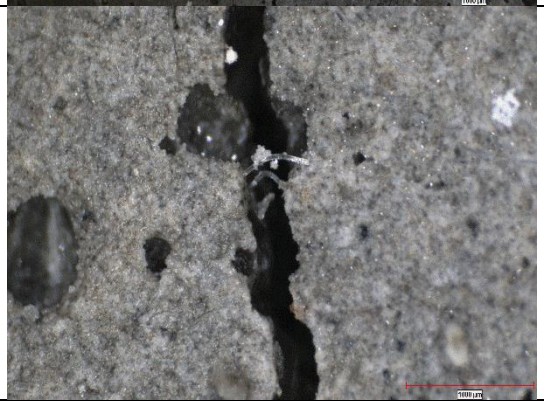
28 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 11 Sep-2P samples cracked at 14 days.

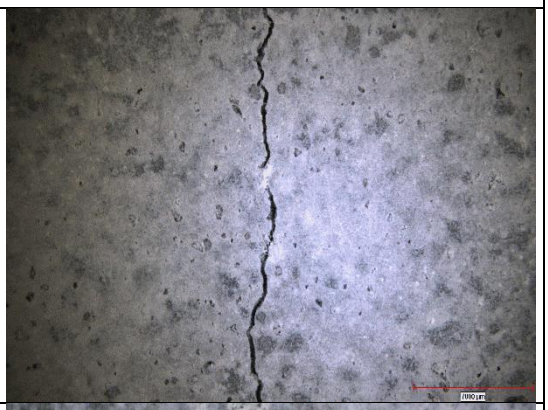
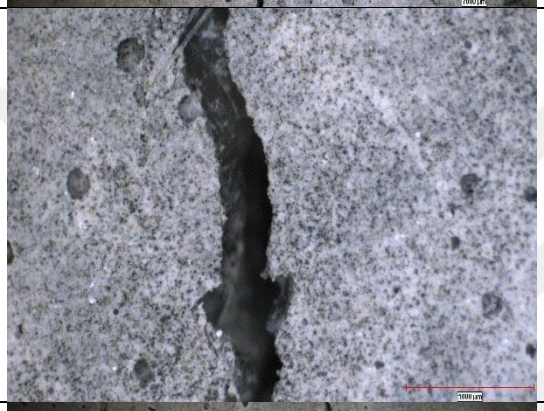
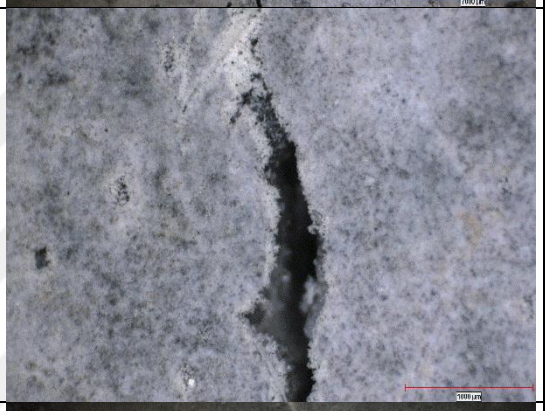
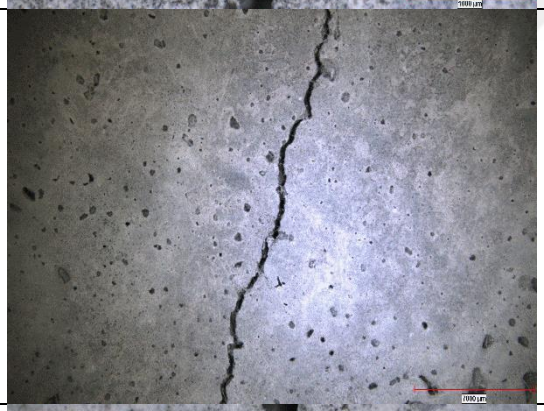
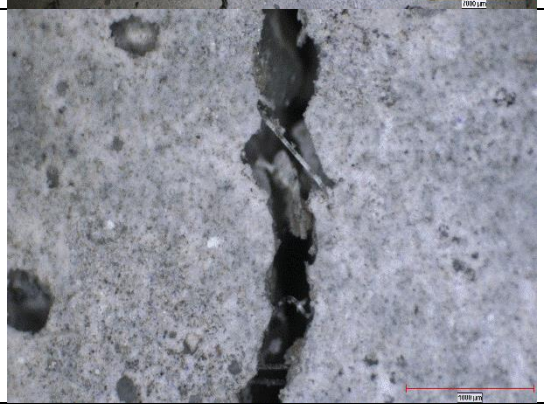
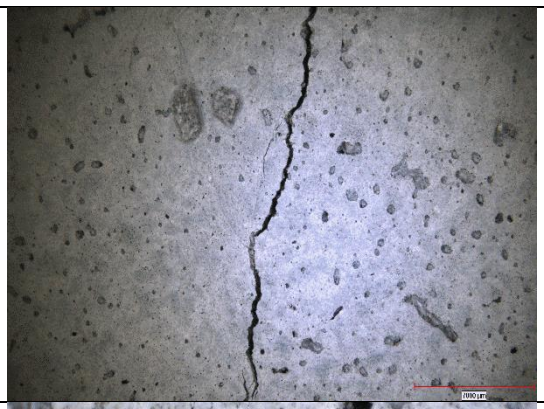
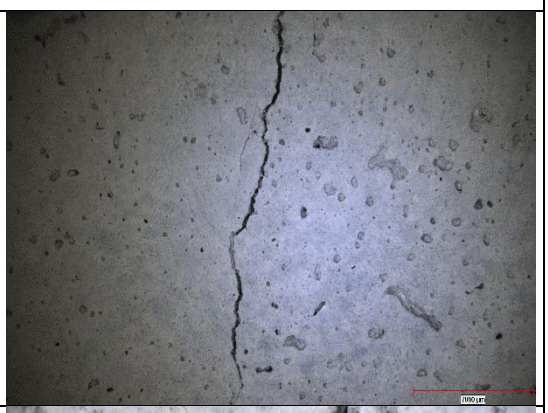
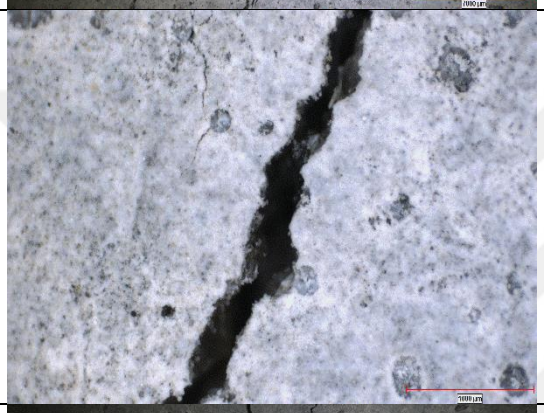
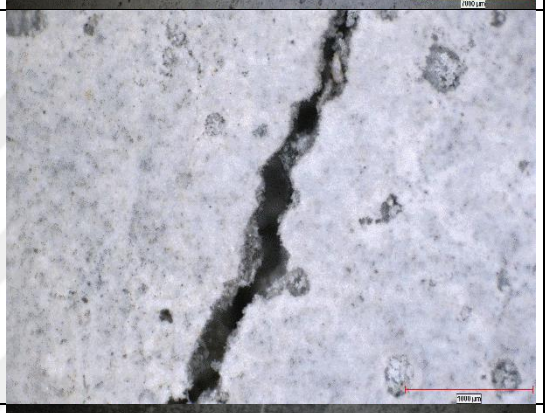
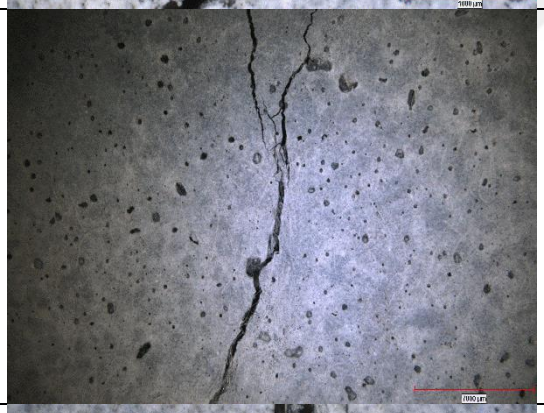
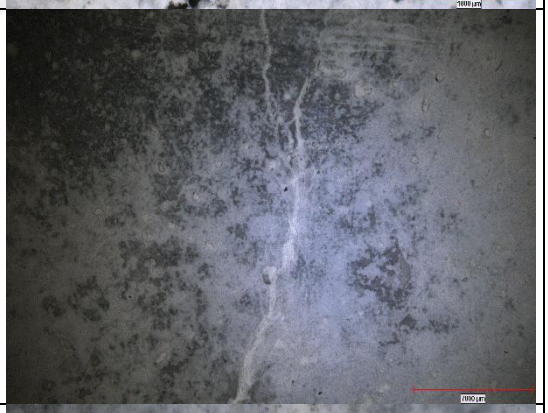
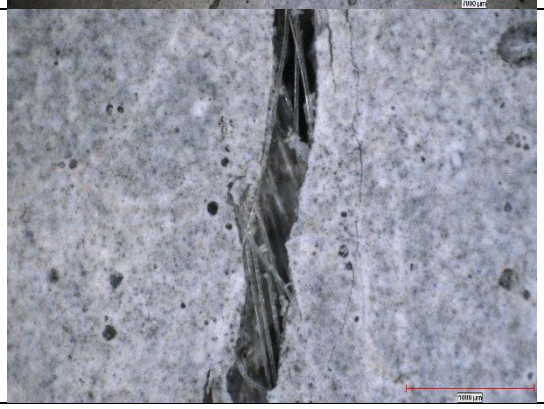
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 12 Sep-2P samples cracked at 28 days.

28 Day	Initial Crack	21st Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

4.3.2. UPV Analysis:

A UPV test was conducted on the Sep-C, Sep-Bac and Sep-2P samples to understand the internal healing mechanism. Transverse time was recorded weekly to construct UPV versus time graph. Figure 4.9 and 4.10 present the UPV records for Sep-C, Sep-Bac and Sep-2P samples for 28 days and the change in UPV before and after healing.

Samples containing sepiolite showed the most consistent trend in terms of correlating stereomicroscopy with UPV. The highest velocity readings were obtained in Sep-Bac and Sep-2P samples that were cracked at 14 and 28 day after casting and further cured in UCSLC medium. Similar to the previous protective barriers the highest velocity reading was obtained in Sep-Bac sample cured in UCSLC medium even compared to Sep-2P sample cured in UCSLC medium. As mentioned, this is directly related to the amount of bacterial cells added to the mix. With the data obtained so far, it is valid to conclude that as the amount of bacterial cell increases the quality of the mortar mix and self-healing efficiency of the additive increase. In addition, nutrient medium curing is required to observe crystal precipitation, except for some samples containing expansive.

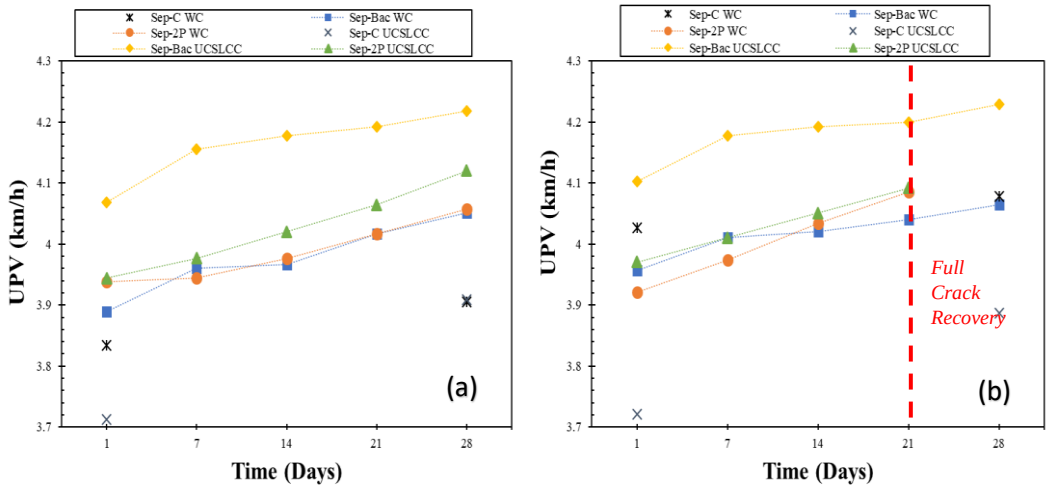


Figure 4. 9 *UPV recordings sepiolite containing samples for 28 days (a) samples cracked at 14-day after mixing (a) samples cracked at 28-day after mixing. SEP: Sepiolite; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing.*

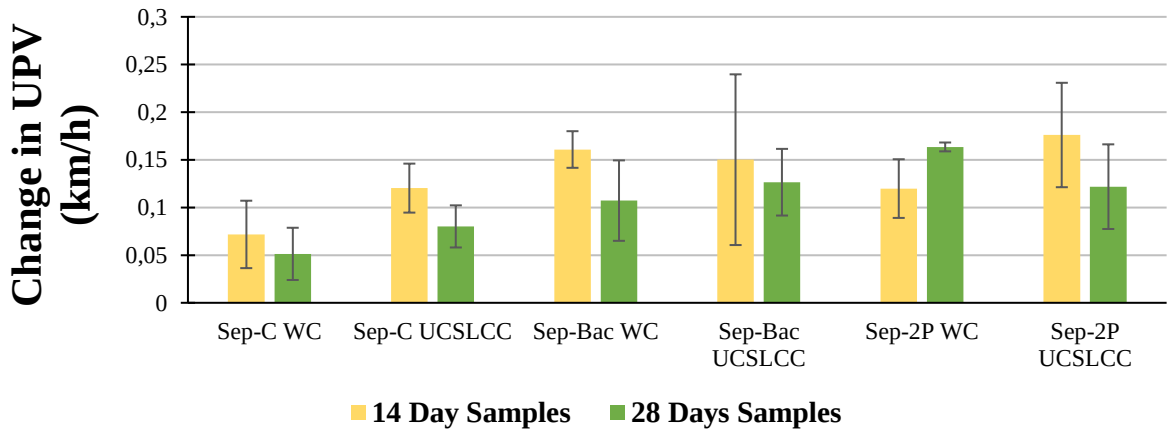


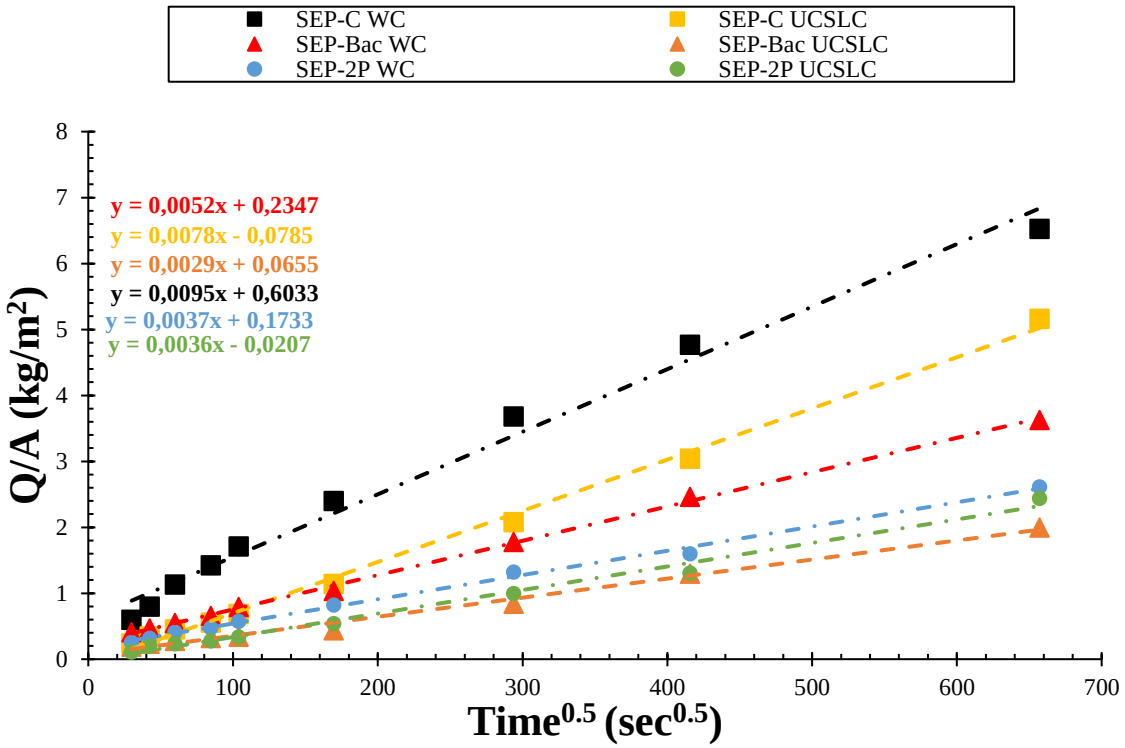
Figure 4. 10 *UPV change of the sepiolite containing samples at the end of curing stage. SEP: Sepiolite; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing. Error Bars represent the standard deviation obtained from triplicates of samples and columns represent the average of 3 samples.*

4.3.3 Water absorption:

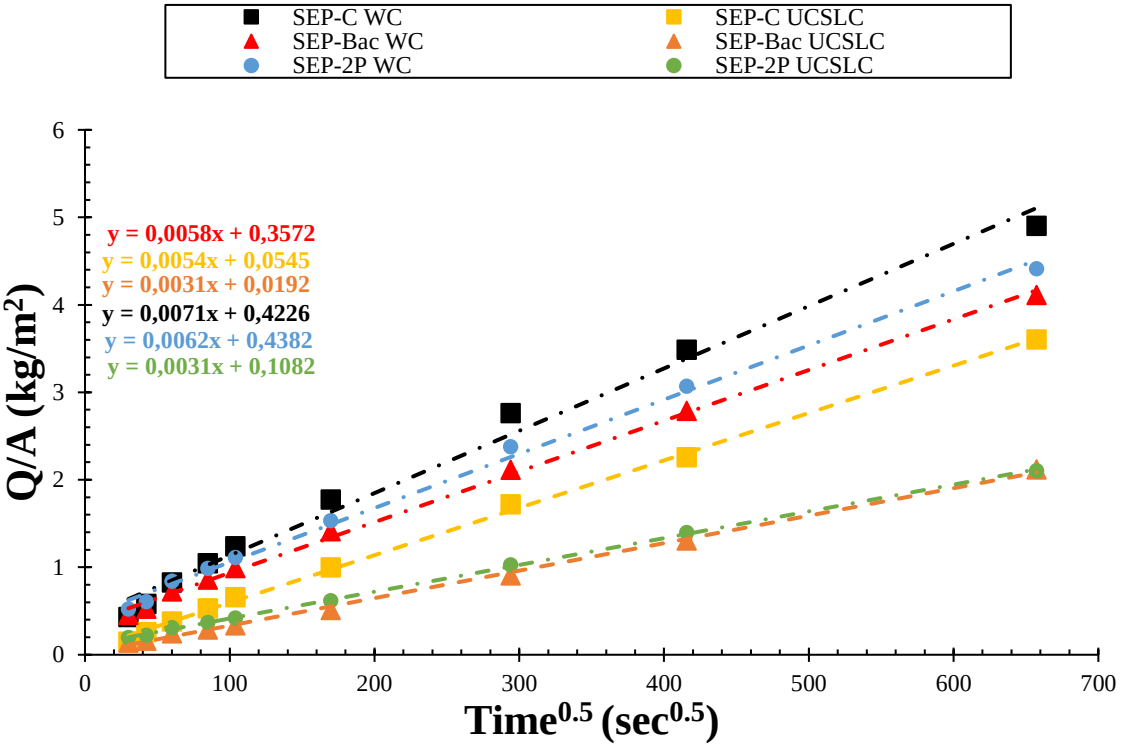
Water absorption test was also conducted for Sep-C, Sep-Bac and Sep-2P samples. Results were in line with the visual inspection. Figure 4.11 shows the water absorption rate of samples as a function of time. The data points represent the average values obtained from triplicates of samples.

It was seen that both 14 and 28 day cracked samples, Sep-Bac and Sep-2P cured in UCSLC medium had the lowest absorption coefficients, k. When the mentioned samples were compared to their counterpart Sep-C samples cured in UCSLC medium, there was approximately 60% decrease in k value in samples cracked after 14 days of mixing. A similar trend was observed when the samples cracked after 28 day of mixing. The difference was a 43% decrease in k value for Sep-Bac and Sep-2P samples cured in UCSLC medium compared to their counterpart Sep-C sample.

It is worthy to note the slight change in water absorption capacity of Sep-C sample cured in UCSLC medium. During, the initial SEM characterization of the protection barriers, a form of mold formations detected in sepiolite (*see* Figure 4.12). This was not observed in any other type of protection material. In fact, throughout the literature it was also known that sepiolite is a suitable bedding environment for various types of microorganisms [77]. Thus, when nutrient provided to Sep-C sample might also induce a type of healing process or some other reaction, resulting with a possible decrease in water absorption. Based on the data obtained, it was clear that presence of these molds did not interfere with the metabolic activity. However, further research has to be done to understand the interaction of these 2 microorganisms and possible use of molds (and sepiolite) as self-healing agents by themselves.



(a)



(b)

Figure 4. 11 *Mass of absorbed water per m^2 for (a) 14 day (b) 28-day samples containing sepiolite. Sep: Sepiolite; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing. Data points represent the average of triplicates of samples.*

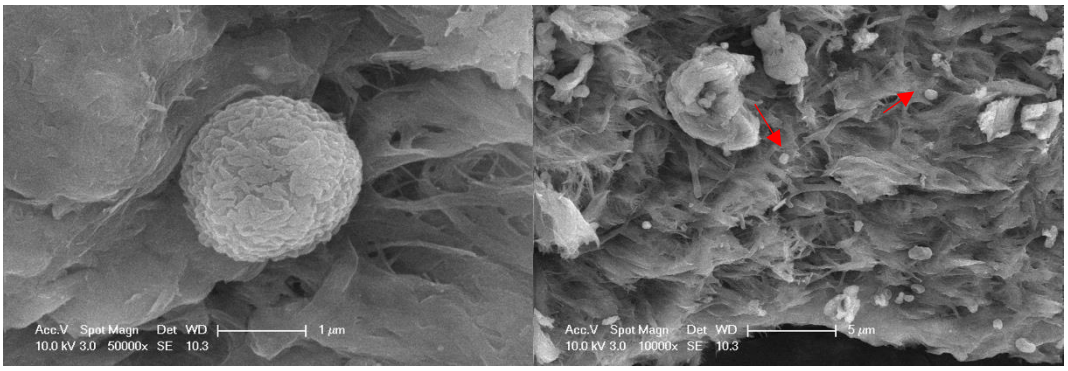
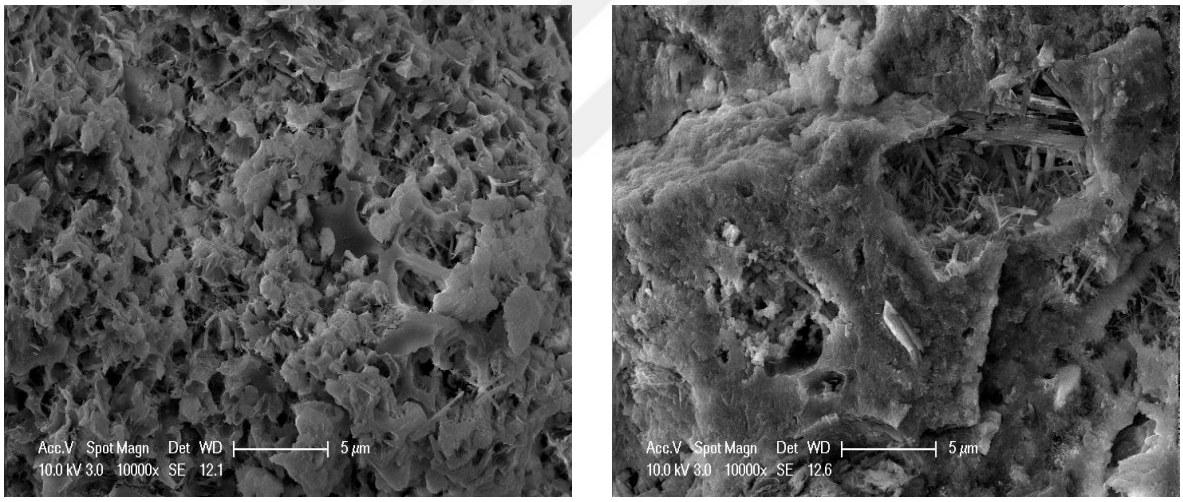


Figure 4. 12 Initial SEM image of sepiolite before immobilization.

4.3.4 Precipitate characterization:

SEM inspection was conducted on the samples collected from the remediated crack walls of Sep-Bac and Sep-2P integrated beams. Images can be seen in Figure 4.13.



(a) (b)

Figure 4. 13 SEM images of the UCSLC cured 14 day cracked Sep-Bac samples.

Similar formations to DE samples were observed at Figure 4.13 (b) where the spherical formations, supposedly amorphous calcium carbonate (ACC), were present. Additionally, needle shaped crystals were detected. However, a unique formation was seen at (a) which could be the sepiolite particles covered with precipitates. Further investigation is needed to understand the true nature of the precipitates.

4.4. Pumice (PUM) as a protective barrier:

4.4.1. Visual crack evaluation:

At last but not the least, self-healing evaluation was done in cracked mortar beams containing bacterial cells immobilized on pumice. Stereomicroscopy analysis was conducted on Pum-C, Pum-Bac and Pum-2P samples to evaluate the healing process. Tables 4.13 to 4.16 present the representative images obtained stereomicroscopy evaluation of Pum-Bac and Pum-2P samples. Images of Pum-C are given in Appendix A.

Similar to the rest of the minerals, and as expected, complete crack sealing was observed for Pum-Bac and Pum-2P samples cured in UCSLC medium. The average crack width healed was 0.35±0.02 mm. In contrast, there was not any precipitation in negative control samples Pum-C regardless of age and curing regime. Interestingly, there was a limited precipitation observed in Pum-Bac and Pum-2P samples cracked after 14 days of mixing and cured in water. First, the precipitation was only observed in samples cracked after 14 days of mixing, not for 28 day cracked samples. This suggests that this mechanism might be related to bacterial viability. A slight precipitation in Pum-2P samples could be explained by presence of limited amount of nutrients absorbed into half of the pumice used. However, there was also a slight precipitation around crack edges in Pum-Bac samples, in which there was not any nutrients provided in the mix, after 28 days of curing in water. Saturated pumice particles might lead to autogenous healing process due to further hydration. But it could also be simply explained by possible presence of ions on bacterial cells due to insufficient washing process.

It should be also mentioned that the rate of crack healing with bacterial cells immobilized on pumice was as slow as it was with DE. The rate of crack healing was faster in samples containing bentonite and sepiolite, which might be related to their possible contribution to the healing process.

Table 4. 13 Pum-Bac samples cracked at 14 days.

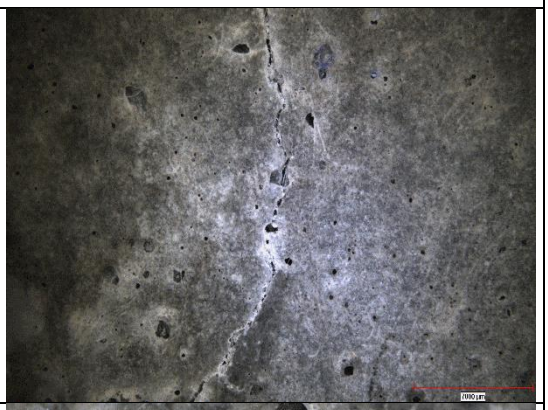
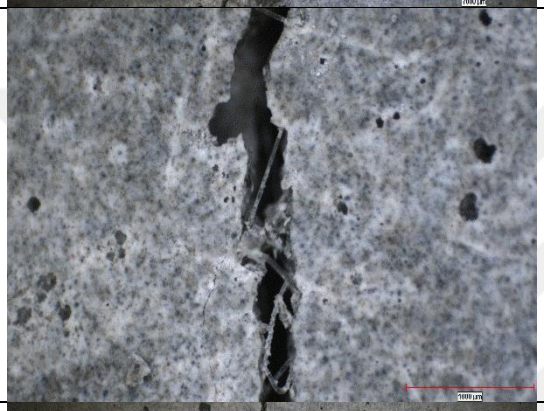
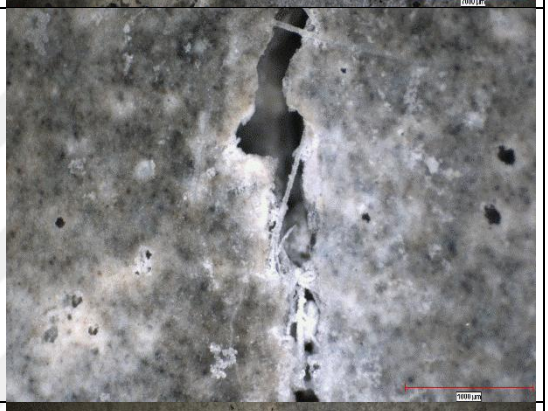
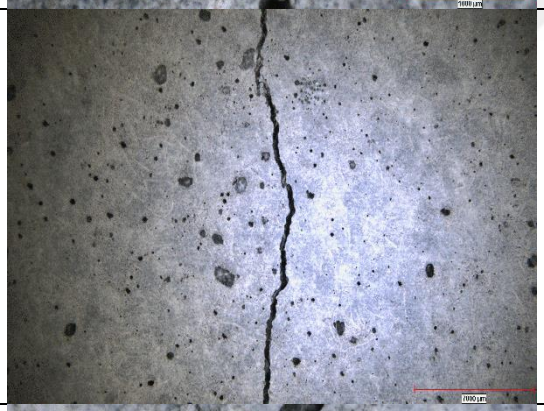
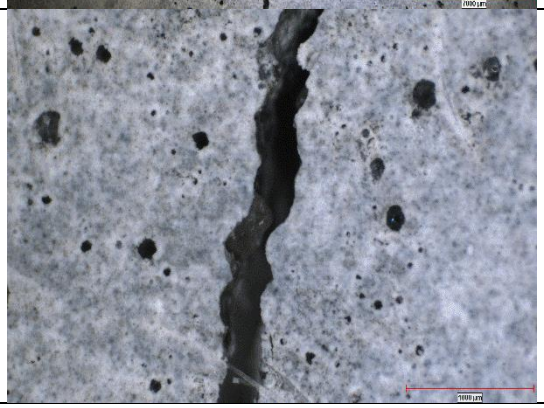
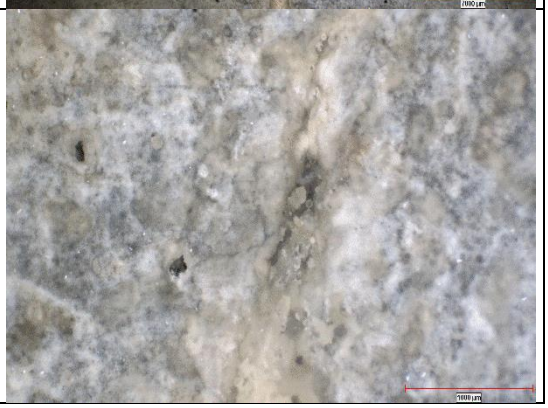
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 14 Pum-Bac samples cracked at 28 days.

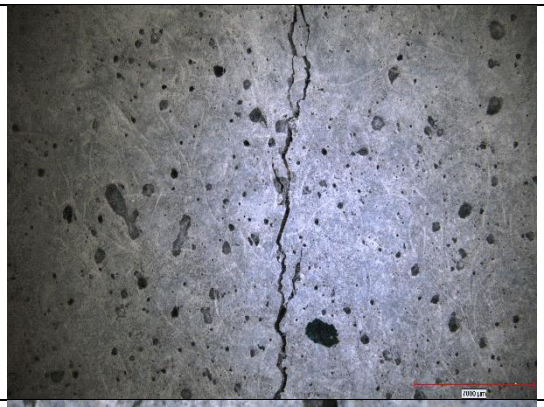
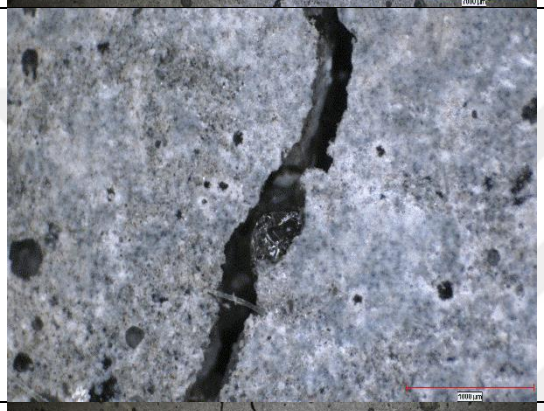
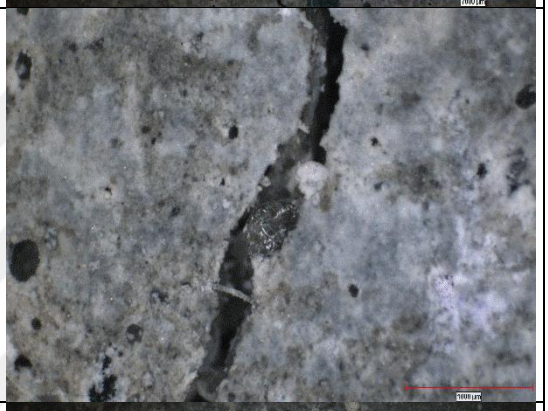
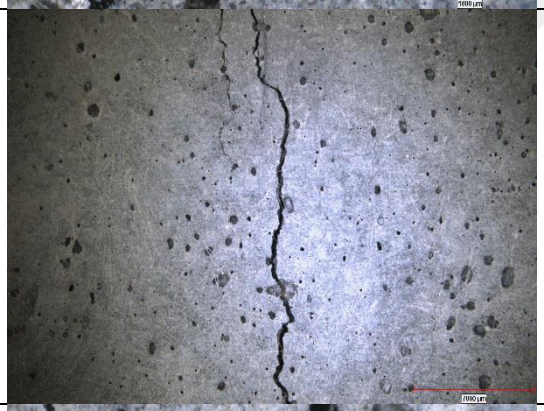
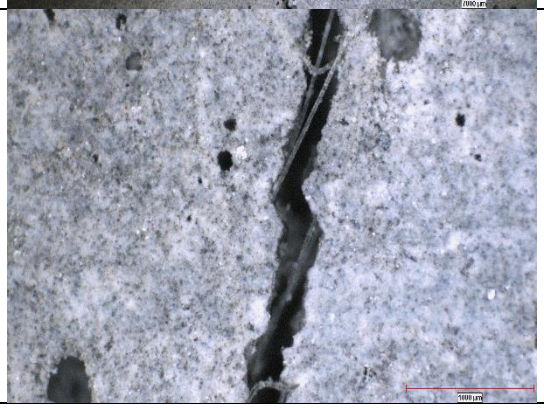
28 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 15 Pum-2P samples cracked at 14 days.

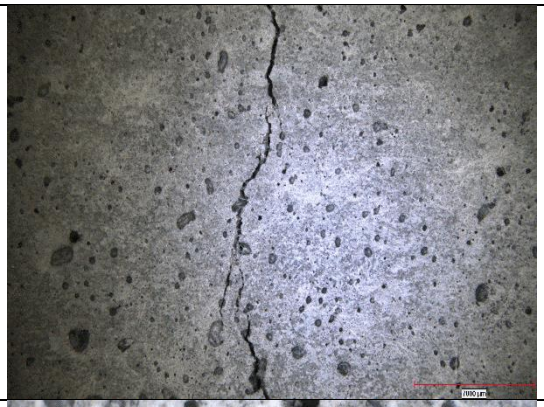
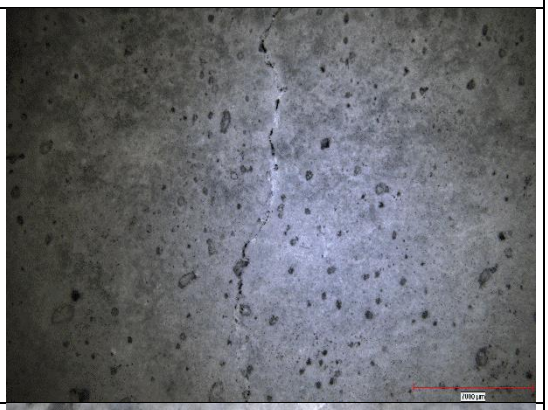
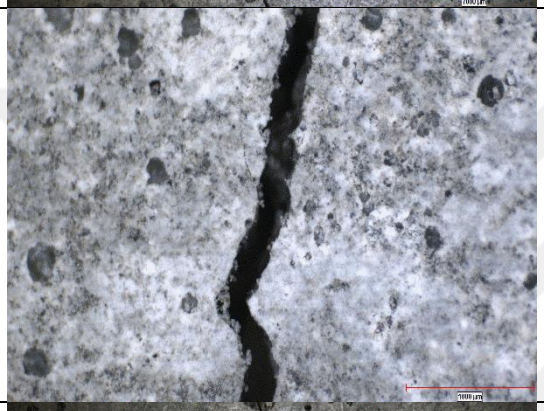
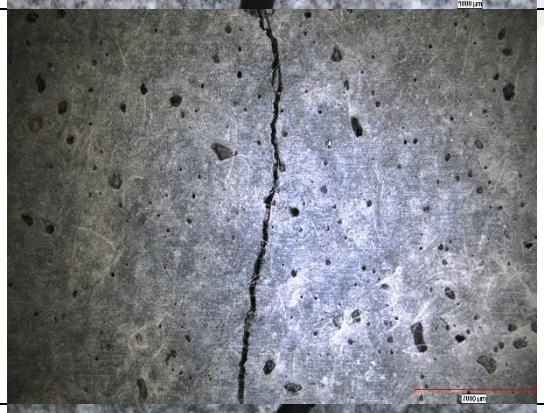
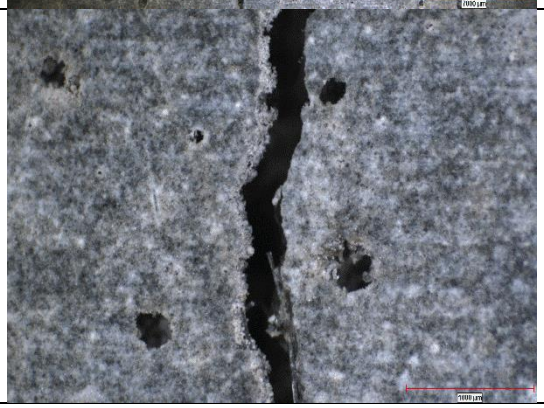
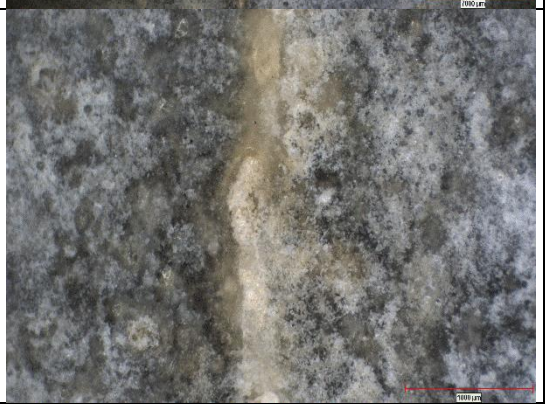
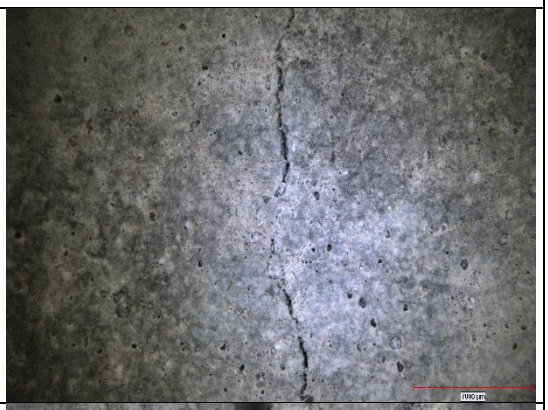
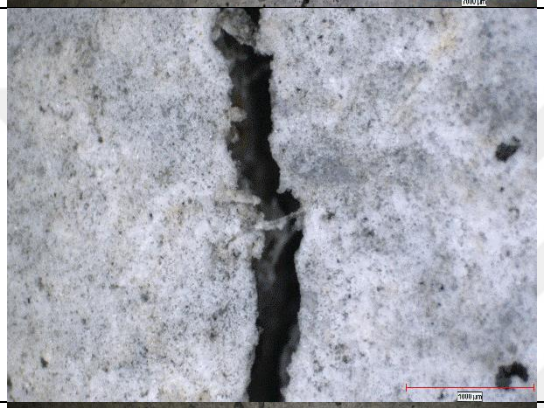
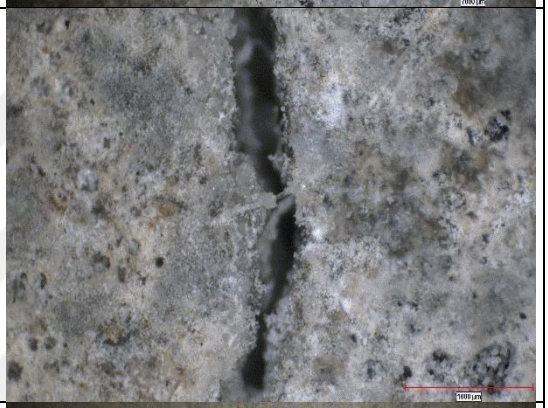
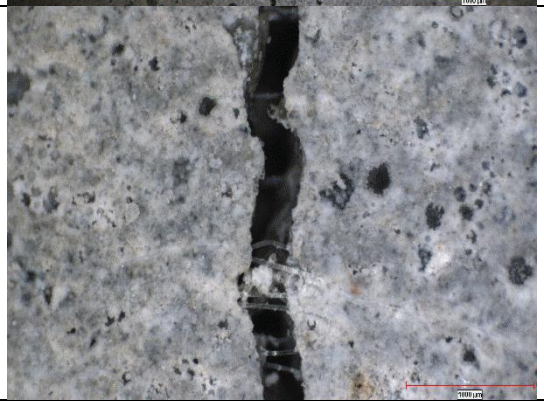
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table 4. 16 Pum-2P samples cracked at 28 days.

28 Day	Initial Crack	21st Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

4.4.2. UPV Analysis:

UPV values and the relative change in velocity for Pum-C, Pum-Bac and Pum-2P plotted as a function of time to understand the change in quality of samples upon healing process. Figure 4.14 and 4.15 present the UPV data obtained from each sample and change in the velocity before and after crack sealing.

For samples cracked at 14 days, there was a significant increase in UPV in Pum-Bac and Pum-2P samples compared to Pum-C regardless of curing regime. The highest change was observed from day 1 of cracking to 7 days of curing. This might indicate that, the cells trigger immediate mineral precipitation within the first few days of curing and then the precipitation rate might substantially decrease. As it was mentioned before, it is known that that higher velocities is an indication of a very good concrete quality, whereas very low velocity ranges are indicative of poor concrete quality [74]. Relatedly, Pum-Bac and Pum-2P samples cracked at 14 days were higher than 4 km/h while it was below 4 km/h for Pum-C samples even after 28 days of curing. It was expected to see a slight decrease in UPV readings in Pum-C sample compared to the control samples prepared with other protection barriers (DE-C, BT-C and Sep-C) due to the use of LWA rather than minerals. Such an increase in UPV due to incorporation of bacterial cells might also be an indication of improvement in concrete quality. Further investigations are being done to understand the influence of cells and protection barriers on compressive strength of mortar.

The change in UPV readings before and after 28 days curing indicates possible mineral precipitation not only crack mouth but rather through the depth of the crack. In general change in UPV was in line with the insight obtained from stereomicroscopy. However, there was a slight variation for samples cracked after 28 days of mixing. Such as, there was full crack sealing observed in Pum-2P sample cured in UCSLC medium, both the change in UPV was

lower than Pum-Bac sample cured in UCSLC medium. As mentioned before, *Bac* samples contain a higher amount bacterial cells, which might be induce a higher density of precipitation. Relatedly, the change in UPV for Pum-Bac samples (28 days cracked) cured in water was on par with Pum-2P (28 days cracked) cured in UCSLC medium. Even though there was not any visual crystal precipitation, this might indicate possible precipitation within the depth of the crack.

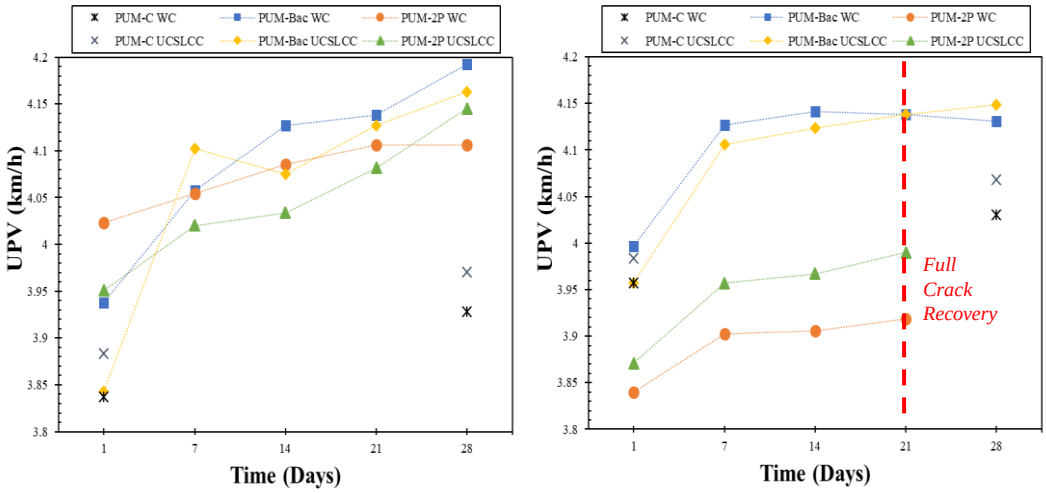


Figure 4. 14 *UPV recordings pumice containing samples for 28 days (a) samples cracked at 14-day after mixing (a) samples cracked at 28-day after mixing. PUM: Pumice; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing.*

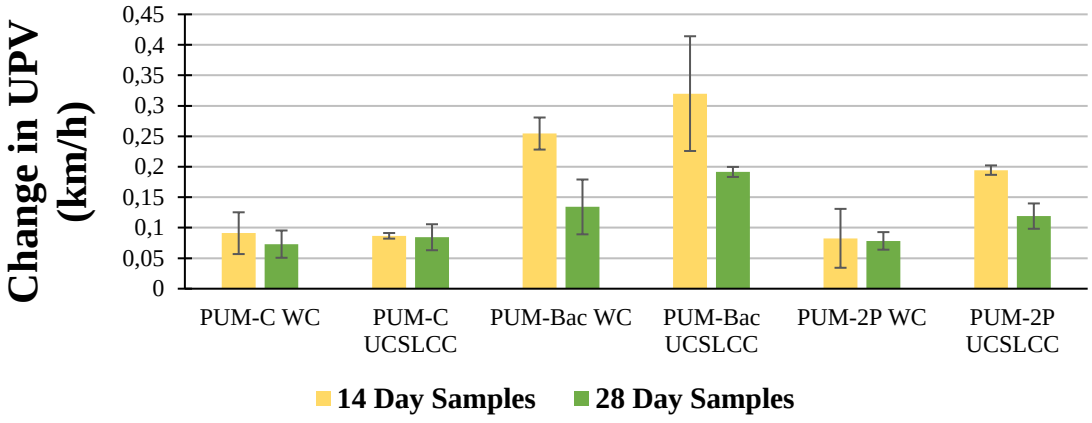
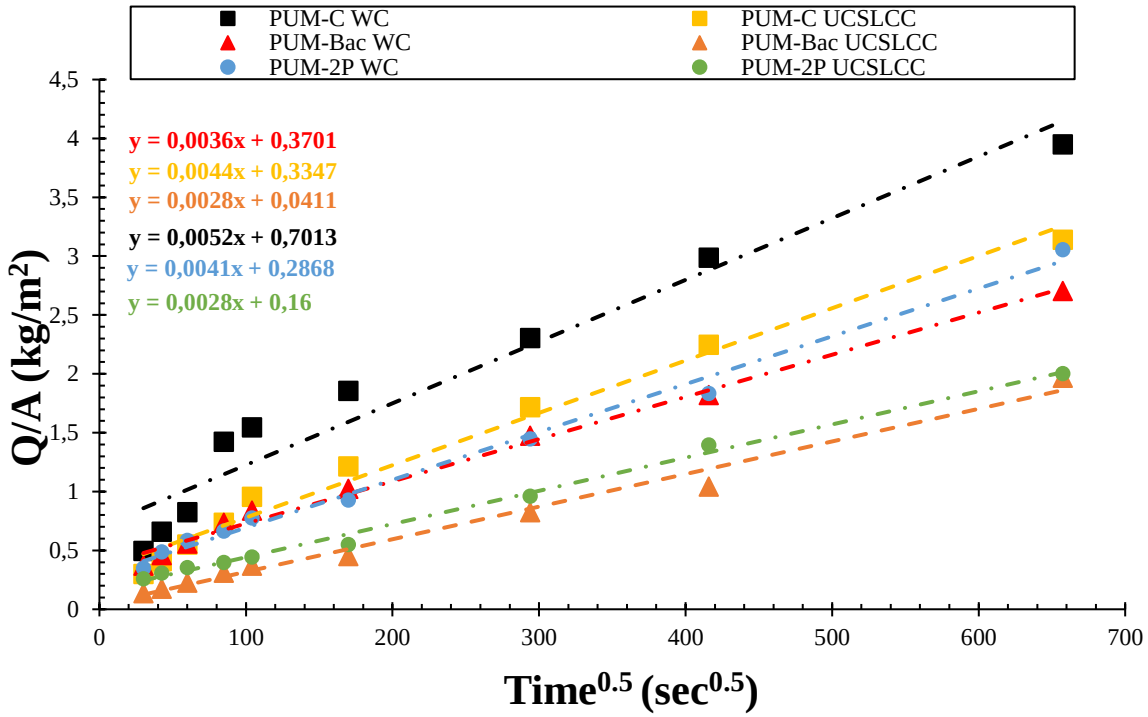


Figure 4. 15 *UPV change of the pumice containing samples at the end of curing stage. PUM: Pumice; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients. WC: Water cured; UCSLCC: Nutrient Medium curing. Error Bars represent the standard deviation obtained from triplicates of samples and columns represent the average of 3 samples.*

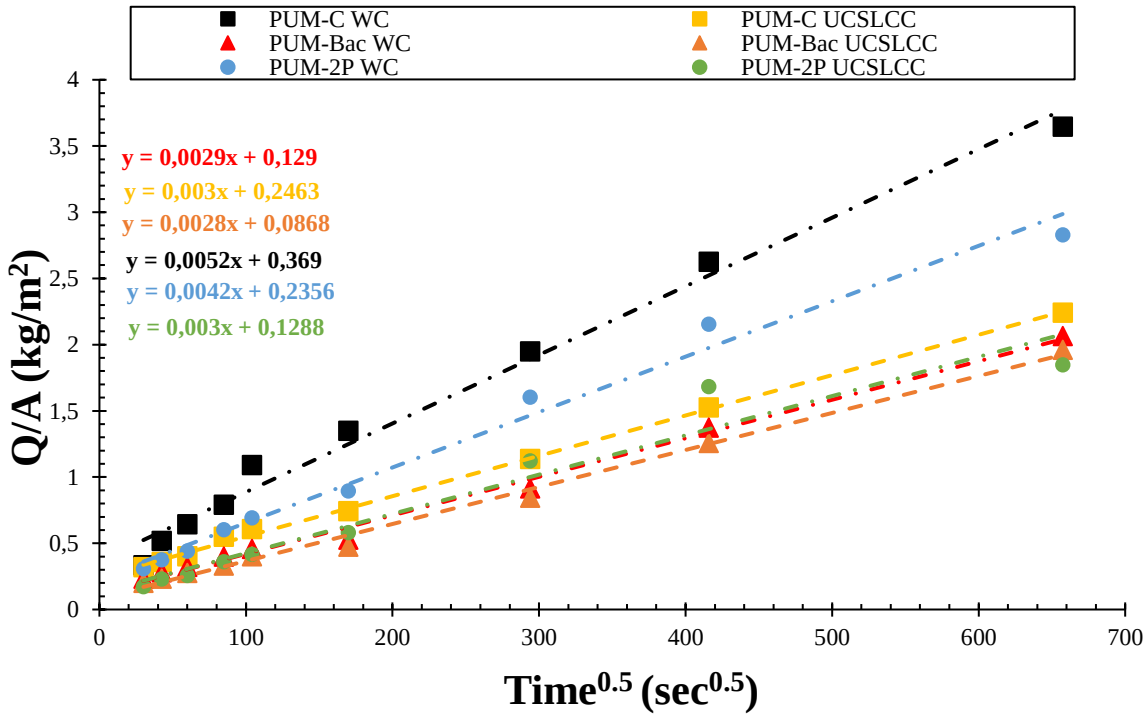
4.4.3 Water absorption:

Water absorption test was conducted to quantify the efficiency of crack sealing for Pum-C, Pum-Bac and Pum-2P samples. Results were interpreted by water absorption coefficient (k). Figure 4.16 presents the results of absorbed water per m^2 as a function of time. The data points represent the average values obtained from triplicates of samples.

For samples cracked at 14 day after mixing, there was a 36% decrease in *k* of Pum-Bac and Pum-2P samples cured in UCSLC medium compared to their counterpart control Pum-C samples. These results were coherent with visual and UPV inspection. Additionally, there was approximately 30% decrease in k-value of Pum-Bac and Pum-2P samples cured in water compared to Pum-C samples cured in water. A similar trend was also observed in samples cracked at 28 day after mixing and cured in UCSLC medium for 28 days. Interestingly, there was a 15% decrease in absorption coefficient in Pum-C samples cracked at 28 day and cured in UCSLC medium compared to its control sample cured in water. Even this was lower than what was observed in samples containing bacteria (almost 45% decrease was observed), it might indicate a contamination of curing medium resulting with a possible (limited) precipitation within the crack. Pumice was used as an aggregate, some type of fine graded pumice can trigger latent hydration reaction in mortar, if they are activated [78]. Even though, the aggregates were sieved and minimum size was limited to 1.18 mm, there might be chance of remained fine particles on pumice that could trigger a reaction with the high pH nutrient medium.



(a)

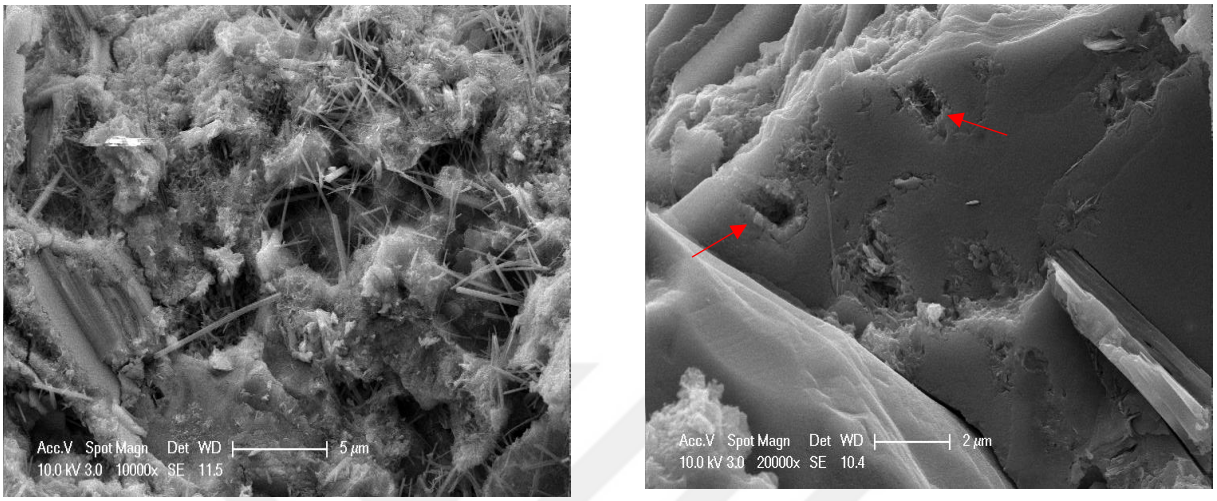


(b)

Figure 4. 16 *Mass of absorbed water per m^2 for (a) 14 day (b) 28-day samples containing pumice. Pum: Pumice; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients -. WC: Water cured; UCSLCC: Nutrient Medium curing. Data points represent the average of triplicates of samples.*

4.4.4 Precipitate characterization:

SEM images were captured for Pum-Bac and Pum-2P samples where crystal precipitation was observed. Images can be seen in Figure 4.17.



(a) (b)

Figure 4. 17 *SEM images of the UCSLC cured 28 day cracked PUM-Bac samples with red arrows highlighting the bacterial presence.*

Needle shaped crystals were observed similar to the DE samples. Calcite formations were seen through the samples collected from the crack surface. Rod shaped indication of bacterial cells were also observed on the crystal formations. Several 1.5-2 µm bacteria beds were spotted in the images. This also proves that MICP was triggered within the cracks when *S. pasteurii* cells were incorporated immobilized on pumice particle.

4.5 Comparative Evaluation of Protective Barriers

4.5.1. Comparison in terms of viability

An important aspect of this study was the evaluation of bacterial viability. Bacteria count of the specimens were conducted by drop plate method as specified at Section 3.5.5. Results were presented as CFU (cell forming units) per gram of mortar. Specimens were especially collected from the beams where the crack remediation was achieved. Figures 4.18 and 4.19 present the results of viability evaluation. Additionally, representative samples were tested in order to see if there was a difference in terms of viability when crack closure did not actualize.

Cells stayed in the mortar for almost 45 and 60 days prior to testing for 14 and 28-day cracked mortar samples, respectively. Since the cementitious matrix is a rough environment for microorganism to stay viable, efficiency of the protective barriers can be better understood through this evaluation.

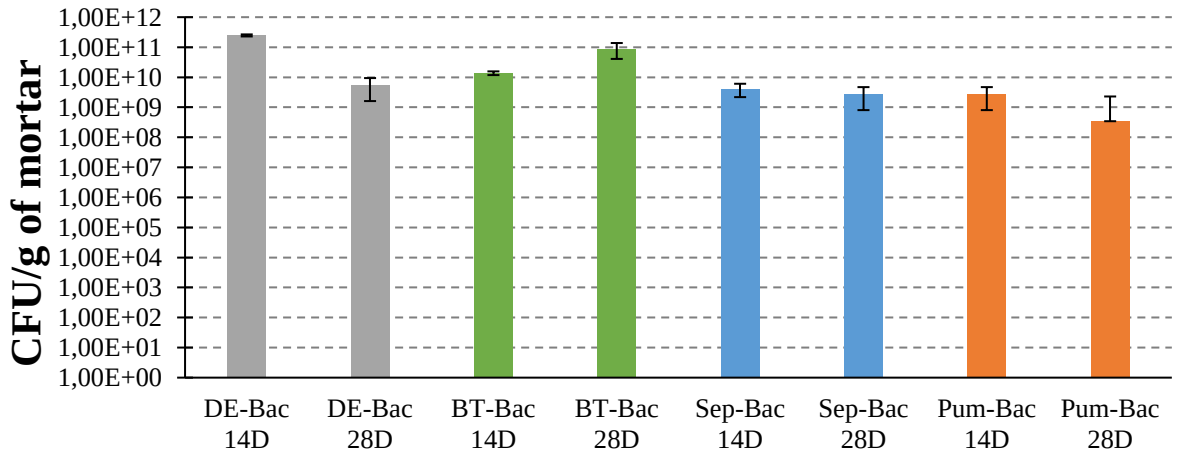


Figure 4. 18 *Bacterial viability evaluation of the UCSLC cured Bac samples. DE: Diatomaceous earth, BE: Bentonite, SEP: Sepiolite PUM: Pumice.*

For *Bac* samples where bacterial cells immobilized through PBS solution, diatomaceous earth and bentonite showed the best performance in terms of keeping the cells viable compared to sepiolite and pumice. However, it should be mentioned that all of the protective barriers were efficient to maintain a high cell concentration to induce $CaCO_3$ even after 60 days of mixing. In fact, previous studies showed that the lowest *S. pasteurii* cell concentration should be in the degree of 10^6 CFU/g of mortar to trigger MICP [79].

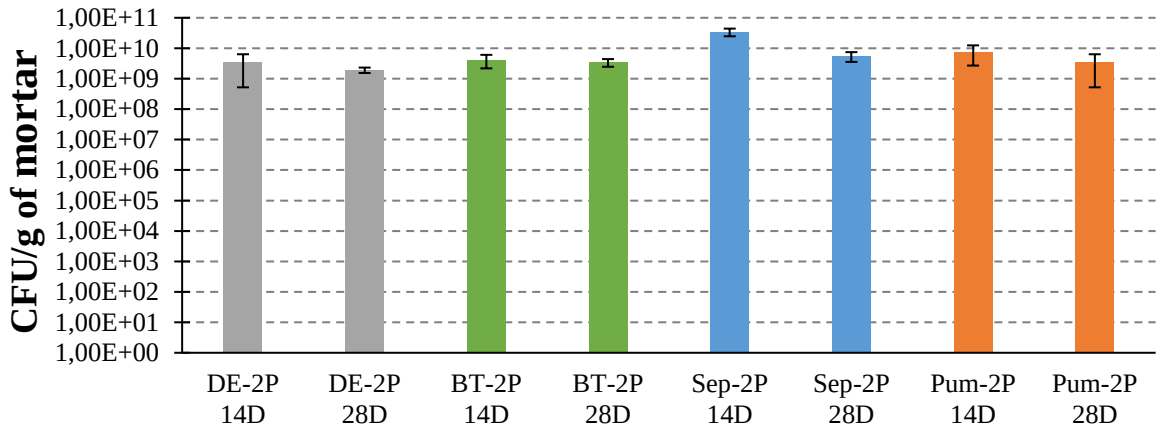


Figure 4. 19 *Bacterial viability evaluation of the UCSLC cured 2 phase samples. DE: Diatomaceous earth, BE: Bentonite, SEP: Sepiolite PUM: Pumice.*

A similar trend was also observed in 2 phase samples however, the performance of protection barriers were almost the same. Compared to Bac samples the remaining viable cell number was significantly lower, at least by 10^3cells per g mortar. This was expected since the quantity of bacteria was slightly lower in 2-phase samples compared to Bac samples (*see* Table 3.2). This also supports the idea of the presence of the UCSLC medium was crucial for the cells. To understand the relationship between cell viability and crack healing, remaining cell concentration was determined in those samples cured in water and exhibit partial crack healing. Results can be seen in Figure 4.20.

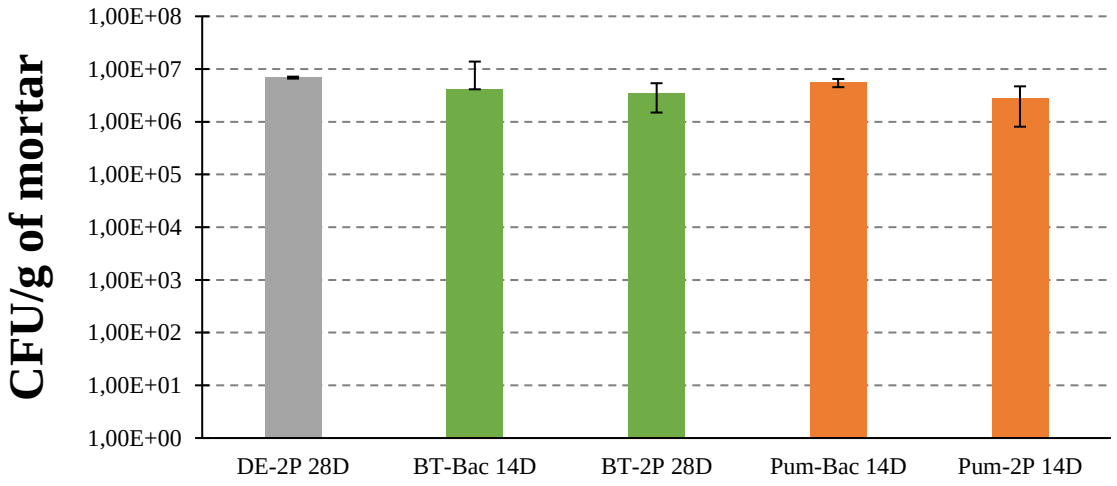


Figure 4. 20 *Bacterial viability evaluation of the water cured samples where the crack closure was observed. DE: Diatomaceous earth, BE: Bentonite PUM: Pumice.*

As expected, the cell concentration was much lower than that of samples cured in UCSLC medium, even for 2P samples. It was seen that when the samples were not supplied with nutrients, concentration was decreases at least by a degree of 10. It should be noted that there is not a direct correlation with the initial cell content used in the mix and final cell concentration obtained after drop plate viable cell counting. However, 2 g of bacterial cells correspond to almost 10^9 CFU/mL grown in 300 mL nutrient medium for 24 hours for *Bac* samples (*see* Figure 3.1). Similarly, for 1 g it is 10^9 CFU/mL grown in 150 mL nutrient medium for 24 hours for *2-phase* samples. This corresponds to a cell concentration of approximately ~10^8 CFU/g of fresh mortar for both samples. Even though, the initial and final cell concentration cannot be directly correlated, number of cells was substantially decreased by water curing whereas the cell concentration was increased in samples cured in nutrient medium. Since the samples were held in ambient conditions before cracking and there is not a suitable environment in hardened cement-based matrix prior to cracking, it is a valid assumption to claim that a cell growth was initiated once nutrients provided upon cracking. This is in line with most of the hypothesis claimed that endospores enable the cells to be dormant for extended periods and when exposed

to a suitable environment again, this reactivates the bacteria into a metabolic state and initiate growth[80]. These results prove that the proposed diatomaceous earth, bentonite, sepiolite and pumice were successful in order to protect the bacteria from extreme environment. Once the cracks were initiated, nutrient medium curing might initiate growth within the crack surface and trigger MICP. However, only water curing is not sufficient to trigger growth. Further evaluation was also conducted on DE-Bac cracked at 28 days and Sep-2P cracked at 14 days cured in water. These sets were especially selected since there were no $CaCO_3$ precipitation was observed in these specimens. In particularly, the cell concentration was even lower 10^5 CFU/g of mortar. This result was align with the discussions present in the literature for the minimal concentrations needed to obtain MICP [29,32]. Thus, it would be correct to conclude that an external source of nutrient in curing solution is not only required to induce MICP but also keep the cells viable.

.

4.5.2. Self-healing efficiency of different protection barriers

This study provides a comparative analysis on possible use of diatomaceous earth, bentonite, sepiolite and pumice as protective barriers for *S. pasteurii* cells to trigger self-healing in cement-based mortars. Table 4.17 summarizes the samples and presence of crack healing. To establish a comparison among the samples tested throughout this study.

Table 4. 17 *Summary of observations for self-healing.DE: Diatomaceous earth, BE: Bentonite, SEP: Sepiolite PUM: Pumice; C: Control samples without any bacteria; Bac: Samples containing bacteria without nutrients; 2P: Samples containing bacterial cells with nutrients. The evaluation is done based on stereomicroscopy analysis, UPV measurement and water absorption test.*

Sample Name	Description	Water Curing		Nutrient Medium Curing	
		14-days	28-days	14-days	28-days
Control	*No protection barrier, no cells*	X	X	X	X
DE-C	*Only water saturated protection barriers without cells*	X	X	X	X
BT-C		X	X	X	X
SEP-C		X	X	X	X
PUM-C		X	X	X	X
DE-Bac	*S. pasteurii cells suspended in PBS (2g) immobized on protection barriers without any nutrients*	X	X	✓	✓
BT-Bac		✓	X	✓	✓
SEP-Bac		X	X	✓	✓
PUM-Bac		✓	X	✓	✓
DE-2P	*S. pasteurii cells suspended in PBS (1 g) immobized on half of the protection barrier and other half of protection barriers were saturated with UCSLC medium*	X	✓	✓	✓
BT-2P		X	✓	✓	✓
SEP-2P		X	X	✓	✓
PUM-2P		✓	X	✓	✓

Upon 28 days of nutrient medium curing, the cracks were almost completely sealed in *Bac* and *2-Phase* samples when they were cured in UCSLC medium. In contrast, there were not any indication of crack remediation in control samples without any bacterial cells.

A critical point of the results obtained is the importance of nutrients for self-healing. As abovementioned crack healing was observed in any sample containing bacterial cells when they were cured in nutrient medium. In addition, partial crack sealing was achieved in 2-Phase

samples when they were cured in water. Results showed that cells require additional nutrient source as urea and calcium acetate either in the mix or as curing regardless of type of the immobilization barrier. Cracks were sealed even in samples including relatively smaller dosage of nutrients and bacterial cells in presence of moisture.

Based on the visual crack evaluation, all of the minerals used were found to be effective in terms of immobilizing the bacterial cells and trigger self-healing. The possible mechanism of these minerals could be attributed to their relatively high capacity to absorb and hold bacterial cells on the surfaces. In addition, it could also be noted that additional nutrients as urea and [Ca^{+2}] source should be provided either during the mixing or in the curing solution. DE was already known to be a good mineral agent to immobilize and protect the cells from high pH environment of cement paste. This was attributed to its relatively higher absorption capacity and high specific surface area which could enable a more homogenous distribution. Since DE is also a microbiological formation, it could also provide a more suitable microenvironment for the bacterial cells compared to cementitious environment and thus bacteria could still decompose urea [28]. However, the remaining 3 protective barriers are introduced to the literature with this study in terms of being protective agent of bacteria.

It should also be noted that, even though all minerals were effective in terms of remediating the cracks. Even though the number of cells were higher in samples containing pumice compared to rest of the samples, it did not lead to a more efficient (or faster) crack remediation. Since pumice have larger particle size, it was used as a sand replacement which was 3 times more than mineral addition. Relatedly, the number of bacterial cells and nutrient solution was also tripled. In theory, it might lead to a more efficient crack healing in pumice containing samples compared to DE, Sep and BT modified samples. However, the remaining particles provide a more homogenous particle distribution in the mortar mix, due to their fine particle size. Thus, efficiency of finer size protection barriers might be higher than use of an aggregate. Fine powder

size lead to a better dispersion compared to pumice which increases the possibility of presence of self-healing agents within the cracked surface. In addition, using higher quantity of bacteria might yield in better performance from diatomaceous earth, bentonite and sepiolite since their performance.

Chapter V

Conclusion and Future Work

5.1 Conclusion

In this research, potential usage of diatomaceous earth, bentonite, sepiolite and pumice as protective barriers of bacteria was investigated. Immobilization of the *Sporosarcina pasteurii* cells onto the DE, BT, Sep and Pum was resolved as a successful attempt. Obtained data shows that this methodology could be used as a self-healing system. Key finding of the study is summarized as follows:

1- Immobilization was achieved for all diatomaceous earth, bentonite, sepiolite and pumice. Except diatomaceous earth, remaining protective barriers were evaluated in terms of their performance as an element of a self-healing system for the first time. Even though the bacterial presence was not observed in the bentonite and sepiolite in the SEM inspection after the immobilization, final results proven that the bacteria were fixed on the minerals and did not detected at the SEM. This result show that, cheap and easy to find natural mineral DE, BT, Sep and Pum can be considered in self-healing systems as carriers due to their high absorption capacity, porous structure and ability to protect bacteria from extreme environments.

2- In order to obtain calcium carbonate precipitation, presence of urea and a calcium source is a must. In 2 phase samples, incorporation of the nutrient media was attempted by immobilizing it onto the DE, BT, Sep and Pum. In UCSLC curing solution 20g/L urea, 10g/L CSL and 10g/L calcium acetate were used. UCSLC cured samples cured in 1-liter batches. However, the incorporated nutrients were 95% less than the curing solution. Increase in the quantity of incorporated nutrients might resolve in a more

effective system. When the samples were cured with water, BT-Bac, Pum-Bac and Pum-2P samples cracked at 14 days and DE-2P and BT-2P samples cracked at 28 days showed limited crack closure.

3- When the samples cured in UCSLC medium showed complete crack remediation regardless of the protective barrier and composition type. All the beams were cured at or before 28 days. BT-Bac samples cracked at 28 days showed the fastest closure time as 14 days. However, this shortened curing period resulted in a increase in the mentioned samples water absorption. These results showed that the even the surface closure was observed, internal healing might need a prolonged curing period.

4- Efficiency of the protective barriers were evaluated with a viability test. Result showed that all DE, BT, Sep and Pum was successful in order to protect the bacteria. Cell concentrations were still sufficient for MICP after 45 and 60 days of suspension in the concrete matrix.

5.2 Future work

This research proved the usage of DE, BT, Sep and Pum. However, possible improvements and modifications can still be applied. Recommended areas for further research are as follows:

1- It was seen that the 2 phase UCSLC cured samples acquired crack remediation. However, this behavior was very limited in the water cured samples. Attempting the same methodology with an increased proportion of incorporated nutrients in 2 phase samples might resolve in an efficient crack closure in the water cured samples which would be a more practical method. Further research is needed to optimization of the 2-phase methodology.

2- Bacterial viability was tested by the means of concentration. Conducting oxygen consumption test might increase the insights about the cell's viability in the matrix. Additionally, evaluating the ureolytic activity of the immobilized cells through urea decomposition rate might be conducted in order to see the protective barriers effect at different pH values, temperature, compositions etc.

3- Further investigation on the morphology of the precipitates are required. In order to understand the crystals true nature through XRD and FTIR inspection.

4- The samples with the crack closure showed a decrease in the water absorption coefficient. However, further investigation is needed in order to see the corrosion resistance of the cured samples.

5- The effects of the used bio-agents on the mechanical properties of the matrix is still unknown. Parameters such as compressive strength and setting times must be evaluated in order to see the used protective barriers, nutrients and the bacteria effect on the concrete.

6- In literature, it was discussed that an increase in the magnesium (Mg) content in the environment resolves in a more stable calcium carbonate morphology through catalyzing the transformation of the amorphous calcium carbonate (ACC) to calcite[81]. Integration of Mg into the methodology that was used for this study by using a high Mg content cement or simply adding it into the nutrient medium might increase the crack closure performance of the system by resolving in a more stable precipitate.

Appendix A

Table A.1 DE-C samples cracked at 14 days.

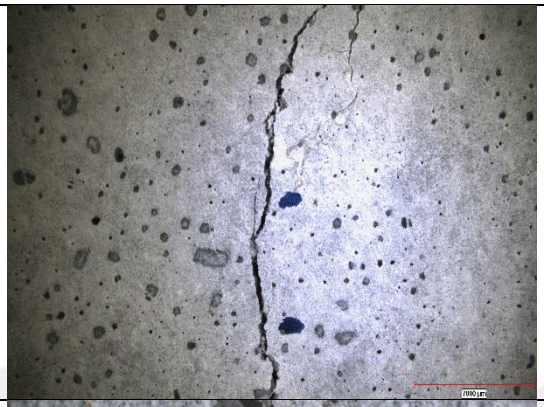
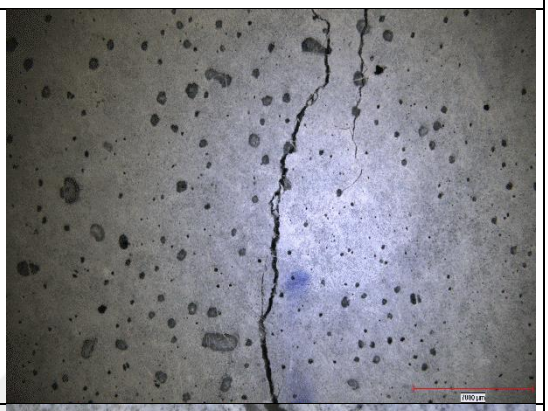
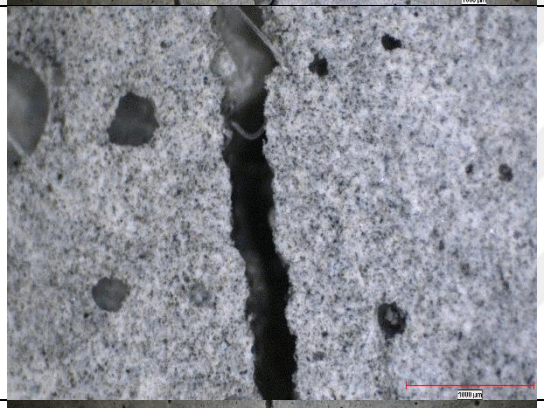
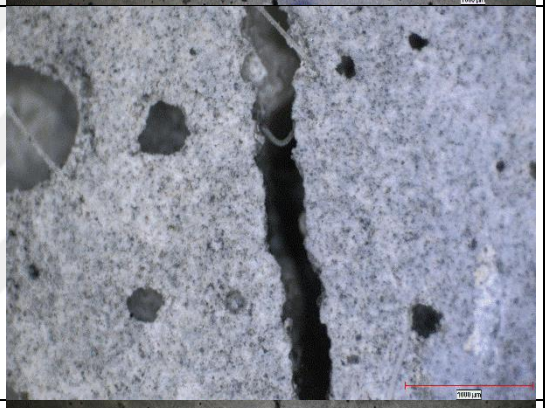
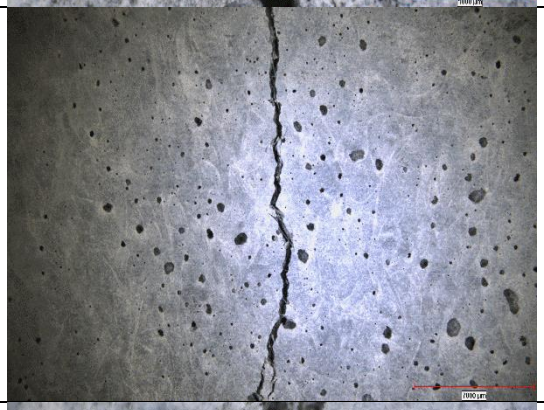
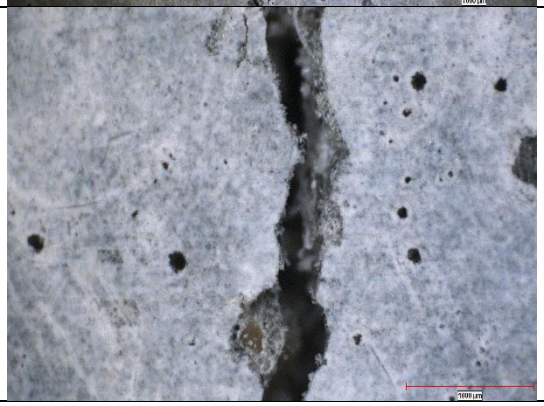
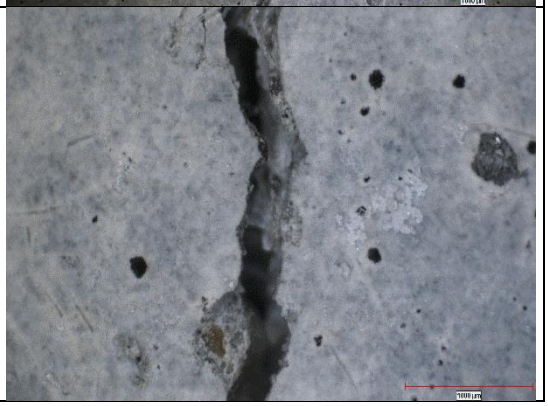
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table A.2 DE-C samples cracked at 28 days.

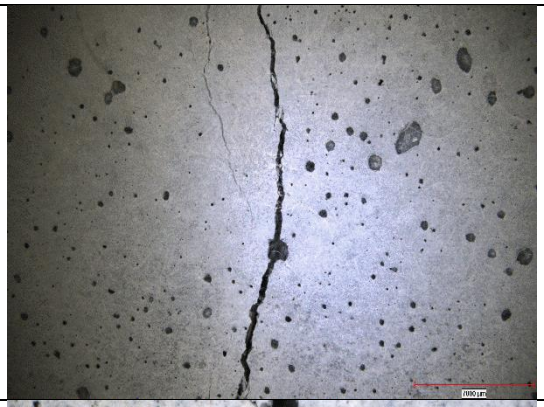
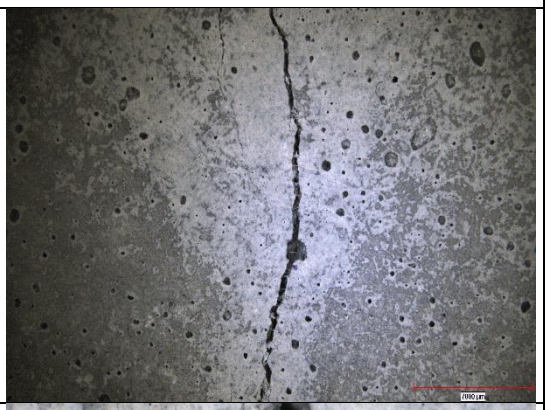
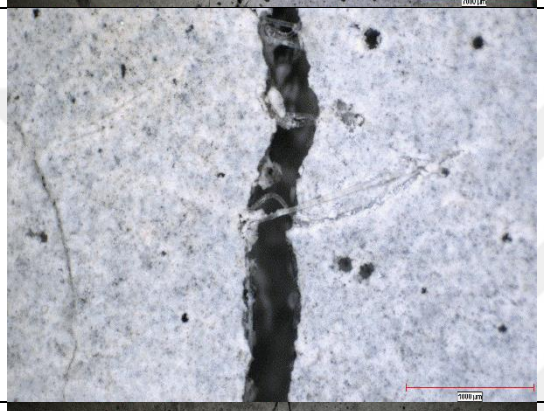
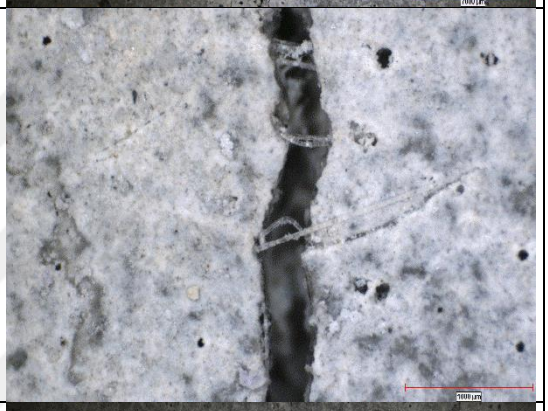
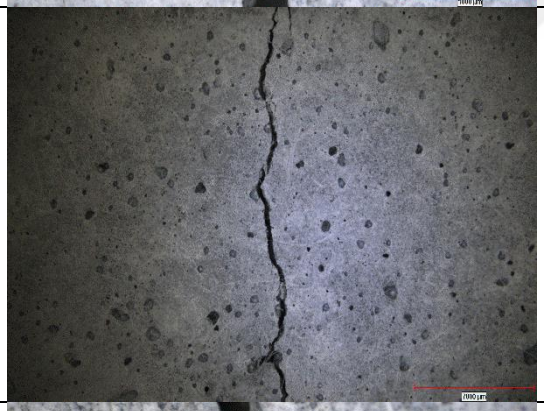
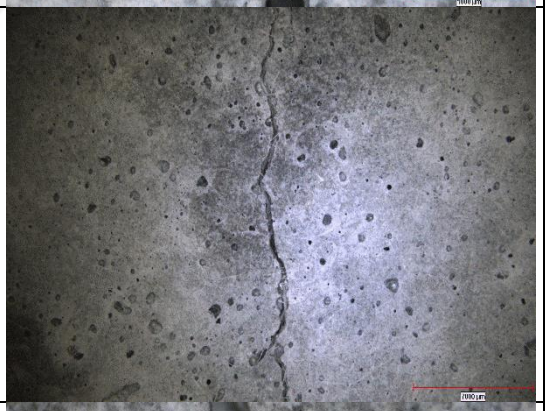
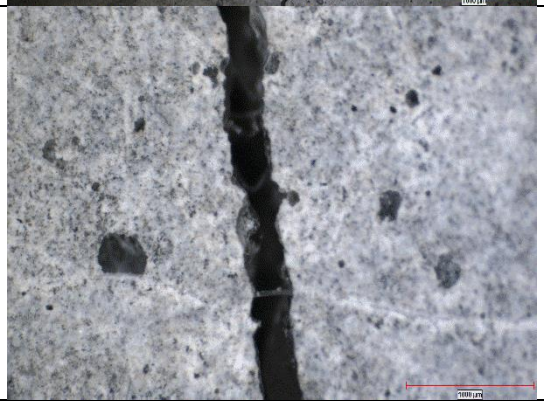
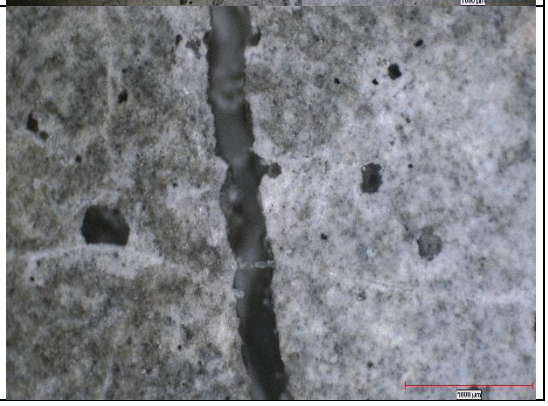
28 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table A.3 BT-C samples cracked at 14 days.

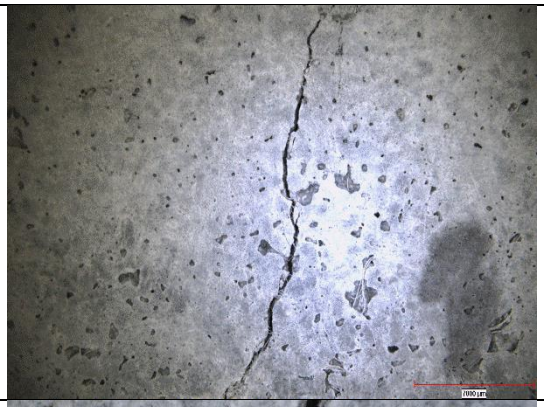
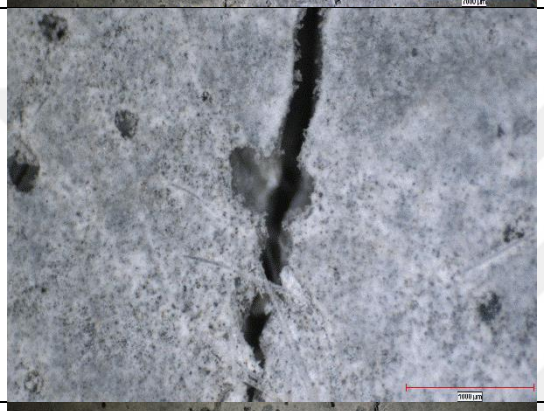
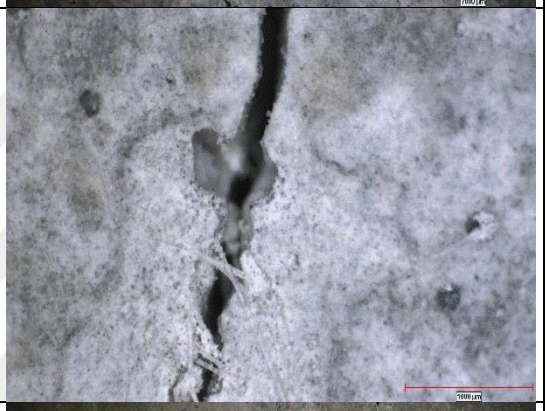
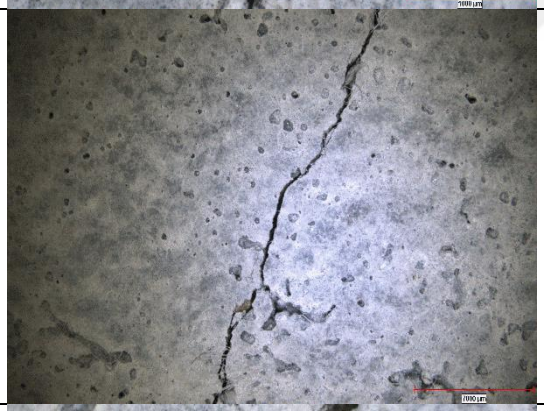
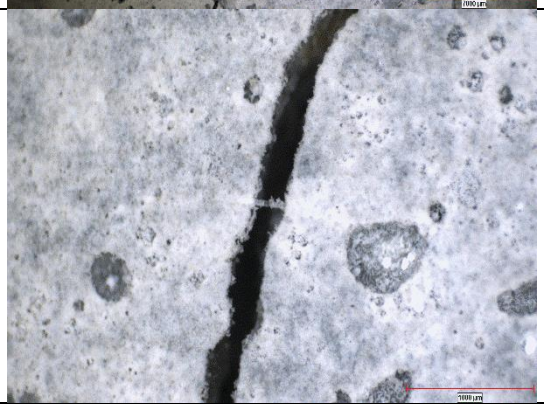
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table A.4 BT-C samples cracked at 28 days.

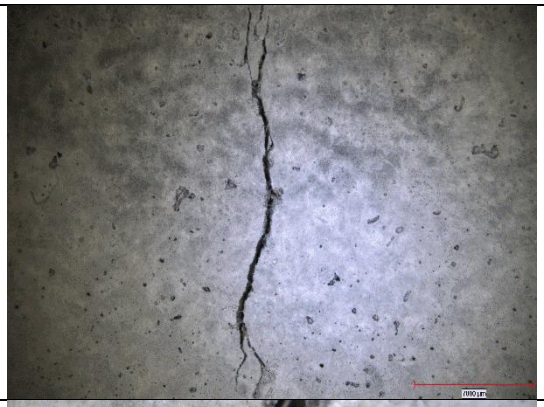
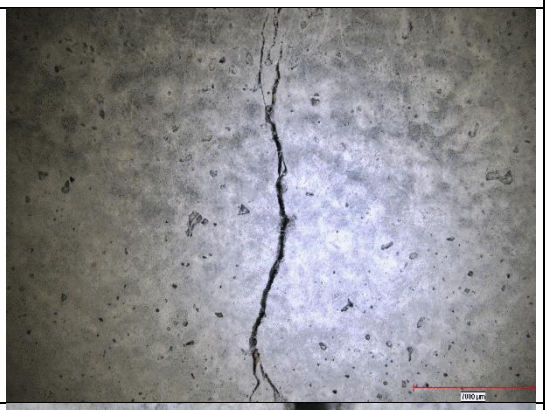
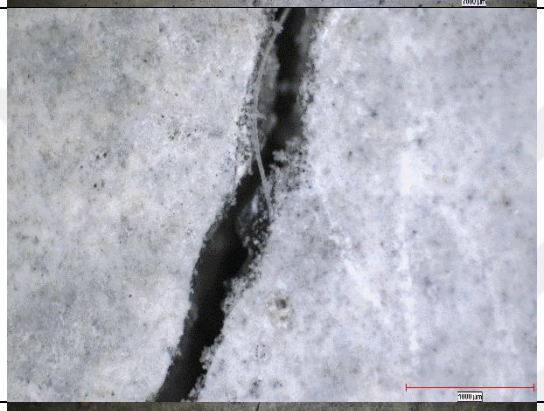
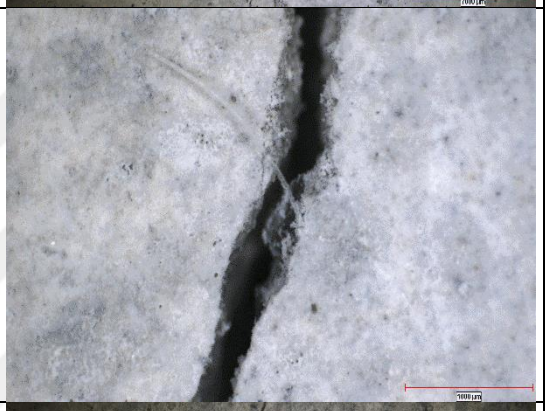
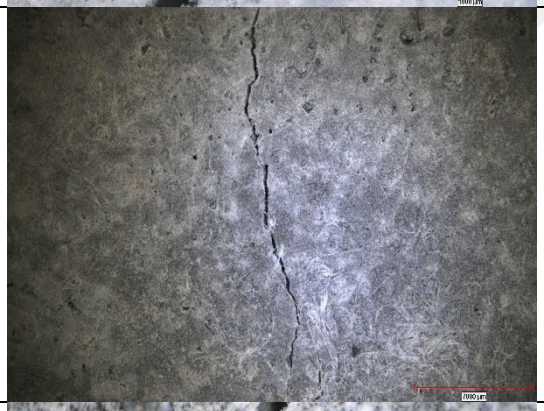
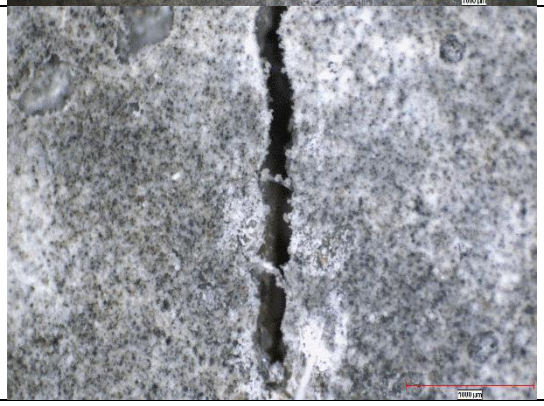
28 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table A.5 Sep-C samples cracked at 14 days.

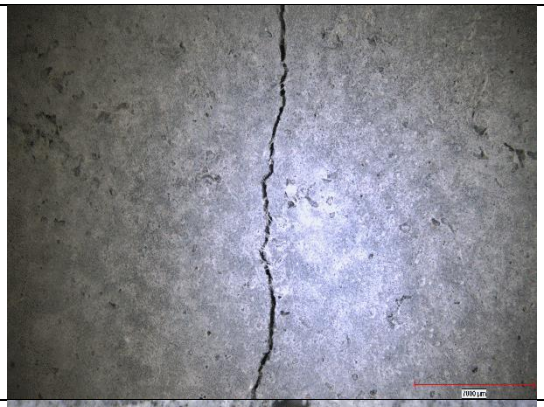
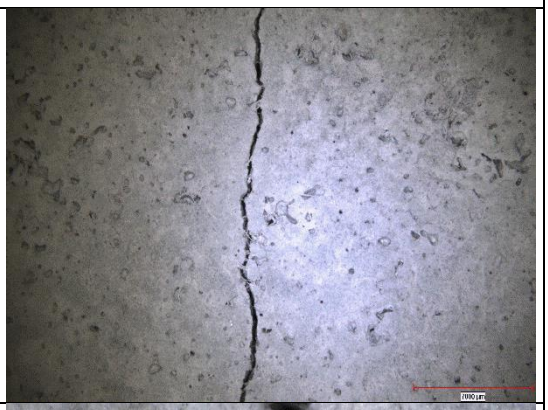
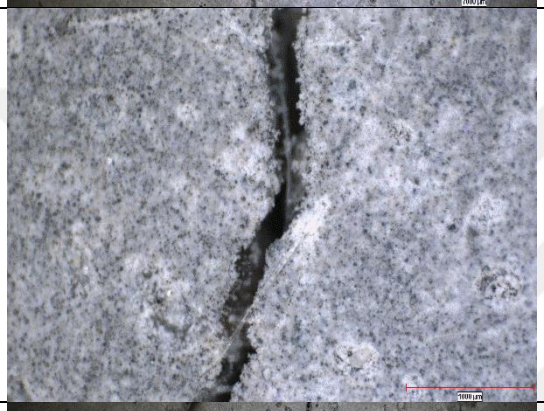
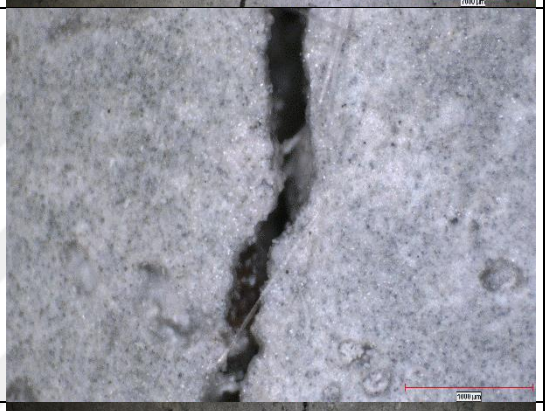
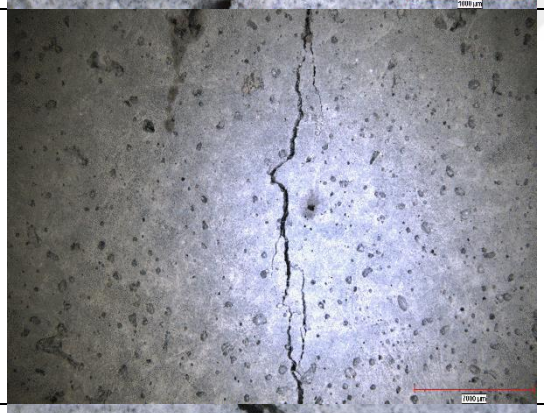
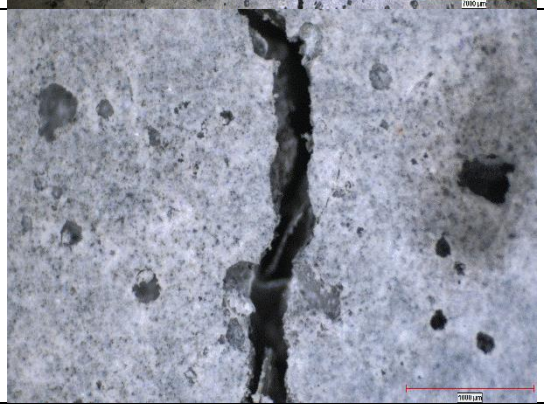
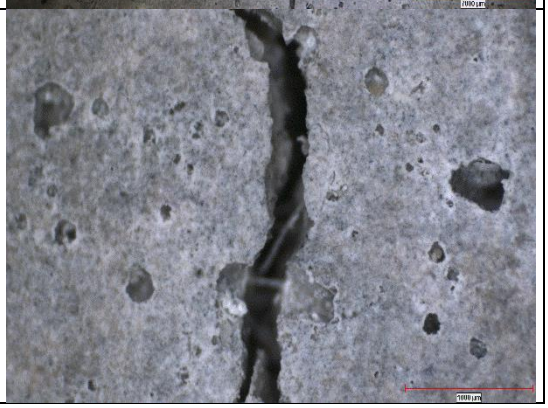
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table A.6 Sep-C samples cracked at 28 days.

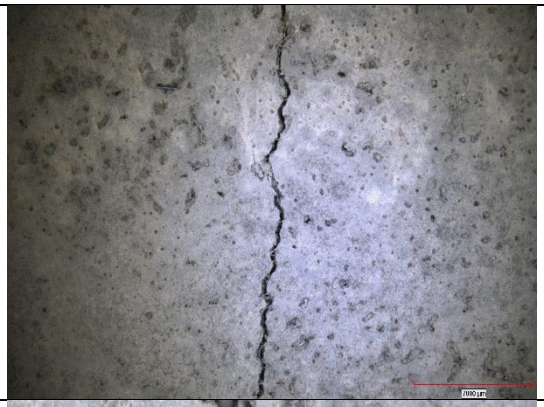
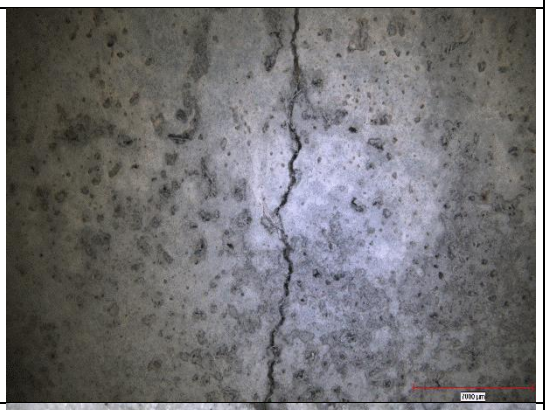
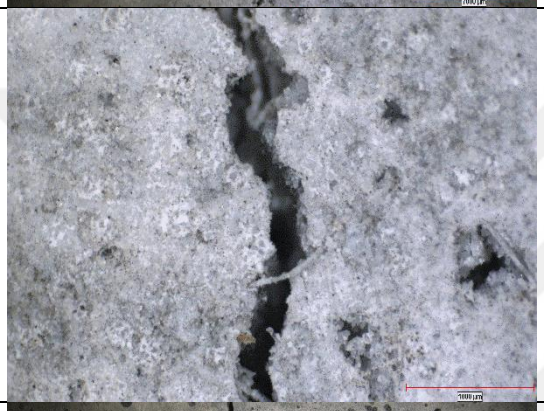
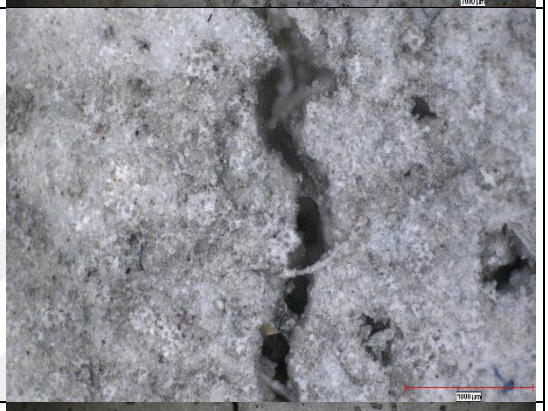
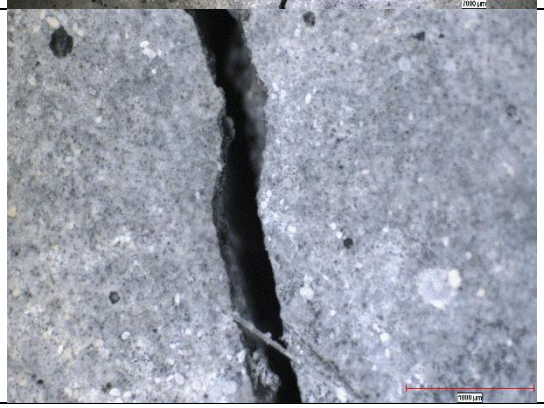
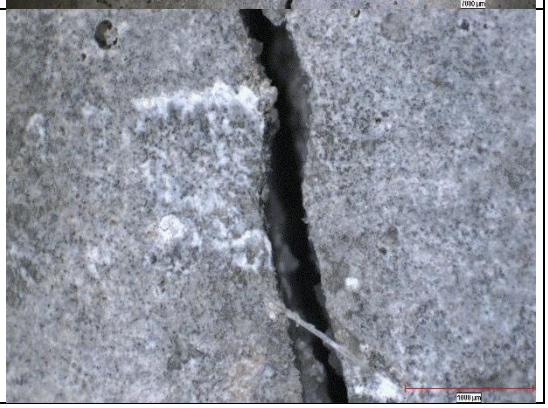
28 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table A.7 Pum-C samples cracked at 14 days.

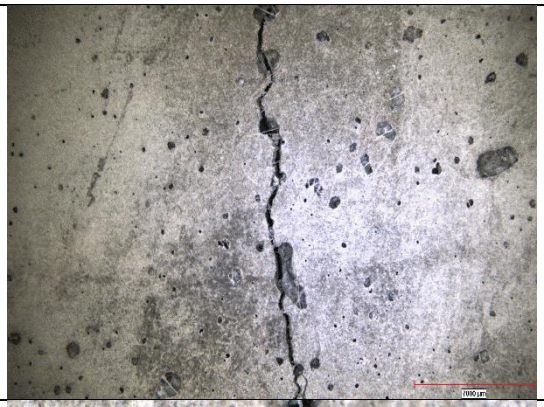
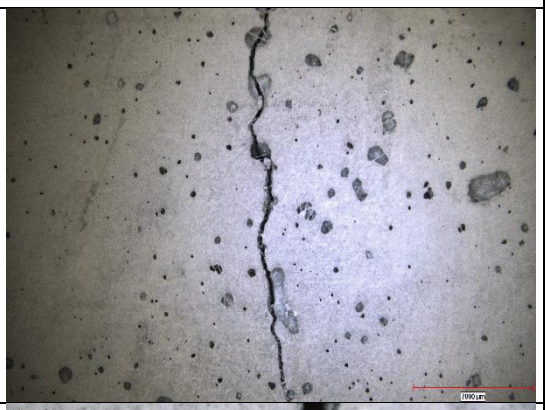
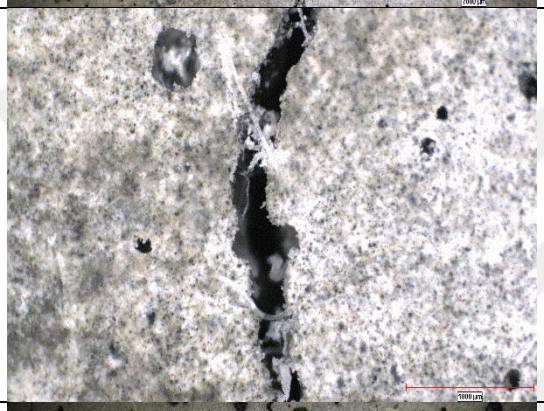
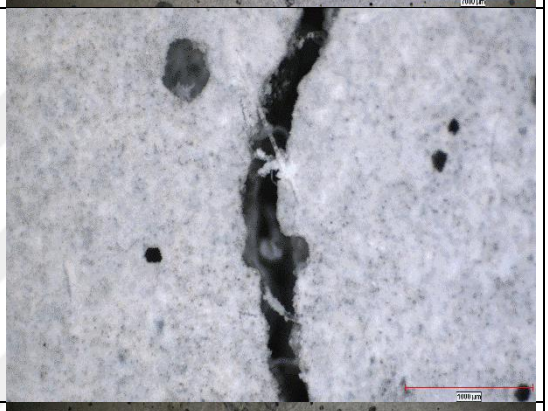
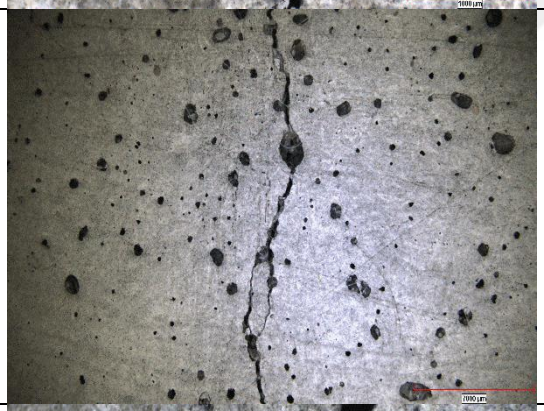
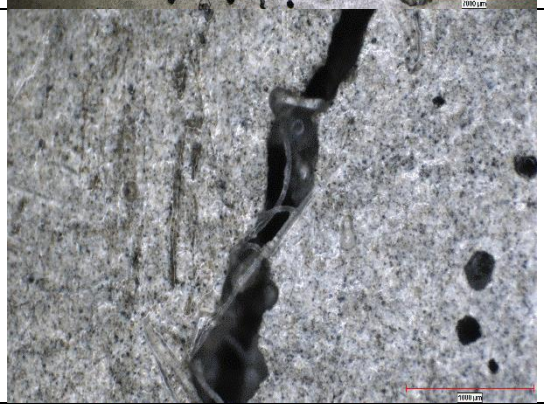
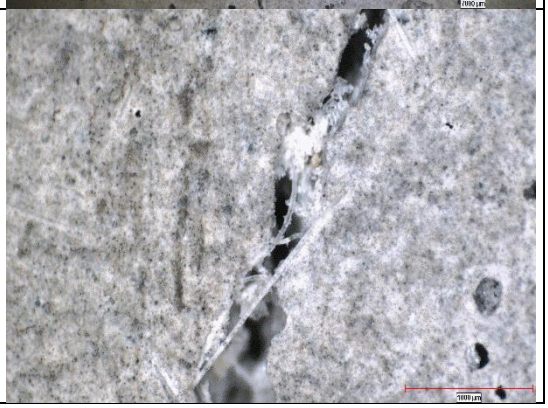
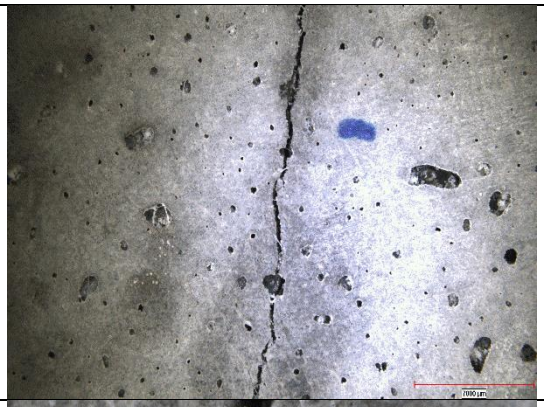
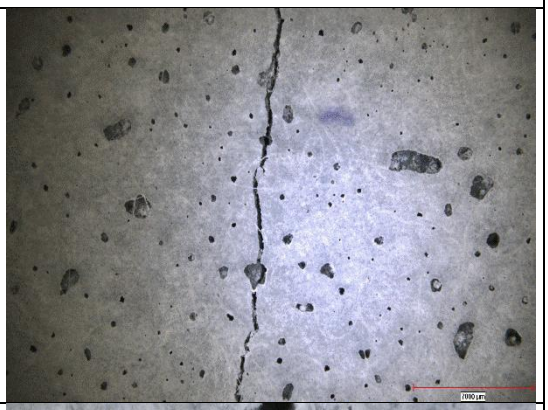
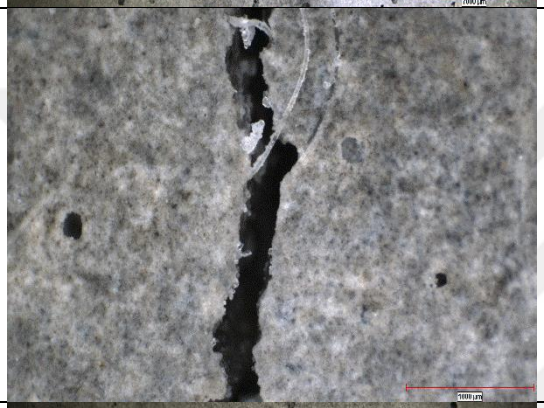
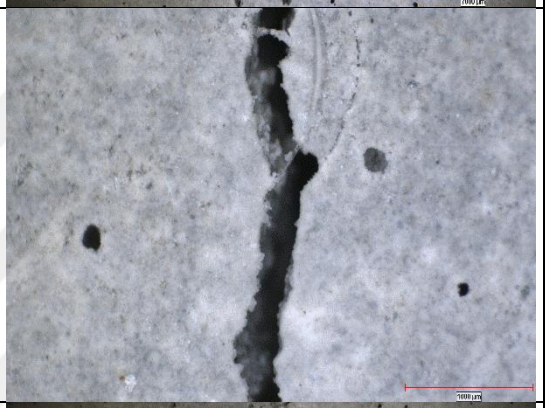
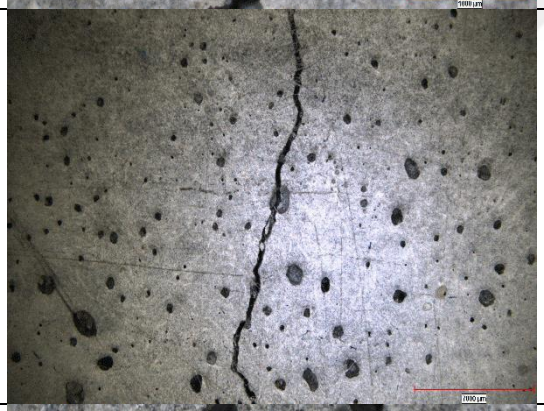
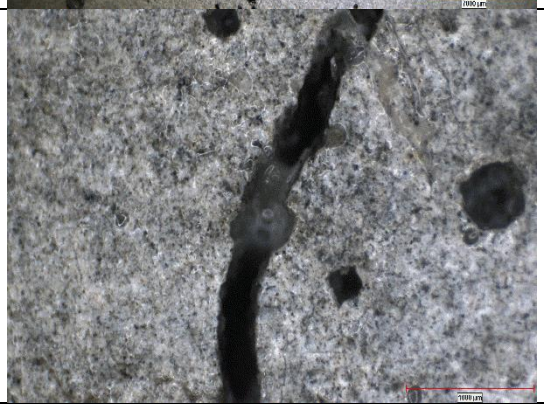
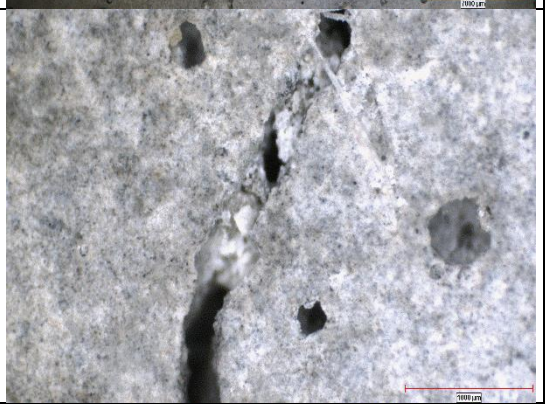
14 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

Table A.8 Pum-C samples cracked at 28 days.

28 Day	Initial Crack	28th Day of Curing
Water Cured Sample 6.7X		
Water Cured Sample 50X		
UCSLC Cured Sample 6.7X		
UCSLC Cured Sample 50X		

References

[1] X. F. Wang *et al.*, "Evaluation of the mechanical performance recovery of self-healing cementitious materials – its methods and future development: A review," *Constr. Build. Mater.*, vol. 212, pp. 400–421, 2019.

[2] K. L. Scrivener, V. M. John, and E. M. Gartner, "Eco-efficient cements: Potential economically viable solutions for a low-CO2 cement-based materials industry," *Cem. Concr. Res.*, 2018.

[3] M. Stajanca and A. Estokova, "Environmental Impacts of Cement Production," *Tech. Univ. Kosice, Civ. Eng. Fac. Inst. Archit. Eng.*, pp. 296–302, 2012.

[4] Z. Wu, H. S. Wong, and N. R. Buenfeld, "Influence of drying-induced microcracking and related size effects on mass transport properties of concrete," *Cem. Concr. Res.*, 2015.

[5] D. Darwin *et al.*, "CAUSES, EVALUATION, AND REPAIR OF CRACKS IN CONCRETE STRUCTURES.," *J. Am. Concr. Inst.*, 1984.

[6] K. Van Tittelboom, N. De Belie, W. De Muynck, and W. Verstraete, "Use of bacteria to repair cracks in concrete," *Cem. Concr. Res.*, vol. 40, no. 1, pp. 157–166, 2010.

[7] J. Wang, K. Van Tittelboom, N. De Belie, and W. Verstraete, "Use of silica gel or polyurethane immobilized bacteria for self-healing concrete," *Constr. Build. Mater.*, vol. 26, no. 1, pp. 532–540, Jan. 2012.

[8] V. C. Li and E. Herbert, "Robust self-healing concrete for sustainable infrastructure," *J. Adv. Concr. Technol.*, vol. 10, no. 6, pp. 207–218, 2012.

[9] W. De Muynck, N. De Belie, and W. Verstraete, "Microbial carbonate precipitation in construction materials: A review," *Ecol. Eng.*, vol. 36, no. 2, pp. 118–136, 2010.

[10] S. van der Zwaag, "An Introduction to Material Design Principles: Damage Prevention versus Damage Management," 2007.

[11] C. Edvardsen, "Water permeability and autogenous healing of cracks in concrete," *ACI Mater. J.*, 1999.

[12] W. Ramm and M. Biscoping, "Autogenous healing and reinforcement corrosion of water-penetrated separation cracks in reinforced concrete," *Nucl. Eng. Des.*, 1998.

[13] K. Van Tittelboom and N. De Belie, "*Self-Healing in Cementitious Materials—A Review*," *Materials*, pp. 2182-2217, 2013.

[14] W. Du, J. Yu, Y. Gu, Y. Li, X. Han, and Q. Liu, "Preparation and application of microcapsules containing toluene-di-isocyanate for self-healing of concrete," *Constr. Build. Mater.*, vol. 202, pp. 762–769, 2019.

[15] M. Ismail, A. Toumi, R. François, and R. Gagné, "Effect of crack opening on the local diffusion of chloride in inert materials," *Cem. Concr. Res.*, 2004.

[16] S. V. Zemskov, H. M. Jonkers, and F. J. Vermolen, "Two analytical models for the probability characteristics of a crack hitting encapsulated particles: Application to self-healing materials," *Comput. Mater. Sci.*, 2011.

[17] T. D. P. Thao, T. J. S. Johnson, Q. S. Tong, and P. S. Dai, "Implementation of self-healing in concrete–Proof of concept," *IES J. Part A Civ. Struct. Eng.*, 2009.

[18] S. K. Ramachandran, V. Ramakrishnan, and S. S. Bang, "Remediation of concrete using micro-organisms," *ACI Mater. J.*, 2001.

[19] S. Mann, *Biomineralization: Principles and Concepts in Bioinorganic Materials Chemistry*. New York: Oxford, 2001.

[20] D. A. Bazylinski, "Biologically Controlled Mineralization in Prokaryotes," *Rev. Mineral. Geochemistry*, 2003.

[21] N. Hearn, "Self-sealing, autogenous healing and continued hydration: What is the difference?," *Mater. Struct.*, vol. 31, no. 8, p. 563, 1998.

[22] V. Wiktor and H. M. Jonkers, "Quantification of crack-healing in novel bacteria-based self-healing concrete," *Cem. Concr. Compos.*, vol. 33, no. 7, pp. 763–770, 2011.

[23] H. M. Jonkers and E. Schlangen, "A two component bacteria-based self-healing concrete," in *Concrete Repair, Rehabilitation and Retrofitting II - Proceedings of the 2nd International Conference on Concrete Repair, Rehabilitation and Retrofitting, ICCRRR*, 2009.

[24] H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen, "Application of bacteria as self-healing agent for the development of sustainable concrete," *Ecol. Eng.*, 2010.

[25] Y. Ç. Erşan, F. B. Da Silva, N. Boon, W. Verstraete, and N. De Belie, "Screening of bacteria and concrete compatible protection materials," *Constr. Build. Mater.*, vol. 88, pp. 196–203, 2015.

[26] J. Wang, K. Van Tittelboom, N. De Belie, and W. Verstraete, "Use of silica gel or polyurethane immobilized bacteria for self-healing concrete," *Constr. Build. Mater.*, vol. 26, no. 1, pp. 532–540, 2012.

[27] J. Y. Wang, D. Snoeck, S. Van Vlierberghe, W. Verstraete, and N. De Belie, "Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete," *Constr. Build. Mater.*, vol. 68, pp. 110–119, 2014.

[28] J. Y. Wang, N. De Belie, and W. Verstraete, "Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete.," *J. Ind. Microbiol. Biotechnol.*, vol. 39, no. 4, pp. 567–77, Apr. 2012.

[29] Y. Ç. Erşan, F. B. Da Silva, N. Boon, W. Verstraete, and N. De Belie, "Screening of bacteria and concrete compatible protection materials," *Constr. Build. Mater.*, vol. 88, pp. 196–203, 2015.

[30] M. Alazhari, T. Sharma, A. Heath, R. Cooper, and K. Paine, "Application of expanded perlite encapsulated bacteria and growth media for self-healing concrete," *Constr. Build. Mater.*, vol. 160, pp. 610–619, 2018.

[31] E. Schlangen and C. Joseph, "Modelling of Self-Healing Cementitious Materials," in *Self-Healing Phenomena in Cement-Based Materials: State-of-the-Art Report of RILEM Technical Committee 221-SHC: Self-Healing Phenomena in Cement-Based Materials*, M. de Rooij, K. Van Tittelboom, N. De Belie, and E. Schlangen, Eds. Dordrecht: Springer Netherlands, 2013, pp. 217–240.

[32] T. G. Nijland, J. a Larbi, R. P. J. Van Hees, and B. Lubelli, "Self Healing Phenomena in Concretes and Masonry Mortars : a Microscopic Study," *Proc. First Int. Conf. Self Heal. Mater. 18-20 April 2007, Noordwijk aan Zee, Netherlands*, 2007.

[33] S. R. White *et al.*, "Autonomic healing of polymer composites," *Nature*. 2001.

[34] H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen, "Application of bacteria as self-healing agent for the development of sustainable concrete," *Ecol. Eng.*, 2010.

[35] K. Van Tittelboom, K. Adesanya, P. Dubruel, P. Van Puyvelde, and N. De Belie, "Methyl methacrylate as a healing agent for self-healing cementitious materials," *Smart Mater. Struct.*, 2011.

[36] H. Huang, G. Ye, C. Qian, and E. Schlangen, “Self-healing in cementitious materials: Materials, methods and service conditions,” *Mater. Des.*, vol. 92, pp. 499–511, 2016.

[37] X. Wang, F. Xing, M. Zhang, N. Han, and Z. Qian, “Experimental study on cementitious composites embedded with organic microcapsules,” *Materials (Basel).*, vol. 6, no. 9, pp. 4064–4081, 2013.

[38] E. Mostavi, S. Asadi, M. M. Hassan, and M. Alansari, “Evaluation of self-healing mechanisms in concrete with double-walled sodium silicate microcapsules,” *J. Mater. Civ. Eng.*, vol. 27, no. 12, pp. 1–8, 2015.

[39] V. V. Zuev, J. Lee, S. V. Kostromin, S. V. Bronnikov, and D. Bhattacharyya, “Statistical analysis of the self-healing epoxy-loaded microcapsules across their synthesis,” *Mater. Lett.*, vol. 94, pp. 79–82, 2013.

[40] R. Alghamri, A. Kanellopoulos, and A. Al-Tabbaa, “Impregnation and encapsulation of lightweight aggregates for self-healing concrete,” *Constr. Build. Mater.*, vol. 124, pp. 910–921, 2016.

[41] F. L. Jin, X. Li, and S. J. Park, “Synthesis and application of epoxy resins: A review,” *Journal of Industrial and Engineering Chemistry*. 2015.

[42] B. Dong *et al.*, “Self-healing features in cementitious material with urea-formaldehyde/epoxy microcapsules,” *Constr. Build. Mater.*, vol. 106, pp. 608–617, 2016.

[43] C. Joseph, A. D. Jefferson, B. Isaacs, R. Lark, and D. Gardner, “Experimental investigation of adhesive-based self-healing of cementitious materials,” *Mag. Concr. Res.*, vol. 62, no. 11, pp. 831–843, 2010.

[44] V. C. Li, Y. M. Lim, and Y. W. Chan, “Feasibility study of a passive smart self-healing cementitious composite,” *Compos. Part B Eng.*, 1998.

[45] Z. X. Hu, X. M. Hu, W. M. Cheng, Y. Y. Zhao, and M. Y. Wu, “Performance optimization of one-component polyurethane healing agent for self-healing concrete,” *Constr. Build. Mater.*, vol. 179, pp. 151–159, 2018.

[46] C. Joseph, A. D. Jefferson, and M. B. Cantoni, “Issues Relating To the Autonomic Healing of Cementitious Materials,” *First Int. Conf. Self Heal. Mater.*, no. April, pp. 1–8, 2007.

[47] H. Mihashi and T. Nishiwaki, “Development of engineered self-healing and self-repairing concrete-state-of-the-art report,” *J. Adv. Concr. Technol.*, vol. 10, no. 5, pp. 170–184, 2012.

[48] C. M. Dry, “Three designs for the internal release of sealants, adhesives, and waterproofing chemicals into concrete to reduce permeability,” *Cem. Concr. Res.*, vol. 30, no. 12, pp. 1969–1977, 2000.

[49] H. M. Jonkers, “Self Healing Concrete: A Biological Approach。In: van der Zwaag, S. (Ed.), Self Healing Materials: An Alternative Approach to 20 Centuries of Materials Science.,” *Springer, Dordrecht, The Netherlands*, 2007.

[50] V. Wiktor and H. M. Jonkers, “Quantification of crack-healing in novel bacteria-based self-healing concrete,” *Cem. Concr. Compos.*, vol. 33, pp. 763–770, 2011.

[51] S. Stocks-Fischer, J. K. Galinat, and S. S. Bang, “Microbiological precipitation of CaCO3,” *Soil Biol. Biochem.*, 1999.

[52] K. O. Konhauser, “Diversity of bacterial iron mineralization,” *Earth Sci. Rev.*, 1998.

[53] V. Achal, X. Pan, and N. Özyurt, “Improved strength and durability of fly ash-amended concrete by microbial calcite precipitation,” *Ecol. Eng.*, 2011.

[54] A. Amiri and Z. B. Bundur, “Use of corn-steep liquor as an alternative carbon source for biomineralization in cement-based materials and its impact on performance,” *Constr. Build. Mater.*, 2018.

[55] C. Rodriguez-Navarro, F. Jroundi, M. Schiro, E. Ruiz-Agudo, and M. T. González-Muñoz, “Influence of substrate mineralogy on bacterial mineralization of calcium carbonate: Implications for stone conservation,” *Appl. Environ. Microbiol.*, 2012.

[56] D. Gebauer *et al.*, “Proto-calcite and proto-vaterite in amorphous calcium carbonates,” *Angew. Chemie - Int. Ed.*, 2010.

[57] J. M. Walker, B. Marzec, and F. Nudelman, “Solid-State Transformation of Amorphous Calcium Carbonate to Aragonite Captured by CryoTEM,” *Angew. Chemie - Int. Ed.*, 2017.

[58] I. Tiago, A. P. Chung, and A. Veríssimo, “Bacterial diversity in a nonsaline alkaline environment: Heterotrophic aerobic populations,” *Appl. Environ. Microbiol.*, 2004.

[59] Z. B. Bundur, S. Bae, M. J. Kirisits, and R. D. Ferron, “Biomineralization in self-healing cement-based materials: Investigating the temporal evolution of microbial metabolic state and material porosity,” *J. Mater. Civ. Eng.*, vol. 29, no. 6, pp. 1–8, 2017.

[60] A. Amiri, M. Azima, and Z. B. Bundur, “Crack remediation in mortar via biomineralization: Effects of chemical admixtures on biogenic calcium carbonate,” *Constr. Build. Mater.*, vol. 190, pp. 317–325, 2018.

[61] S. S. Bang, J. J. Lippert, U. Yerra, S. Mulukutla, and V. Ramakrishnan, “Microbial calcite, a bio-based smart nanomaterial in concrete remediation,” *Int. J. Smart Nano Mater.*, vol. 1, no. 1, pp. 28–39, Mar. 2010.

[62] J. Y. Wang, H. Soens, W. Verstraete, and N. De Belie, “Self-healing concrete by use of microencapsulated bacterial spores,” *Cem. Concr. Res.*, vol. 56, pp. 139–152, Feb. 2014.

[63] J. Y. Wang, D. Snoeck, S. Van Vlierberghe, W. Verstraete, and N. De Belie, “Application of hydrogel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete,” *Constr. Build. Mater.*, vol. 68, pp. 110–119, Oct. 2014.

[64] J. Y. Wang, N. De Belie, and W. Verstraete, “Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete,” *J. Ind. Microbiol. Biotechnol.*, vol. 39, no. 4, pp. 567–577, 2012.

[65] V. Achal, A. Mukherjee, and M. S. Reddy, “Effect of calcifying bacteria on permeation properties of concrete structures,” *J. Ind. Microbiol. Biotechnol.*, vol. 38, no. 9, pp. 1229–1234, 2011.

[66] ASTM C136, “Standard Test Method for Sieve Analysis of Fine and Coarse Aggregates,” *Annu. B. ASTM Stand.*, pp. 3–7, 2006.

[67] ASTM, “Test method for slump of hydraulic cement concrete,” *ASTM Int. West Conshohocken, PA*, pp. 12–15, 2005.

[68] ASTM-C230, “Standard Specification for Flow Table for Use in Tests of Hydraulic Cement 1,” *Annu. B. ASTM Stand.*, pp. 4–9, 2010.

[69] ASTM C305, “Standard Practice for Mechanical Mixing of Hydraulic Cement Pastes and Mortars of Plastic Consistency,” *ASTM lnternational*, 2011.

[70] ASTM C597, “Standard Test Method for Pulse Velocity Through Concrete ASTM C 597,” *ASTM Int.*, p. 4, 2016.

[71] “Commission 25-PEM Protection et érosion des monuments - Essais recommandés pour mesurer l’altération des pierres et évaluer l’efficacité des méthodes de traitement/recommended tests to measure the deterioration of stone and to assess the effectiveness of ,” *Matériaux Constr.*, 1980.

[72] L. Prieto-Borja, A. Conde, M. A. Arenas, J. J. de Damborenea, and J. Esteban, “Influence of exposure time on the release of bacteria from a biofilm on Ti6Al4V discs using sonication: An in vitro model,” *Diagn. Microbiol. Infect. Dis.*, vol. 89, no. 4, pp. 258–261, 2017.

[73] B. Herigstad, M. Hamilton, and J. Heersink, “How to optimize the drop plate method for enumerating bacteria,” *J. Microbiol. Methods*, vol. 44, no. 2, pp. 121–129, 2001.

[74] V. S. Ramachandran and J. J. Beaudoin, *HANDBOOK OF ANALYTICAL TECHNIQUES IN CONCRETE SCIENCE AND TECHNOLOGY Principles, Techniques, and Applications*. 2001.

[75] A. R. Suleiman, A. J. Nelson, and M. L. Nehdi, “Visualization and quantification of crack self-healing in cement-based materials incorporating different minerals,” *Cem. Concr. Compos.*, vol. 103, no. October 2018, pp. 49–58, 2019.

[76] W. De Muynck, K. Cox, N. De Belie, and W. Verstraete, “Bacterial carbonate precipitation as an alternative surface treatment for concrete,” *Constr. Build. Mater.*, 2008.

[77] G. Ranalli *et al.*, “The use of microorganisms for the removal of sulphates on artistic stoneworks,” in *International Biodeterioration and Biodegradation*, 1997.

[78] P. . Saamiya Seraj, “Evaluating Natural Pozzolans for Use as Alternative Supplementary Cementitious Materials in Concrete,” p. 176, 2014.

[79] Z. B. Bundur, A. Amiri, Y. C. Ersan, N. Boon, and N. De Belie, “Impact of air entraining admixtures on biogenic calcium carbonate precipitation and bacterial viability,” *Cem. Concr. Res.*, 2017.

[80] B. Zhang, Z. B. Bundur, P. Mondal, and R. D. Ferron, “Use of biomineralisation in developing smart concrete inspired by nature,” *Int. J. Mater. Struct. Integr.*, vol. 9, no. 1–3, 2015.

[81] E. Loste, R. M. Wilson, R. Seshadri, and F. C. Meldrum, “The role of magnesium in stabilising amorphous calcium carbonate and controlling calcite morphologies,” *J. Cryst. Growth*, 2003.

Curriculum Vitae

PERSONAL DATA

Name: Mustafa Mert

Surname: Tezer

Date of birth: 12/08/1995

Email: mert.tezer@ozu.edu.tr

EDUCATION

09/2017– 01/2020 **Master of Science (MSc.)**

Engineering Faculty Civil Engineering Department

Ozyegin University, Istanbul, Turkey

GPA: 3.67/4.00

Thesis: Development of a bio-additive for self-healing mortar

Advisor: Assist. Prof. Zeynep Başaran Bundur

09/2013– 06/2017 **Bachelor of Science (BSc.)**

Civil Engineering Department, Ozyegin University

GPA: 2.53/4.00

PROFESSIONAL EXPERIENCE

09/2017 –01/2020 **Graduate Research Assistant**

Engineering Faculty/ Civil Engineering Department

Ozyegin University, Istanbul, Turkey

09/2015 –01/2020 **Undergraduate/Graduate Teaching Assistant**

Engineering Faculty/ Civil Engineering Department

Ozyegin University, Istanbul, Turkey

CE 205- Construction Materials

07/2016-09/2016 **Business Information Technologies Intern** -*PepsiCO Istanbul/Turkey*

06/2016-07/2016 **Intern-***Balkar Engineering Istanbul/Turkey*

07/2015-08/2015 **Intern-** *Agaoglu Corporate Group Maslak 1453 Istanbul/Turkey*

RESEARCH PROJECTS

09/2017 –01/2020 **Development of a two-phase biological additive for self-healing cement-based mortar,** *TÜBİTAK-1001 Project (118M327),* Ozyegin University Istanbul, Turkey, Graduate Research Assistant

Work Description: Mineral characterization, Bacteria analysis and growth, Performance analysis of the mortar with bio-additive and Sealing performance evaluation

PUBLICATIONS and PROCEEDINGS (* indicates the presenter)

1. *Mustafa Mert Tezer*, Zeynep Başaran Bundur "Use of natural minerals as protective barriers of bacteria for self-healing mortar" 3rd International Congress on Bio-Based Building Materials. June 26-28 Belfast, UK*
2. *Mustafa Mert Tezer, Zeynep Başaran Bundur* "Two-part bio-based self-healing repair agent for cement-based mortar" DBMC - 15th International Conference on Durability of Building Materials and Components Barcelona, Spain, 30 June-3 July 2020*
3. *Mustafa Mert Tezer, Ilgın Sandalcı, Zeynep Başaran Bundur* "Biologically modified minerals for self-healing mortars" RM4L2020 – Resilient Materials 4Life International Conference Churchill College, Cambridge – UK, 14-17 September 2020 (Submitted)*
4. *Mustafa Mert Tezer, Zeynep Başaran Bundur "Immobilization of bacterial cells on natural minerals for self-healing mortars" will be submitted to ASCE Journal of Materials in Civil Engineering by February 2020*
5. *Mustafa Mert Tezer, Zeynep Başaran Bundur "Use of diatomaceous earth as a protective barrier for bacterial self-healing mortar" Dokuz Eylül University Faculty of Engineering Journal of Science and Engineering (Submitted) (In Turkish)*