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Assessment of performance of bio self-healing mortar using diatomaceous earth and silica fume

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ASSESSMENT OF PERFORMANCE OF BIO SELF-HEALING MORTAR USING
DIATOMACEOUS EARTH AND SILICA FUME

BY

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"Aim at perfection in everything, though in most things it is unattainable. However, they who aim at it, and persevere, will come much nearer to it than those whose laziness and despondency make them give it up as unattainable"

-Lord Chesterfield

This work is dedicated to my mother who passed away during the course of this study after bravely fighting a long battle with cancer.

ACKNOWLEDGEMENT

ABSTRACT

Cracking represents a major threat for the integrity and performance of structures. There are many factors that initiate cracking that are related to environment, properties of concrete itself and several other variables that are difficult to control. Repairing becomes inevitable for concrete structures; however it could be costly and time consuming. Self-healing concept has been introduced to construction materials in order to enhance their performance and extend their service life with less repair.

The objective of this study is to assess the performance of Portland cement mortar incorporating self-healing Bacillus *Pseudofirmus* bacteria using Diatomaceous earth (DE) to immobilize precursor and bacteria in mortar and lowering the pH level of mortar by using silica fume to provide a suitable growth environment for bacteria to generate limestone. The specimens were prepared at three different bacteria dosages and three DE dosages in addition to burned Bentonite dosages and w/c of 0.5. Cracking of specimens was induced by load percent concept after 3 days and tests were performed at 7, 14 and 28 days of curing. The testing scheme for the bacteria viability and mortar included sporulation tests, compression test, indirect tension test, rapid chloride permeability test, chemical soundness test and ultrasonic pulse velocity in addition to the effect of mixing techniques on strength. Results were compared against ordinary Portland cement mortar that includes 15% silica fume. Micro analysis of the healed crack surface of the different specimens was performed and a parametric study was conducted to select the optimum dosage of bacteria, DE and mix design combination as well.

This work reveals that self-healing bacteria is promising technique in minimizing cracking and enhancing mortar physical and mechanical properties such as increasing compressive and tensile strength of mortar and decreasing permeability of mortar. The SEM Pictures shows that using bacteria enhances mortar through precipitating calcite in the voids and cracks of mortar. It is recommended to expand this work to cover more dosages of bacteria, different types of self-healing as well as concrete specimens.

Keywords: Self-Healing, Bacteria, Diatomaceous earth, Silica Fumes, Mortar.

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LIST OF NOTATIONS

- ASTM: American Society for Testing and Materials

- DE: Diatomaceous earth

- EHT: Extra High Tension

- LB: Luria Broth

- PU: Polyurethanes foam

- SEM: Scanning Electron Microscope

- w/c: Water-to-Cement Ratio

CHAPTER 1
INTRODUCTION

1.1 BACKGROUND

Portland Cement Concrete or so- called "Man-Made Rock" and referred to in the industry by the word "Concrete" only, is a composite material that consists of three main constituents, which are water, Aggregates both fine and course, and cement binder (Jonkers, 2011). The basic reaction that takes place to form concrete is when cement reacts with water to form of gel material that gets hardened with time by the matrix that the cement develops to bind ingredients (Neville 2011, Page et al. 2007). There are other constituents of concrete, which are admixtures that can be used to optimize its purpose or facilitate its use (Neville 2011, Konsta-Gdoutos (Ed.) 2006).

There are basic properties for concrete such as workability, compressive strength, tensile strength, and ductility that are critical to the integrity of concrete structures and must be assessed and controlled in order to secure safety and functionality of concrete structures (Neville 2011, Page et al. 2007).

The main characteristic of concrete, however, is its compressive strength. Strength in general is the material's capability to resist compressive stress without reaching the point of failure. The importance of strength characteristics comes from the fact that other properties can be deduced from it. Compressive strength is measure after 28 days by a uniaxial compression test. There are different factors that affect the strength characteristic of concert such as water-to-cement ratio (w/c) which when reduced, compressive strength increases; aging of concrete that as time progresses compressive strength increases due to different reasons that include later reactions with the different compounds on cement hydration process; aggregate sizes and the use of different admixtures in the mix design (Neville 2011, Page et al. 2007, Reinhardt 1985).

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Another main characteristic of concrete is elasticity. Elasticity of concrete is determined empirically as 40% of the ultimate strength of concrete and is in the range of 30 to 50 MPa (Onwuka 2013, Neville 2011).

Dimensional stability is another characteristic of concrete which refers to the dimensional change of concrete over time (Neville 2011, Mehta 1993). Dimensional stability is governed by shrinkage and creep (Shen et al. 2016). Shrinkage occurs after curing and such characteristic compromises the advantages of concrete as it will crack the concrete block and expose any reinforcement inside the block to the atmosphere (Shen et al. 2016); on the other hand creep is a deformation due to constant load with respect to time (Neville 2011, Page et al. 2007, Reinhardt, 1985). Creep develops rapidly at the beginning and decreases in magnitude with time as 75% of the ultimate creep takes place at the first year of loading (Neville 2011, Mehta 1993, Sakata et al. 2013).

1.2 Deteriorations of concrete and their Impacts

Defects in concrete can be due to poor production of concrete or to the surrounding environment; nonetheless, the common causes include the following (Neville 2011, Mehta 1993):

- Physical causes
  - Surface wear
  - Cracks
- Chemical causes (Wang 2016)
  - Alkali-aggregate reaction
  - Sulfate attack
  - Steel Corrosion

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1.2.1.   Physical Factors

(1) Surface wear

Surface wear happens due to abrasion, erosion or cavitation (Liu 2007) .Abrasion happens mostly with concrete structures such as pavements and industrial floors by traffic and is due to dry attrition (Liu 2007). Erosion have the same concept of abrasion regarding what happens to concrete surface except that its due to fluids containing solid particles and the most affected concrete structures of such deterioration are concrete pipes and canal lining Lawrence (2004). Cavitation is defined as the loss of concrete mass due to vapor bubbles that forms due to rapid changes of pressure on the liquid and their subsequent collapse and such deterioration leads to erosion of concrete and is mostly in concrete pipes and concrete structures that contain liquids (Somerville, 2008).

(2) Cracks
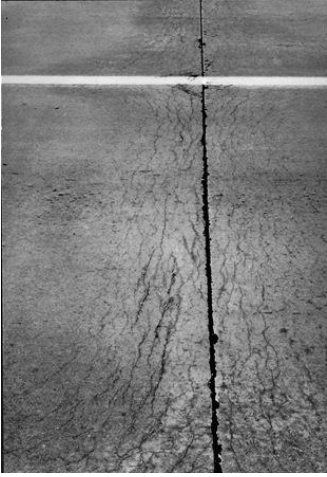
Cracks are the most common defect in concrete structures as there are many different reasons for their development (Rossello 2004). The reasons for cracks development are diverse and difficult to control them all at the same time (Wang 2016). For instance, cracks in the different concrete applications happen due to plastic or dry shrinkage, creep, freeze-thaw cycling, temperature, fatigue, overloading, chemical reactions, concrete crazing, long-term shrinkage, steel corrosion induced cracking, improper construction joint, soil settlement and errors in design and detailing (Rossello 2004).
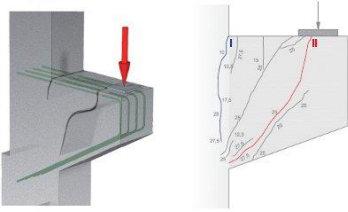
The following table 1-1 lists the different crack causes, reasons and shows some pictures of such cracks.

Table 1-1: Concrete and Mortar Cracks

Crack cause	Reason	Picture
Plastic shrinkage	Rapid loss of water while concrete is in the plastic form before it hardens. Such incident creates internal tensile stresses in the weak stiffening plastic concrete which results in shallow cracking. The crack width in this case is a hair line and it can extend through the thickness of concrete (Shen et al. 2016).	 (NRMCA 2014)
Dry shrinkage	Concrete is mixed with excessive water to hydrate the cement, Thus water evaporates after concrete is dried which causes concrete to shrink and accordingly create internal tensile stresses that develops cracks (Shen et al. 2016).	 (NRMCA 2014)

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Long-term shrinkage	Shrinkage is a cement paste property that alone, it would shrink up to about 1%; on the other hand, aggregates provide resistant to shrinkage and reduces the shrinkage percent to about 0.06%. Concrete keeps shrinking over it life time though on a lower scale. However, since resistance exists in reinforced concrete, shrinkage causes cracks due to the internal tensile stresses that develop due to differential shrinkage in concrete between interior and surface concrete leading cracks to take place at the thicker section of concrete (Shen et al. 2016).	 (NRMCA 2014)

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Freeze-thaw cycling	As water increases in volume by 9% when freezes; accordingly, it produces pressure at the concrete and if exceeded the tensile strength of concrete, rupture will occur. The repetition of the freeze and thaw cycles will accumulate disruption of the cement paste that leads to expanding the cracks and compromising concrete integrity (Neville 2011, Mehta 1993).	(NRMCA 2014)
Temperature	Temperature variations compiles concrete to expand and contract the length change for concrete (which could vary depending on the type of concrete) is of 5mm for 10m of concrete and, in case of retrain existence to such movement, cracks take place in the concrete mass (Somerville, 2008).	(NRMCA 2009)

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Overloading	Change of the structure use, earthquake, early removal of formwork and impact are examples of overloading that induces tensile stress inside concrete that steel bars cannot withstand; thus, initiation and development of wide cracks (Thanoon et al. 2005).	 (PEEM 2008)
Chemical reactions	- Alkali-aggregate reaction - Sulfate attack - Steel Corrosion (Mehta and Monteiro 1993).	 (Federal Highway Administration 2011)

Concrete crazing	When the top surface of concrete losses water rapidly. They form a web of cracks that are extremely fine and usually not repaired (Suyun and Oh 2013).	 (Gromicko and Shepard 2006)
Errors in design and detailing	• The existence of resistant members to concrete masses that are subjected to dimensional instability due to changes in volume by temperature as an example. • Insufficient contraction joints. • Substructure deferential movement that results in initiating cracks especially at window and door openings (Somerville, 2008).	 (NRMCA 2012)

1.2.2. Chemical causes

(1) Alkali-aggregate reaction

The fact that aggregates might contain forms of silica, it would be possible for it to react with alkali hydroxide in concrete which would form a gel that if hydrated, will swell and, accordingly produce and expansive pressure which would damage concrete (Mehta and Monteiro 1993).

Concrete damage in this case is severe and of a material impact on the concrete structure as the concrete starts cracking in a random pattern. Usually, cracks appear near areas that are more exposed to water (Reinhardt 1985). Existence of cracks of concrete exposes the concrete structure to such severe damage that would be reduced significantly if concrete was to be maintained dry and not exposed to water (Neville 2011, Page et al. 2007).

On the other hand, dedolomtization is another form of alkali- aggregate reaction that takes place due to the breaking down of dolomite which is associated with expansive crystallization that compromises concrete's strength and durability (Rundong et al. 2013).

(2) Sulfate aggregate attack

Sulfate aggregate attack is one of the most sever deteriorations that extensively harm the concrete material on an atomic levels as sulfate ions react with the hardened cement paste when water exists. Such reactions are like recrystallization of ettringite, formation of gypsum and declassification of cement paste. Ettringites expands in this formation (Rundong et al. 2013)

(3) Steel corrosion

The fact that concrete has a high pH, contributes as a good environment for steel corrosion (Neville 2011, Reinhardt 1985). When chloride ions enter concrete and reaches to steel, usually due to cracks, and reaches concentration levels of 0.6 to 0.9 kg/m3, it will react with $Fe(OH)_2$ that

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has been formally oxidized due to the specific high pH environment of concrete and forms a water solution and destroy the protective surface of steel (Michel et al 2016). After this stage, steel starts to corrode via an electromechanically process as clarified in the below figure in accordance to the following equations and figure 1-1 the corrosion process (Mehta and Monteiro 1993):

At the anode

$$Fe \rightarrow Fe^{++} + 2e^-$$

At the cathode

$$4e^- + O_2 + 2H_2O \rightarrow 4(OH)^-$$

In the electroyte

$$Fe^{++} + 2(OH)^- \rightarrow Fe(OH)_2$$

$$4\ Fe(OH)_2 + 2\ H_2O + O_2 \rightarrow 4\ Fe(OH)_3$$
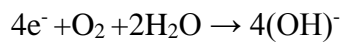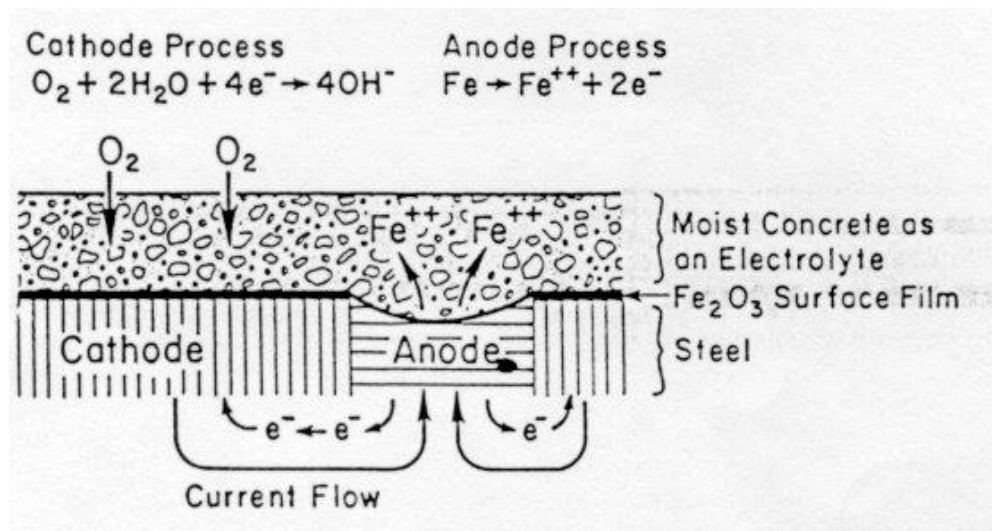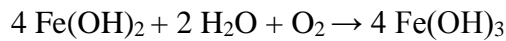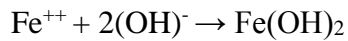
Figure 1-1: Steel Corrosion Process (Mehta and Monteiro 1993)

1.3 Durability

Durability could be simply defined as the ability of concrete to resist deterioration. The main factor that affects durability is the permeability of concrete since an increase in permeability of concrete is followed by many deterioration factors (Dyer, 2014). The main deterioration factors that affect durability characteristic and, thus, concrete serviceability are (Page et al. 2007, Neville 2011):

- Surface wear such as abrasion, erosion and cavitation.

- Cracking due to volume changes, extreme loading and temperature.

- Alkali aggregate reaction

- Sulphate attack

- Steel corrosion (Mehta and Monteiro 1993)

As has been discussed, there are many variables that are interdependent factors that lead to the deterioration of concert. Those factors are extremely difficult to cover at the same time without compromising some of the main concrete characteristics.

Repairing methods for every deterioration of the concrete exists and are effective but at the same time extremely costly and time consuming.

As has been discussed, concrete has to crack in nature; it will inevitably crack due to the many variables that are connected to such physical deteriorating, cracking. Once concrete cracks, the permeability of concrete increases exponentially leading the water to enter concrete which is the main reason for every main chemical deterioration.

Since crack is inevitable, a quick rapid method of repairing must exist to take place once crack occur; thus, increasing the impermeability of concrete without compromising any other

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concrete characteristic. In order to solve this issue, a quick and cost effective solution would be to introduce a self-healing mechanism for the concrete in order to seal cracks once they appear.

Self-healing of concrete is a phenomenon introduced recently in the research which could be divided into two main sectors; the healing that occur by regular concrete which is due to the hydration of the cement particles that have not yet been hydrated and is referred to as autogenous healing of concrete or mortar which happens due to swelling effect, expansion effect and re-crystallization (Jonkers et al. 2010). Another type of healing which includes adding special additives to concrete mix that results in healing and could be referred to as autonomous healing. The second approach of healing involves adding self-healing agents without compromising the initial properties of concrete during the mixing phase of concrete or mortar (Jonkers 2011).

1.4 PROBLEM STATEMENT

Several studies have been conducted recently to examine the effects of using bacteria as a self-healing agent for concrete crack repair. A review of the literature review indicates that using bacteria as a healing agent for concrete cracks has a promising potential. However, few of these studies conducted on this topic have addressed the main four items (environment, bacteria type, immobilization techniques and precursor) in the same study. In addition to the fact that few studies have been conducted in Egypt using the available materials in the market. The construction boom and the increasing need for sophisticated structures for infrastructures and residential compounds in Egypt require innovative materials that could increase the live hood of structures and decrease repair costs especially for large governmental infrastructures.

1.5 OBJECTIVE AND SCOPE

The objective of this work is to study the effect of using a biological matter (bacteria) in cement mortar on sealing surface cracks of micro widths and enhancing its mechanical properties and durability. Also, the study aims at providing liquid compound that could be used while mix design to provide self-healing for mortar to be used in some special applications.

In order to achieve this objective, Bacillus *Pseudofirmus* Bacteria was used as well as three different percentages (5, 10 and 20 percent per weight of fine aggregates) of Diatomaceous earth (DE), fifteen percent per weight of cement of silica fume and calcium lactate with a w/c ratio (0.5). Also, many properties have been tested in order to achieve the above-mentioned objectives which are compressive strength, tensile strength, chemical durability, permeability, the effect of changing the mixing techniques on compressive strength and microstructure analysis using the scanning electron microscope. The results of these tests were compared to ordinary Portland cement mortar with the same w/c ratio (0.5).

CHAPTER 2
LITERATURE REVIEW

2.1 Self-healing concept

Neville, (2002) in his study provided that there are signs that concrete structures have the ability for autonomous healing of micro cracks, specifically those smaller than 0.2 mm. This ability of micro crack healing was found to be mainly related to the composition of the concrete mixtures. Mixtures based on high cement content showed remarkable crack-healing properties. Autogenous self-healing of cracks in concrete mixes of the regularly used cement content or of high cement content mixtures appear to be limited to cracks with a width smaller than 0.2 (Neville 2002, Reinhardt & Jooss 2003, Li & Yang 2007, Edvardsen 1999).

Although high cement content enhances autogenous self-healing of micro cracks, it can't be used only for the purpose of increasing self-healing capacities as current policies supports usage of small amounts of cement in concrete for sustainability reasons as current cement production contributes to about 7% of the CO_2 emissions (Worrell et al. 2001). Thus, Concrete could not be considered as a sustainable material from an environmental viewpoint ((Gerilla et al. 2007).

Crack width is a major factor that self-healing depends on as it was found that self-healing works on small crack widths (micro widths) for attaining complete healing (Edvardsen 1999; Reinhardt and Jooss 2003).

Another type of self-healing concept, Autonomous self-healing, involves the use of special additives in order for concrete to self-heal on its own without further human intervention was investigated in several studies and has different approaches such as: chemical encapsulation at which self-healing is implemented through chemical agents that are contained in microcapsules which have to be speeded uniformly in the concrete matrix so the healing agent would be released

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once crack occurs; expansive agents and geo-materials are used in some studies by dispersing them inside concrete matrix at where they would expand filling cracks when damage occurs also fly ash and blast furnace slag is used under this category of self-healing; glass tubing is another way studied for self-healing at which chemical agent is used that is encapsulated in glass tubes; bacteria is used in the self-healing concept by using it to precipitate calcium carbonate filling cracks (Herbert and Li 2013). Among these promising approaches of autonomous self-healing, using bacteria has clearly been an interesting topic for many researchers and demonstrated clear outcomes of attaining improved compressive strengths as well as decrease in permeability of concrete or mortar.

In the work Li and Yang (2007) spores of specific alkali-resistant bacteria related to the genus Bacillus were added to the concrete mixture as self-healing agent. These spores germinated after activation by crack ingress water and produced copious amounts of crack-filling calcium carbonate based minerals through conversion of precursor organic compounds which was added to concrete mixture on purpose. However, in that study it was found that the bacterial self-healing capacity was limited to 7-days cured concrete only which is relatively young aged concrete, as viability and related activity of bacterial spores directly (unprotected) embedded in the concrete matrix was restricted to about two months.

A previous study by Ghosha et al. (2005) considered self-healing with aerobic microorganism (Bacillus *pasteurii* and Pseudomonas *aeruginosa*) showed a significant improvement of about 18% in compressive strength of cement mortar. Also in to work of RamKrishnan et al. (1999), Scanning Electron Microscopy (SEM) also confirmed the role of microbiologically induced precipitation within the mortar matrix. Self-healing concept was addressed in the work of Fathy et al. (2014) by the use of encapsulated sodium silicate that was embedded in mortar specimens and showed effective self-healing.

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2.2 Biological Self-healing mechanism

Mortar matrix seems an aggressive environment for any life form to inhabit because of its' high alkalinity and dryness. A good analogy would be 'finding a life form on Mars'. However, this is not really the case as from the perspective of microbiology. Bacteria is found in extreme tough environments such as rocks, beds of oceans, ultra base environments and deep inside earths' crust (Jorgensen and D'Hondt 2006; Fajardo-Cavazos and Nicholson 2006; Dorn and Oberlander 1981; DelaTorre et al. 2003; Pedersen et al. 2004; Sleep et al. 2004). The Fact that bacteria can resist such high alkaline environments is due to its ability to form spores (Sagripanti and Bonifacino 1996). Such ability allows the bacteria to survive harsh conditions such as mechanical and chemical stresses by forming a thick surface layer around itself and reduction of the metabolic activity. This ultimately increases the livelihood of bacteria up to 200 years in certain types (Schlegel 1993).

One Biological self-healing mechanism, involves forming calcium carbonate by using ureolytic bacteria of the genus Bacillus based on the enzymatic hydrolysis of urea to ammonia and carbon dioxide. Since the value of the equilibrium pK is constant, the reaction would integer the pH increase to alkaline conditions and accordingly forming bicarbonate and carbonate ions, which precipitate with calcium ions to form calcium carbonate minerals (Bang et al. 2001; Ramachandran et al. 2001; Rodriguez-Navarro et al. 2003; De Muynck et al. 2005; Dick et al. 2006). None the less, this mechanism involved a high risk of reinforcement corrosion due to production of massive amounts of ammonia (Neville 2011).

Another Biological self-healing mechanism explains that the main process of bacterial crack self-healing is that the bacteria themselves act mainly as a catalyst, and transform a precursor compound to a suitable filler material. The newly produced composites such as calcium carbonate-based mineral precipitates should then act as a kind of bio-binder which effectively seals the

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cracks. Thus for effective self-healing, both bacteria and a bio-binder precursor compound should be integrated in the concrete mixture without negatively affecting other required concrete qualities (Jonkers, 2011).
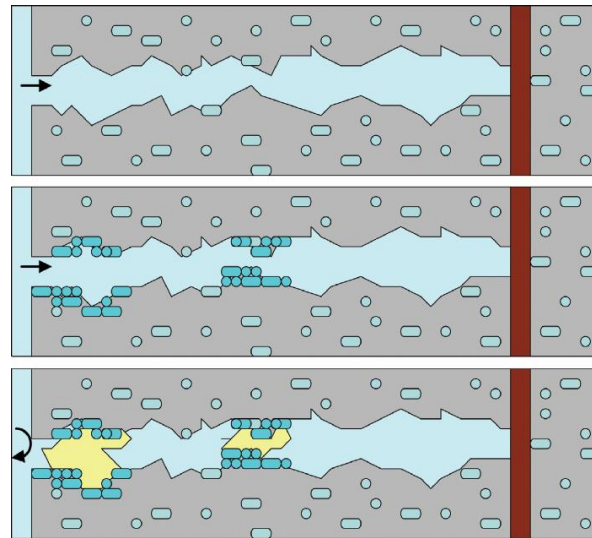
**Figure 2-1: Scinario of Self-Healing bu
Bacteria (Jonkers, 2011)**

The mechanism used in generating calcite is performed using the following reaction that takes place inside the concrete or mortar matrix:

$CaC_6H_{10}O_6 +6O_2 \rightarrow CaCO_3 +5CO_2 +5H_2O$
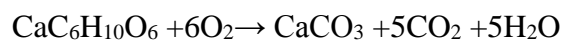
In this reaction the bacteria would work as a catalyst in degrading calcium lactate. Another reaction takes place which could be considered as an indirect approach in generating calcite which follows the following reaction:

$5CO_2 +Ca(OH)_2 \rightarrow 5CaCO_3 +5H_2O$
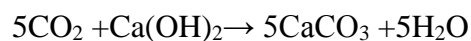
In the above reaction (Indirect approach) carbon dioxide would react with with portlandite ($Ca(OH)_2$) at the surface of crack generating calcite (Jonkers,2011).

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2.3 Bacteria Used in previous studies

In the work of Ghosha et al. (2005), a thermophilic, anaerobic microorganism from the hot spring of Bakreshwar, India, categorized under the Shewanella species was used. This iron reducing microorganism was cultured anaerobically in a modified medium (pH 7.5) before adding to the cement– sand mortar mixture. Also, E. coli microorganisms grown in standard Luria Broth (LB) medium having (pH of 7.2) were also used to study their effect on mortar was used and Different cell concentrations resulted from the bacterial growth culture by serial dilution method.

Jonkers and E. Schlangen (2007) used Alkaliphilic spore-forming bacteria Bacillus *cohnii* DSM 6307, *sporosarcina pasteurii* DSM 33, Bacillus *halodurans* DSM 497 and Bacillus *pseudofirmus* DSM 8715 purchased from Braunschweig, germany. Jonkers work involved using different concentrations of the different types of bacteria in his study and advised that Bacillus *pseudofirmus* DSM 8715 were the most effective strains in self-healing of concrete.

Wang et al. (2012) study involved the use of Bacillus *sphaericus* LMG 22557 from Belgian Co-ordinated Collections of Micro-organisms, Ghent; however, this type of bacteria was based on decomposing urea to generate calcium carbonate and such approach involves producing large amounts of carbon dioxide relatively. The same type of bacteria was used in a study done by Gandhimathi et al. (2012) as well. Bacillus sphaericus and one Bacillus lentus were used in the work of Dick et al. (2006) that involved urea degradation to induce precipitation of calcite.

2.4 Precursors used in previous studies

Different precursor compounds have been used in order to induce self-healing mechanism since it would work as an energy and carbon source for the different strains of bacteria. In the

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study of Worrell et al. (2001), it was found that various organic bio-cement precursor compounds such as yeast extract, peptone and calcium acetate resulted in a remarkable decrease of compressive strength. The only exception appeared to be calcium lactate what actually resulted in a 10% increase in compressive strength compared to control specimens. Jonkers et al. (2007) used yeast extract as a precursor. In his preceding study Jonkers (2009) investigated the use of Calcium glutamate in addition to peptone, calcium lactate and reached the same conclusion as Worrell (2001). calcium acetate was used by Jonkers and Thijssen (2010) and concluded that it has an adverse effect on compressive strength.

Other studies following the mechanism that involves forming calcium carbonate by using ureolytic bacteria used urea as a precursor as in the work of Gandhimathi et al. (2012), however.when urea was used as a precursor, the buffer used was phosphate solution. None the less, the mechanism of this reaction of urea CH_4N_2O and water yields CO_2 and ammonia (NH_3) which has an adverse effect on concrete matrix. Urea used as a precursor with yeast extract also in the work of Wang (2012) and RamKrishnan et al. (1999).

2.5 Immobilization techniques used in previous studies

The main purpose of immobilization is to protect bacteria inside concrete or mortar matrices. Immobilization could be done using many different materials in order to attain such purpose. Different studies have investigated different immobilization materials for bacteria to induce self-healing. For example, expanded clay particles were used by Jonkers (2011). However, such approached of immobilization contributed to a decrease in the samples compressive strength.

Another immobilization technique used is Physicochemically versatile PU has been used to immobilize Bacillus pasteurii which was successful in stabilizing the metabolic activity for a longer period of time by decreasing the rate of enzymatic activity. However, this reduced the rate

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of calcite precipitation and, accordingly, concluded that the performance, at the end, is equally effective whether the bacteria was immobilized or not (Bang et. al. 2001).

Diatomaceous earth was used in a study by Wang et al. (2012) conducted on Bacillus Sphaericus as a protective immobilization technique to measure ureolytic activity in concrete matrix showed promising results, yet due to the ureolytic activity, the generation of ammonia indicated an adverse effect of such mechanism.

Silica gel was used as a vessel of immobilization of bacteria in the work of Tittelboom et al. (2010); none the less, this technique involved ejecting the silica gel containing bacteria inside cracks which contradicts with the concept of self-healing.

2.6 Buffer used in previous studies

Buffers are the medium that is used to suspend bacteria in before adding it to the mortar or concrete mix design. There are several buffers used in previous studies in order to keep bacteria alive before adding it to the mix designs. Phosphate buffer was used by RamKrishnan et al. (1999), De Muynck (2010), Gandhimathi et al. (2012), Fajardo (2006). The use of phosphate in such studies is due to the fact that bacteria was put in its vegetative, not germinated, form. Water is used as a buffer to suspend spores of bacteria in it as in the study of Jonker and Thijssen (2010).

2.7 Crack inducement used in previous studies

Crack inducement method that has been used in different studies applied controlled application of compressive tensile strength as in Jonkers (2011). Another method used by Fathy et al. (2014) is load percent method at which specimens were cracked by applying a load that is 80% of the ultimate load that specimens can withstand; Wang et al. (2012) used the same method as Fathy et al. (2014) as well.

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In the study of Tittelboom et al. (2010), crack creation was implemented using two methods which are the standardized cracks methods and the realistic crack method. The standardized crack method involved using a thin copper plate of 0.3mm thickness in fresh concrete paste up to a specific depth and were removed during de-molding specimens after 24 hours which resulted in prisms with narrow groves of same depths and widths. On the other hand, the realistic cracks method was obtained by split tensile strength as Jonkers (2011).

2.8 Tests performed and results of previous studies

There are different types of tests used in order to assess the different mechanical properties of self-healing induced by bacteria in previous studies. Bang et al. (2001) have performed compressive test to project the effect of crack remediation by PU-encapsulated B. *pasteurii* for different dosages of bacteria cells and his results shows that the highest compressive strength attained was when he used a bacteria dosage of 5×10^9 at the age of 7 days; however with longer ages (28 days) the increase in strength was found to be marginal in comparison with the 7 days gain of strength because PU polymer was found to get less rigid with periods longer than 7 days in Urea-CaCl2 medium.

In the work done by Gandhimathi, et al. (2012), the effect of the use of the bacteria Bacillus *spharecius* was assessed by Compressive strength and split tensile strength tests; and his results showed that the percent increase in compressive strength of cracked specimen with 10 ml and 20 ml addition of bacillus *sphaericus* are 13.07% and 13.75 % respectively and the tensile strength results of bacterial concrete when adding the same amount of bacteria to every one liter of buffer (phosphate) for the split tensile strength are 3.15% and 7.25% respectively.

Jonkers (2011) investigated the mechanical properties of concrete by the use immobilizing bacteria in concrete by using compressive strength test and permeability test and concluded that

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when calcium lactate is used as a precursor with bacteria such as Bacillus *Pseudofirmus* a 10 % increase in compressive strength occurs. On the other hand, the permeability test showed that bacterial specimens had a much lower permeability after two month of self-healing and curing in comparison to control specimens that were cured as well for two months.

Ghosha et al. (2005), provided that when using a thermophilic, anaerobic microorganism belonging to the *Shewanella* species, compressive strength of mortar specimens increases to 25% at 28 days at cell concentrations of 10^5 cells/ml for all ages. E. *coli* microorganisms were also used, however, no significant improvement in compressive strength of mortar was witnessed. Permeability test, ultrasonic pulse velocity test in addition to Thermogravimetric analysis were performed in the study of Tittelboom et al. (2010) at which he used B. *sphaericus* bacteria and sol-gel in his study. Tittelboom et al. (2010) concluded that the use of bacteria decreased permeability of concrete specimens and Thermogravimetric analysis revealed that the precipitation of calcium carbonate crystals was due to bacteria which enhanced the gel matrix in filling cracks. Ultrasonic pulse velocity test resulted in an increase in the pulse velocity in bacterial specimens indicating that cracks has been bridged.

Ramakrishnan et al. (2001) compared compressive strength, stiffness and modulus of rupture of specimens containing bacteria (Bacillus *pasteurii*) and without bacteria. It was concluded that compressive strength, stiffness and modulus of rupture improved when bacteria was used ad documented his findings by SEM pictures showing mineral precipitation on cracks.

A study by C. C. Gavimath, B. M. Mali, V. R. Hooli, J. D. Mallpur , A. B. Patil , D.P.Gaddi, C.R.Ternikar investigated the use of dormant bacteria of B. *sphaericus* in concrete and depend on activating the dormant bacteria crack occurs and water enters the crack. Compressive strength and

tensile strength tests were performed and the study concluded that specimens with bacteria have significantly improved compressive strength of bacteria.

2.9 Effect of pH on Mortar matrix

Few studies have tackled the issue of the effect of pH on the biological additive in concrete or mortar. Of these studies, a study done by Sagripanti and Bonifacino (1995) investigating the effect of hi pH on alkali resistant bacteria which found out that the high value of pH results in keeping spores inactive and not to transform to vegetative form and, accordingly, low metabolic activity and less biomineralization activity.

According to Jonkers and Schlangen (2008), pH value of mortar or concrete specimens is between 11-13; however, such high values keeps the bacteria dormant inside mortar and reduces its capacity to precipitate calcite. Furthermore, in the study done by Wang et al. (2012), indicated that bacteria activity would be dramatically decreased when bacteria is added to an environment with pH value higher than 12 thus a use of DE was introduced in order to protect bacteria from the high pH value of concrete matrix and such approach was successful as 60% of urea was decomposed due to immobilizing bacteria by DE in comparison to only 5% of urea decomposed when bacteria was put in the concrete matrix without DE protection.

2.10   SEM results of previous studies

The scanning electron microscopic is a device that is used to generate pictures of materials on the micro and Nano-scale. Only some studies have generated SEM pictures for their work to document healing process. For example Jonkers (2009) generated Plate-like aggregate formation pictures precipitated in a concrete matrix due to the use of bacteria; he also showed bacteria in its spore form and vegetative form in Jonkers et al. preceding study in 2010 as shown in figure 2-2. Bang et al. (2001) showed SEM pictures showing microorganisms embedded in PU matrix and

precipitated calcite. SEM pictures of Dick et al. (2006) shows a dense and homogeneous layer of calcite produced by B. *sphaericus* strain. In RamKrishnan et al. (1999) study, a B. Pasteur ii is shown spread around calcite crystals. In the study done by Wang et al. (2012), SEM pictures acquired shows bacteria immobilized by DE as shown in figure 2-3.
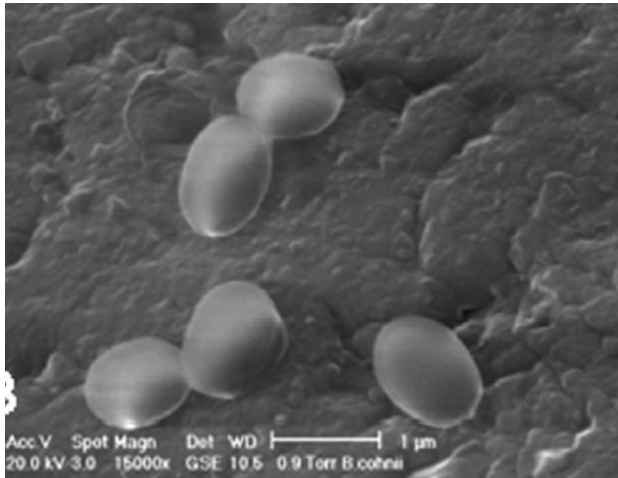
Figure 2-3: SEM Picture showing Bacterial spores in concrete matrix (Jonker, 2009)
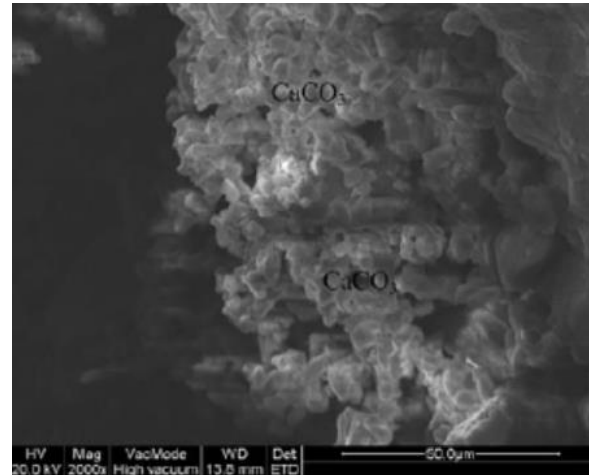
Figure 2-2: SEM Picture dhowing bacteria immobilized by DE (Wang. J. Y, 2012)

2.11   Energy-dispersive X-ray spectrum

RamKrishnan et al. (1999) have used the technique of Energy-dispersive X-ray spectrum in order to assure the precipitation of calcite and concluded that the abundance calcium project by the test is due to the precipitation of calcium carbonate which is a rough conclusion to make as the concept of the Energy-dispersive X-ray spectrum is identifying element not compounds so the calcium abundance is a must in the case of concrete or mortar is it is a huge part of all concrete ingredients.

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2.12 Self-healing using bacteria is a step towards green industry

Applying self- healing concept in the concrete industry is of crucial importance towards sustainable development of the industry and is a huge step in attaining green industry for the following reasons and outcomes (Jonker 2010; Worrell et al. 2001):

1- Attaining one of the most important goals of sustainable development which is conserving natural resources used for the cement industry and other repairing methods raw materials.

2- Helping in decreasing concrete industry's carbon foot print due which is a major contributor to the total carbon foot print of the world industries

3- Reducing the costs needed for repairs and maintenance of sutures.

4- Contributing to longer service life of the structure.

5- Increasing the durability of mortar or concrete through decreasing their permeability.

6- Reducing the efforts needed for repairing parts of structures that are difficult to physically reach or extremely expensive.

7- Decreasing energy consumption of construction industry.

CHAPTER 3
EXPERIMENTAL WORK

3.1 GENERAL

The experimental program associated wtih this study was prepared to assess the mechanical properties of using Bacillus *Pseudofirmus* bacteria as a catalyst to induce calcite when fed on calcium lactate as a precursor and immobilized by (DE) or burned Bentonite in a reduced pH environment, mortar matrix, by replacing a percentage of cement with silica fume. Bacteria was acquired from the DSMZ (German Collection of Microorganisms and Cell Cultures) and cultured according to the supplier recommendation. Bacteria was then forced to make spores in the lab to use it as part of the mix design proportions for specimens. Mortar mixtures were prepared using three different concentrations of bacteria as well as (DE) and a 15 percent per weight of cement of silica fume. Control mixtures for the different mixtures has been designed without adding bacteria and DE. 16 types of mortar mixes were prepared for this work to account for the comparison analysis of the study. Table 3-5 lists the mixing proportions of all mixtures prepared for this study.

3.2 MATERIALS

3.2.1.   Bacteria

Bacillus *Pseudofirmus* DSM 8715 strains were purchased form the DSMZ (German Collection of Microorganisms and Cell Cultures), Braunschweig, Germany isolated from lake bank soil in Germany. Table 3-1 lists present bacteria data and Figure 3-1 shows bacteria strains in their stock form.

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Table 3-1: Bacteria Details

Domain	Bacteria
Phylum	Firmicutes
Class	Bacilli
Order	Bacillales
Family	Bacillaceae
Genus	Bacillus
Species	Bacillus Pseudofirmus
Full Scientific Name	Bacillus pseudofirmus Nielsen et al. 1995
Groth Tempreature	30
Tempreature range	mesophilic
Biosaftey Level	1
GC-Content	39.6 mol%
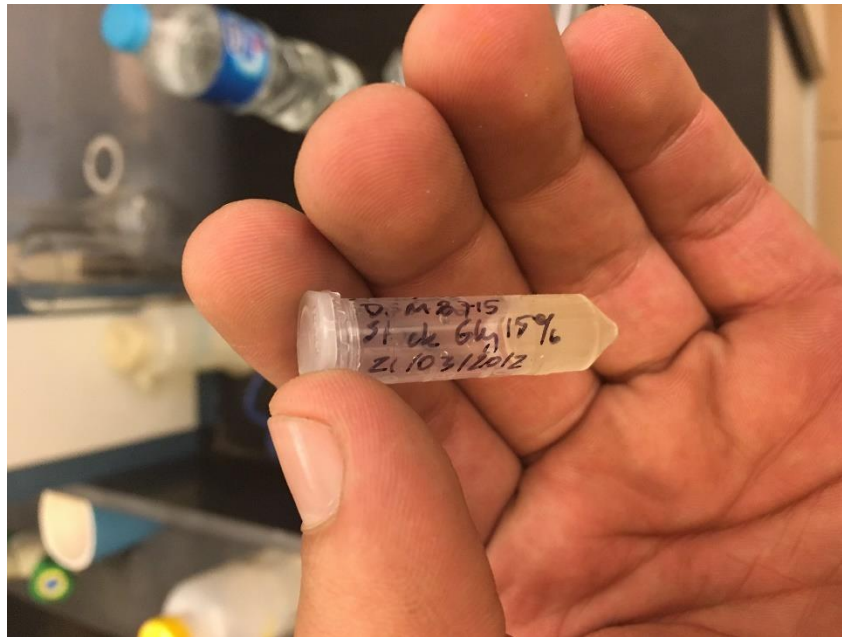
Figure 3-1: Bacteria Strains in Stock Form

3.2.2.   Nutrients

Nutrient broth consisted of beef extracts and peptone. Table 3-2 lists the amounts of nutrient broth ingredients per 1 liter.

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Table 3-2: Difco™ Nutrient Broth Approximate Formula Per Liter

Beef Extract	3.0 g
Peptone	5.0 g

3.2.3. Culturing Chemical Compounds

Na-sesquicarbonate solution that consists of sodium carbonate and sodium bicarbonate is used during the cultural phase.

3.2.4. Spore-Forming Chemical Compounds

Several chemical compounds have been used for the spore-forming phase which are listed in table 3-3

Table 3-3:Spore-Forming Chemical Compounds

Minerals Compounds	Trace Element Solution Compounds
$FeSO_4.7H_2O$	$MnSO_4$
$CoCl_2.6H$	Sodium Citrate
$MnCl4.4H_2O$	Yeast Extract
$ZnCl_2$	Na HCO_3
$NaCl_2.6H_2O$	Na_2CO_3
$Na_2MoO_4.2H_2O$	Distilled de-ionized water
$CuCl2.2H2O$	$(NH4)_2SO_4$
H_3BO_3	Powder agar
Na_2EDTA	
Na HCO_3	
Na_2CO_3	
NH_4Cl	
$KHPO_4$	
$CaCl_2$	
KCl	
KNO_3	

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3.2.5. Precursor

Calcium lactate salt ($C_6H_{10}CaO_6$) is used in it powder form with a molar mass of 218.22 g/mol and density of 1.494 g/cm^3 as a precursor for bacteria vegetation. Figure 3-1 shows the calcium lactate particles in its powder form balanced.

Figure 3-2: Clacium Lactate

3.2.6. Portland cement

Ordinary Portland cement manufactured by one of the local companies in Egypt has been used with a particle nominal size of 1 μm and with the following properties as listed in table 3-4. Figure 3-3 shows cement particles in its powder from.

Table 3-4 Properties of Portland cement

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Property	Test result	ASTM C 150	ES. 1-4756/2009	Test conducted according to
Normal Consistency	22.4%	-	-	ASTM C 187
Initial Setting Time	2 hrs, 12 min	> 45 min	> 60 min	ASTM C 191
Final Setting Time	2 hrs, 58 min	< 6hrs, 15 min	-	ASTM C 191
Blaine Fineness	314.3 m^2/kg	> 280 m^2/kg	-	ASTM C 204
Specific gravity	3.15	3.14-3.16	-	ASTM C 188

Figure 3-3: Cement

3.2.7. Fine aggregate

The sand used in this study is standard sand passed from sieve# 20 and retained on sieve# 30. This type of sand has been chosen according to ASTM C190 and to minimize variations due to supply of sand throughout the experimental work. This sand attained a fineness modulus of 2.80 and a saturated surface dry specific gravity of 2.60. The absorption of fine aggregate was experimentally measured at 0.73%. Figure 3-4 shows fine aggregates used.

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Figure 3-4: Fine Aggregates

3.2.8. Water

Mili-Q water that is ultra-purified and ionized water has been used for bacteria preparation and tap water was used for both mixing and curing. Figure 3-5 shows the device used for generating purified water used.
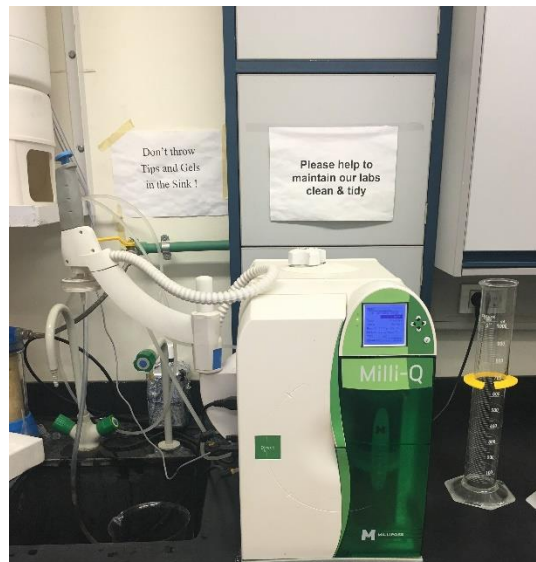
Figure 3-5: Mili-Q device used for generating purified water

3.2.9. Silica Fume

The silica fume used in this study was brought from a local manufacturer. This type of silica-fume has grain size 0.15 μm, bulk density 0.5 kg/l and specific surface 20 m^2/g. It contains extremely fine (0.15 μm) latently reactive silicon dioxide (minimum of 90%). Figure 3-6 shows the silica fume used.

Figure 3-6: Silica Fume

3.2.10. Bentonite

Bentonite has been purchased from a local supplier in Egypt and was acquired from the New Wadi Governorate in Egypt passing sieve #200 and with a free swell of 60% by volume and having a Pozzolanic nature. Figure 3-7 shows the used Bentonite in this study. Betonite used was burned in the oven on a temperature of 400°C in order to force it to lose its water section property and can form an immobilization protection for bacteria and storage for calcium lactate after its volume increased.

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Figure 3-7: Bentonite

3.2.11. Diatomaceous earth (DE)

Diatomaceous Earth (DE) which is a porous sedimentary fossils of diatoms with a particle size ranging from 1µm to 1 mm. (DE) is chemically inactive. DE for this study was acquired form company Dicalite Europe NV, Belgium. Figure 3-8 shows the DE with the use of SEM.

Figure 3-8: Diatomaceous earth (DE)
under SEM

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3.3 Experimental Procedure

The procedure of experimental work in this study can be divided into four phases at which phase I includes culturing and spores-forming of bacteria; phase II includes the preparation of mortar mix designs and Specimens; phase III includes crack inducement and phase IV includes performing tests.

3.3.1   Phase I

Phase I of the experimental work tackles only the biological part of the study at which the bacteria (Bacillus *Pseudofirmus*) strains imported from Germany would be cultured in order to increase the number of bacteria strains to be used in the various study experiments and tests. After acquiring the amount of bacteria needed, the bacteria was forced to form spores.

3.3.1.1 Culturing Bacteria

After the bacteria stock was imported from Germany, it was put in a -80°C fridge in order to reserve it for further use. Culture media was prepared by adding 8g of nutrient Broth to a 0.9 liter of ionized water generated from a Mili-Q water filter. The container containing the nutrient broth media and water was sterilized by autoclaving on a 120°C and a pressure of 1.5 bar for 20

Figure 3-9: Autoclave device
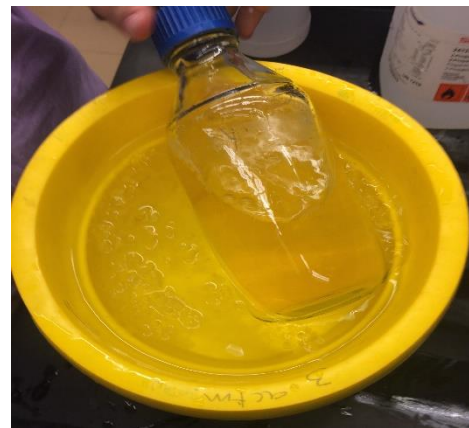
Figure 3-10: Media container after satirizing

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minutes. Figure 3-9 shows the Autoclave and Figure 3-10 shows the media container after satirizing. The media was then cooled to room temperature 25°C.

Na-sesquicarbonate solution was prepared by adding 4.2 g $NaHCO_3$ and 5.3 g Na_2CO_3 to 0.1 liter of ionized Milli-Q water which was dissolved using a magnetic stirrer equipment as shown in figure 3-11 the solution was further filtered using a 0.2 micrometer filter under a laminar flow cabinet as shown in figure 3-12 to insure no bacteria from the surrounding environment interfere in the experimental work.
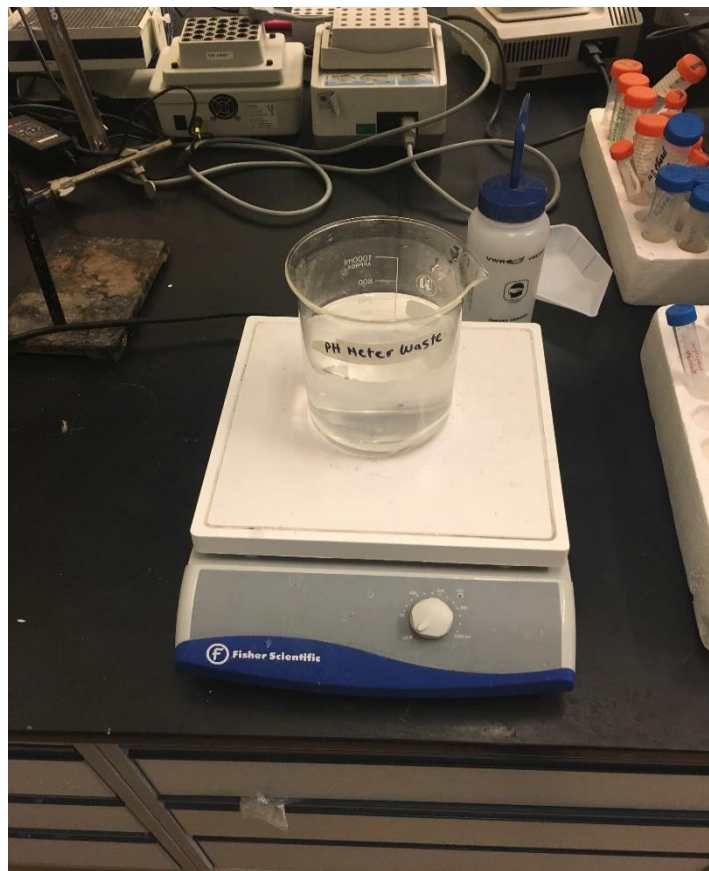
Figure 3-11: Magnetic stirrer equipment

Figure 3-12: Laminar flow cabinet
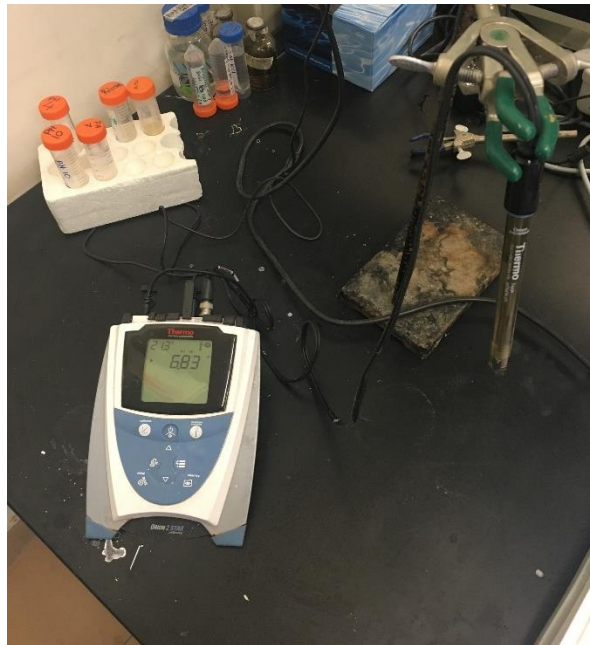
Figure 3-13: pH meter

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Na-sesquicarbonate solution that was prepared was added to the culturing media in order to attain a pH level of 9.7 which is the optimum environment for the Bacillus *Pseudofirmus* growth. The pH level was measured using pH meter as shown in figure 3-13.

Three falcon tubes with a volume of 50 ml were then used to pour 0.45 ml of the growth media in one of them. Bacteria stock was quickly brought from the fridge and a loop was used to extract bacteria from its plates and suspend the strains in the falcon tube that has the growth media. The media was then divided on the three falcon tubes equally and were left in an incubator with 30°C and a shaking rate of 150 rpm for 15 hours to initiate bacteria growth.

The growth was investigated with visual inspection when noticing that the medium turned turbid as shown in figure 3-14. Sample of the media with the bacteria was then taken to investigate the growth rate using Spectrophotometer equipment which was 0.9. Figure 3-15 shows Spectrophotometer equipment used in this study.

Figure 3-14: Turbid media

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Figure 3-15: Spectrophotometer equipment

Containers of one liter capacity was then autoclaved and the 1 liter growth media was then divided on them under the laminar flow cabinet and the falcon tubes containing the bacteria was poured in each of them and were left in an incubator with 30°C and a shaking rate of 150 rpm for 15 hours to initiate bacteria growth in larger amounts. Figure 3-16 shows the incubator shaker used.

The above procedure was repeated for ten liters of the culturing media and bacteria was then counted using Hemocytometer equipment shown in figure 3-17.

Figure 3-16: Incubator shaker
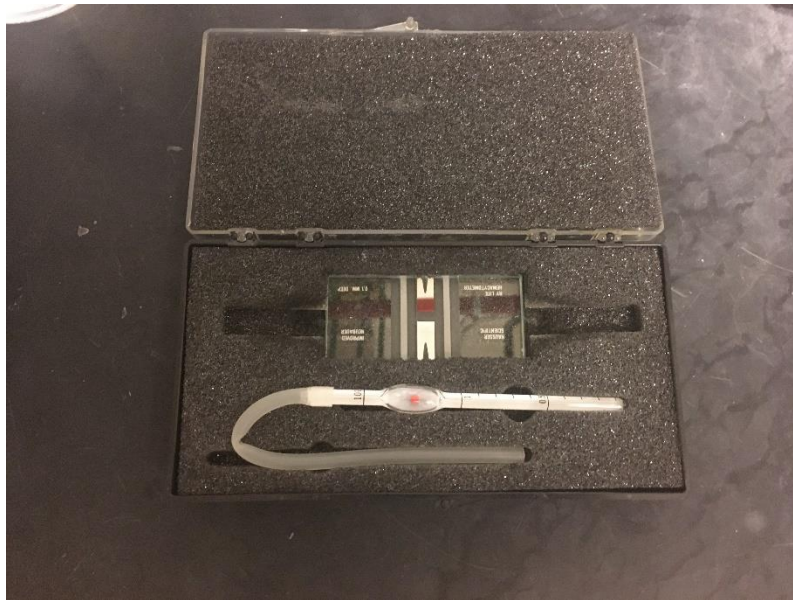
Figure 3-17: Hemocytometer

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3.3.1.2 Spore-Forming

Spore-forming compounds with weights a listed in table 3-3 were balanced and filtered using a filtration pump shown in figure 3-18. Bacteria in the growth media was harvested using a centrifuging equipment shown in figure 3-19 that revolves with a rate of 10,000 rpm for 10 minutes and then the bacteria pellets were suspended in the spore-forming media and left in an incubator with 30°C and a shaking rate of 150 rpm for 15 hours. Samples of the spore forming bacteria was then harvested and put under microscope to ensure sporulation of the bacteria. Figure 3-20 shows harvested bacteria pellets. Spore-forming media that includes bacteria spores was then centrifuged with a rate of 10,000 rpm for 10 minutes and bacteria plates were suspended in tap water to be ready for use in mortar mix designs.

Figure 3-18: Filtration pump

Figure 3-19: Centrifuging equipment
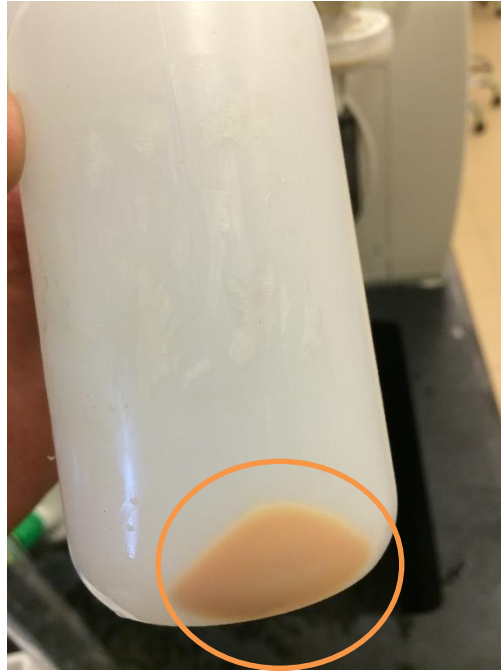
Figure 3-20: Harvested bateria Pellets

The viability of spores were tested on plates that contained a media of agar and calcium lactate. Spores of bacteria were counted using Hemocytometer and divided into three dosages of $3x \ 10^8$, $6x \ 10^8$ and 10^9 cm-3.

3.3.2 Phase II

Phase II of the experimental work deals with mainly the portions of mix designs as well as the molds that specimens will be casted in in order to conduct the different tests on them later in the experimental work in phase IV. In phase II, the mix designed were piloted to test the different effects of the different portions of bacteria and DE on the mechanical propertied of mortar specimens.

3.3.1.1 Preparation of mix designs

Mortar specimens' mix designs were all prepared at a w/c ratio of 0.5 in order to stabilize the w/c ratio parameter and its effect on specimens strength. Silica fume proportion was also fixed in all specimens' mix design to 15% per weight of cement in order to reduce the pH level of the mortar matrix. Cement and silica fume to fine aggregates ration was as well fixed to be 1:3.

Sixteen types of mix designs were prepared for this study. Type I which is the control mix design consisted of only cement, silica fume, water and fine aggregates. Types II, III and IV consisted of cement, silica fume, water, fine aggregates, calcium lactate and low dosage of DE in addition to three dosages of bacteria. Types V, VI and VII consisted of cement, silica fume, water, fine aggregates, calcium lactate and medium dosage of DE in addition to three dosages of bacteria. Types VIII, IX and X consisted of cement, silica fume, water, fine aggregates, calcium lactate and high dosage of DE in addition to three dosages of bacteria. Types XI, XII and XIII consisted of cement, silica fume, water, fine aggregates, calcium lactate and dosages of burned Bentonite. Types XIV, XV and XVI consisted of cement, silica fume, water, fine aggregates, calcium lactate and dosages of burned Bentonite in addition to bacteria. Table 3-5 lists the weights of the constituents of every type in the experimental work and their labeled codes.

Table 3-5: Mixing proportions for all mortar mixtures prepared for this study

Mixture	Mixture I.D.	Cement (g)	Silica Fume (g)	Water (g)	Fine Aggregate (g)	Diatomaceous earth (DE) (g)	Bentonite (g)	Calcium lactate (w/w) ratio	Bacteria (cm-3)	(w/c) Ratio
Type I	C1	425	75	250	1375	0	0			0.5
Type II	BD1A	425	75	250	1306.25	68.75	0	5%	3×10^8	0.5
Type III	BD1B	425	75	250	1306.25	68.75	0	5%	6×10^8	0.5
Type IV	BD1C	425	75	250	1306.25	68.75	0	5%	1×10^9	0.5
Type V	BD2A	425	75	250	1237.5	137.5	0	5%	3×10^8	0.5
Type VI	BD2B	425	75	250	1237.5	137.5	0	5%	6×10^8	0.5
Type VII	BD2C	425	75	250	1237.5	137.5	0	5%	1×10^9	0.5
Type VIII	BD3A	425	75	250	1168.75	206.25	0	5%	3×10^8	0.5
Type IX	BD3B	425	75	250	1168.75	206.25	0	5%	6×10^8	0.5
Type X	BD3C	425	75	250	1168.75	206.25	0	5%	1×10^9	0.5
Type XI	C2	425	75	250	1306.25	0	68.75	0%	0	0.5
Type XII	C3	425	75	250	1237.5	0	137.5	0%	0	0.5
Type XIII	C4	425	75	250	1168.75	0	206.25	0%	0	0.5
Type XIV	BB2	425	75	250	1306.25	0	68.75	5%	1×10^9	0.5
Type XV	BB3	425	75	250	1237.5	0	137.5	5%	1×10^9	0.5
Type XVI	BB4	425	75	250	1168.75	0	206.25	5%	1×10^9	0.5

Mixture I.D. Meaning

C: Control

B-D-1-A: Bacteria-Diatomaceous earth- Low percent of Diatomaceous earth (1=Low, 2= Medioum, 3= High) - Low percent of Bacteria (A=Low, B= Medioum, C= High)

B-B-2: Bacteria- Burned Bentonite- Low percent of Bentonite (2= Low, 3= Medium, 4= Hig

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3.3.2.1 Preparation of Mortar Specimens

Different mortar specimens were prepared for the purpose of study. Specimens were designed depending on the test to be performed on the different specimens which includes compressive strength test, tensile strength test, rapid chloride permeability test and chemical soundness test in addition to ultrasonic pulse velocity test and micro analysis using SEM.

Standard cubes of size (50x50x50) mm were prepared for the compressive strength to be tested after 7, 14 and 28 days as shown in figure 3-21. Cylindrical specimens were prepared for the indirect tension test with a diameter of 5 cm and a length of 10 cm as shown in figure 3-22. Cylindrical specimens with a diameter of 10 cm and a length of 5 cm were prepared for the rapid chloride permeability test as shown in figure 3-23. And standard cubes of (50x50x50) mm were prepared for chemical soundness test.

Figure 3-21: Mortar Cubes casted in molds

Figure 3-22: Indirect Tension test cast in molds

Figure 3-23: Rapid Choloride Permeability test Specimens cast in molds

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Three different mixing techniques were used in order to figure out the optimum mixing technique for the study. Technique X performed by adding cement, silica fume, DE or burned Bentonite and fine aggregates in their dry status and conduct a dry mixing then add the water with calcium lactate and bacteria. Technique Y performed by mixing water with calcium lactate and bacteria at the beginning with silica fume and DE or burned Bentonite and the adding the rest of the materials. Technique Z performed by mixing water that included calcium lactate and bacteria with the DE then adding the rest of the ingredient gradually while mixing and finally add the rest of the water.

3.3.3 Phase III

Phase three of the experimental work deals with the crack inducement procedure that was followed.

At the beginning a specimen of every experiment was loaded until failure in order to measure the ultimate load at fracture after 3 days of curing all samples in a moist room of 95% moist. Specimens were then loaded using a universal testing machine until 80% of the ultimate load in order to induce micro cracks in specimens and test the effects of using bacteria in them. All specimens were then put in the curing room until 7 days at which tests were undertaken on a number of the specimens. Then the remainder of the specimens were tested after 14 and 28 days of curing.

3.3.4 Phase IV

Phase four includes the testing procedures that have been performed on all specimens in order to figure out the final results of the study which included compressive and tensile strength test in addition to ultrasonic pulse velocity and chemical soundness tests. This phase also includes procedures used for the micro study using the SEM equipment.

3.3.4.1 Viability of spores test

After bacteria was harvested and suspended in tap water to be used in the mix design, culture plates that consisted of agar and calcium lactate media was prepared and bacterial spores were put on it and was left into an incubator to test its growth after two days. The purpose of the test is to see spores forming colonies on the culture plates and assure that spores could transform to vegetative phase and accordingly produce calcite.

3.3.4.2 Compressive strength test

Compressive test was conducted on the age of 7, 14 and 28 of the healing specimens. Cubes were loaded on a universal testing machine as shown in figure 3-24 and figure3-25 and loaded until failure and results were taken and tabulated.

Figure 3-24: Cube Loaded

Figure 3-25: Cube fracture after uplying ultimat compressive load

3.3.4.3 Tensile strength test

Cylindrical specimens assigned for indirect tension test were taken from the curing room and a universal testing machine shown in figure 3-26 was used to load the specimens after 7 days of curing and again after 28 days of curing and results were tabulated.

Figure 3-26: Indirect Tension Test

3.3.4.4 Ultrasonic pulse velocity test

Ultrasonic pulse velocity test was performed using the apparatus shown in figure 3-27 at the age of 14 and 28 days on the cracked specimens.
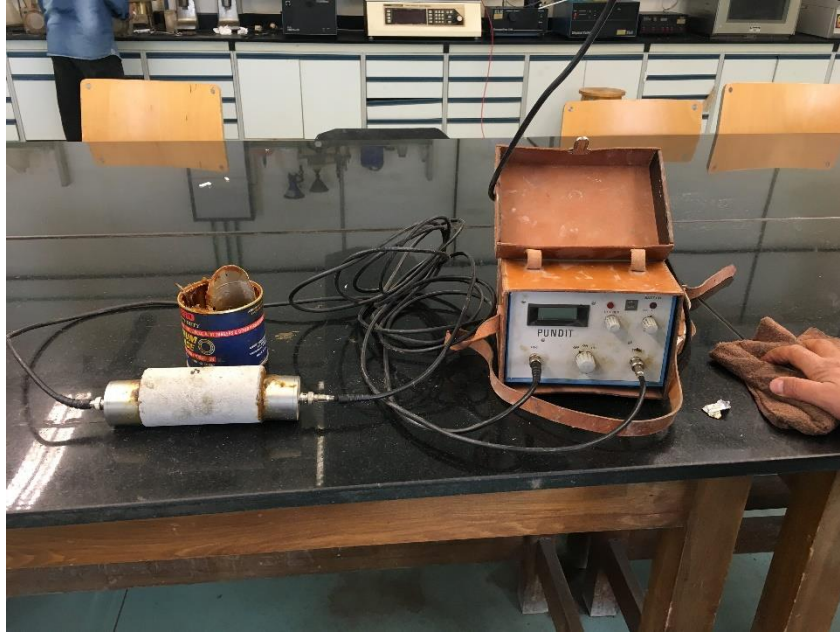
Figure 3-27: Ultrasonic pulse velocity test

3.3.4.5 Rapid chloride permeability test

This test was conducted in accordance with the ASTM C 1202-97. The set up used for

this test are shown in figure 3-28.

Figure 3-28: Rapid chloride permeability test set up

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3.3.4.6 Chemical soundness test

Chemical soundness test was conducted on the cubical specimens assigned fort this test and were submerged in a magnesium sulfate and sulfuric acid compounds. Figure 3-29 shows the test set up.

Figure 3-29: Chemical soundess test set up

3.3.4.7 SEM inspection

The micro study inspection was performed using the Scanning Electron Microscope at which the voltage of the SEM (EHT) was set at eight kV and the detector used was the secondary electron detector working at a distance between the electron's gun and the sample ranging from 2-15 mm. samples from the compressive strength specimens were taken after chopping them in order to be used for this experiment.

CHAPTER 4
RESULTS AND ANALYSIS

4.1 PREAMBLE

This chapter introduces the results and analysis of the experimental work implemented for this study. The results and analysis are divided into bio results and hardened mortar results depending on the tests performed on the different types of specimens, mixtures and mixing techniques. There were 16 different mixtures in this study that were deigned in order to study the different mechanical properties of the mortar specimens and testing the feasibility of using bacteria with the prepared set up in mortar. Mixtures designed included different dosages of bacteria, DE and burned Bentonite with three different mixing techniques for comparison and analysis purposes. Tests were conducted for the compressive strength after 7, 14 and 28 days of curing and tensile strength after 28 days of curing as for the ultrasonic pulse velocity, chemical soundness tests and SEM inspection. All results were analyzed with the basis of the control specimens C1 for DE specimens and C2, C3 and C4 for burned Bentonite specimens. The difference between the bacteria dosages, DE and burned Bentonite was as well investigated by studying their effects on the mechanical properties of the mortar specimens prepared.

Bio Results

4.2 Viability of Spores

After spores were put on agar plates that contained calcium lactate, the appearance of few colonies started to appear as shown in figure 4-1 after two days which is attributed to the enhancement of growth conditions created to mimic the food-enriched environment that the bacteria would be exhibited to inside the mortar matrix. The growth noticed by eye inspection indicates that spores have actually transformed from its idle phase to a vegetative bacterial phase when the environment was suiting its growth and increased in number.

This test provided us with an indication that the culturing and sporulation processes that we have worked in were successful.

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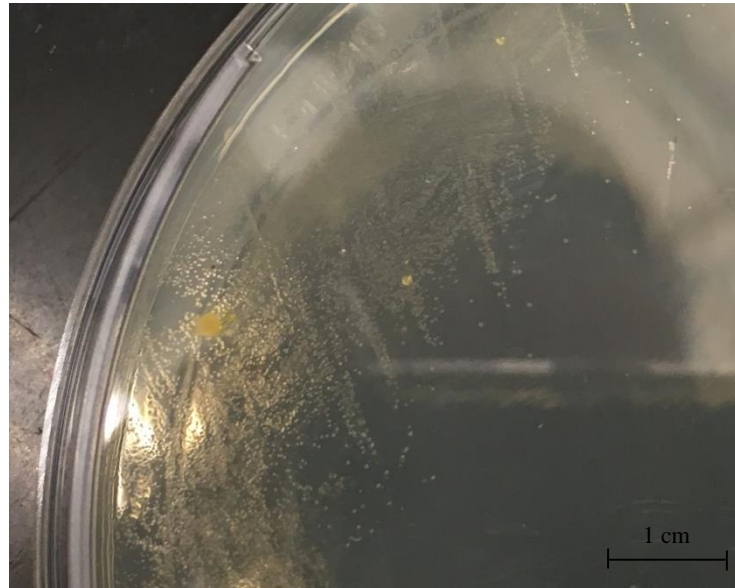
Figure 4-1:Appearance of few colonies after two days

Hardened Mortar Results

4.3 Compressive strength

The compressive strength tests were conducted on cement mortar cubes of size (50x50x50) mm after 7, 14 and 28 days. Table 4-1 lists the Compressive strength results and figure 4-2 demonstrates the compressive strength results of the 16 mortar mixtures. The percent increase of specimens' compressive strength is listed in table 4-2. The percentages of increase for compressive strength calculated by comparing each specimen to the control specimen C1 for DE specimens and C2 for Bentonite specimens and figure 4-3 demonstrates the percent increase among the different mortar mixtures.

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The effect of age on compressive strength is illustrated in Figure 4-4. The effect of mixing technique on the compressive strength of specimens is illustrated in figure 4-6.

Table 4-1 lists all mixtures having a higher compressive strength than the control mixture C1 at all ages except with the mixtures that included burned Bentonite in them which were drastically lower in the compressive strength than control specimen C1 which is likely due to the bentonite swelling properties that still had effect even after burning it. For instance, the mixture BD2C recorded a strength of 24.8 MPa at 28 days and mixture BD1C recorded a strength of 25 MPa after 28 days of curing; however, the control mix C1 yielded a strength of 24 MPa after 28 days while the mixture BB2 had a compressive strength of only 9.5 MPa .

Results also revealed that specimens containing bacteria and DE has a higher compressive strength than their control specimens C1 which is leading to a conclusion that the addition of bacteria to the mix design is what attributed to the increases in compressive strength which is the evident case with all mixtures that included bacteria in comparison to their control samples.

Results also had the same conclusion with the samples that contained burned Bentonite in their mixtures as illustrated by figure 4-2 despite the fact that the compressive strength is low relatively, samples with bacteria has exposed that their compressive strength is higher than their control samples C2, C3 and C4. For example specimen with BB3 mixture I.D. has a compressive strength of 8.7 MPa while it control specimen has a compressive strength of 7.5 MPa at the age of 28 day and the only parameters that changed were adding the bacteria and its food, calcium lactate, thus, the increase in the compressive strength is likely due to the effect of bacteria in closing cracks induced or further created by the use of Bentonite.

Specimens that had higher concentrations of bacteria is found to have the highest compressive strength when stabilizing the DE contents. Such that is specimen BD2C has a

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compressive strength of 24.4 MPa and 24.8 MPa after 14 and 28 days respectively and on the other hand, Specimen BD2A has a compressive strength of 23.4 MPa and 24.6 MPa after 14 and 28 days respectively. These results lead to a conclusion that the dosage of bacteria is a main factor that contributes to the compressive strength of mortar.

The fact that the use of burned Bentonite as an immobilization system for protection bacteria inside the mortar matrix revealed that it has a severe adverse effect on the compressive strength property on mortar and, accordingly, was not used in the rest of the study tests. On the other hand, the use DE as an immobilization material and a storage for the calcium lactate have shown positive effect on compressive strength of mortar and was further analyzed.

The compressive strength in the bacterial batches, containing high percent (15%) of DE, appears higher than that of the ones containing 5 and 10% in all bacterial dosages. The higher compressive strength in BD1C, BD2C, and BD3C, is likely due to the fact that DE has decreased the amount of water content on the mix design and accordingly increased the compressive strength. Thus, the compressive strength in this study was function of two variables due to the use of DE and Bacteria.

Table 4-1: Compressive strength results at 7, 14 and 28-day

Mixture	Mixture I.D.	Compressive Strength Results of Cracked Specimen (MPa)		
		7-day	14-day	28-day
Type I	C1	18.5	23.0	24.0
Type II	BD1A	19.5	23.5	24.3
Type III	BD1B	18.8	23.4	24.4
Type IV	BD1C	19.7	23.9	25.0
Type V	BD2A	18.7	23.3	24.4
Type VI	BD2B	19.4	23.4	24.6
Type VII	BD2C	19.9	24.1	24.8
Type VIII	BD3A	20.1	23.3	25.6
Type IX	BD3B	20.6	23.7	26.0
Type X	BD3C	21.1	24.7	26.8
Type XI	C2	6.8	8.2	9.1
Type XII	C3	5.9	7.5	8.4
Type XIII	C4	5.1	6.3	7.1
Type XIV	BB2	7.0	8.6	9.5
Type XV	BB3	6.1	7.9	8.7
Type XVI	BB4	5.3	6.5	7.4

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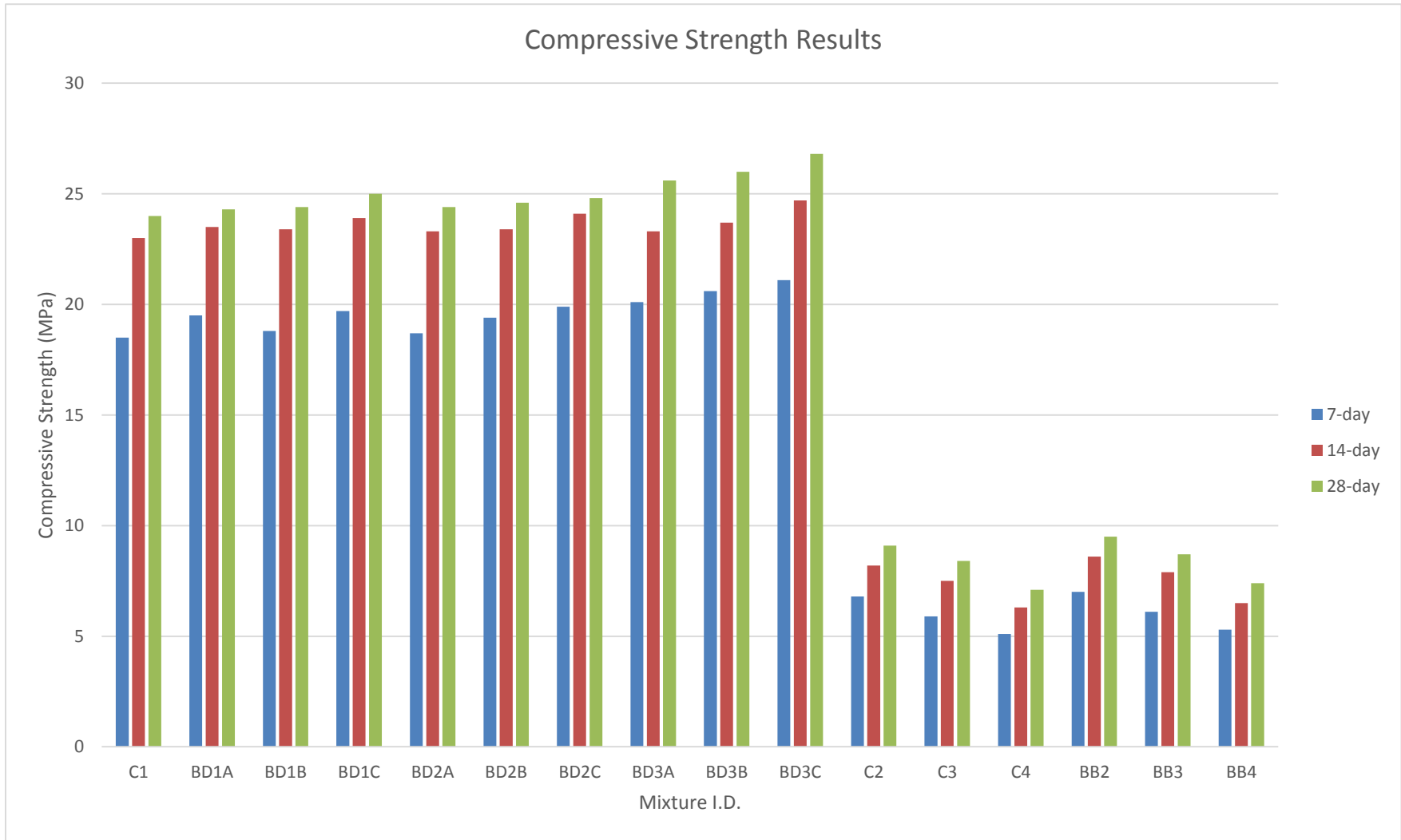
Figure 4-2: Compressive strength results at 7, 14 and28-days

Percent increase of compressive strength

Table 4-2 listing percent increase and figure 4-3 illustrates that the percent increase in the compressive strength is highest when 1×10^{-9} dosage of bacteria and high dosage of DE is used with a percent increase with respect to the control Mixture C1 of (12%) at 28 days and slightly decreased to 7% at 14 days and 12% increase after 7 days for specimen BD3C. The reason behind the increase of the compressive strength could be attributed to as following depending on the trends of increase.

1- An increase is seen among type II, III and IV which included the same amount Of DE, however, different dosages of bacteria. It is seen that the highest percentage of increases among the three types is the one containing the highest dosage of bacteria, which could be concluded that when stabilizing the DE percentage, the main factor contributing to the strength increase is the dosage of bacteria which is directly proportional to the increase of compressive strength

2- An increase is also witnessed as the percent of the DE percentages increases. For example the percent increase after 28 days in specimen BD3A that has a 15% DE of the weight of sand is 7% while the percent increase in BD2A that has a 10% DE of the weight of sand is 2% and specimen BD1A that has a 5% DE of the weight of sand is 1%. Although the dosage of the bacteria is being stabilized in this example, the compressive strength increases, which concludes that the compressive strength increases when the percent of DE increases. The fact that the increase in the use of DE increases the compressive strength is likely to be traced back to two main facts. First, the increase in the compressive strength could likely be due to the fact that the increased amount of DE results in mores absorption to water as DE are porous and has higher percent of absorption than regular sand and,

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accordingly, its use could have altered the w/c ratio to a lower value resulting in increasing the value of the compressive strength. Second, the greater use of the DE introduced more volume to saving the calcium lactate and bacteria and accordingly higher number of bacteria was able to work on sealing cracks.

It is clear from figure 4-3 that the percent increase is highest at the age of 7 days as listed in table 4-2 which mainly indicates the bacteria is working more, precipitating calcite, at the time the beginning of crack initiation. For example the percent increase of specimen BD3B is 11% at the age of 7 days while the same specimen has a percent increase of 7% at the age of 28 days. This observation could be likely due to the fact that the number of bacteria at the early age of the crack is high and calcium lactate is abundant; thus, bacteria is more effective in sealing cracks at early ages while its capacity decreases with time according to the results revealed could be attributed to two main aspects; one, many bacteria have either sporulated due to the lack of precursor or dies; two, the growth rate of bacteria inside mortar specimen is relatively slow and, accordingly resulting in less effective sealing for cracks at prolonged ages. This trend should be investigated further for a better explanation.

The percent increase in compressive strength in the burned Bentonite specimens is not as high as the DE and that is likely to be due to the fact that burned Bentonite has a huge adverse effect on compressive strength which has resulted in an unclear trend and it is recommended to conduct further studies on it to better explanation.

Table 4-2: Percent increase in compressive strength from control mix

Mixture I.D.	Percent increase in compressive strength		
	7-day	14-day	28-day
BD1A	5%	2%	1%
BD1B	2%	2%	2%
BD1C	6%	4%	4%
BD2A	1%	1%	2%
BD2B	5%	2%	3%
BD2C	8%	5%	3%
BD3A	9%	1%	7%
BD3B	11%	3%	8%
BD3C	14%	7%	12%
BB2	3%	5%	4%
BB3	3%	5%	4%
BB4	4%	3%	4%

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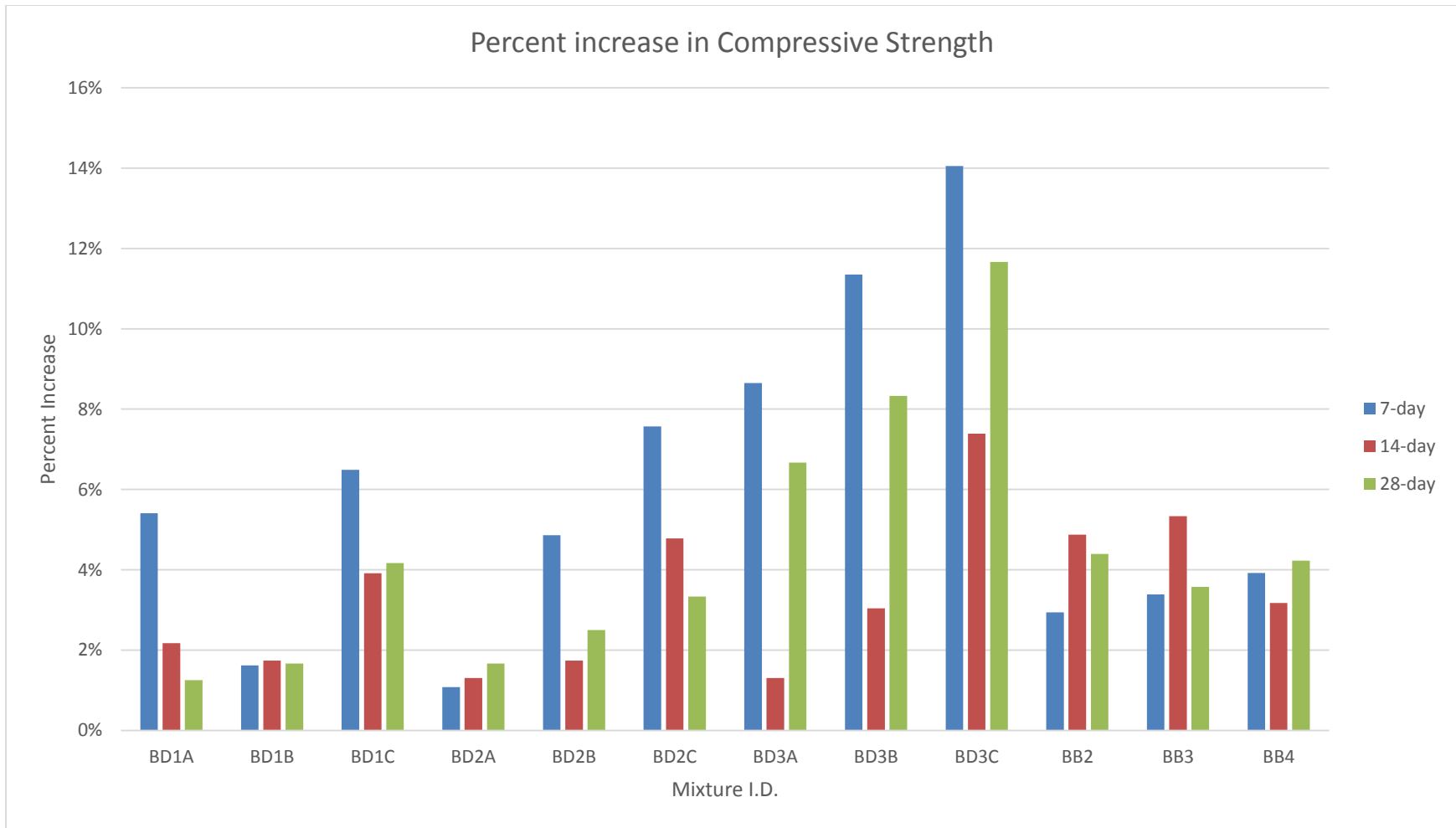
Figure 4-3: Percent increase in compressive strength at the ages of 7, 14and 28-days

Ageing effect on the compressive strength

It could be concluded from figure 4-4 that the compressive strength constantly increases at the early ages for all the cement mortar mixtures except the ones that use burned Bentonite. It could be also concluded that the mortar mixture BD3C had the highest compressive strength at all ages which confirms that compressive strength increases with both increasing the dosage of bacteria and DE. The increase of compressive strength with time is likely due to the original properties and chemical reactions of cement in mortar. On the other hand the specimens containing burned Bentonite exhibit the same trend which could be also explained as due to the property of cement hardening with time as well however, with lower variations.
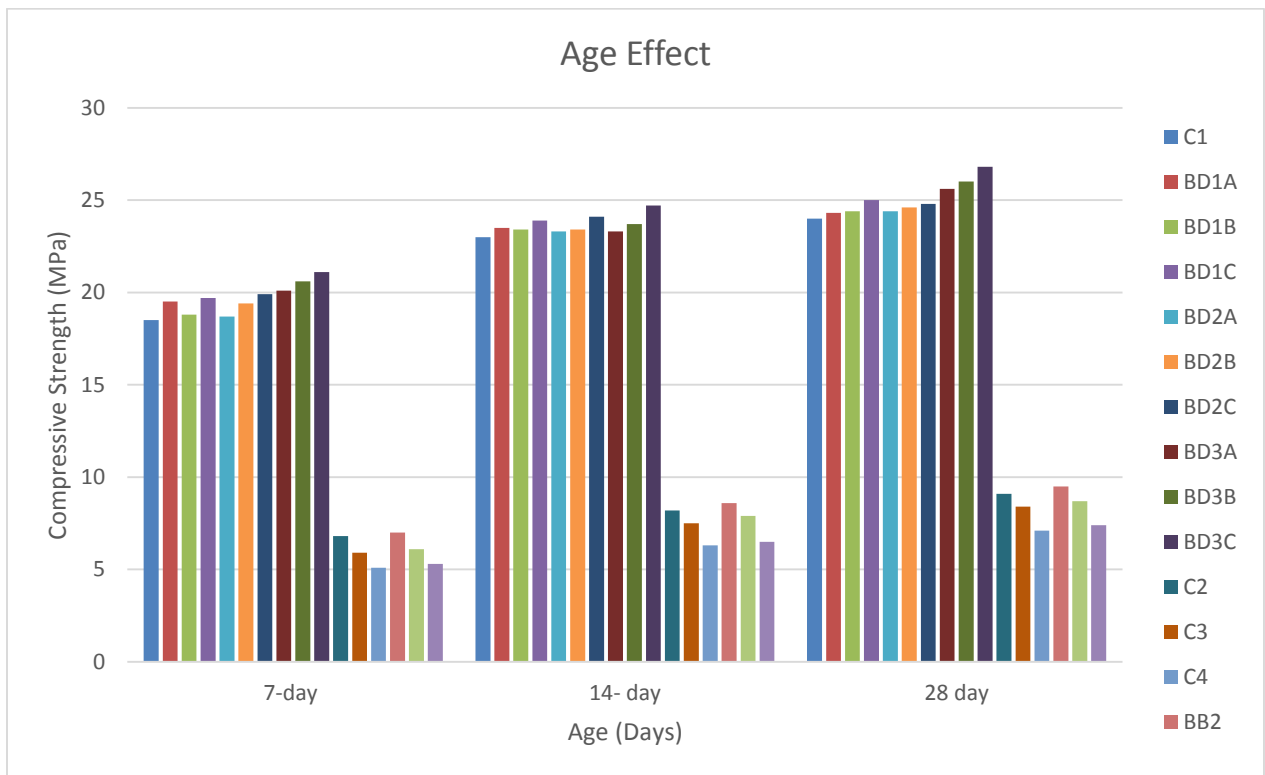

Figure 4-4: Effect of age on compressive strength

The effect of the bacteria dosage on compressive strength

The main effect that is entitled to the recovery of mortar specimens and their compressive strength is the number of bacteria dosage. By examining the different cement mortar cubes prepared for this study with the use of different dosages of bacterial spores, it can be seen from figure 4-5 representing the different mortar specimens and bacterial dosages. Mortar specimens that contained higher dosages of bacteria displayed high compressive strength which is a rational outcome that is attributed to the fact that more bacteria has survived the mechanical stresses, heat of hydration and was able to work as a catalyst in producing more amounts of calcite for sealing cracks which directly contributes to the increase in the compressive strength. Also, the rationality goes back to the fact that the dosage of bacteria is high and accordingly the growth rate is higher.

However, the time of healing for bacterial specimens with high dosages was less than of lower dosages as we observed that the sealing using calcite by the bacteria was still occurring on the mortar cubes for longer time than the ones containing the high dosages which agrees with Jonkers (2010) findings. For example, the time of crack sealing for mortar cubes with mixtures BD2B was more than the one with mortar mixture of BD2C which could be explained due to the fact that for the bacteria to produce more calcite, it needs higher calcium lactate content because when it is finished then bacteria will not be able to produce calcite; thus, when bacteria dosage is higher, the amounts of calcium lactate used is higher with less time than the ones with lower dosages and accordingly the time of healing of cracks is more with lower bacteria dosages which could contribute different than the scope of the study results on the longer run.

As demonstrated by figure 4-5, there is a small variation in the compressive strength results among mixtures using 3×10^8 cm^{-3} and 6×10^8 cm^{-3} and 5 and 10 percent of DE. For example, the difference in compressive strength between BD2A and BD1A is 0.1 MPa and between BD1B and

BD2B is 0.2 MPa which is likely due to the fact that the difference in the number of bacteria is not high relatively. On the other hand the difference is much larger when the number of bacteria increases to 1 x 10^9 cm^{-3}; for instance, the gap between BD3A and BD1A is 1.3 MPA; thus, the effect of bacteria on compressive strength is clear when the number of bacteria is highly increased. This analysis confirms that the there is a relation between the number of bacteria and the increase in the compressive strength; however, the trend should be investigated on more dosages of bacteria and further studies should be conducted to acquire a relationship describing this phenomenon.

Another interesting results demonstrated by figure 4-5 is the high variation of compressive strength existing between specimens that includes 15% of DE in their mixtures and the other specimens having the same number of bacteria. For example, the variation is clear between specimens having the same number of bacteria such as BD1C, on one hand, and BD1A and BD1B on the other hand and the same trend is evident in within every cluster of specimens holding the same number of bacteria which is likely due to the effect of DE that takes place when its' percentage increases to 15% and such could be contributed to the same reason explained earlier of altering the w/c ration by the use of DE.

The fact that BD1C has a higher compressive strength than BD2C is an unclear trend and is recommended to conduct further study to better explain it.
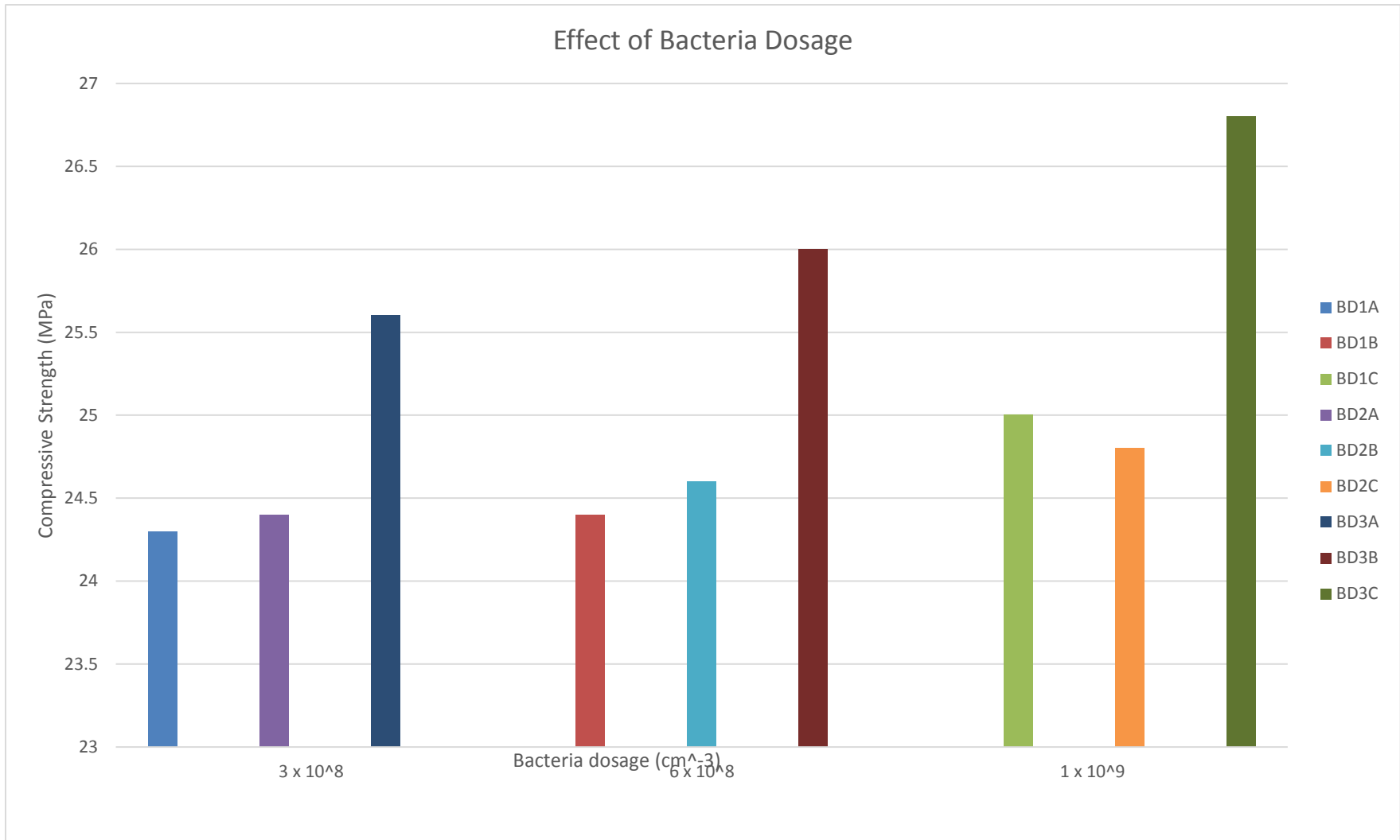
Figure 4-5: Effect of Bacteria Dossage on Compressive Strength

Figure 4-6 demonstrates that the mixing technique that resulted in higher compressive strength was mixing technique Z. For example, for mixture I.D. BD2B prepared with mixing technique (Z) recorded a strength of 24.6 MPa which is slightly higher than (X) 24.4 MPa and (Y) 24 MPa. The same result could be detected at which mixing technique (Z) gives the highest strength with all other mixtures. This result would likely be due to the fact that bacteria would work perfectly when it is immobilized by the DE so when the water that contained calcium lactate and bacteria was first mixed with the DE, most of the bacteria was well immobilized and accordingly were well protected from the mortar matrix environment and, thus, bacteria was effective in sealing micro cracks and, accordingly, increasing the compressive strength.

Furthermore, adding water at the end allowed all components of the mix to share it fairly after the DE have absorbed its water at the beginning due to its high porosity.

It should be noted that mixing techniques have not been conducted on the burned Bentonite specimens, and was not further investigated due to its negative effect on the compressive strength.

Based on the results compared in figure 4-6, it is recommended to use mixing technique (Z) which includes mixing water first with DE while conducting a dry mixing for all other components then adding the dry components to the wet DE and adding the other half of the water at the end and mixing in order to have higher compressive strength results. It is also recommended to add the water at the end in order to lessen the attraction of water by the silica fume due to its surface area and give a fair advantage of sharing water with all components.
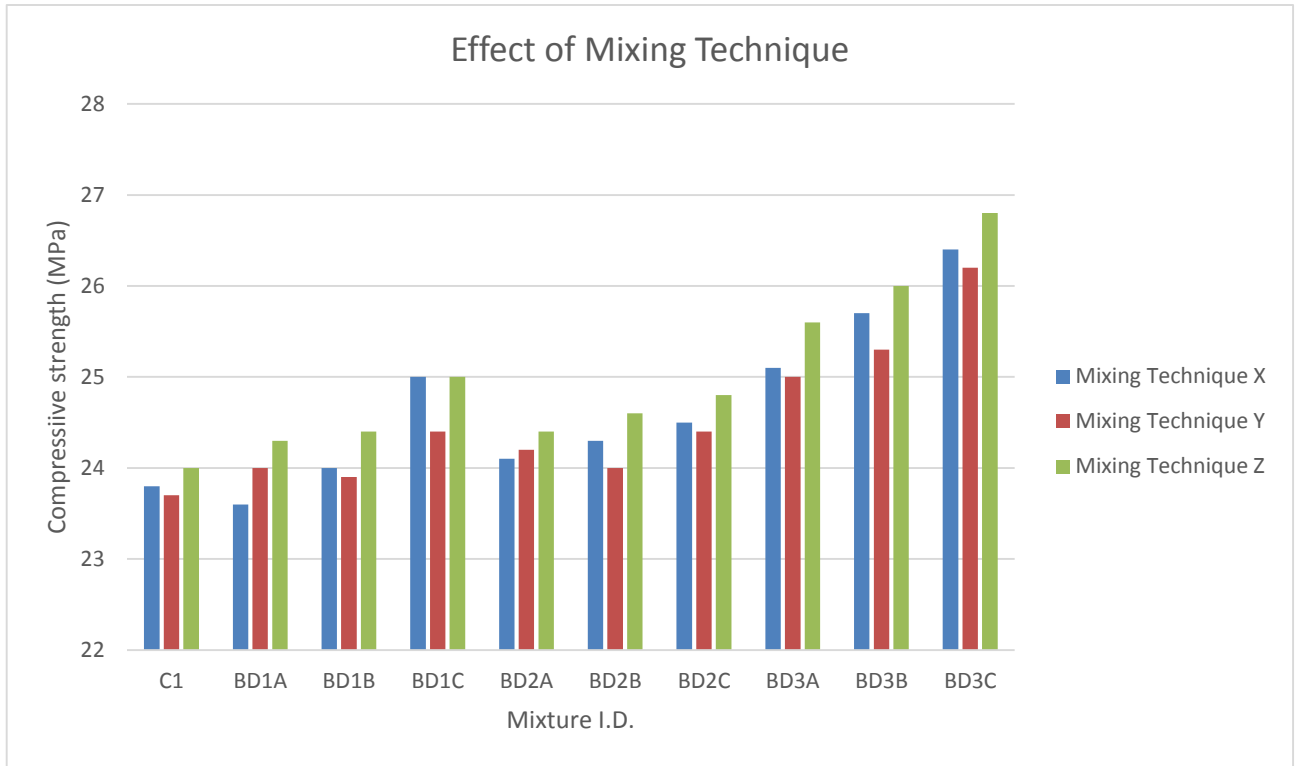
Figure 4-6: Effect of Mixing Technique on Compressive Strength

4.4 Tensile strength

Only the DE specimens and control specimen were subjected to indirect tension test at which a Universal Testing Machine was used to induce pressure on the middle line of the specimen in order to transfer it as tension pressure on the specimen as shown in figure 3-27. Specimens were tested after 28 days. Analysis were made on the tensile strength of specimens as well as the percent increase and the effect of bacterial dosages used on tensile strengths of specimens. The tested specimens showed a unified fracture mode of the specimens. Table 4-3 lists the tensile strength of the different mixtures as well as the ratio of tensile strength to compressive strength. Figure 4-7 also compares the tensile strengths of the different specimens.

Table 4-3 Tensile strength results and ratio between tensile and compressive strength

Mixture I.D.	Tensile Strength Results for Cracked Specimens	
	Tensile Strength (MPa)	Ratio of Tensile Strength to Compressive Strength
C1	1.9	7.92%
BD1A	1.93	7.94%
BD1B	2	8.20%
BD1C	2.03	8.12%
BD2A	1.91	7.83%
BD2B	1.98	8.05%
BD2C	2.1	8.47%
BD3A	1.95	7.62%
BD3B	2.07	7.96%
BD3C	2.14	7.99%
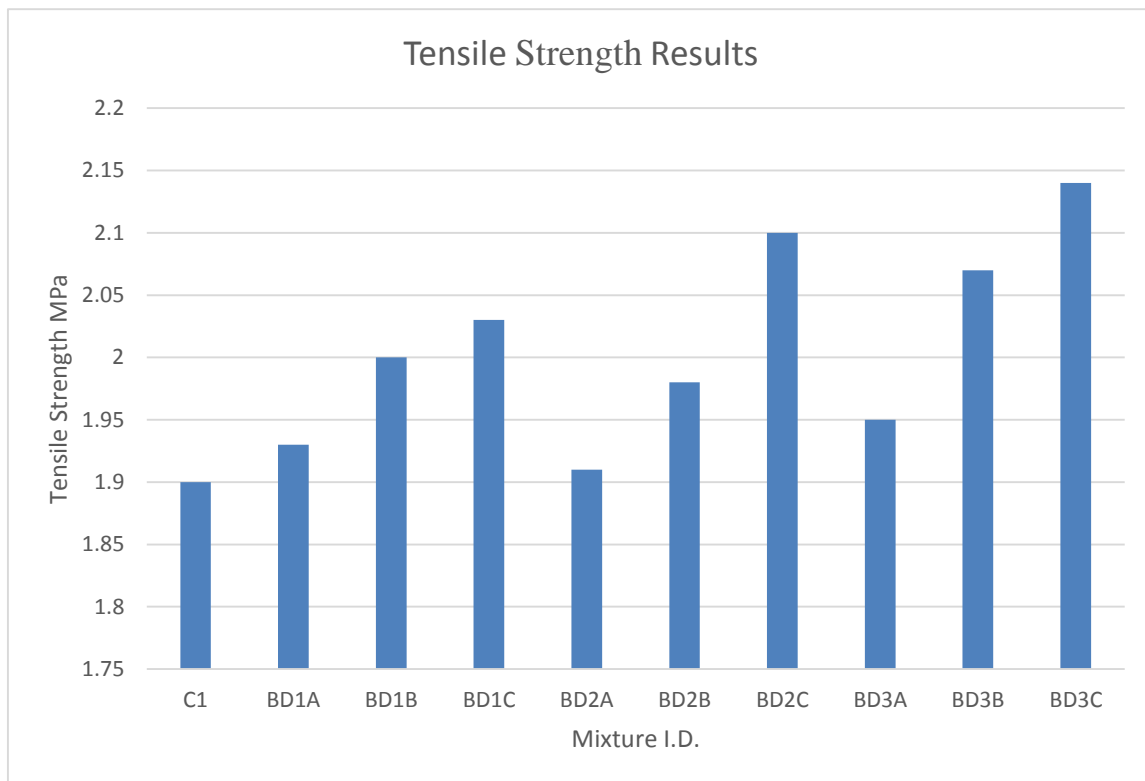
Figure 4-7 Tensile strength results at the age of 28-day

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According to figure 4-7, the tensile strength increases when adding DE and dosage of bacteria in all specimens which indicates a positive effect of such bio additive on the tension strength of mortar. For example, mixture BD3A showed higher tensile strength (1.95 MPa) than the control specimen (1.9 MPa).

The tensile strengths between different dosages of DE is obvious and supports the previous analysis made in the case of compressive strength which is that the dosage of bacteria is the key element for healing. For example, the tensile strength of mixture BD1C (2.03 MPa) and mixture BD2C (2.1 MPa) which is higher due to the use of higher amount of DE.

The ratio of tensile strength to compressive strength indicates that the relation between both strength are proportional and are due to the biological additive to the mortar.

Percent increase in tensile strength

Table 4-8 lists the percent increase of tensile strength with respect to the control specimen mixture C1. Indicating that the highest percent of tensile strength increase is correlated with the highest dosages of bacteria and DE which concludes that the tensile strength increase is likely due to the increase in DE and bacteria dosages that are directly proportional to it.

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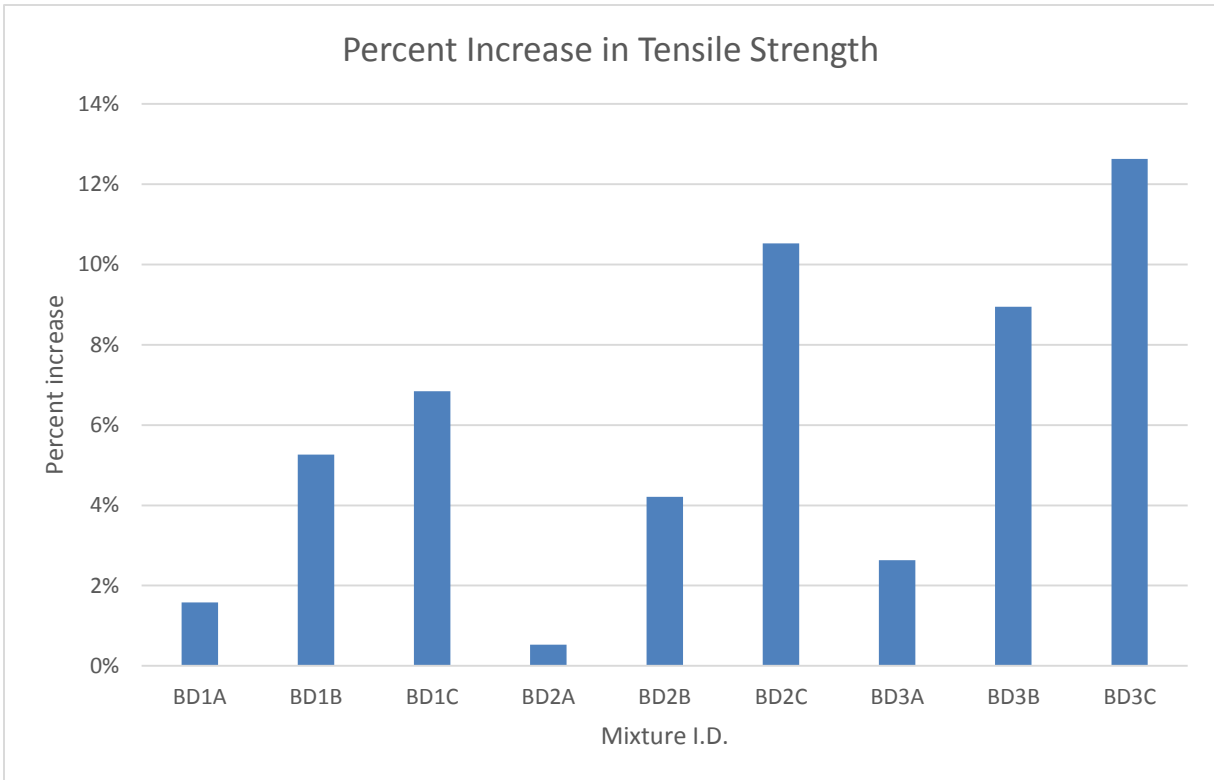
Figure 4-8: Percent increase in Tensile Strength

The effect of the dosage of bacteria on tensile strength

It is shown from figure 4-9 that the trend of increased tensile strength is correlated with the increase of the dosage of bacteria which also confirms with the compressive strength test results. For example the mixture BD3A gave a tensile strength of 1.95 MPa while mixture BD3B gave a tensile strength of 2.07 MPa and BD3C have the highest tensile strength of 2.14 MPa and the only parameter that changed among these mixtures is the dosage of bacteria; thus, the increases in the tensile strength is attributed to the higher dosage of bacteria which could be analyzed by the fact that sealing the induced cracks of

the different specimens is likely what contributed to the overall integrity of specimens and accordingly higher tensile strength.
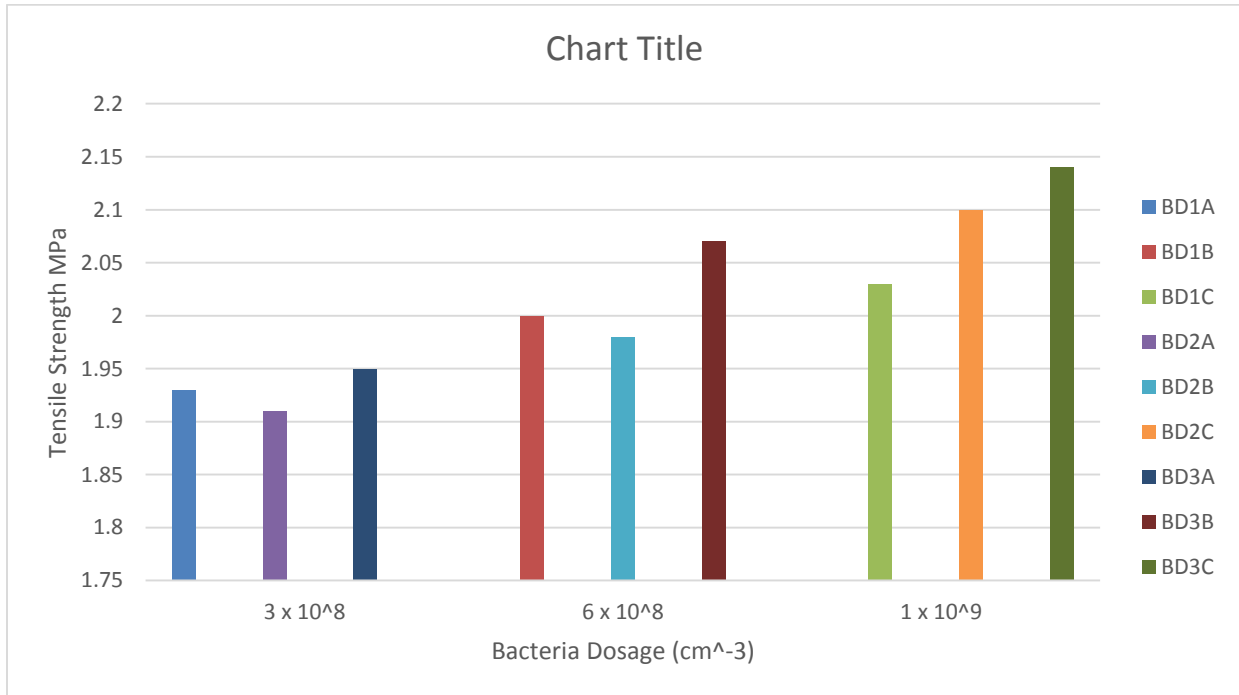
Figure 4-9: Effect of Bacteria Dosage on Tensile Strength

4.5 Rapid Chloride Permeability

In order to measure the effect of sealing cracks on the permeability of specimens, rapid chloride permeability test was utilized to test chloride penetration resistance of the cement mortar specimens. The mixtures that have been used in this test are the control mixture C1 and the mixture that had the highest results in compressive strengths with respect in their domains which are mixtures: BD1C, BD2C and BD3C. The test results at the age of 28 days are listed in table 4-4 and compared in figure 4-10. All specimens that included bacterial dosages exhibited less permeability to chloride in comparison to the control specimen mixture C1. For example, the control specimen mixture recorded charges of 2676 Coulombs while it dropped to 1636 Coulombs with the specimen of mixture BD2C

and further decreased to 1235 with specimen mixture BD3C. The reduction in chloride permeability is likely to be correlated to the effect of healing by bacteria that had closed surface cracks with calcite.

Table 4-4: Rapid chloride permeability test results

Mixture I.D. of Cracked Specimens	Charges Passed (Coulombs) after healing	Permeability class
C1	2676	Moderate
BD1C	2140	Moderate
BD2C	1636	Low
BD3C	1235	low
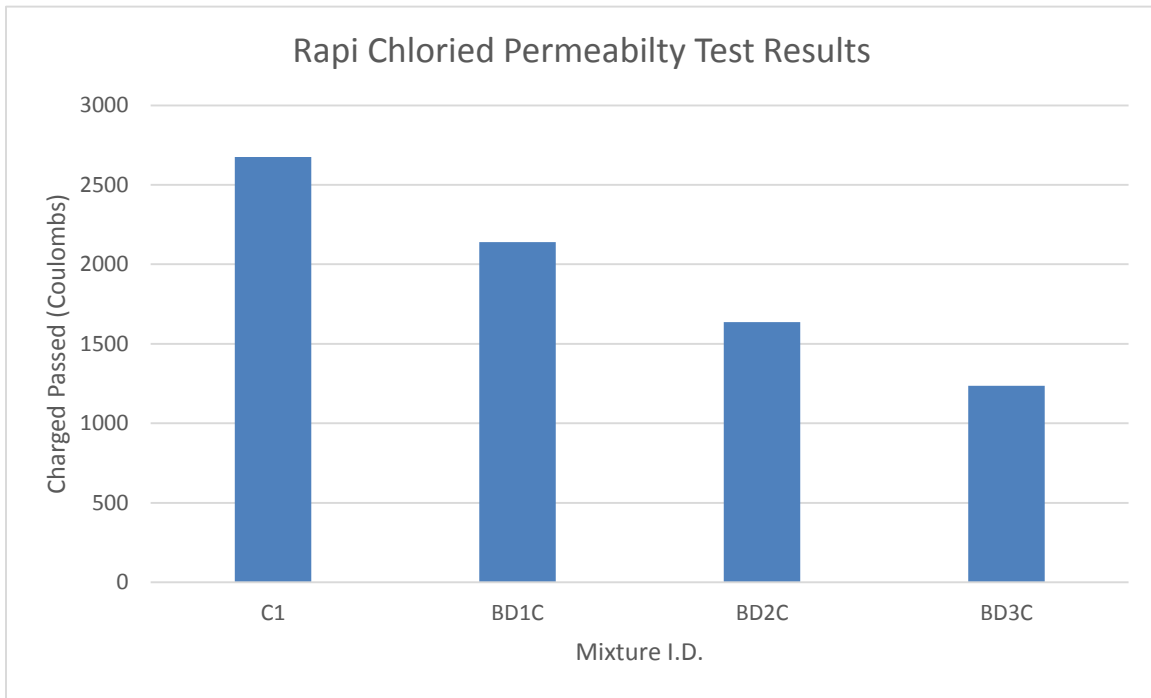
Figure 4-10: Rapid chloride permeability test results

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It is noticed that the permeability values for the different bacterial specimens are close to each other which could be explained by bearing in mind the fact that most of the surface cracks were sealed in the different bacterial specimens which was a factor that compromised a well comparison between the effects of different dosages of bacteria on the permeability of specimens. The trend is not clear and it is recommended to conduct further studies on this issue for a better understanding especially at early ages of the mortar specimens to indicate the effect of the dosages of bacteria since at earlier ages not all surface cracks would be closed by the bacteria work.

4.6 Chemical durability

Chemical durability test was conducted only on the control mortar specimens of mixture C1 and the DE mixtures in order to evaluate the soundness of the mortar specimens using bacteria and DE when exposed to magnesium sulfate solution and subjected to cycles of wetting and drying for durations up to 28 days. The test has been conducted on a cracked mortar specimen cubes of size (50x50x50) mm. the specimens were soaked for four cycles with a duration of 5 days per cycle with a two days drying gap in order to expedite the chemical attack on the cement mortar cubes.

The results of the chemical durability test is listed in table 4-5 and demonstrated in figure 4-11 which records the mass loss percent due to the exposure to magnesium sulfate solution.

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Table 4-5: Mass loss percentage due to exposure to magnesium sulfate

Test Day	C1	BD1A	BD1B	BD1C	BD2A	BD2B	BD2C	BD3A	BD3B	BD3C
28 days	3.30%	2.70%	3.30%	2.55%	3.60%	3.69%	3.45%	2.40%	2.91%	3.30%
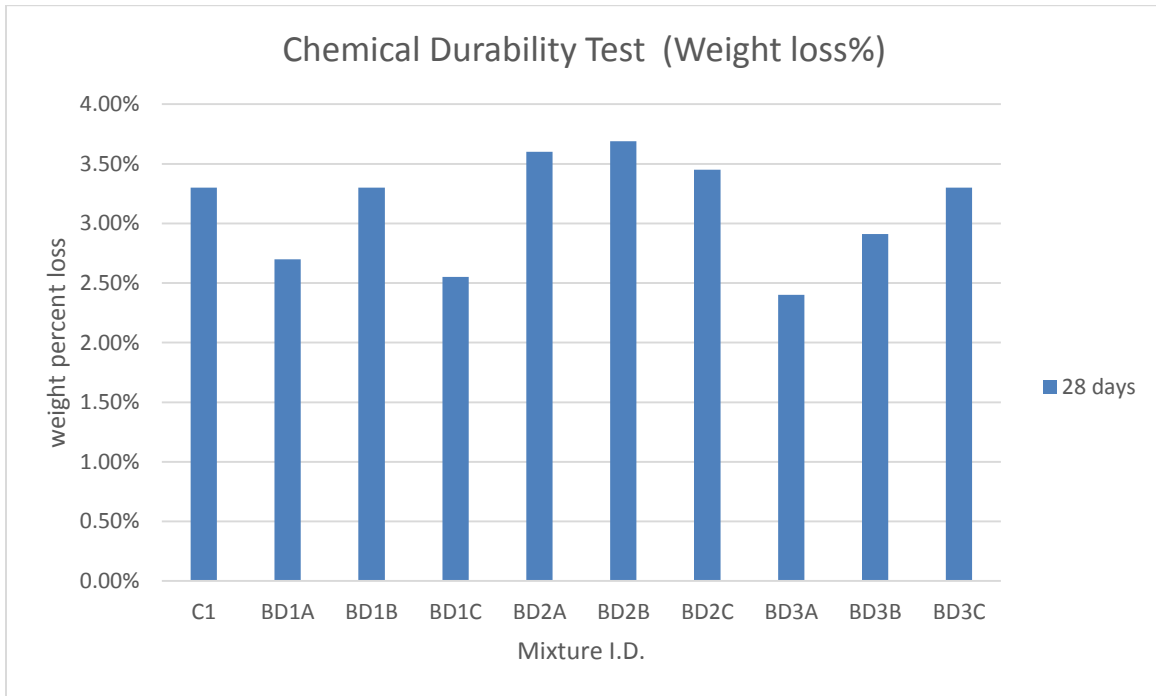
Figure 4-11: Mass loss percentage due to exposure to magnesium sulfate

It can be noticed from table 4-5 and figure 4-11 that the mass losses in the different mortar cubes are close to each other and the trend is not clear. It is also noticed that the results are close to the control mixture specimen result indicating that they have approximately behaved the same under the attack of the magnesium sulfate solution. This observation could be explained based on the bacteria response to the chemical attack as follows; bacteria has not transformed from the spores form to the vegetative form, thus, did not attain its objective in sealing cracks or contributing to the resistance of the chemical attack since the magnesium sulfate solution is a harsh environment for the bacteria to

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increase metabolism. On the contrary, magnesium sulfate solution is used to force bacteria to sporulation and, accordingly, extremely lowers it metabolic activity which is what likely to be happened in this test.
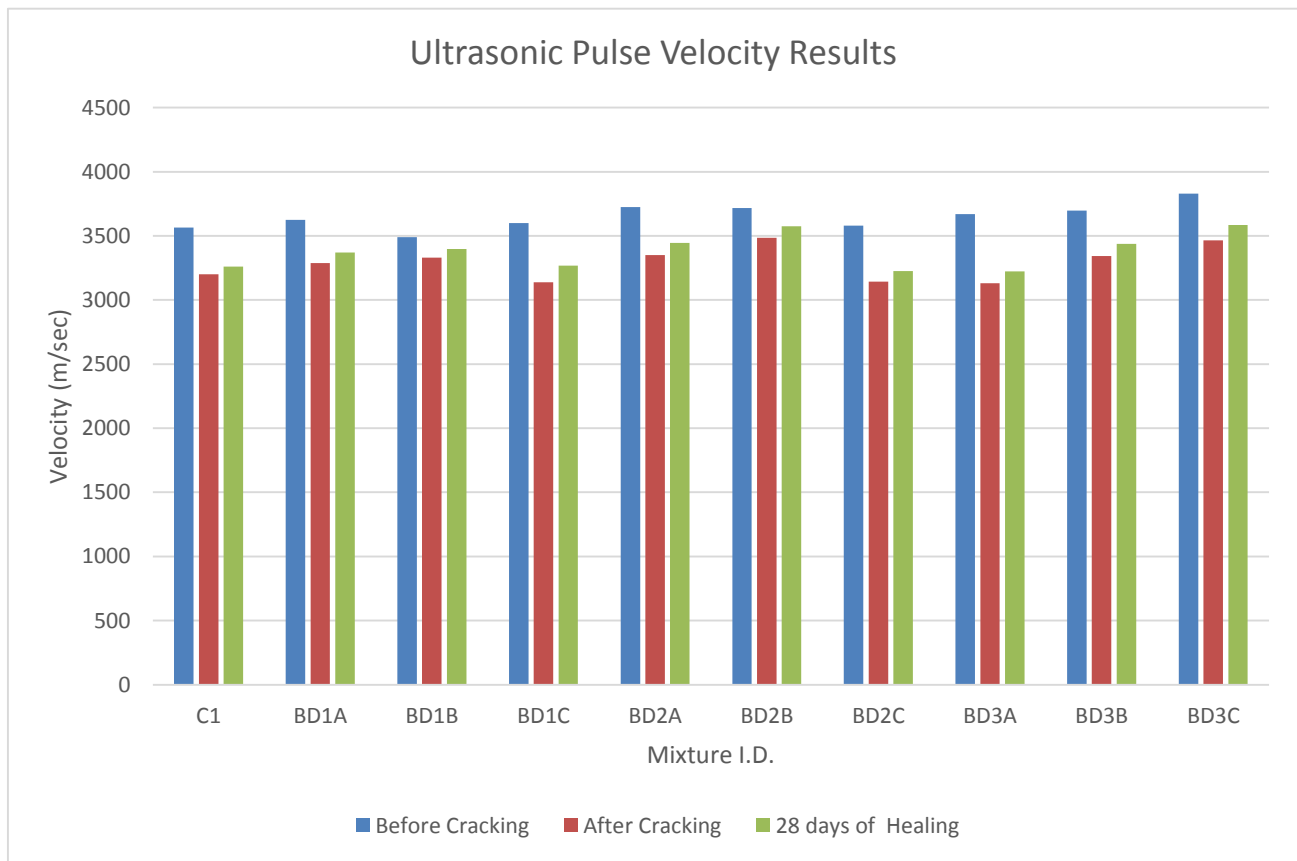
4.7 Ultrasonic Pulse Velocity test

Ultrasonic pulse velocity test was conducted on mixtures that included DE only and the control mixture specimen C1. The test was conducted before inducing cracks of the specimens then after inducing cracks and was conducted at the age of 28 days after inducing cracks.

Table 4-6 lists the values obtained from the ultrasonic pulse velocity test and demonstrated in figure 4-12. It its well noticed that specimens containing bacteria had higher velocity than the control specimen after healing which is likely due to bacteria increasing the solidity of the mortar specimens by inducing calcite in voids and cracks. The effect of the higher dosage of bacteria was obvious as such specimens has higher velocities. Although figure 4-12 shows that specimens with higher concentration of DE has higher velocities than the ones with less concentration, the values are close which may not accurately infer that the that more healing happened to specimens with higher values of DE and bacteria concentration; none the less, such neighboring results is due to the effect of the increased number of DE as such fossils are porous and would contain water and food, calcium lactate, inside of them, they might affect the judgment and analysis of the ultrasonic pulse velocity test. For example, the velocity recorded for specimen BD3C 3670 m/sec is close to the velocity recorded for specimen BD2C 3580 m/sec. thus it is recommended to conduct further studies for better understanding of the effect of DE on the results of the ultrasonic pulse velocity test.

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Table 4-6: Ultrasonic Pulse Velocity Results (m/sec)

Test Day	C1	BD1A	BD1B	BD1C	BD2A	BD2B	BD2C	BD3A	BD3B	BD3C
Before Cracking	3564	3626	3490	3600	3724	3717	3580	3670	3698	3830
After Cracking	3200	3289	3330	3138	3350	3485	3143	3130	3343	3464
28 days of Healing	3260	3370	3397	3267	3444	3575	3226	3223	3438	3584

Figure 4-12: Ultrasonic Pulse Velocity of cracked specimens after 28 days of healing

4.8 Visual Inspection of Healed Mortar Specimens

Picture (A), figure 4-13, shows that the effect of healing is minimal with low dosage of bacteria and low concentration of DE. While picture (B) shows that specimen with medium dosage of bacteria and high medium concentration of DE healed the surface cracks and voids in the specimen which indicates that most of the bacterial concentration was at the surface of the specimen and, due to the abundance of oxygen, higher metabolic activity occurs. On the other hand picture (C), figure 4-13, represents the higher dosage of bacteria and DE in the mixture design and the specimen's cracks are fully healed, revealing that the higher the dosage of bacteria and DE, the higher the rate and amount of healing.
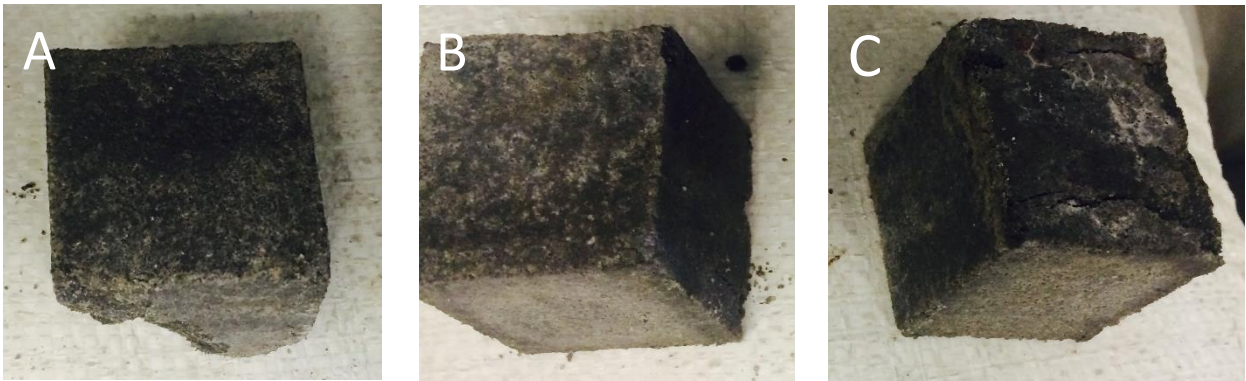

Figure 4-13: A: Specimen BD1A, B: Specimen BD2B presenting precipitation of calcite on the surface of the cube; C: Specimen BD3C showing healing of few surface Cracks.

4.9 Micro-study examination

SEM photos were taken at the age of 28 days for the different mortar specimens. Specimens studied are the control specimen C1 and the Diatomaceous earth (DE) specimens in addition to the burned Bentonite specimens. SEM analysis was used to confirm the results of the other properties tested and in order to have inspect on a micro level the effect of the bacteria inside the mortar matrix.

Figure 4-14 shows the surface of the mortar specimen with mixture C1 (Control mixture) at which no bacteria or DE was used in it at the age of 28 days. It is clear from the picture that the material is almost homogeneous. Figure 4-15 shows a zoom in of the control specimen C1 surface which is a regular picture of mortar specimen with silica fume. It was clear that control specimen pictures using the SEM exhibited a lot of voids in them in comparison to the specimens that had bacteria in them.
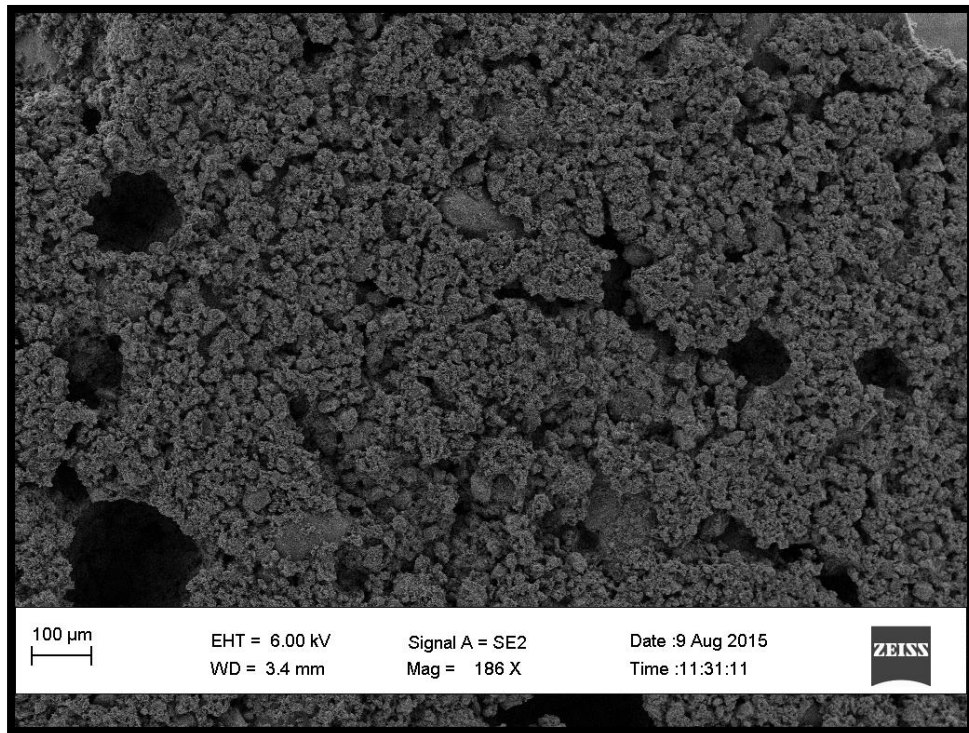
Figure 4-14: SEM Picture of C1 at 28 days

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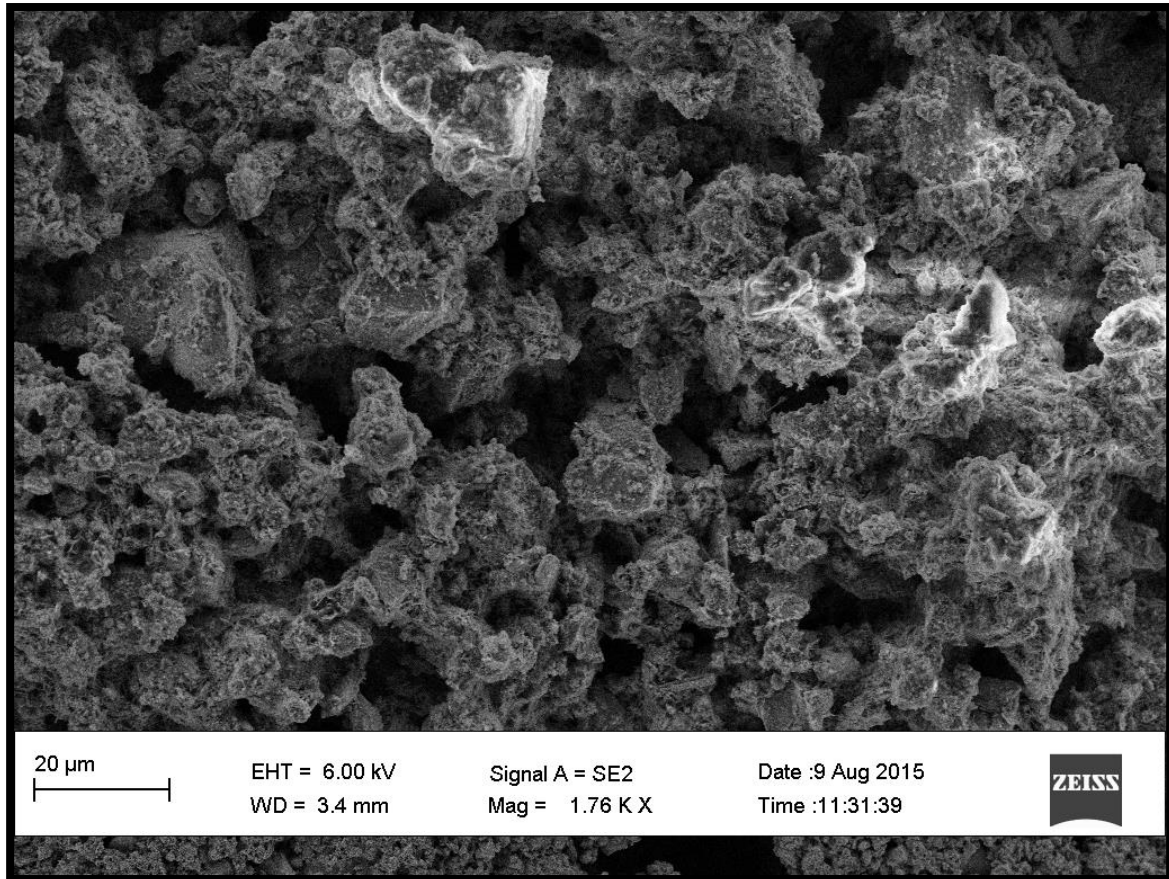
Figure 4-15: SEM Picture of C1 at 20 micrometer at 28 days

Figure 4-16 shows cluster of Bacillus *Pseudofirmus* DSM 8715 strains in its vegetative form using the SEM. The shape of the bacteria when in vegetative form is a rod-like with a length dimension of about 1 micron and a width of half a micron on average. Figure 4-17, figure 4-18 and figure 4-19 show a burned Bentonite particle and close up images of it that were investigated using the SEM for its surface. The surface of the particle is not porous at all in an analogy what happened to Bentonite when burned is like inflating a balloon. There are no bores seen on the surface of and accordingly it could be inferred that the main purpose of including burned Bentonite in the concrete matrix, to work as a carrier for the bacteria and calcium lactate, could not be true since it has no porosity.

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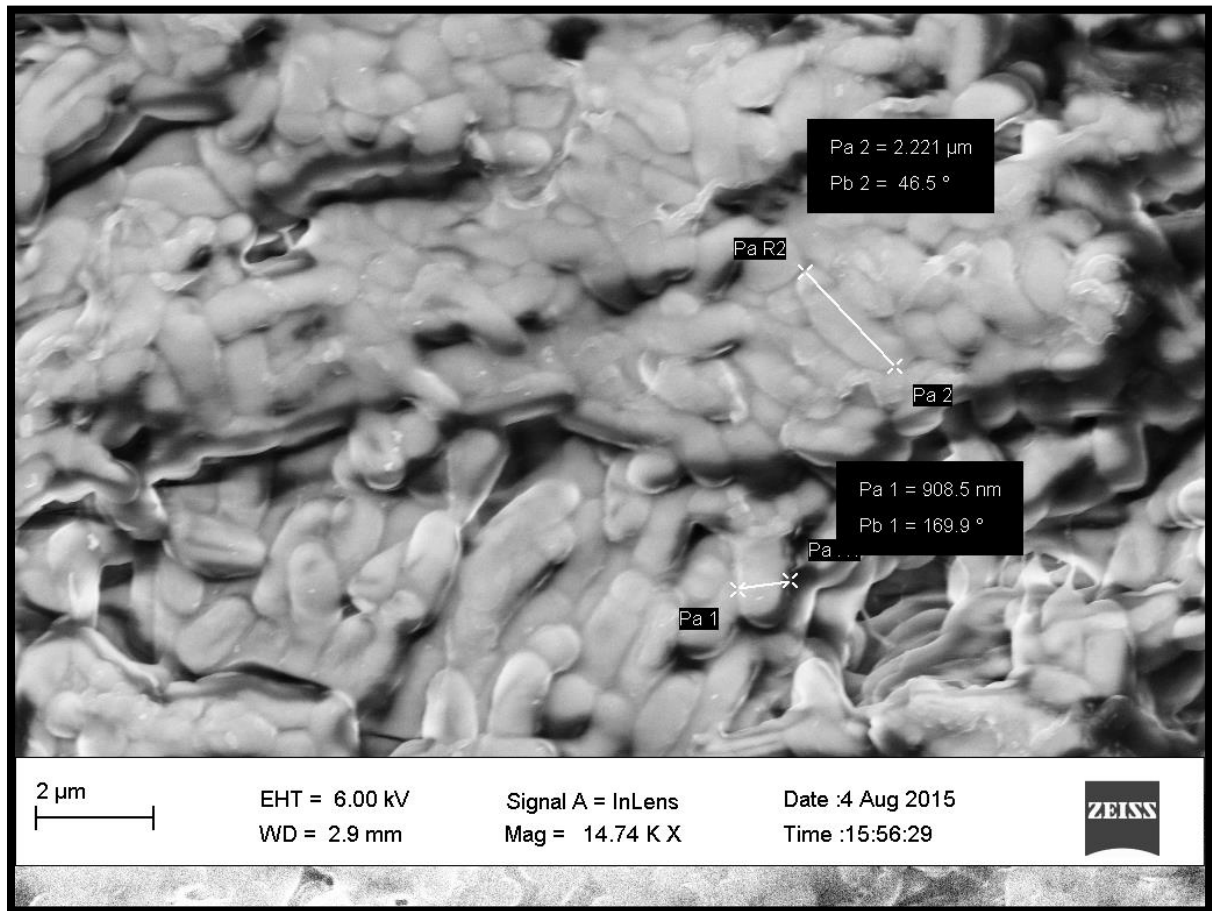
Figure 4-16: SEM Picture of a cluster of Bacillus Pseudofirmus DSM 8715 strains
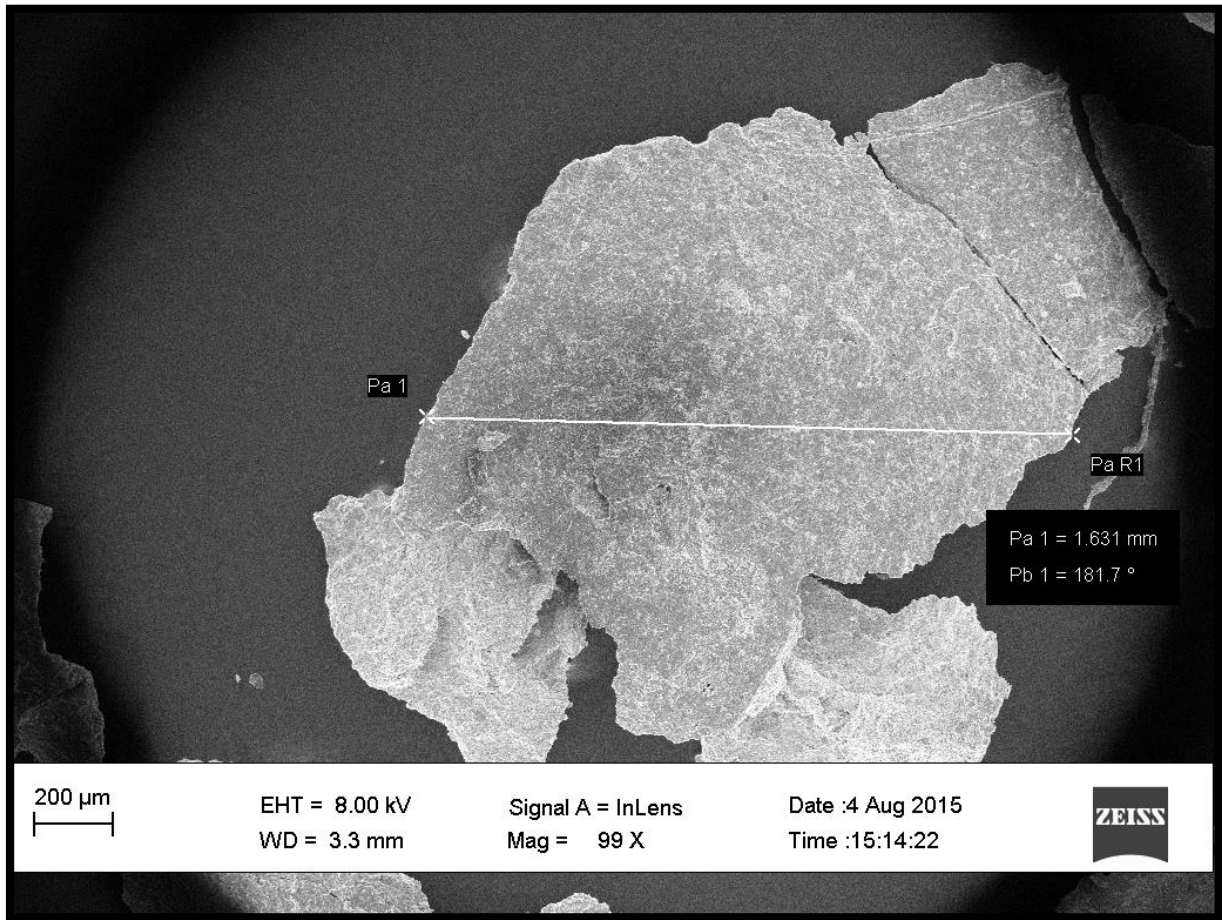
Figure 4-17: SEM Picture showing a dimension of Burned Bentonite Particle
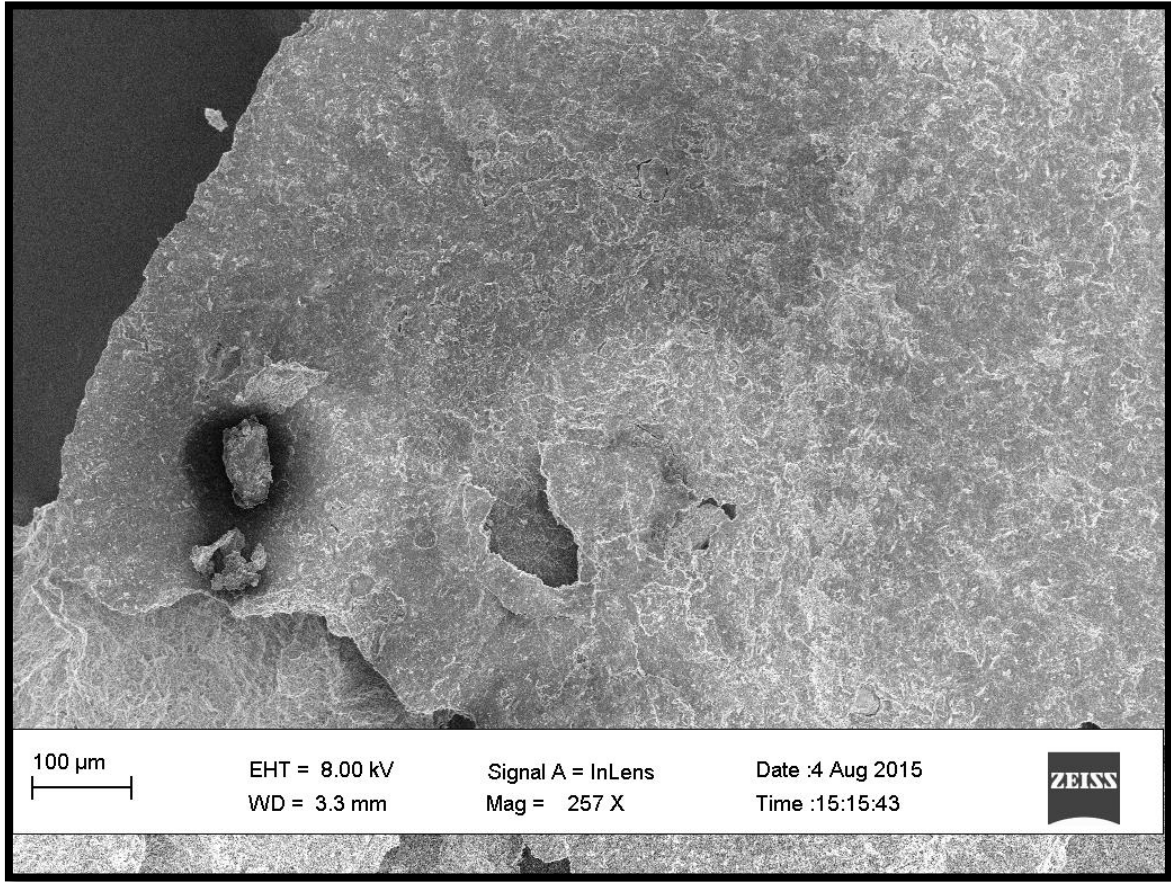
Figure 4-18: SEM Picture of Burned Bentonite Particle
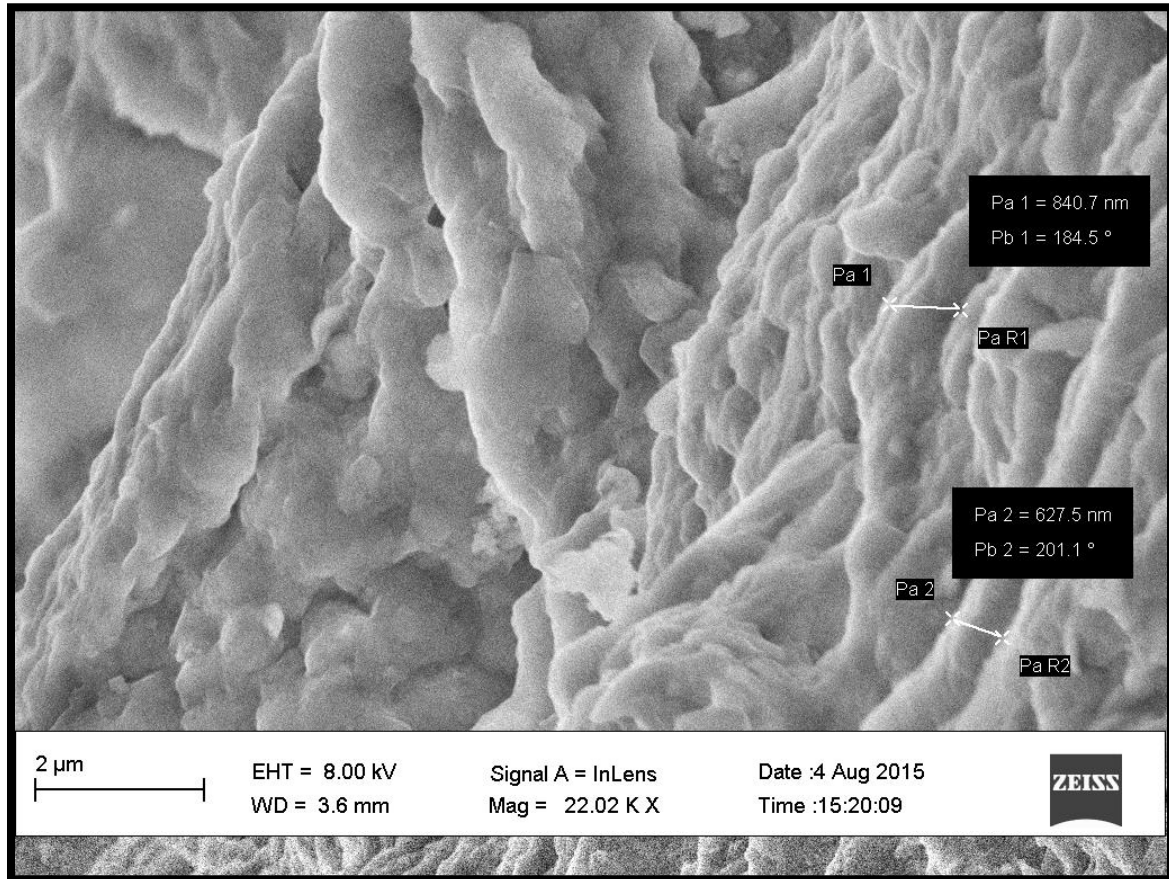
Figure 4-19: SEM picture of a burned Bentonite surface

Figure 4-20 shows the spread of bacteria over a mortar particle. The following figures 4-21 and 4-22 shows a close up images of the SEM for bacteria in its vegetative form over mortar. In figure 4-21, the bacteria concentration shown is high and this bacteria shows that it has transformed to vegetative form in the mortar specimen in high efficiency after it was embedded as spores during the mix design and such indicates that the fact that pH level was reduced due to the silica fume as well as having water acceding the mortar mix during curing through cracks and having calcium lactate provided a good environment for spores to transform to vegetative form and induce growth.

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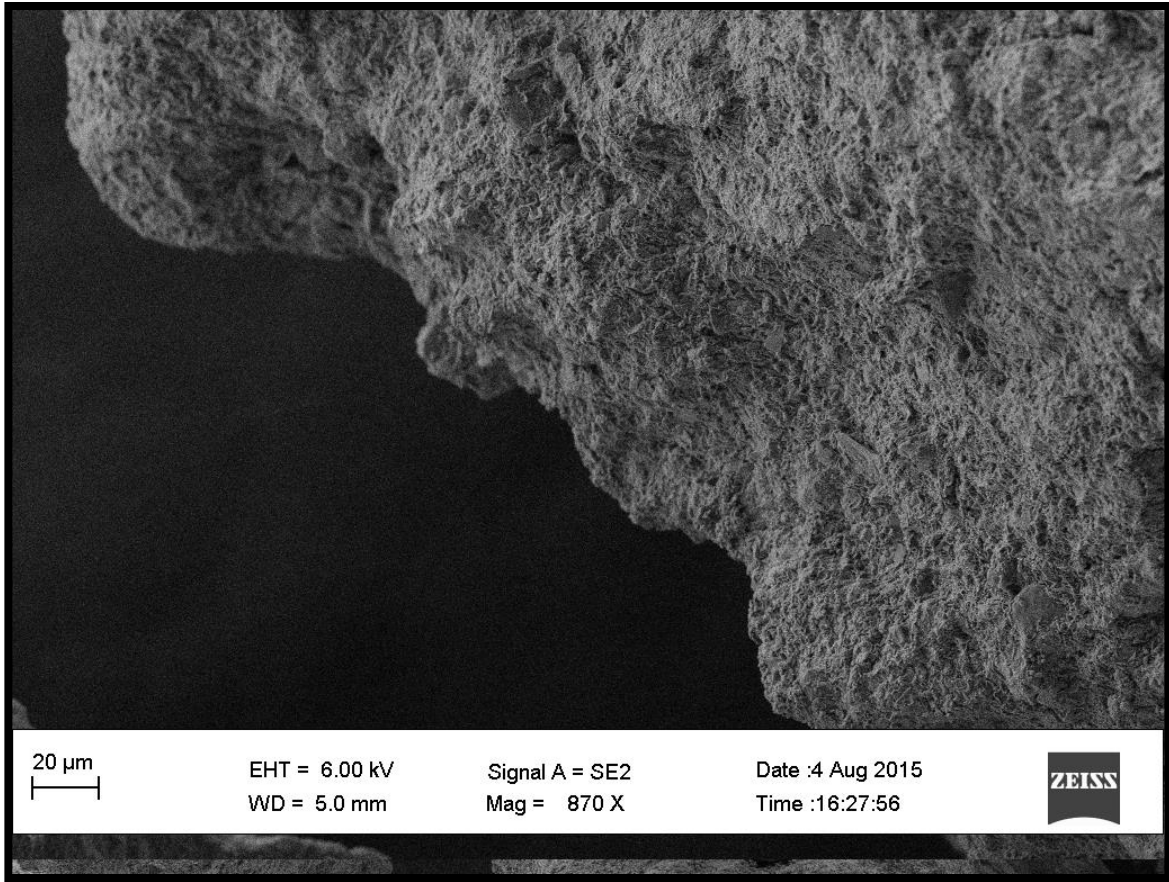
Figure 4-20: SEM Picture of a mortar particle with a spread of bacteria on its surface –BD1C

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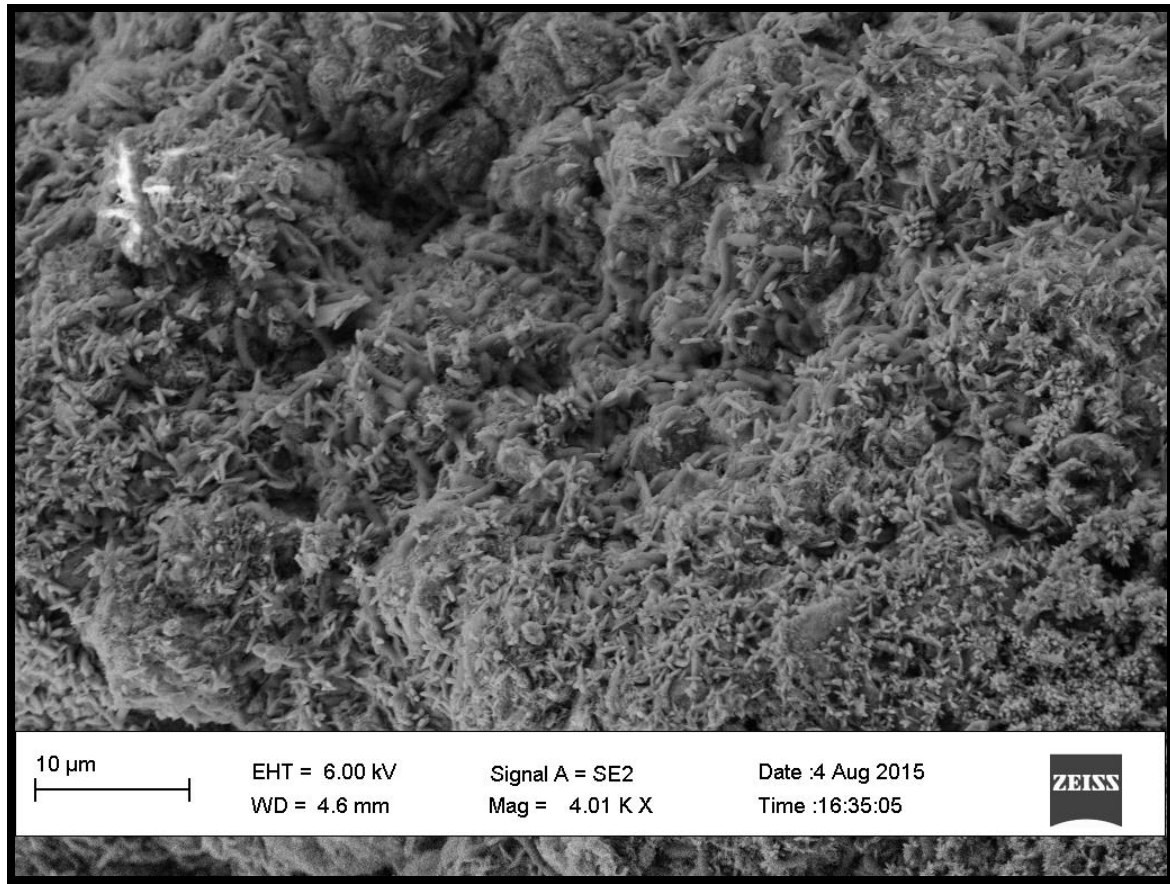
Figure 4-21: SEM picture of bacteria on the surface of a mortar particle BD1C
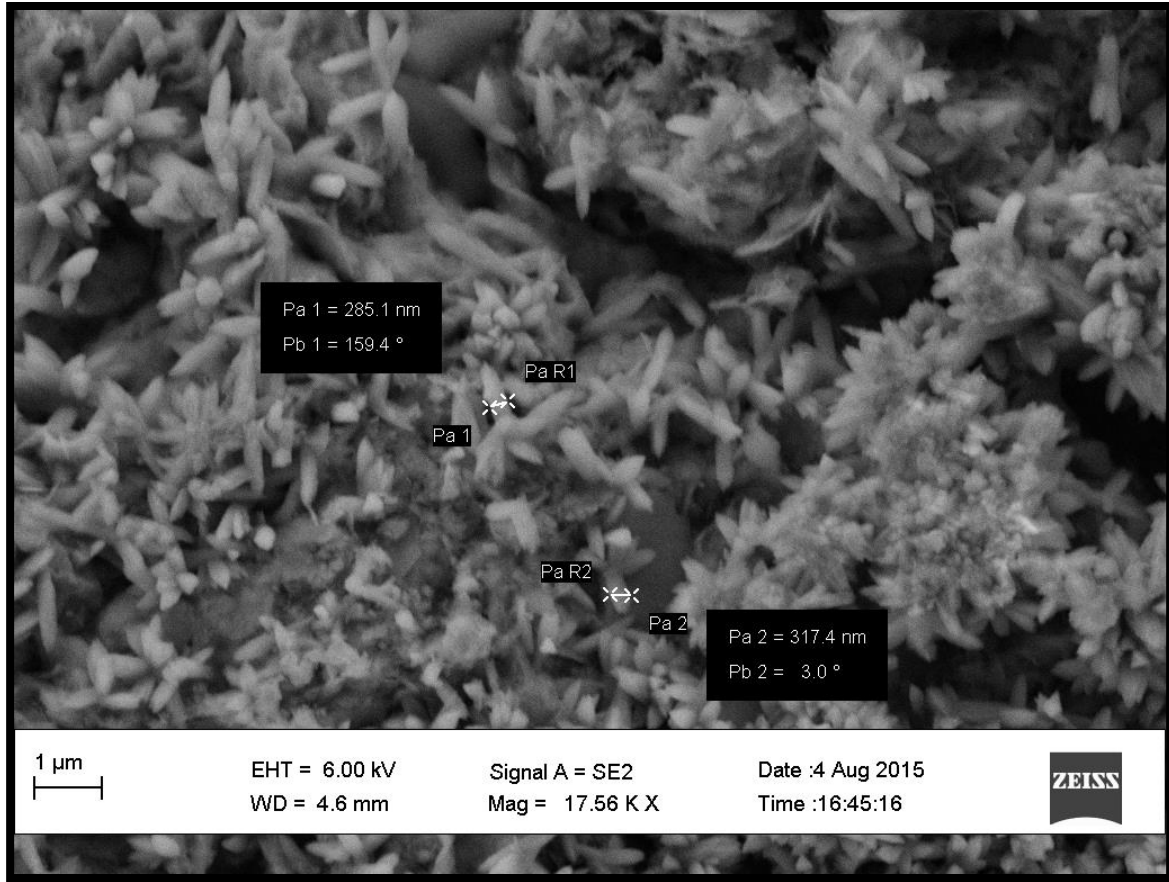
Figure 4-22: SEM picture of vegetaive bacteria on a mortar particle BD1C

Figure 4-23 shows SEM picture of spores of bacteria on a mortar specimen BD3C and shows that some of the spores are embedded in groves of DE and showing crystal like shapes that would be of calcium lactate dissolved particles around DE.
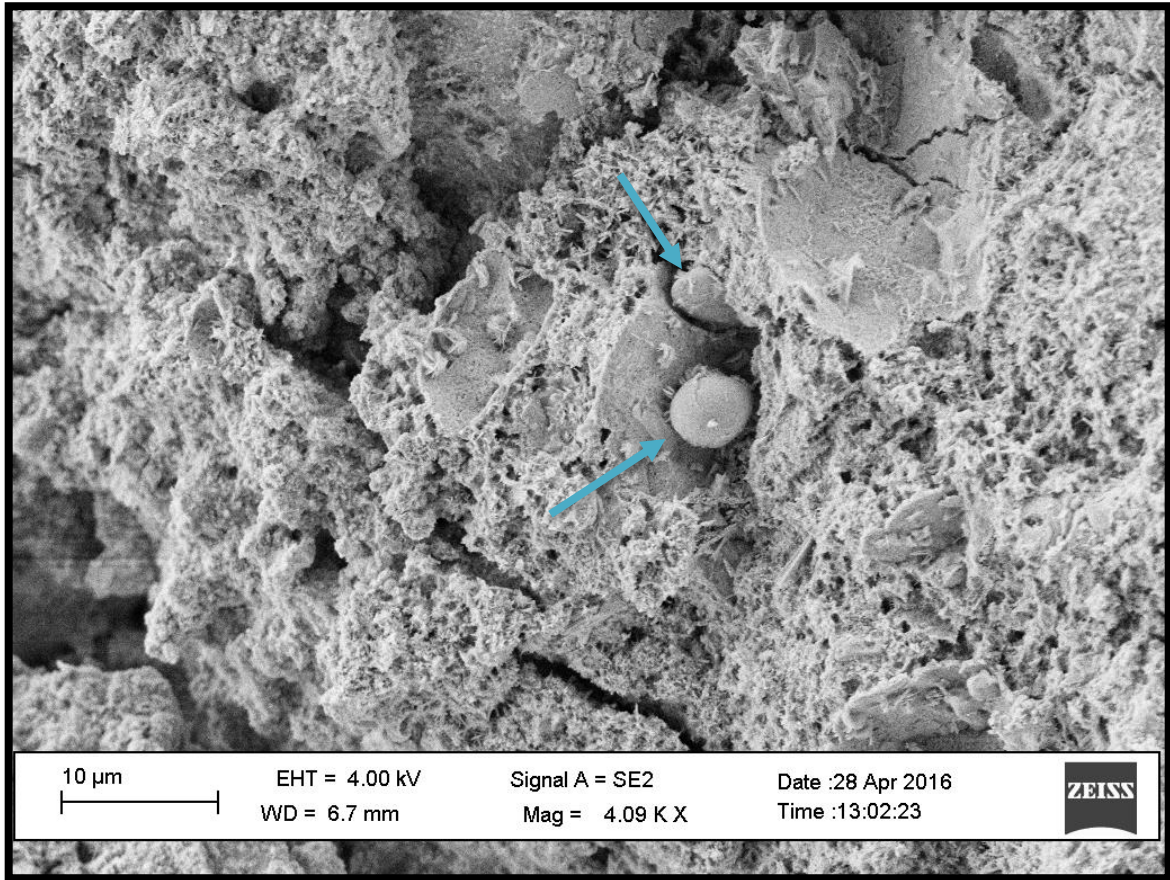
Figure 4-23: SEM picture of BD3C

Figure 4-24 shows SEM picture of BD2C that has vegetative bacteria in addition to spores as and precipitations of calcite. Figure 2-25 shows SEM picture of BD3C of at which many spores have transformed to vegetative form and calcite precipitations near the vegetative bacteria. It also show dormant bacteria at the same location which could be a reverse transformation of bacteria due to lack of calcium lactate at longer ages.

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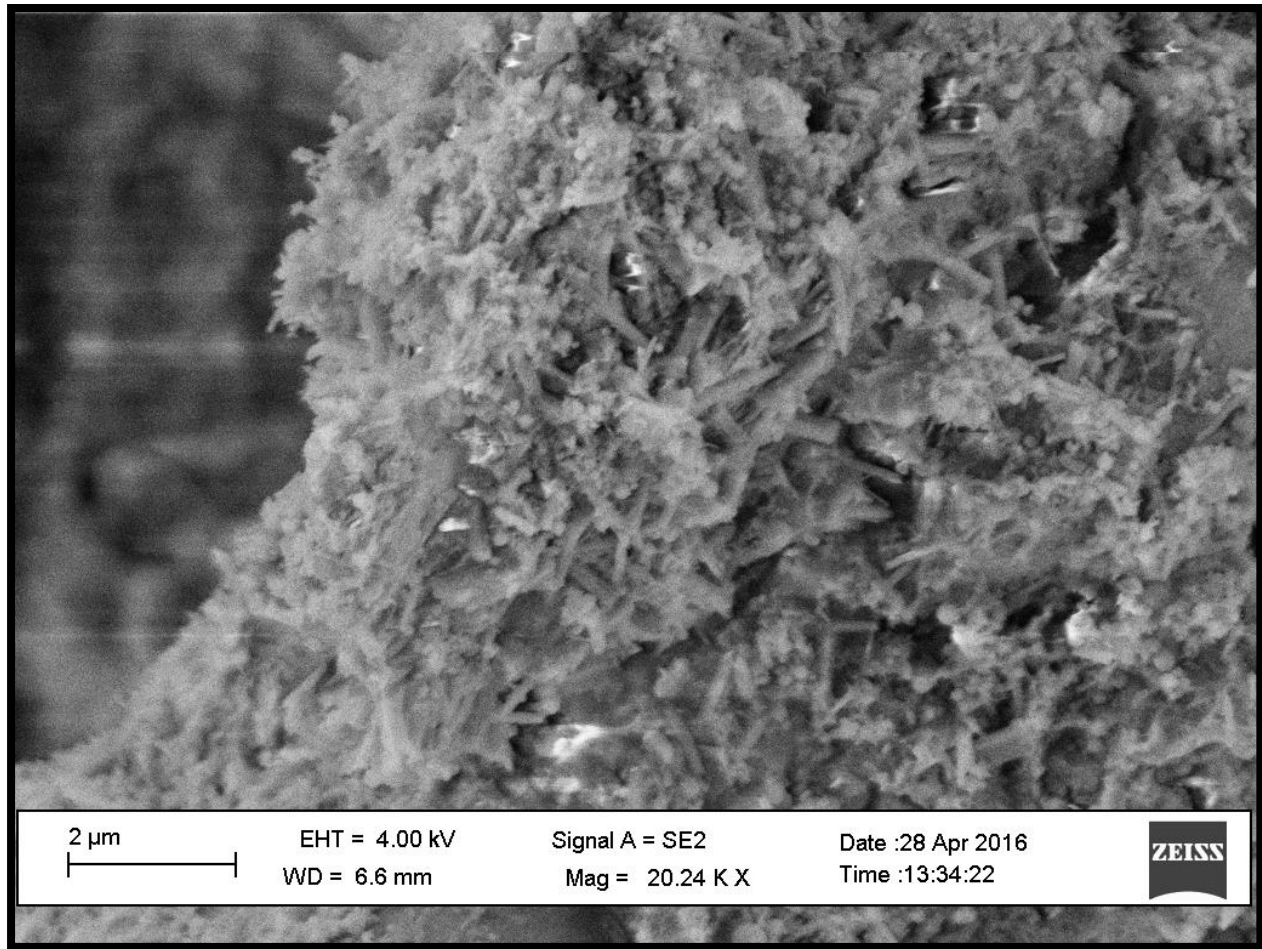
Figure 4-24: SEM picture of BD2C

101
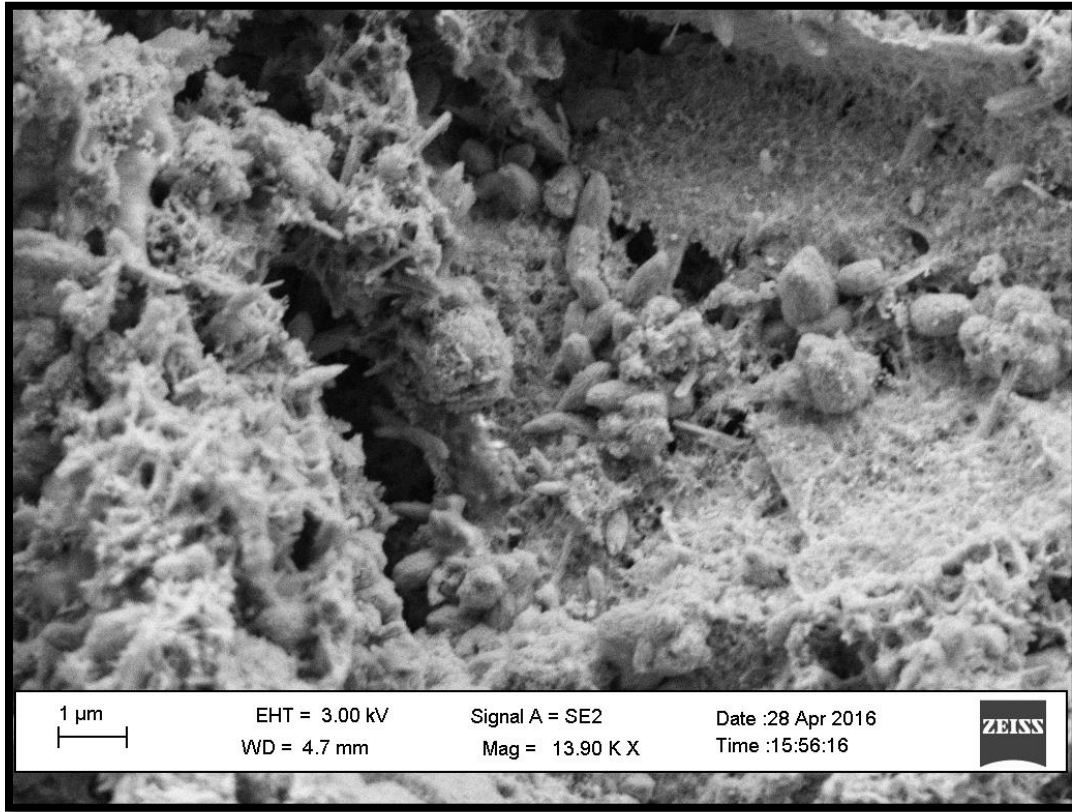
Figure 4-25: SEM Picture of BD3C

In summary, the SEM pictured have showed that control specimens having no bacteria in them has more groves that the ones having bacteria in them indicating that bacteria have worked on closing such voids. Burned Bentonite after being studied using the SEM, on the contrary, of its purpose to carry bacteria was found to have a surface that does not let neither bacteria nor calcium lactate to be protected by it. SEM pictures have shown that the environment of the mortar matrix that has been engineered to be suitable for bacteria to transform to a vegetative form was successful and bacteria was able to feed on calcium lactate and precipitate calcite.

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CHAPTER 5
CONCLUSIONS AND RECOMMENDATIONS
5.1 Conclusions and Major Findings and Other

Based on the materials, equipment and other parameters associated with this study, the following can be concluded:

- The rate of autonomous healing using bacteria is higher than the autogenous healing that is free of bacteria.

- Incorporating both bacteria and Diatomaceous earth in mortar mixtures generally enhances their mechanical properties.

- The Increase of dosage of bacteria was found to have a positive effect on the Calcite precipitation of bacillus *Pseudofirmus* in the concrete matrix as more viable bacteria would have a higher cumulative effect on mortar specimens healing

- Introducing the Diatomaceous earth in the design mix has offered a protective environment for bacteria from the high pH of the cement mortar as well as from the heat of hydration and provided a storage for calcium lactate and prevented from wasting such a relatively expensive material that is vital for the healing process but not letting it react with matrix chemical compounds.

- The use of bentonite after burning in oven at 400 °C have not contributed positively to the mix design as it had a huge adverse effect on the compressive strength. And SEM inspection showed that the bentonite only expands like a balloon and does not offer voids for protecting bacteria or conserving calcium lactate.

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- Decreasing the pH level of mortar specimens by replacing 15% of cement with silica fume enhanced the environmental conditions for higher percent of spores to transform to functioning cells.

- The specimens that included high dosages of bacteria exhibited the highest percentages of increase in the tensile strength.

- Incorporating bacteria had a positive effect on decreasing the permeability of specimens when conducted the rapid chloride permeability test.

- The analysis of chemical soundness test offered that self-healing mortar using bacterial approach perform poorly as the chemical compounds toughen the environment for bacteria to transform and decrease its metabolism. Thus, decreasing the precipitation of calcite.

- In terms of mixing techniques and its effect on compressive strength, mixing water first with Diatomaceous earth while conducting a dry mixing for all other components then adding the dry components to the wet Diatomaceous earth and adding the other half of the water at the end was found to have the most positive effect on compressive strength.

- Increase in velocity in the ultrasonic pulse velocity test was found highest with specimens including highest dosages of bacteria

- The Micro study conducted on cement mortar mixtures demonstrated that the enhancing in mechanical properties of mortar specimens that included bacteria is primarily because of the efficiency of bacteria in sealing cracks with calcite.

- The trend of strength increase and improvement in properties associated with the use of bacteria and Diatomaceous earth in mortar suggests an enhancement in concrete that uses bacteria and Diatomaceous earth as well. However, the predicted improvement and its extent remain to be investigated by a separate experimental work.

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5.2 Work Limitations

- The preparation of bacteria has been time and effort consuming and had to go through many trial and error cycles in order to finally acquire the required numbers of bacterial dosages needed.

- The scarcity of references on this topic made it challenging to compare the results against reference data.

- There is evidence that the Diatomaceous earth used was not pure and might have absorbed water before using it in the experiments from the atmosphere.

- The rigorous work in the preparation of bacteria made it difficult to conduct more tests with different mixing techniques on the tensile strength and rapid chloride permeability tests.

- As often encountered in experimental work, random error encountered in the tensile strength and rapid chloride permeability tests due to the limited number of specimens used.

5.3 Recommendations for Bacteria Supplier

- It is recommended to supply bacteria with the optimum protocol for culturing it with detailed information for its further use.

- It is of utmost importance to provide the optimum way to be used in spore forming the bacteria with the exact quantities of all elements and compounds needed.

- It is crucial for the suppler to present a mechanism for testing whether bacteria have formed spores or not.

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5.4 Recommendations for Future Research Work

Similar to common experimental work, there are several recommendations that need to be considered by future work as well as by the construction industry, this is incudes, but is not limited to the following:

- This work need to be expanded upon and further validated through investigations covering various types and higher dosages of bacteria, different precursor compounds and immobilization techniques.

- Conducting further studies to better explain the adverse effect of using of burned Bentonite as immobilization technique for bacteria in mortar.

- Future studies should cover long term testing as well as exposure conditions.

- Rapid chloride permeability test should be avoided as it will have significant adverse effect on the transformation of spores to vegetative cells; permeability test using only water should be considered.

- Further studies should tackle the applicability of using self-healing mechanism for structures immersed in sea water.

- Investigating the rate of healing of bacteria using ultrasonic pulse velocity for higher number of samples.

- Experimenting on a larger models or prototypes is an essential step for the widespread and adoption of this relatively new technique.

- The construction industry needs to consider this new technique in structures of strategic nature as well as those where detection of damage, accessibility or repair effectiveness are in doubt.

- Studies should be conducting on the effect of using bacteria in grout materials.

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- Feasibility studies must be conducted in order to evaluate if it is economic to add bacteria and Diatomaceous earth (DE) to cement mortar and concrete or not and to discover the possible potential applications for incorporating a bio additive in concrete especially and in construction materials in general.

- The rapid chloride permeability tests that have been conducted in this study should be further examined and validated.

- Further studies should be carried out on the negative effects of Bacteria and Diatomaceous earth (DE) on human health and how these effects could be mitigated.

- Codes of practice should be introduced for testing construction materials with bacteria to determine how to choose the suitable percentage to be added to concrete and the appropriate water to cement ratio for each application.

5.5 Recommendation to concrete Application

- More quality control on the Bacteria number is needed to avoid excessive trial and error cycles in its production

- Companies that manufacture special mortars and repair grouts need to consider the use of bacteria in marketed products for sealing micro cracks upon extensive testing.

- It is recommended for companies considering production to include bacteria and calcium lactate initially in the mixing water that would be used for concrete production.

- It is recommended for future production to perform initial mixing of bacteria, Calcium Lactate and Diatomaceous earth then mixing the rest of the mixture materials.

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- Because small number of bacteria may have profound impact on cement mortar, it is essential to implement rigorous quality control on all stages of mortar/concrete manufacturers

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