ROLE OF MIXING OF TWO BACTERIA ON COMPRESSIVE STRENGTH, WATER ABSORPTION AND MICROSTRUCTURE OF MORTAR CUBES

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Title

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Major/minor cracks is inevitable in concrete because of its lower tensile strength and different load and non-load factors. Addition of bacteria in mortar is an emerging concept. Despite the fact that the live cells has proven to be beneficial towards enhancement of several concrete properties, the trend of increment in the compressive strength has not been significant with addition of single bacteria. This study introduces a new approach of mixing two bacteria: *B Subtilus* and *B Megaterium*, and investigating the role of the microbes on compressive strength, water absorption and SEM analysis. The results demonstrated an increment of compressive strength by 18.09 % when two bacteria's were mixed. Also, cubes with *B Megaterium* absorbed 17.03% less water than normal cubes. This new method of mixing bacteria can potentially solve major/minor concrete cracking issues, could be economical in the long run, and is an environment friendly approach.

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LIST OF ABBREVIATIONS

ACI.....American Concrete Institute.

ASCE .....American Society of Civil engineers.

ASTM .....American Society for testing and materials.

CTM.....Compression testing machine.

NC.....Normal cube.

BS.....*Bacillus Subtilus.*

BM .....*Bacillus Megaterium.*

BS+BM .....*Bacillus Subtilus+ Bacillus Megaterium*.

Div.....Mix.

ATCC.....American type culture collection.

Cells/ml.....Number of cells per milliliter.

NB.....Nutrient broth.

SEM .....Scanning electronic Microscope

XRD .....X-ray diffraction.

EDX .....Energy-dispersive X-ray spectroscopy

OPC.....Ordinary Portland cement.

E coli .....Escherichia coli.

et al.....others.

Fig .....Figure.

LWAC .....Light weight aggregate concrete.

MICP.....Microbiologically induced calcite precipitation.

Mm.....millimeters.

KN.....Kilo newton.

Mpa .....Mega Pascal.

gms.....grams.

KN/mm^2.....Kilo newton per millimeter square.

rpm.....revolutions per minute.

WA.....Water absorption

$CaCl_2$.....Calcium chloride

$CacO_3$.....Calcium carbonate.

CO_2.....Carbon dioxide.

1. INTRODUCTION

1.1. Background

Concrete is a composite material which comprises of cement, coarse aggregates, fine aggregates, water and sometimes admixtures, fibers or other cementitious materials. In the construction world, concrete is taken for granted not only because it is a predominant material, but also because it is highly sustainable, can be cast into any shape and size, is fire resistant, is susceptible to all kind of weathers and is highly economical (Mindess, 2003). In addition to 1.5 billion tons of cement that is being consumed today, the concrete industry is annually consuming 9 billion of tons of sand and rock together with 1 billion tons of mixing water. (Mehta, 2006) However, this most commonly used building material has some fatal drawbacks. The most predominant problem with concrete is that it cracks. Numbers of concrete, especially ones exposed to industrial and urban environments, chemicals and seawater reports premature detoriation. Research done has shown that it is the presence of cracks, not their widths that has the greatest influence on the durability of concrete (R.T.L Allen, 1998)

The formation of major/minor cracks has grabbed attention of many researchers. Many conventional methodologies have been investigated such as reducing water content in the mix. Furthermore gels and resins have been used to fix the crack and replacing concrete with green concrete has been used as well – all of those have been proven not to last long. Conventional methods have a number of disadvantages including degradation over time, a need for constant maintenance, potentially expensive materials, mismatch color of concrete, and concerns with environmental pollution. Therefore, bacterial concrete has been proposed as an environmental friendly crack repair technique.(Van Tittelboom, De Belie, De Muynck, & Verstraete, 2010). The history of microbial technique backs a decade, Gollapudi et al, were the first to introduce

this novel technique in fixing cracks with environmentally friendly biological processes. (Types, 1995). Addition of bacteria to the mix is one of the preferred method not just because it improves the strength but also because it has been found to be very helpful in various aspects: healing abilities of major/minor cracks, environment friendly, cost effective in long terms, and overall improving the properties of the cementitious material present in the mix. Bacterial concrete can be defined as a product that will biologically produce lime stone as a byproduct of process like photosynthesis, sulfate reduction and urea hydrolysis that helps in self-healing of cracks to some extent and mostly increase the compressive strength of the concrete. However, the main role of those microbes in carbonate precipitation is still not clear (Van Tittelboom et al., 2010).

It has been hypothesized that almost all bacteria are capable of $CaCO_3$ production because precipitation occurs as a byproduct of common metabolic processes such as photosynthesis, sulfate reduction, and urea hydrolysis(Chahal, Siddique, & Rajor, 2012a). Bacteria suspended at a certain concentration was found to be effective in remediating major/minor cracks, increasing compressive strength (Ramachandran, Ramakrishnan, & Bang, 2001). The ability of these bacteria to precipitate calcite layer under favorable condition, and their capability to survive in dormant state for 100 of years, regardless the environmental condition makes this bacterium the most commonly used one (Chahal, Siddique, & Rajor, 2012b). From enhancement in durability of cementitious materials to improvement in sand properties, from repair of monuments, to increment of compressive strength, from being environment friendly to being cost effective bacterial concrete is successful in all aspects.

One potential solution to increase the strength of the concrete is increment of compressive strength. Despite of the advancements in the part of using fly ash, and other chemical admixtures, several methodologies, there is still a critical need to solve the issue of

concrete and improve its strength without affecting the environment. (De Muynck, De Belie, & Verstraete, 2010).This study is based on the two most commonly used bacteria. *Bacillus* bacteria are well known for their capability of surviving in adverse environmental conditions.

1.2. Problem statement and purpose of study

Over the past decade, addition of different ureolytic bacteria, in concrete/mortar mix with varying concentrations of 10^3-10^8 cells/ml has been investigated. Several researchers have focused on property enhancement of mortar/concrete with a single high bacterial concentration technique which was not quite successful. Addition of bacteria in mortar is an emerging concept and despite the fact that these live cells has proven to be beneficial towards enhancement of several properties like flexural strength, tensile strength, compressive strength, water absorption and rapid chloride properties of concrete/mortar, the trend of increment in the compressive strength has not been reported in a very significant amount. The prime strategy of mixing of two bacteria in mortar cubes relies on calcite precipitation, and how this could contribute towards enhancement of properties of mortar/concrete. None of the researchers in the past have explored how mixing of two bacteria in a single mortar cube could contribute towards the properties of the concrete.

The development of an effective methodology by mixing of two bacteria for solving major/minor issues associated with concrete and mortar may be able to reduce repair and maintenance cost substantially, without adversely affecting the environment and economy. Bacteria in concrete is a complex method and has not been defined in a proper way. The purpose of this study are to perform experimental studies to improve the compressive strength and durability of mortar by mixing of two bacterial cells. Live bacteria (*B Subtilus* and *B*

Megaterium) cells that are well known to be able to produce calcite, of calcium carbonate crystal, and their benefit in the mortar cubes are used in this study.

1.3. Research objective and research goals

The major goal of this research is to explore the novel role of mixing of bacterial cells and using it as a mortar admixture to increase the mechanical strength and water absorption of mortar in an environmental friendly way.

Figure 1.1 is a schematic representation of the goal this research is trying to accomplish. The main objectives of this research has been summarized as follows:

1. To determine the significance of mixing two bacteria on properties of mortar cubes: on compressive strength and water absorption properties.
2. To determine if mixing of two bacteria could be beneficial and significantly reduce the maintenance cost of mortar/concrete.
3. To investigate the role of bacteria's on the mortar cubes through SEM.

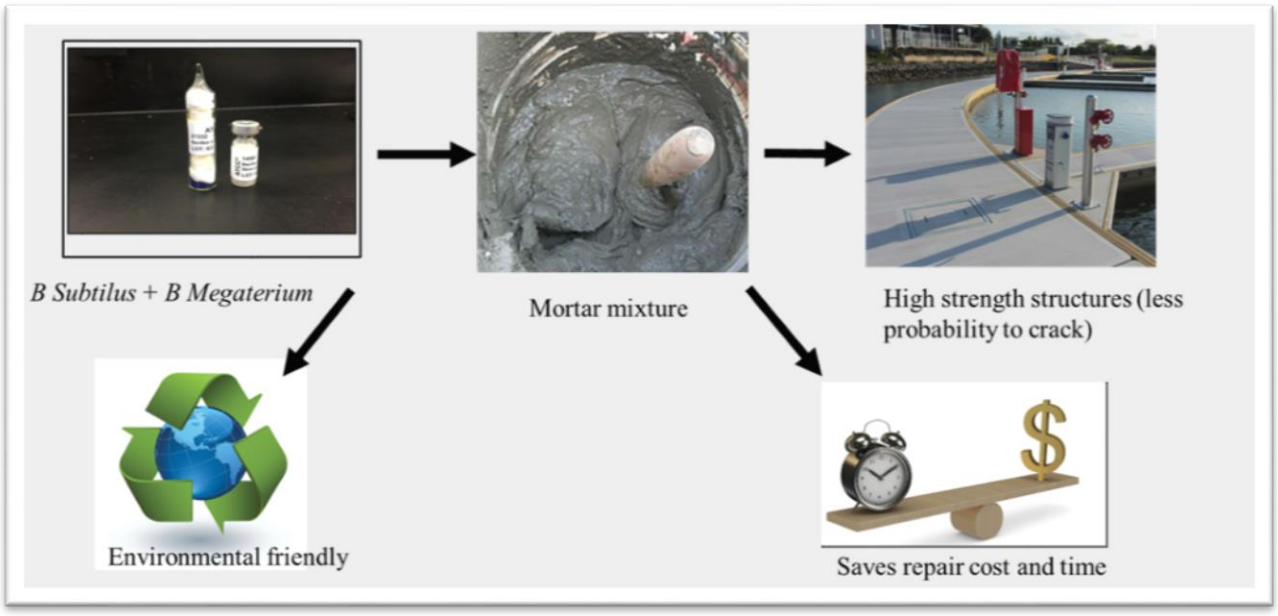

Figure 1.1. Schematic representation of research goal.

1.4. Research methodology

This research was carried out mostly to find out the effect of two different bacteria and how they influence the characteristics of a mortar cube when they are mixed together. The major objective of this research is to perform theoretical and experimental studies of mixing bacteria and how they influence the characteristics of mortar cubes. Theoretical studies of all the previous literatures was done to determine what bacteria was suitable for mixing together. Experiments were designed likewise for four different mixes. The study was narrowed down to compressive strength and water absorption test, and SEM analysis on respective mixes. The results were analyzed and then a conclusion with some final recommendations for future were made.

The steps involved in the research is explained in the steps below and also in the figure 1.1:

1. Literature review: The literature review was carried out by looking up and collection of articles, papers, and thesis performed on addition of bacteria in concrete/mortar. The previous literature was studied theoretically in details.
2. Narrow scope of study: The past studies talked about studies in several types of bacteria and analysis of various properties. The scope of study was narrowed down to what bacteria worked the best, what concentration of bacteria is to be used, and what studies needed to be carried out.
3. Experimental design: The next important step was design of experiment. The experiment was designed using ASTM C 109 M. Bacteria was prepared in the laboratory, and mortar cubes were casted, and. Four different mixes were designed for a comparative study of mortar cubes with no bacteria, mortar cubes with Bacteria 1, mortar cubes with Bacteria-2 and Mortar cubes with Bacteria (1+2).

4. Experimental conduction: Compressive strength tests were carried out on these cubes using CTM on 1 day, 3 days, 7 days, 14 days and 28 days respectively. Water absorption tests were carried out on the cubes of all Mix on 28^{th} day using respective ASTM.
5. Analysis of results: The difference in the compressive strength and water absorption properties of these mortar cubes on different days and different mix were compared and analyzed from the results obtained. The results were explained with SEM images from the samples obtained at the 28^{th} day.
6. Conclusion and recommendations: On the basis of results and analysis, the research was concluded with some further recommendations for future researches.

Figure 1.1 explains the step by step procedure followed in this research.

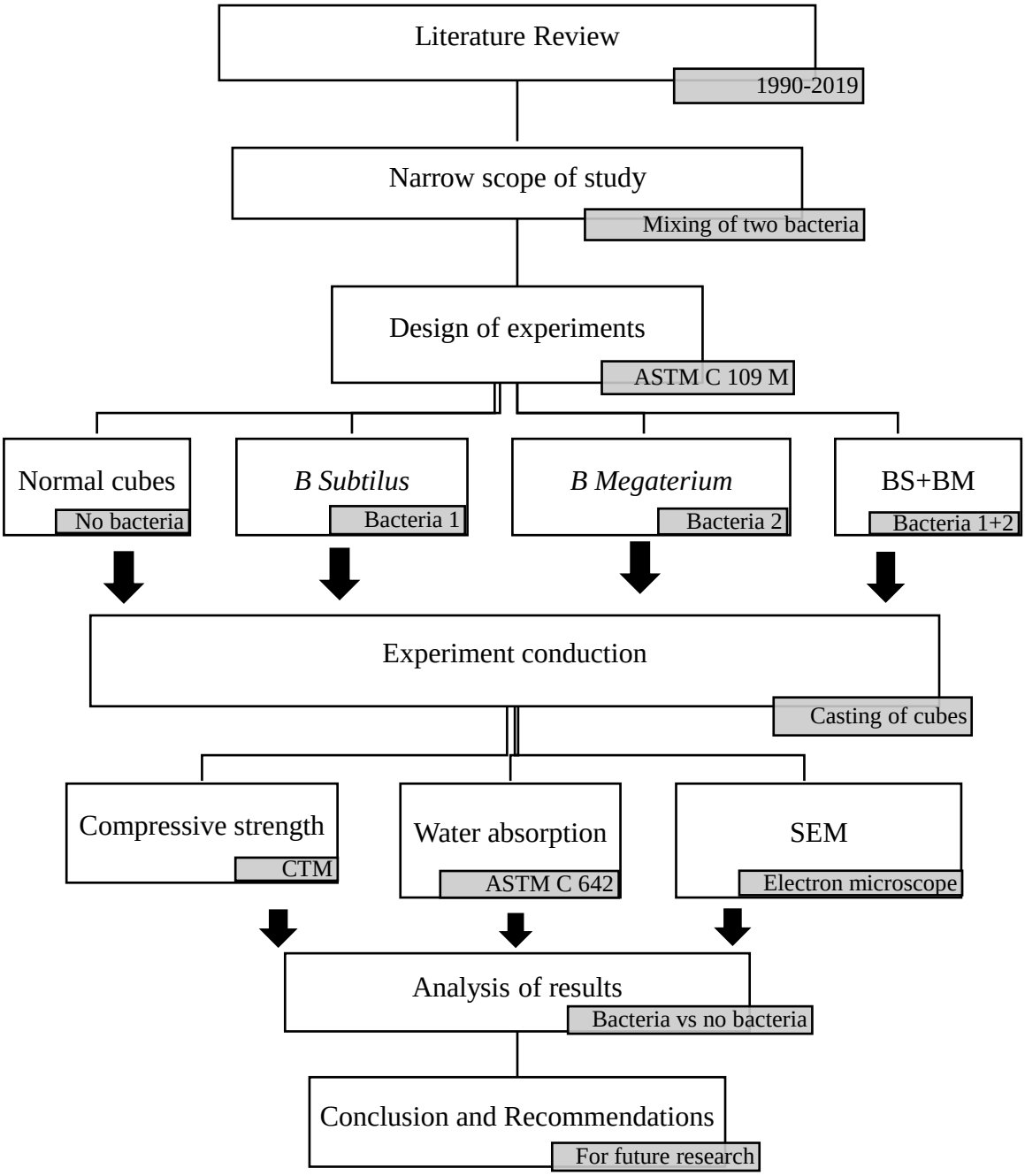

Figure 1.2. Research methodology.

1.5. Content organization

This research is classified into a total of five chapters. Chapter one is the introductory art, background, the purpose of the study and also the problem statement this research is focused on.

It also explains in details how the research was carried out. Chapter two discusses about previous literatures and researches and provides an overview of the need of this research. Chapter three discusses the scope of work, the materials and methodology and experimental design. Chapter four discusses all the results obtained from the tests carried out i.e. the compressive strength test using CTM, water absorption tests, and the SEM analysis is discussed briefly. Chapter five is a comparative study of all the experiments and analysis. Chapter six concludes the entire research and provides recommendations for future research respectively.

2. LITERATURE REVIEW

2.1. General

Bacterial concrete has been an issue of interest for several reasons. From being able to increase the compressive strength of the concrete to healing the cracks in a concrete structure and being able to lower the cost of maintenance/repair, it certainly is a factor that can potentially solve a lot of existing issues in construction industry.

The literature review presents the current state of research on role of microbes and bacteria on compressive strength and water absorption of mortar cubes. While, the researches in the past revolved around factors like types of bacteria, carrier compounds, life of bacteria, activation of bacteria, remediation of cracks and fissures, strength and durability properties, bacteria in hot and humid environment and in wet conditions and introduction technique. (Ramachandran et al., 2001).(De Muynck et al., 2010) (Chahal et al., 2012a). It has been observed that, bacteria has been beneficial in the concrete mix, but not in a significant amount. Also, none of the researchers in the past have explored the idea of mixing of two bacteria in a single mortar mix could contribute towards the properties of the concrete.

From the literature review, the various types of bacteria used in mortar/concrete is shown in the figure below.

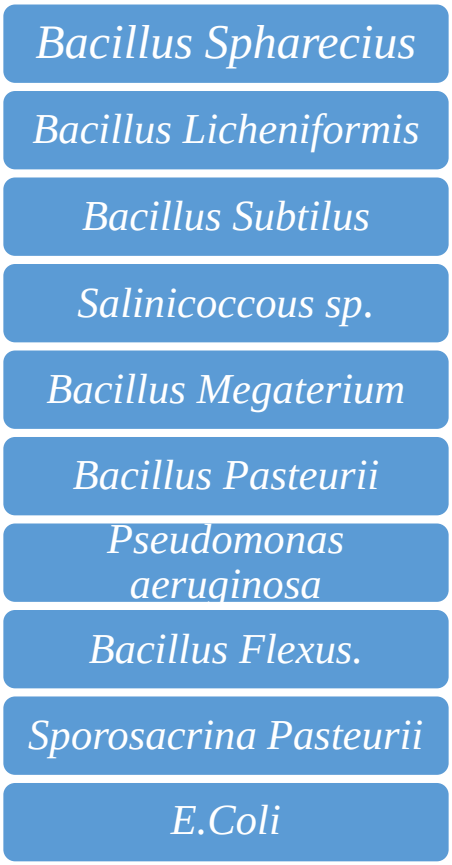

Figure 2.1. Various types of bacteria used in concrete.

Fig 2.1 shows different types of bacteria that has been most commonly used in the past in concrete/mortar to enhance the properties. Of all the bacteria, the researchers has concluded that *Bacillus* bacteria has the tolerance to high alkaline environment, has capability to form endospores and has a capability to survive in dormant state for 100 of years(Wiktor & Jonkers, 2011). Analysis of properties like compressive strength, water absorption, flexural strength, rapid chloride permeability and several others were performed, and when added at a certain concentration. The comparative study of previous literatures concluded how *Bacillus Subtilus* and *Bacillus Megaterium* were the two bacteria that altered the properties in a significant way. Therefore, this literature review explains in details about past experiments on *Bacillus Subtilus*, *Bacillus Megaterium* and few other bacteria that has been used in the previous experiments.

The literature review has been divided into three parts on the basis of different bacteria's used in the previous literatures:

2.1.1 Previous research on compressive strength of mortar cubes using bacteria

2.1.2 Previous literatures on analysis of water absorption using bacteria and

2.1.3 Previous literatures on SEM analysis of bacterial mortar/concrete cubes.

2.1.1. Previous research on compressive strength of mortar cubes using bacteria

2.1.1.1. Bacillus Subtilus

B Subtilus is one of the most used and effective bacteria in concrete/mortar. (Sunil Pratap Reddy, Seshagiri Raob, Aparnac, & Sasikalac, 2010) analyzed the compressive strength at 3 days, 7 days and 28 days for different cell concentrations and it was observed that the compressive strength of cement mortar showed a significant increase by 16.15% for cell concentration of 10^5 cells per ml of mixing water. The author also concluded that *Bacillus Subtilis* genes are safe to use, not very difficult to culture and grow and can widely be used in improving the performance characteristics of concrete. (Jonkers, Thijssen, Muyzer, Copuroglu, & Schlangen, 2010) found that alkali-resistant spore-forming bacteria related to the genus *Bacillu*s represented promising candidates for application as self-healing agent in concrete and probably other cement-based materials. The author mentioned that the bacterial spores directly added to the cement paste mixture remained viable for a period up to 4 months. The cement stone incorporated bacterial spores are able to convert incorporated calcium lactate to calcium carbonate-based minerals upon activation by crack ingress water.

(Vempada & Reddy, 2011)used three different kinds of bacteria and of the three, *B Subtiluis* has offered a substantial improvement in compressive strength of cement mortar. The greatest increase reaches to 19.26% at 28 days for 10^5 cells/ml. Out of all isolated cultures

developed and tested, it was observed that, *Bacillus Subtilus* has offered the substantial improvement in compressive strength of cement mortar. The author also suggested that the choice of microorganism is important if mortar compressive strength has to be improved.

(Fedko, 2012) observed that the cubes mixed with *B Subtilus* had lowest compressive strength at 7 days and 35 days compared to the water based specimens. At laboratory conditions, *B Subtilus* showed a lower compressive strength, 11% at 7 days, 8% at 35 days and 16 % at the age of 56 days. (Reddy, 2004)found out that addition of *Bacillus Subtilis* bacteria improves the hydrated structure of cement in concrete for a cell concentration of 10^5 cells per ml of mixing water. The addition of bacteria increased the compressive strength of concrete. The compressive strength is increased nearly 23% at 28 days for ordinary, standard and high grades of concrete when compared to controlled concrete. (Pei, Liu, Wang, & Yang, 2013)also confirmed that the cell walls of *B. Subtilis*, accelerated $CaCO_3$ formation and the bacterial cell walls significantly increased compressive strengths of concrete by 15% concluding how it could act as a promising concrete admixture with benefits in enhancing mechanical performance and improving other carbonation-related properties. (Mondal & Ghosh, 2018) added *B Subtilus* in concentration 10^3, 10^5 and 10^7 cells per ml, and it was seen that higher the concentration of cell, higher is the precipitation amount and rate, and the strength improvement was seen at 10^5 cells per ml at all ages which is almost 27 % in comparison to control specimens. (Nain et al., 2019) also performed a research on the compressive strength by addition of *B Subtilus* and determined an increment by 14.36 % respectively at the 28^{th} day. The author also mentioned that the microorganism demonstrated a positive role in not only enhancing the strength of concrete but also facilitates self-healing of cracks.

2.1.1.2. Bacillus Megaterium

(Varenyam Achal, Pan, & Özyurt, 2011) performed a compressive strength of cement mortar cubes with fly ash addition and the samples were tested at 3, 7 and 28 days. It was found that higher the concentration of fly ash, lower was the compressive strength with all the samples (with or without bacterial cells). The experiment demonstrated an improvement of 21 % in compressive strength at 28 days with respect to control specimens (without fly ash). Specimens containing 10 % fly ash by bacterial cells showed an increment of 19 %, and at 20 % fly ash concentration in mortar enhanced 14 % compared to control specimens.(Krishnapriya, Venkatesh Babu, & G., 2015) annalyzed bacterial concrete casted with *B Megaterium*, yielded compressive strength which amount to 12.01 %, compared to control concrete specimens. The bacterial strains exhibited high urerease activity, they formed endospores and precipitated Calcium carbonate. (Mirza et al., 2016) performed a research with five different concentration of cells, ($10X10^5$) to ($50X10^5$) cfu/ml and it was found that strength of higher grade of structural bacteria increased compared to lower grade due to precipitation of calcite. The maximum rate of strength development was 24%. (Nain et al., 2019)performed a research on the compressive strength by addition of *B Megaterium* and other bacteria. The author mentions that *B Megaterium* demonstrated the highest strength and also slower strength gains in *B. Megaterium* when compared others i.e. 22.58 % respectively on the 28^{th} day.

2.1.1.3. Others

(Ramachandran et al., 2001)observed at lower concentration *B Pasteurii* increased the compressive strength of mortar cubes. Cubes with live or dead cells mass decreased as cell concentrations and curing time increased. This was attributed to the fact that cells got good nourishment in the start cause mortar was still porous, and upon calcite precipitation mortar was

less porous, plugging the flow of nutrients to the bacterial cells. (Van Tittelboom et al., 2010)used silica gel to protect *B Sphaericus* the high pH in the concrete. Protection of the bacteria against this gel matrix seemed very effective as $CaCO_3$ crystals were precipitated inside the matrix which was not the case if bacteria were used without immobilization in silica gel.

(Vempada & Reddy, 2011)used *Bacillus Pasteurii, Salinicoccous sp and Bacillus Subtilus* for study of the compressive strength of mortar cubes, and concluded that the strength increased at all levels of microorganism addition except for E.*coli* where observed changes in compressive strength is almost nothing at all ages and for all cell concentrations. The author suggests that the choice of microorganism is important if mortar compressive strength has to be improved. (Chowdhury, Mandal, Sarkar, Majumdar, & Chattopadhyay, 2012)noted that compressive strength of mortar cubes augmented with bacterial cells (BKH1)Protein (Bioremediase) at every stages of curing, compared to control specimens were higher.40.6 % of strength after 28 days of curing and 41.8 % after 120 days of curing was observed by addition of bacterial cells in cement sand mortar directly at the concentration of 10^5 cells per ml. (Fedko, 2012) mentioned the highest compressive strength was shown by cubes embedded with *S Pasteurii* bacteria. At laboratory conditions *S Pasteurii* specimens had higher compressive strength, 46 % at 7 days, 25 % at 35 days and 26 % at 56 days compared with water based specimens. (Chahal et al., 2012a)performed an analysis where cement was replaced by 5% and 10 % of silica fume by weight. In silica fume concrete, at 28 days there was an improvement of compressive strength with 5 % silica fume and 10^5 cells/ml bacterial cells. *(Sporosacrina Pasteurii).* Drastic improvement in compressive strength was seen with 10% silica fume and 10^5 cells/ml bacterial cells. Increase of compressive strength is mainly due to consolidation of pores

inside the cement mortar cubes with $CaCO_3$ precipitation which plugs the pores within the mortar.

(Jagadeesha, Prabhakara, & Pushpa, 2013)indicated that there was an improvement in the compressive strength of cubes which were reduced with time. Among *B Flexus, B Pasteurii and B Spharecius*, cubes treated with *B Flexus* bacteria which is not reported for calcite precipitation has shown maximum compressive strength than other two bacterial strains and control cubes .Improvement in compressive strength reaches a maximum at around 18 %as compared to control specimens. (Krishnapriya et al., 2015)experimented concrete casted with *B Lincheniformis* and *B Flexus* and observed that the samples yielded a compressive strength 10.06% and 6.1 %. The author suggested that all these bacteria's exhibited high urerease activity, they formed endospores and precipita*ted* Calcium carbonate. (Siddique at al 2016) used Alkaliphilic, alkali tolerant (AKRR) and the cement was substituted with 5, 10 and 15 % silica fume in concrete by weight and at 28 days, nearly 10-12 % increase in compressive strength was observed on incorporation of bacteria in Silica Fume concrete. At 28 days to 56 days all specimen exhibited higher strength. This was due to continuous hydration of cement and pozzolanic action of Silica fume in concrete. (Hosseini Balam, Mostofinejad, & Eftekhar, 2017b)researched and found that specimens remediated with bacteria exhibited higher compressive strength at all curing days. The specimens treated with bacteria exhibited (about 38 % increase) and a faster trend in increasing compressive strength. Using bacteria in LWAC makes denser and less permeable microstructure.

All the previous literatures mentions how the use of bacteria to enhance the compressive strength of mortar cubes has been very successful over the past years. The most effective of all the bacteria used was observed to be genes of *Bacillus*. They have proven to increase the

compressive strength of mortar cubes in a significant way when added at a certain concentration. The authors in the past has revolved around the factors like different concentrations of bacteria, different carrier compounds or replacing cement by Fly ash, LWAC etc. concept that has never been explored by previous researchers is mixing of two bacteria's in the mortar cube. The main research goal is to analyze the mixing two bacteria on compressive strength of the mortar cubes.

2.1.2. Previous research on water absorption of mortar cubes using bacteria

2.1.2.1. B Subtilus

(Mondal & Ghosh, 2018) presented a research where presence of bacteria at mortar cubes reduces water absorption at all ages. Moreover, the water absorption decreases with increase in bacterial cell concentration. At 10^7 cells/ml the water absorption reduced by 27 % at 28 days when compared to control specimens.

2.1.2.2. B Megaterium

(Varenyam Achal et al., 2011) used *B Megaterium* and in 7 days, cubes amended with fly ash (0 %, 10 % and 20 %) with bacterial cells absorbed 3.5 times less water than control cubes. Cubes containing 40 % fly ash mortar cubes, absorbed two times less water than control specimens. (Hosseini Balam et al., 2017b) concluded water absorption of the sample submerged in water and Urea- $Cacl_2$ solution decreased over time. Water absorption of LWAC specimens was observed to significantly reduce. The reduction was attributed to the calcium carbonate that filled the pores of the specimens.

2.1.2.3. Others

(Chahal et al., 2012a)used *Sporosacrina Pasteurii* on water absorption capacity of fly ash concrete and observed that it decreases with increase in bacterial concentration. Maximum reduction of water absorption was significantly influenced by addition of bacteria and reduction

was in a range of 50- 70 % in sorptivity coefficient of specimens at 28, 56 days. Bacteria cause change in microstructure of specimen, decreasing the water transport properties of the specimen absorbed with 10^5 cells/ml for all fly ash concretes. Concrete with 10 % fly ash concrete showed a minimum water absorption 3.25 % (minimum).

Water absorption test is necessary to determine the increase in resistance towards water penetration in concrete/mortar. All the previous researches demonstrated that presence of bacteria decreased the water uptake compared to the ones with no bacteria. Water absorption contributes towards durability of the concrete/mortar. The bacterial action deposition can seal the pores, voids, and micro cracks. This research is based on determining if the mixing of two bacteria in the mortar cubes decreases the water uptake in mortar cubes.

2.1.3. Previous literatures on SEM analysis of bacterial cubes

2.1.3.1. Bacillus Subtilus

(Vempada & Reddy, 2011)concluded that improvement of hydrated structure of cement sand mortar was seen using bacteria of 10^5 cells/ml. Cracks were sealed by crystalline materials grown over the surface due to microbial activity of the bacteria.(Reddy, 2004)concluded a deposition of a layer of calcite crystals on the surface of the specimens resulted in a decrease of permeability of water and other liquids in concrete. (Fedko, 2012)performed an analysis that the water based specimens had a porous structure, while both media based and bacterial specimens had more crystalline structure, and reduced porosity. (Mondal & Ghosh, 2018) also concluded that the precipitation of $CaCO_3$ both at the surface and inner matrix of mortar samples was observed, which indicated that even in the absence of external Calcium sources, free Calcium oxide already present in the cement can serve as calcium source. The precipitation layer produced acts as a shield to the mortar, and can protect the inner matrix, and also prevent

harmful substances from entering into the mortar. The precipitation of $CaCO_3$ both at the surface and inner matrix of mortar samples was observed, which indicated that even in the absence of external Calcium sources, free Calcium oxide already present in the cement can serve as calcium source. The precipitation layer produced acts as a shield to the mortar, and can protect the inner matrix, and also prevent harmful substances from entering into the mortar. (Nain et al., 2019)concluded that upon visual inspection, the specimen incubated with bacterial water, showed the presence of Calcium Carbonate, and has feasibility to manage micro cracks and enhance the strength of the concrete.

2.1.3.2. Bacillus Megaterium

(Varenyam Achal et al., 2011) performed an analysis with *Bacillus Megaterium* in mortar and concrete specimens and by visual inspection by SEM, a dense growth of calcite crystals embedded with bacterial cells was observed in the specimens. This deposition served as a barrier to harmful substances from entering the sample and thus improved impermeability. (Krishnapriya et al., 2015)confirmed the presence of distinct calcite crystals in bacterial concrete that has increased the compressive strength and contributed to crack healing. (Mirza et al., 2016)performed an analysis with five different concentration of cells, ($10X10^5$) to ($50X10^5$) cfu/ml and through SEM analysis it was conformed that microbial calcite precipitation was more in 30 X 10^5 cells/ml which was also confirmed by EDX and XRD analysis.(Nain et al., 2019)confirmed upon visual inspection, the specimen incubated with bacterial water, showed the presence of Calcium Carbonate, and has feasibility to manage micro cracks and enhance the strength of the concrete.

2.1.3.3. Others

(Ramachandran et al., 2001)performed a SEM analysis and samples showed calcite crystals grown all over the sand particles. On closer observation, it was found that Calcium Carbonate crystals were well developed near the surface of the crack. This behavior evidenced by SEM suggests that microbial remediation is more effective in shallow crack. (Chahal et al., 2012a)used *Sporosacrina Pasteurii* and the SEM analysis revealed distinct Calcite crystals embedded in concrete. High Calcium amounts in it confirmed that calcite was present in the form of calcium carbonate due to bacteria. (Vempada & Reddy, 2011)used *Bacillus Pasteurii, B Subtilus* and *Salinicoccous sp* for the improvement of hydrated structure of cement sand mortar was seen using bacteria of 10^5 cells/ml. Cracks were sealed by crystalline materials grown over the surface due to microbial activity of the bacteria. (Krishnapriya et al., 2015) added *Bacillus Megaterium, Bacillus Licheniformis and Bacillus Flexus* and the presence of distinct calcite crystals was confirmed in bacterial concrete that has increased the compressive strength and contributed to crack healing. Siddique et al 2016 used *Alkaliphilic/Alkali tolerant (AKRR5) and S*EM analysis revealed the presence of calcite in the samples incorporating bacteria. The formation of Calcium Silicate hydrate (CSH) and portlandite (CH) and pores was observed in all concrete samples. SEM analysis showed dense microstructure of concrete and less pores and voids in bacterial silica fume concrete.

Scanning electron microscope (SEM) analyzes calcite precipitation in concrete/mortar and explains the importance of this analysis to determine the role of microbes in the concrete. All the previous researches concluded that upon visual inspection that concrete has capability to manage micro cracks and can contribute to enhance the strength of the concrete. None of the previous researchers has performed a SEM analysis on mixing of two mortar cubes in a single

mixture. Through this research, an investigation of mixing *B Subtilus and B Megaterium* in a mortar cube will be performed through SEM analysis.

2.2. Summary

The history of addition of microbes in the concrete/mortar is several decades old. From the literature review it was clear that addition of bacteria in mortar is an emerging concept and despite the fact that these live cells had proven to be beneficial towards enhancement of several properties like flexural strength, tensile strength, compressive strength, water absorption and rapid chloride properties of concrete/mortar, the trend of increment in the compressive strength reported was not quite significant. None of the researchers in the past have explored how mixing of two bacteria in a single mortar cube could contribute towards the properties of the concrete. Different bacteria's *B Subtilus, B Megaterium*, and few others were seen to be used in the past in separate mixes, and have improved properties of concrete/mortar. This research studies the role of mixing of two bacteria's on the properties of mortar mix. The prime strategy of mixing of two bacteria in mortar cubes relies on calcite precipitation, and how this could contribute towards enhancement of properties of mortar/concrete. Live bacteria (*B Subtilus* and *B Megaterium*) cells that are well known to be able to produce microbial bio mineralization, was used in this research to determine their role in the properties of mortar cubes.

3. PROCEDURE AND TEST PREPARATION

3.1. Scope

To meet the goal of this research, we define the scope of work as follows. Two types of bacteria: *B Subtilus* and *B Megaterium* were used for the experiments. All experiments were performed on normal room temperature. The use of bacteria in the mortar cubes was done by adding bacterial media prepared in the laboratory with mixing water. Mortar cubes were casted into 4 Mix:

- Mix 1-Mortar cubes with no bacteria.
- Mix 2: Mortar cubes with only *B Subtilus.*
- Mix 3: Mortar cubes with only *B Megaterium.*
- Mix 4: Mortar cubes with *B Subtilus + B Megaterium*.

3.2. Materials

3.2.1. Cement, sand and water

Ordinary Portland cement (OPC-Type 1) was used for this experimental work and it was stored in a fresh and dry condition in the laboratory. Figure 3.1 shows OPC Type 1. Sand used in this experimental procedure was all purpose sand. The sand was stored in a dry and fresh condition. Figure 3.2 shows a sample of all-purpose sand. Clean potable water was used for the mix. It was not only used for mixing the mortar, but also for curing the cubes. Table 3.1 shows the details of cement, sand and water.

Figure 3.1. Ordinary Portland cement (Type 1).

Figure 3.2. All-purpose sand.

Table 3.1

Details of materials used

Materials used	Sources	Properties
Cement	Ordinary Portland cement, Type 1	Color: Gray; Specific gravity: 3.15
Sand	All Purpose Sand #1152:	Color: White to tan Specific gravity: 2.70.
Water	Clean potable water	Color: Colorless Specific gravity :1

3.2.2. Bacteria and media of growth

Bacillus genes are known to be able to survive in extreme conditions and are also able to increase the strength and durability of the concrete. Therefore, *B Subtilus* and *B Megaterium* was used in this experimental procedure. Difco Nutrient broth was used as a media to grow the bacteria. It is a powdered substance as shown in figure 3.4. The table 3.2 gives the details of bacteria and the media used.

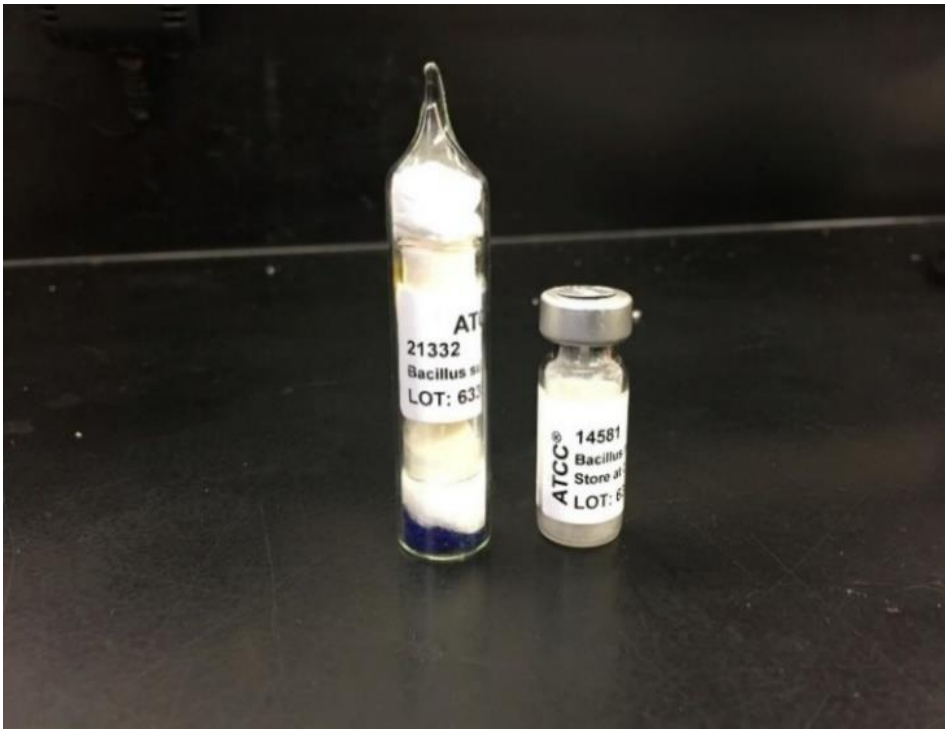

Figure 3.3. *B Subtilus* and *B Megaterium* before growth (ATCC 21332 and ATCC 14581).

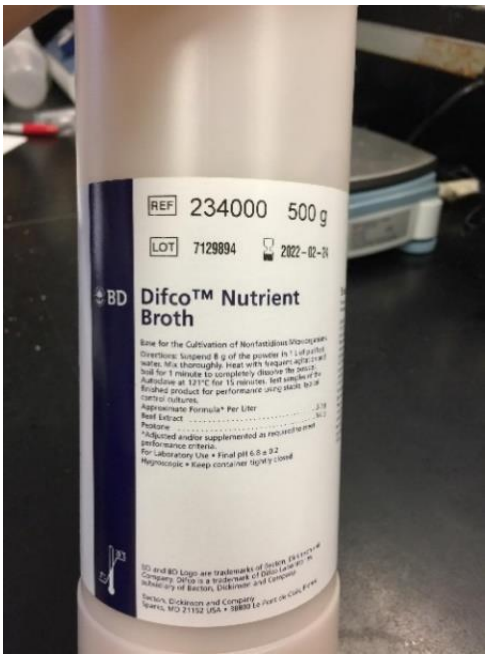

Figure 3.4. Media powder for growth (BD 2340000).

Table 3.2

Details of bacteria and the media for preparation

Bacteria /Media	Sources	Properties
B Subtilus	www.atcc.org (ATCC 21332)	Biosafety Level : 1 Storage Conditions: Frozen: -80°C or colder
B Megaterium	www.atcc.org (ATCC 14581)	Biosafety Level: 1 Storage Conditions: Frozen: -80°C or colder
Media (BD Difco nutrient broth)	www.atcc.org (BD 2340000)	Biosafety Level: 1

3.3. Procedure

3.3.1. Preparation of media

Media preparation of bacteria is one of the simplest step in growing a bacterium. For preparing 1000 ml of sample, take a flask, Suspend 8 grams of powdered mixture in clean water. Mix it thoroughly. Autoclave the mixture at 121 degrees Celsius for 15 minutes. The samples prepared are cooled down and is ready to be used for bacteria growth. (as instructed in the bottle).

The contents per liter of the media as indicated in the bottle are:

- Beef extract-3.0 grams.
- Peptone-5.0 grams.

The media could be adjusted or supplemented as per requirements. For laboratory use, the pH of the media should be maintained at 6.8+-0.2.

Figure 3.5. Preparation of media.

3.3.2. Preparation of bacteria

Stock cultures of *B Subtilus* and *B Megaterium* were suspended in media (Difco Tm Nutrient broth) prepared overnight and stored. The mixture is covered shaken well and left in an incubator at required temperature (T=37 degree Celsius) for 24 hours. Figure 3.6 shows the bacteria in an incubator. The bacteria can be stored in the freezer and is now ready to be used in the mortar mix. The conditions for growth of bacteria in the laboratory is shown in the table 3.3.

Figure 3.6. Incubation of bacteria in an incubator.

Table 3.3

Conditions required for bacterial growth in the laboratory

Bacteria	Media for growth	Temperature/ RPM
B Subtilus	B Difco NB medium	T=37-degree celcius,125 rpm
B Megaterium	B Difco NB medium	T=30-degree celcius,125 rpm

3.3.3. Bacterial growth

It is necessary to determine the growth of bacteria, and the number of microorganisms present in the sample prepared. To determine this, a serial dilution method was performed. For this, plates were prepared as shown in the figure 3.7. The agar plate is prepared by mixing growth medium with agar and then autoclaving to sterilise. Once the agar has cooled to ~50^{o}C approximately 15ml is poured into a sterile Petri dish and left to set. The dish is set aside overnight to cool.

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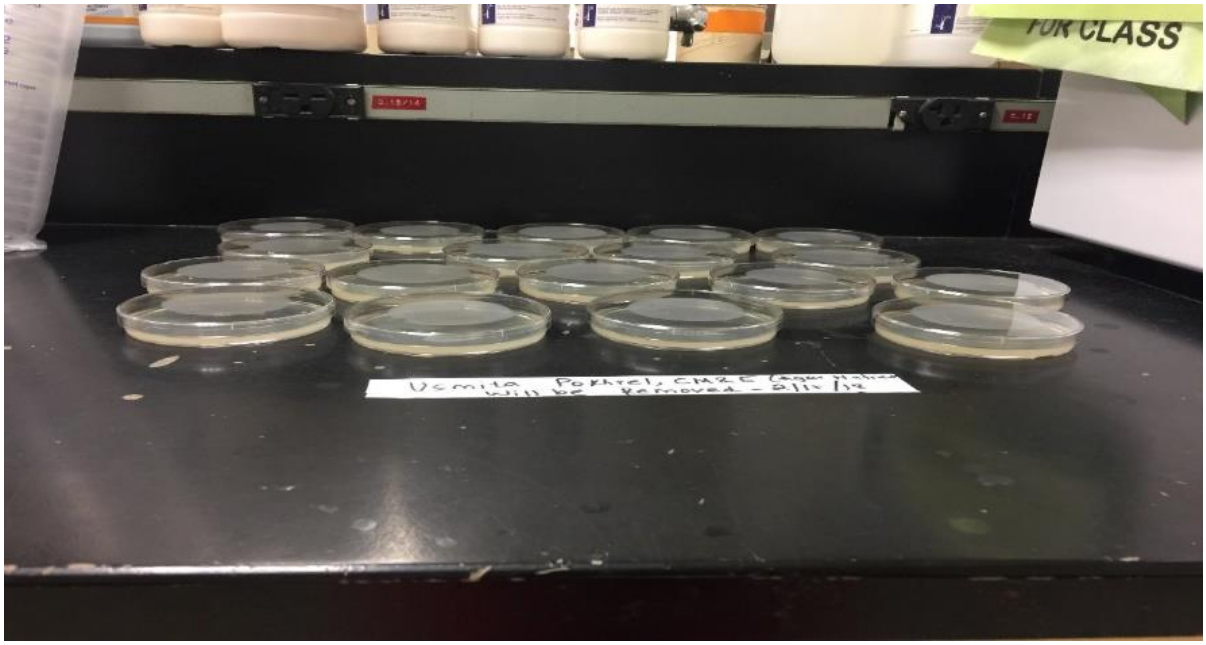

Figure 3.7. Preparation of plates.

The serial dilution method is one of the most fundamental microbiological techniques which is also known as plate counting method. This method is used to determine the number of viable (i.e. living) cells in a sample. There are several steps to the technique and all the steps must be carried out carefully in order to obtain accurate results and to avoid contamination of the samples.

Step one: Diluting the sample

The Nutrient broth prepared with B *Subtilus* and *B Megaterium* has millions or even billions of microorganisms per millilitre of sample which would be almost impossible to count. Therefore, dilution of the sample is carried out. For this method, 1ml of bacterial sample added to 9 ml of a suitable diluent (e.g. sterile buffer), and the sample and diluent are mixed together. This new sample (Dilution One) has a concentration (number of microorganisms per ml)

$1/10^{th}$ that of the original sample. Then, 1ml of dilution one is added to another 9ml of diluent to make dilution two. Dilution two now has a concentration $1/10^{th}$ that of Dilution 1 and $1/100^{th}$ that of the original sample. This process is repeated until we have a series of dilutions. Dilution of bacteria using buffer solution is shown in Figure 3.8.

Step two: Plating the sample

To find out how many viable cells are in each of the dilutions, samples were spread into the plates prepared and kept overnight. 0.1ml of sample is pipetted onto the agar plate and spread around using a sterile glass rod. This is repeated until there are 2 or 3 replicate plates for the original sample and for each dilutions.

Step three: Incubating the plates

Once all of the plates have been prepared they are left to dry and then moved to an incubator at a suitable growth temperature for the bacteria to grow and to form a visible colony of microorganisms. After the incubation period the plates are ready to be counted to determine how many microorganisms were present in the original sample. The plates form bacterial colonies as shown in Figure 3.9.

Step four: Counting the colonies

The plates will have different numbers of colonies depending on the dilution of the sample The plated with too many colonies would be impossible or very difficult to count and the plates with small number of colonies is easy to count, but the results are prone to error. Therefore, the aim would be to count plates with between 25 and 250 colonies. The results noting the dilutions that had between 25 and 250 colonies and how many colonies there were on these plates were recorded.

Step five: Determining how many viable organisms were in the original sample

In this step the results obtained from Step 4 was used. Taking in account the amount by which the sample was diluted in Step 1 and the volume that we put onto the plate in Step 2.The total amount of microorganism is calculated using the following formula:

Number of microorganism = Plate count X Dilution factor

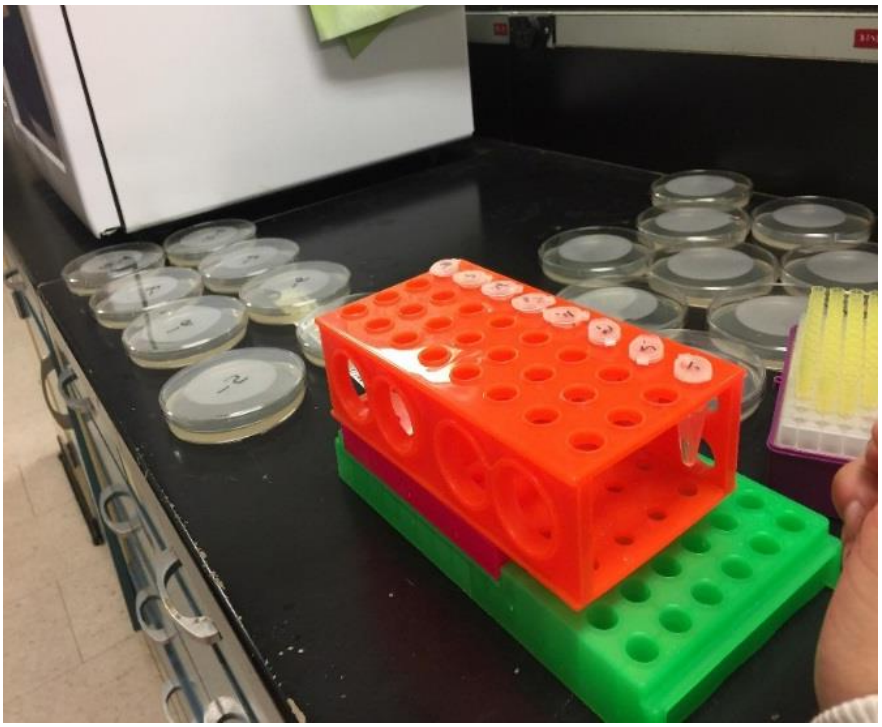

Figure 3.8. Dilution of bacteria using buffer solution.

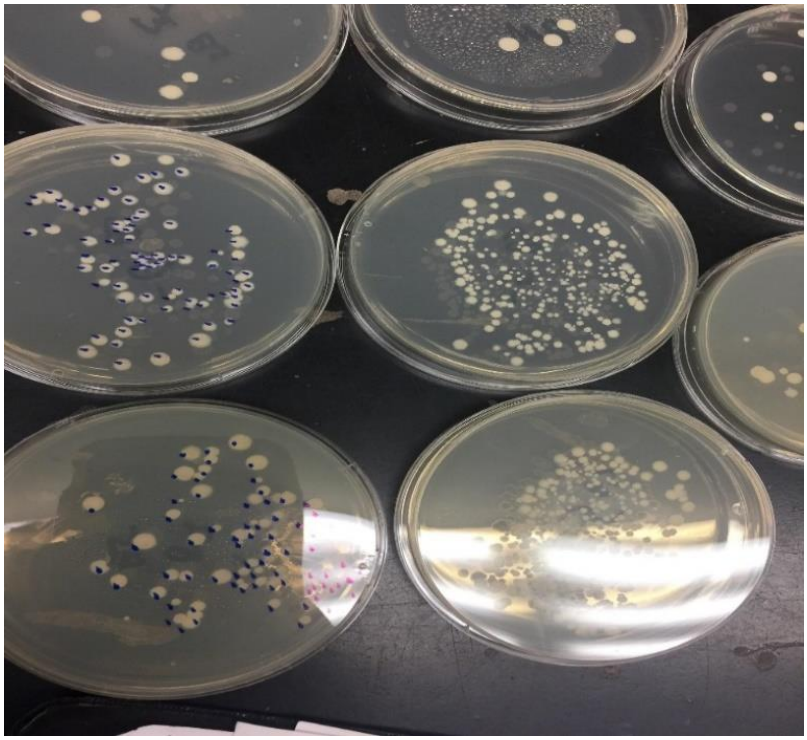

Figure 3.9. Bacterial colonies.

3.3.4. Experimental setup

Compressive strength of mortar cubes was determined to check if the use of these two bacteria is beneficial in the mortar cubes. Mortar cubes of 51mm*51mm*51mm is casted for this. The casting procedure is explained below.

Firstly, the molds are cleaned, oiled and greased and set aside ready to use as shown in figure 3.10

Figure 3.10. 51mm *51mm molds oiled and greased for casting of mortar cubes.

The required amount of cement and sand are weighed and transferred in a mixer. It is mixed until a homogenous color is obtained. The mixture is turned on for a minute, and this procedure is repeated 2-3 times with a pause. The required amount of water is added, and the mixing procedure is repeated. The mixture procedure and respective weights were followed according to mixing standards using ASTM C109 M (ASTM, 2010).The mixture is now casted in the clean molds, in layers followed by tamping after layers. The molds are set aside for 24 hours under room temperature and demolded after that. For bacterial mix, the water is mixed with water and media containing bacteria.

Total 80 samples of cubes were casted, and a 3 sample of each Mix is tested using CTM. The sample is cured under water and are tested using Compression Testing machine in standard number of days i.e. 1 day, 3 days, 7 days, 14 days and 28 days respectively. The samples cured for 28 days were also used to analyze the difference in resistance towards water penetration of the molds prepared both with bacteria and without bacteria using.(ASTM C 642-06, 2008). NC is a representation for Mix 1, normal cube, BS represents Mix 2 i.e. the cube with *B Subtilus*, and

BM represents Mix 3, the cube with *B Megaterium* and BS + BM represents Mix 4 the cube with *B Subtilus* and *B Megaterium.* Figure 3.11 shows mortar cubes casted with no bacteria and *B Subtilus* for all days of testing. Similarly, figure 3.12 shows mortar cubes casted with *B Megaterium* and *B Subtilus* plus *B Megaterium* for all days of testing.

Figure 3.11. Mortar cubes of Mix 1(NC) and Mix 2 (BS).

Figure 3.12. Mortar cubes of Mix 3(BM) and Mix 4(BS+BM).

The cubes are demolded from the molds after 24 hours and are set to be cured in clean water as shown in figure 3.13. The cubes are removed from water on the respective days of testing. Figure shows the cubes cured and ready for testing.

Figure 3.13. Cubes set for curing.

Figure 3.14. Mortar cubes of all 4 Mix ready to be tested.

4. TESTS AND RESULTS

4.1. General

This chapter discusses the experimental results of the research. The strength and durability improvement of mortar cubes were evaluated by addition of bacteria in the mix. First, the samples were weighed for any relevant weight difference on respective days before testing. The strength test was conducted as per C 109 M. (ASTM, 2010)to determine the compressive strength of the cubes at 1,3,7,14 and 28 days respectively in four different Mixes i.e. Mix 1, with no bacteria, Mix 2 with *B Subtilus*, Mix 3 with *B Megaterium* and Mix 4 with *B Subtilus and B Megaterium* respectively. Also, water absorption test was carried out on the 28^{th} day for all four Mix to verify the durability of the mortar cubes referring (ASTM C 642-06, 2008).

4.2. Weight analysis

Before carrying out any tests, the weights of the mortar cubes were measured to determine any changes due to addition of bacteria and with mixing water. The respective weights of the cubes at respective days were carried out by using a weighing machine which is adjusted to zero as shown in figure 4.1 and the respective weights are shown in figure and is shown in the Table 4.1.The weight shown is the average of three samples at least, and the weight analysis were carried out on the respective days

Figure 4.1. Weighing machine scaled to 0.0 grams.

Table 4.1

Weights of four mixes on respective days before testing

	Curing Time (Days)				
Types of mix	1	3	7	14	28
Mix 1: Normal cubes (NC)	297.06g	299g	297.9 g	297.1g	298.1 g
Mix 2*: B Subtilus* (BS)	294.7g	295.8 g	293.7	295.5 g	295.7 g
Mix 3*: B Megaterium* (BM)	291.0 g	295.5g	296.5g	297.4g	293.9 g
Mix 4: *B Subtilus +B Megaterium* (BS+BM)	292.1g	292.3g	294.2 g	293.5g	294.5 g

4.3. Compressive strength test

Compressive strength is one of the most important properties contributing to concrete durability. The compressive strength test was carried out using destructive method, in a

compression testing machine subjected to a loading as per C109/C 109 M. (ASTM, 2010) The compressive strength is computed using the following formula:

$$\text{Compressive strength} = \frac{\boldsymbol{Normal\ Load\ (kN)}}{\boldsymbol{Area\ (mm^2)}}$$

The molds were tested at 1 day, 3 days, 7 days, 14 and 28 days respectively. Figure 4.2 shows the placement of the 51 mm *51 mm mortar cubes in CTM before the sample fails. Figure 4.3 shows the sample in the machine how it failed after application of load.

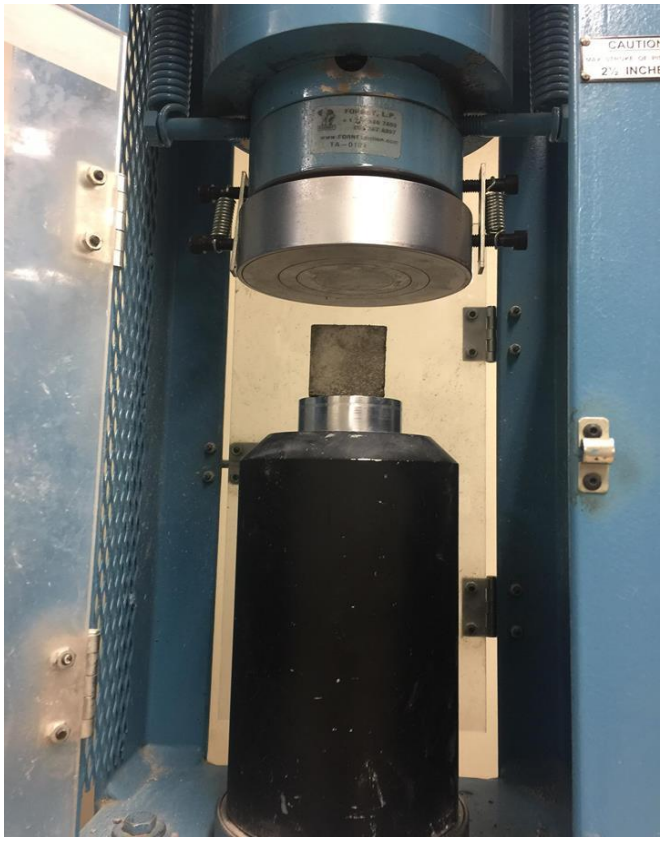

Figure 4.2. 51mm *51mm sample placed in CTM.

Figure 4.3. Failure of sample after application of load.

The tables below show the Compressive strength in Mpa for all four mixes for all the respective days i.e. 1 day, 3 days, 7 days, 14 days and 28 days. The compressive strength values are determined by using a CTM and at least 3 samples for each test. Table-4.2 shows the compressive strength values obtained for the mortar cubes for Mix 1 i.e. mix with no bacteria, figure 4.4 shows the graphical representation of Mix 1 and 4.5 shows the variation of compressive strength with respect to days.

Table 4.2

Compressive strengths in Mpa of mortar cubes (Mix 1)

	Curing Time (Days)				
Compressive strength(KN/mm^2)	1	3	7	14	28
Normal Load(KN)	75.25	78.2	84	88.5	101.28
Area of the cubes(mm^2)	2601	2601	2601	2601	2601
Compressive strength= Normal Load/Area	0.02893	0.0300	0.03229	0.03402	0.03893

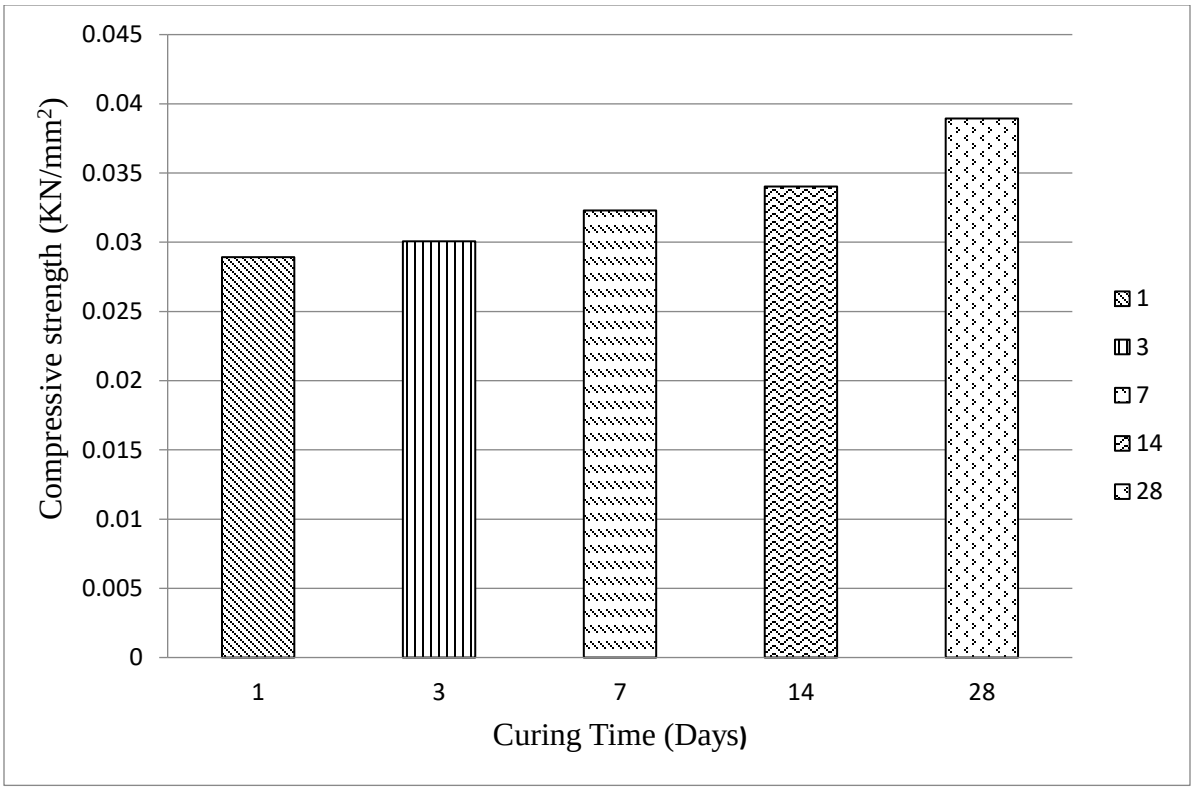

Figure 4.4. Graphical representation of the compressive strength (Mix 1).

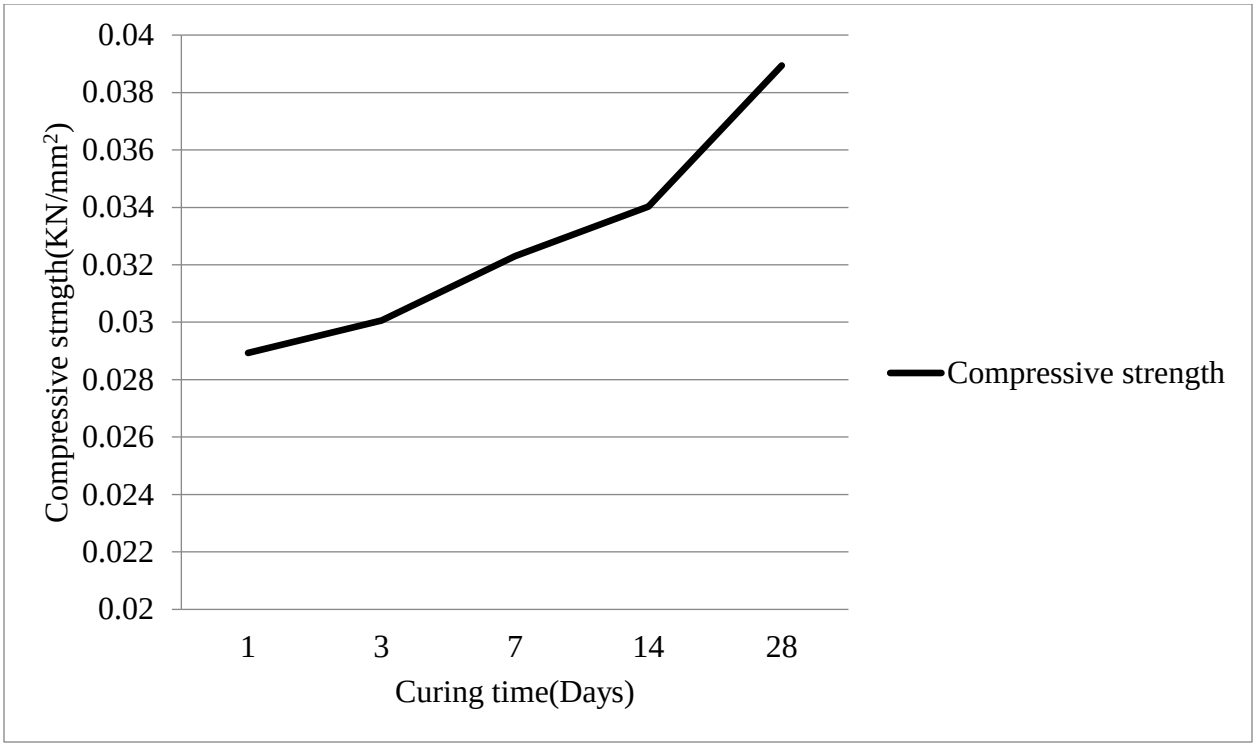

Figure 4.5. Variation of the compressive strength (Mix 1).

Table-4.3 shows the compressive strength values obtained for the mortar cubes for Mix 2 i.e. *B Subtilus* mortar cube and figure 4.6 shows the graphical representation of Mix. Figure 4.7 demonstrates the variation of compressive strength with respect to days. (Mix 1 vs Mix 2). The values demonstrated in the table and graphical representation is an average of at least three samples tested on each testing day. It is also observed that Mix 2 has slightly higher compressive strength compared to Mix 1 for all curing days.

Table 4.4

Compressive strengths in Mpa of mortar cubes (Mix 2)

	Curing Time (Days)				
Compressive strength(KN/mm^2)	1	3	7	14	28
Normal Load(KN)	78.6	81	90.60	95	105.6
Area of the cubes(mm^2)	2601	2601	2601	2601	2601
Compressive strength= Normal Load/Area	0.03021	0.03114	0.03483	0.03652	0.04059

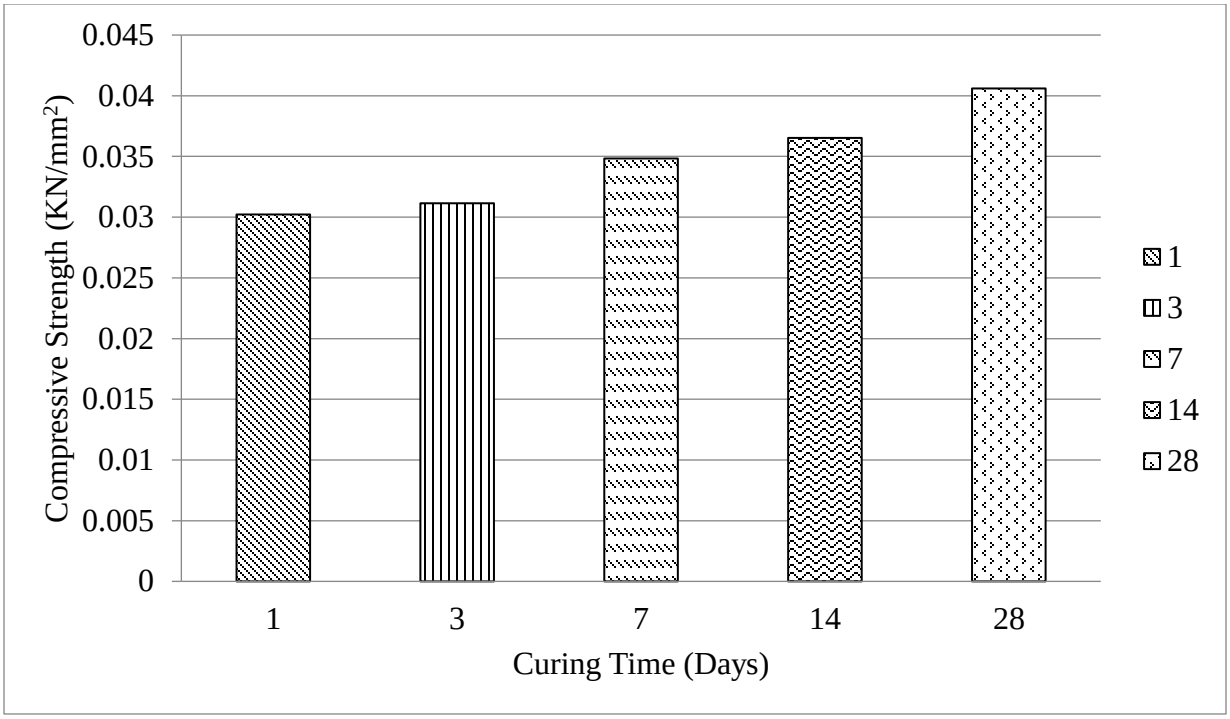

Figure 4.6. Graphical representation of the compressive strength (Mix 2).

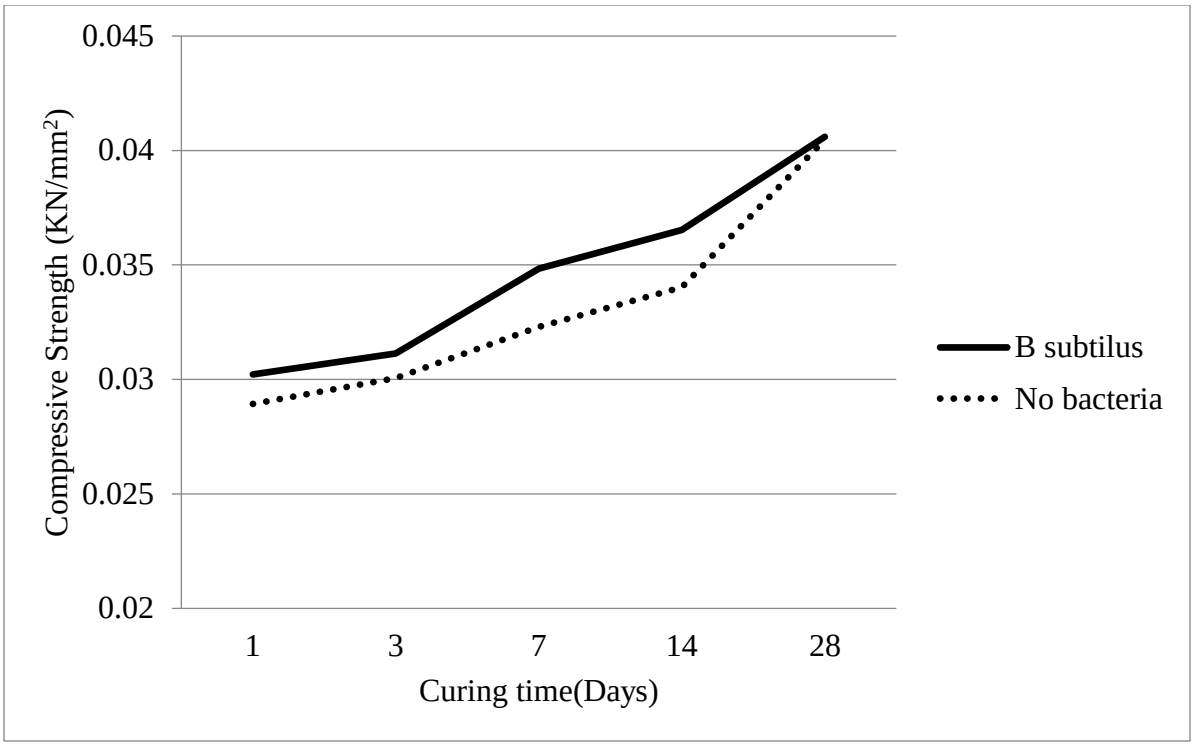

Figure 4.7. Variation of the compressive strength (Mix 1 vs Mix 2).

Table-4.4 shows the compressive strength values obtained for the mortar cubes for mix 3 i.e. *B. Megaterium* mortar cube and figure 4.8 shows the graphical representation of Mix 3 and 4.9 shows the variation of compressive strength with respect to days. (Mix 1 vs Mix 3). It can be observed that Mix 3 performed better than Mix 1 in all curing days. Also, mix 3 performed better than Mix 2 in7, 14 and 28 days and this is because *B Megaterium* has slower strength gaining capacity

Table 4.4.

Compressive strengths in Mpa of mortar cubes (Mix 3)

	Curing Time (Days)				
Compressive strength(KN/mm^2)	1	3	7	14	28
Normal Load(KN)	76	80	93.4	98.8	113.8
Area of the cubes(mm^2)	2601	2601	2601	2601	2601
Compressive strength= Normal Load/Area	0.02921	0.03075	0.03590	0.03798	0.04363

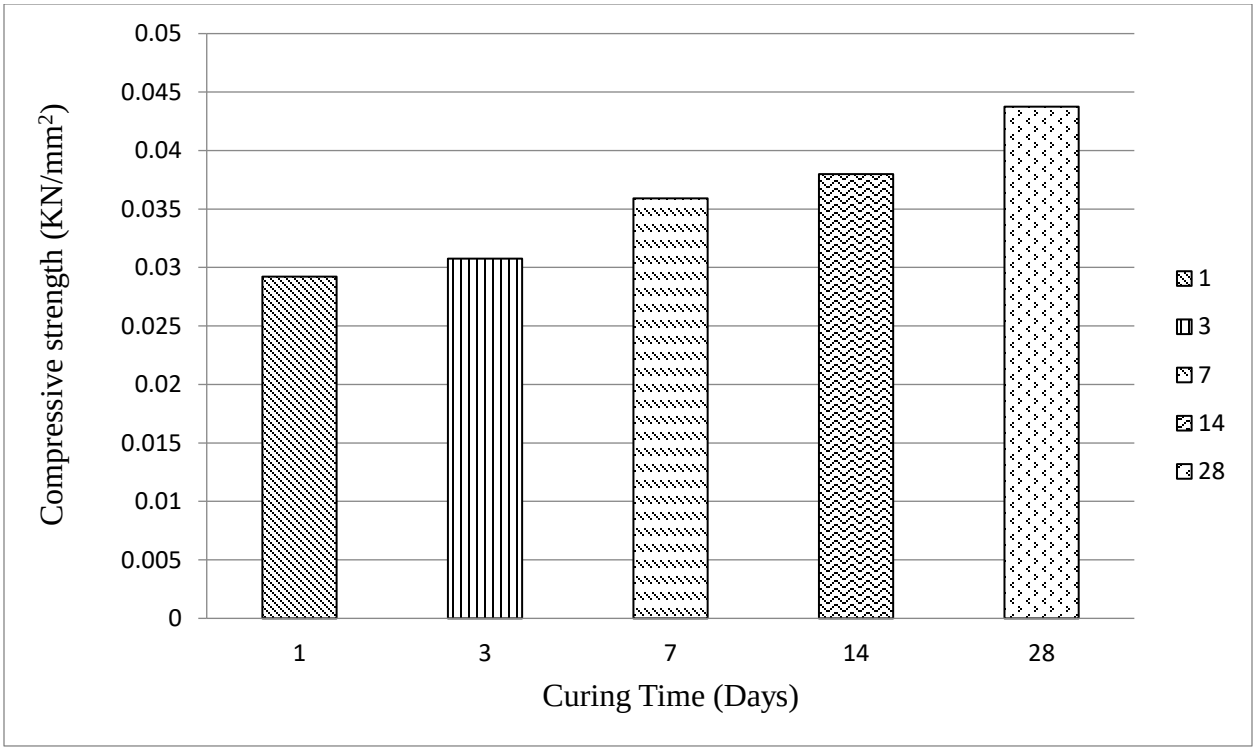

Figure 4.8. Graphical representation of the compressive strength (Mix 3).

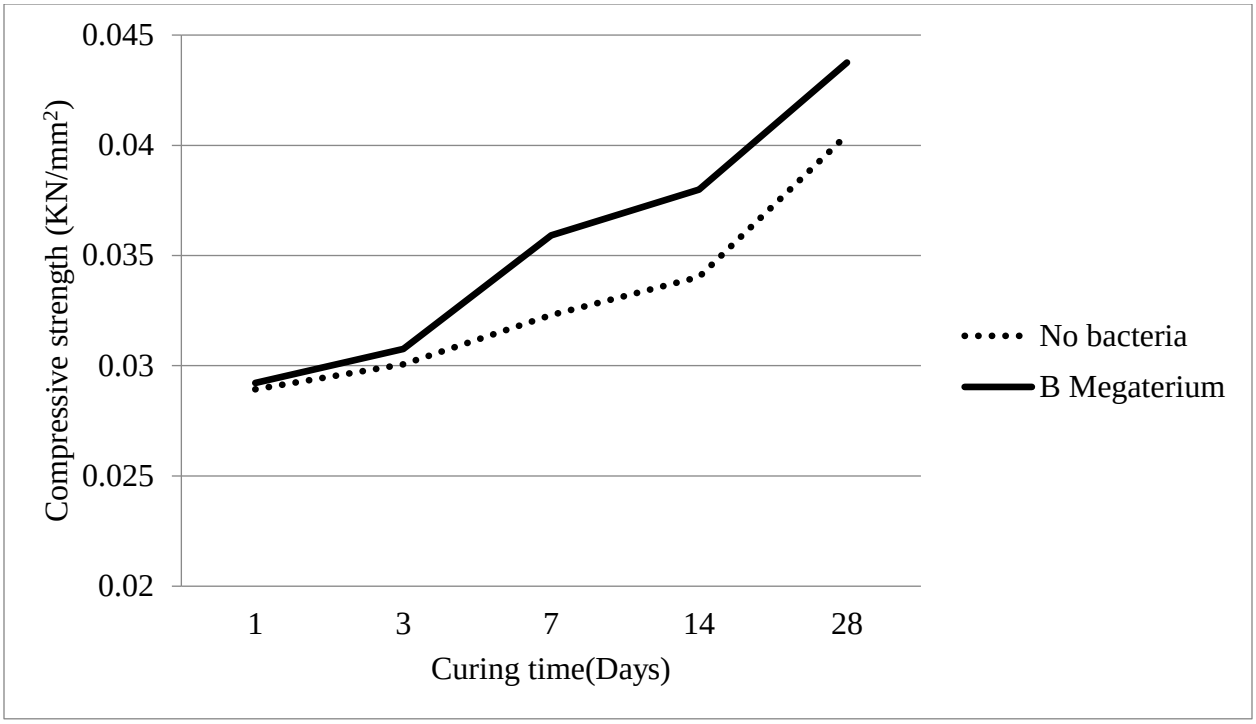

Figure 4.9. Variation of the compressive strength (Mix 1 vs Mix 3).

Table-4.5 shows the compressive strength values obtained for the mortar cubes for Mix 4 i.e. *B Subtilus + B Megaterium* mortar cube. Figure 4.10 shows the graphical representation of Mix 4 and 4.11 shows the variation of compressive strength with respect to days. (Mix 1 vs Mix 4). From the data obtained, it can be observed that the mixing of bacteria in the Mix 4 demonstrated the highest compressive strength in all the curing days compared to Mix 1, Mix 2 and Mix 3.

Table 4.5

Compressive strengths in Mpa of mortar cubes (Mix 4)

	Curing Time (Days)				
Compressive strength(KN/mm^2)	1	3	7	14	28
Normal Load(KN)	81.3	85	91.2	99.5	119.5
Area of the cubes(mm^2)	2601	2601	2601	2601	2601
Compressive strength= Normal Load/Area	0.03125	0.03267	0.03506	0.03825	0.04594

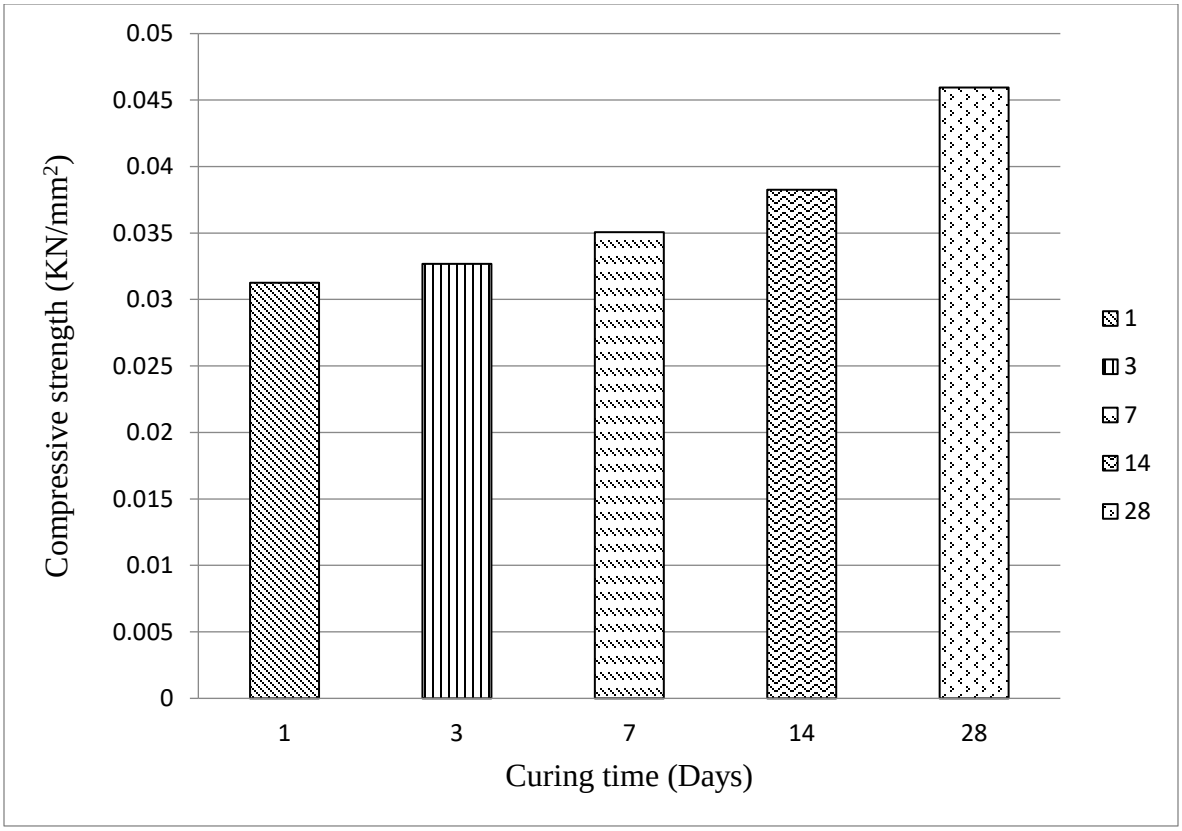

Figure 4.10. Graphical representation of the compressive strength (Mix 4).

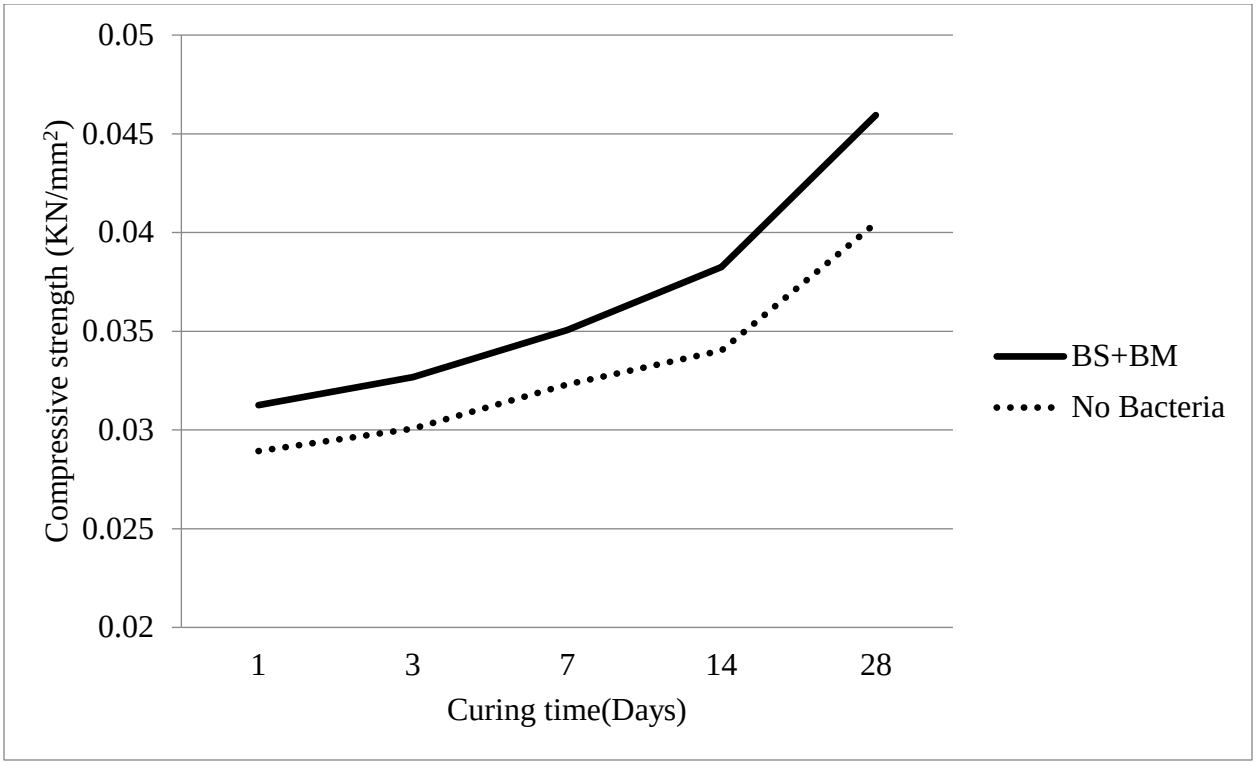

Figure 4.11. Variation of the compressive strength (Mix 1 vs Mix 4).

4.4. Water absorption test

Water absorption test is one of test conducted to determine the increased resistance towards water penetration in concrete as per (ASTM C 642-06, 2008). Cubic molds of 51 mm in size were prepared in all four mixes. And the samples were subsequently cured for 28 days in water. After curing, the surfaces of samples were dried, and their saturated mass were determined after immersion. For this purpose, the specimens were oven dried at 115 ±5-degree Celsius. The water absorption is calculated by using the following formula:

$$\text{Absorption after immersion} = \frac{B-A}{A} \, x \, 100$$

Where A is the mass of oven dried sample in air and B is the mass of the samples after immersion with a dry surface.

The table below shows the towel dry weight of samples cured for 28 days , an oven dried weight and water absorption in percentage for all Mixes. The graphical representation in 4.12 explains how Mix 3 uptakes the least amount of water compared to the other mortar cubes.

Table 4.6

Water absorption rate (%) of all mixes, all in grams

Weight/ Mix	Towel dry	24 hours in the oven (115+_5 °C)	Water absorption rate (%)
Normal (Mix 1)	297.43 g	272.63	9.10
B Subtilus (Mix 2)	295.2 g	271.63 g	8.70
B Megaterium (Mix 3)	293.9 g	273.30 g	7.55
BS+BM (Mix 4)	294.5 g	273.67 g	7.62

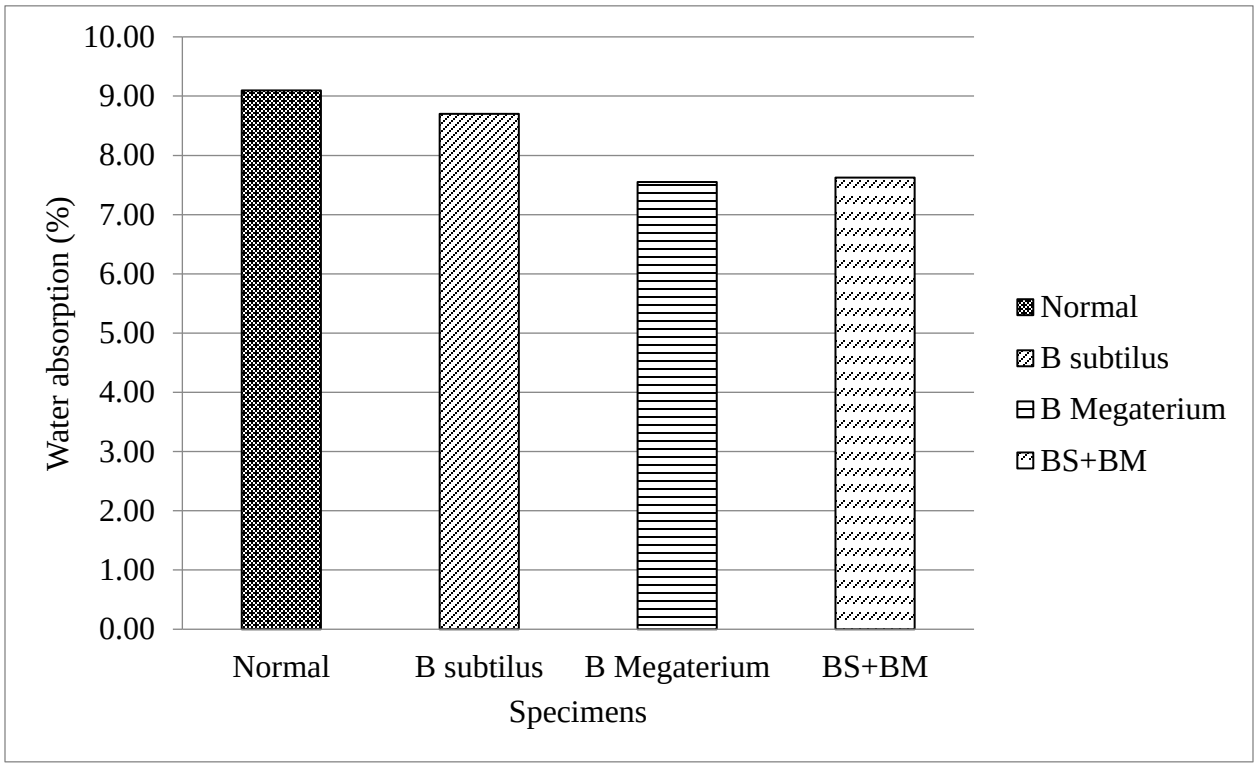

Figure 4.12. Graphical representation of the water absorption rate (All Mixes).

4.5. SEM analysis

To investigate the specimen's and whether the change in compressive strength of the mortar cubes and its reduction in water permeability could be attributed to the presence of bacteria, some deteriorated parts of the broken specimens of 28-day compressive strength were chosen to be visualized by SEM.

SEM micrographs of sample of all four different Mixes-(Mix 1: mortar cubes with no bacteria, Mix 2 mortar cubes with *B Subtilus*, Mix 3: mortar cubes with *B Megaterium* and Mix 4- mortar cubes with *B Subtilus and B Megaterium* are shown in figure. Fig 4.13 shows a Scanning electron micrographs of 28 days samples for mix 1, mortar cubes with no bacteria; Figure 4.14 is the SEM images for mix 2, mortar cubes with *B Subtilus*; Figure 4.15 shows the images of mix 3, mortar cubes with *B Megaterium* and Figure 4.16 shows images of mix 4, mortar cubes with *B Subtilus + B Megaterium*.

On a closer observation of the images, it was seen that the images with bacteria were seen to be densified and have negligible number of pores and spaces then the one with no bacteria, and as per previous researches it is associated with presence of Calcium carbonate. Calcium carbonate formation can be seen in Mix 1 samples as well. The formation of $CaCO_3$ in samples with no bacteria is quite different i.e. it is formed due to carbonation of Calcium hydroxide which is one of the major hydration products of cement. The amount of calcium carbonate produced is very less and dissolves in water compared to bacterial specimens, where $CaCO_3$ is directly produced as a result conversion of calcium lactate directly to calcium carbonate which is insoluble in water, and due to metabolic action with CO_2 it reacts with calcium hydroxide on the spot and doesn't let it wash away, producing more Calcium carbonate. Formation of similar crystalline structure was also confirmed by the previous authors(Hosseini Balam, Mostofinejad,

& Eftekhar, 2017a) . Calcite was present in the form of Calcium Carbonate due to bacteria. The deposition of calcite serves as barrier to harmful substances and thus improves impermeability. Densification, and filling up of voids was clearly observed which made the matrix more compact, blocking the ingress of harmful materials inside the mix, and resulting in the better strength of the mix. Careful inspection of the SEM images reveals a denser microstructure with lower amounts of pores for the specimens.

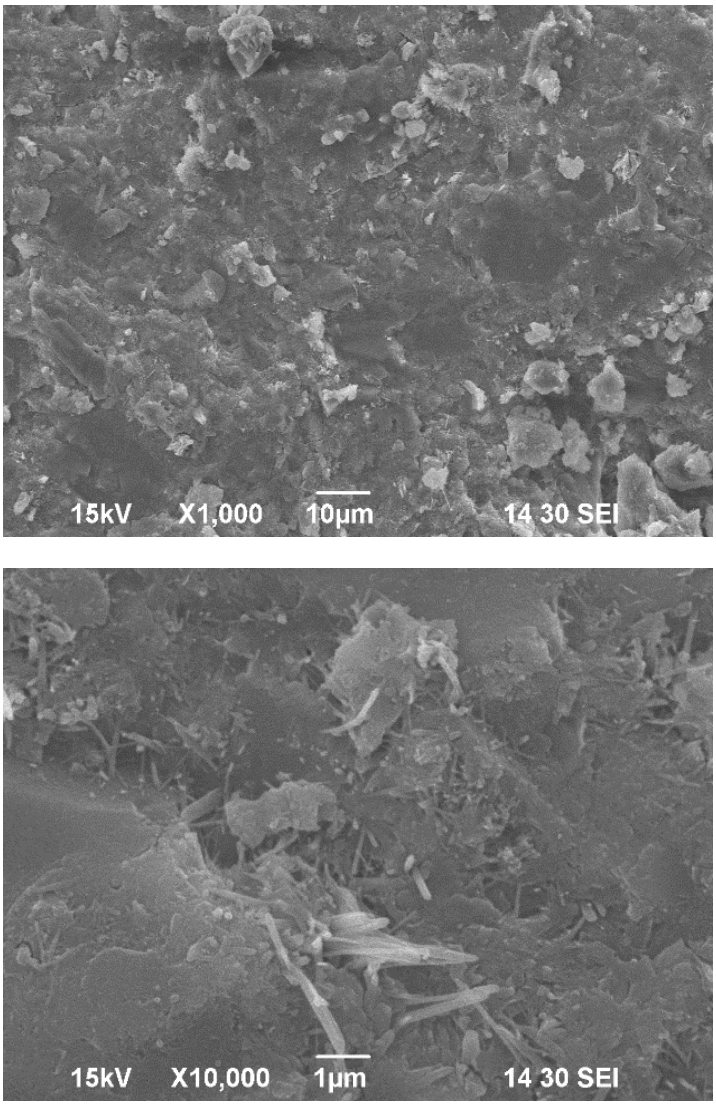

Figure 4.13. Scanning electron micrographs of 28 days samples: Mix 1.

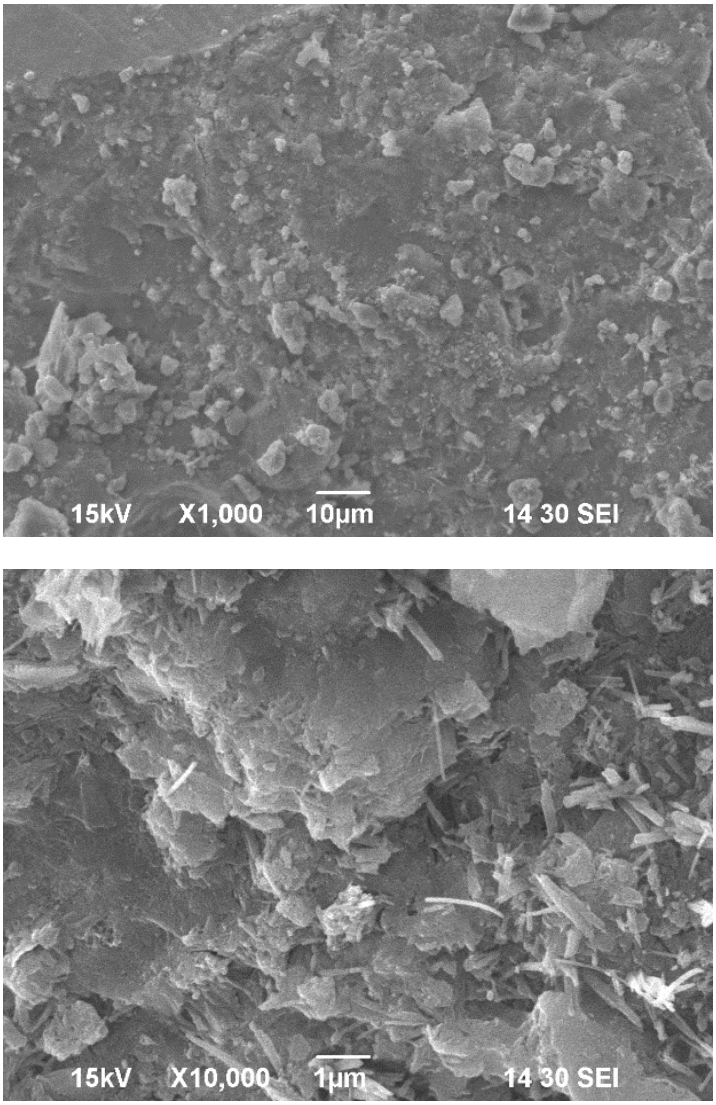

*Figure 4.14. S*canning electron micrographs of 28 days samples: Mix 2.

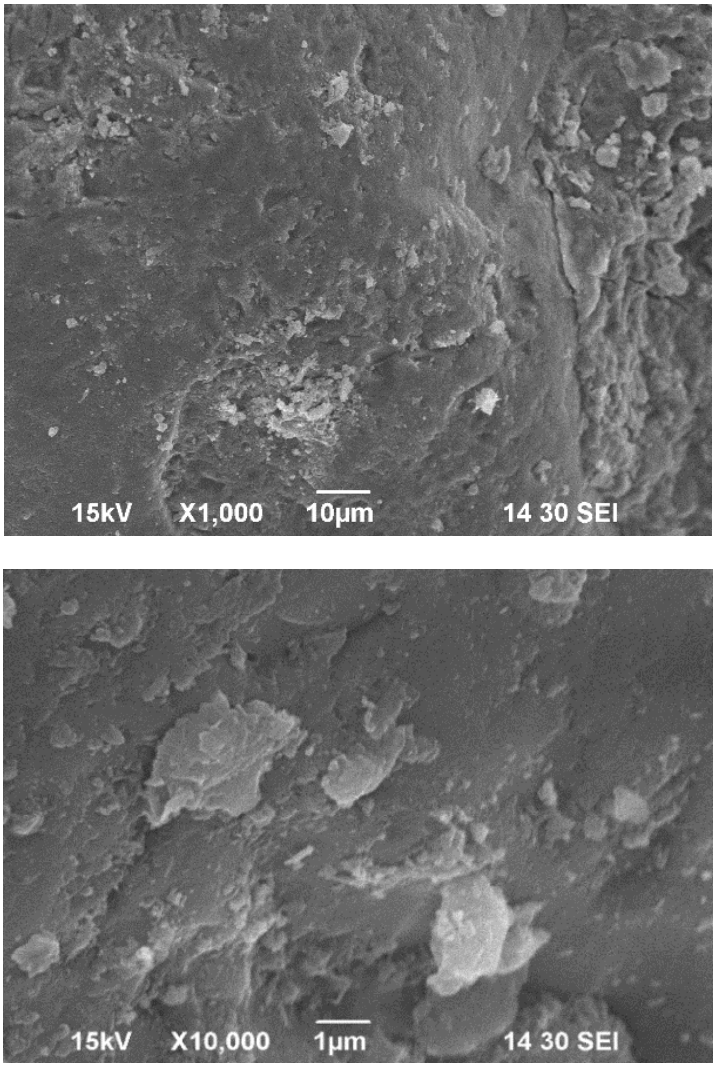

Figure 4.15. Scanning electron micrographs of 28 days samples: Mix 3.

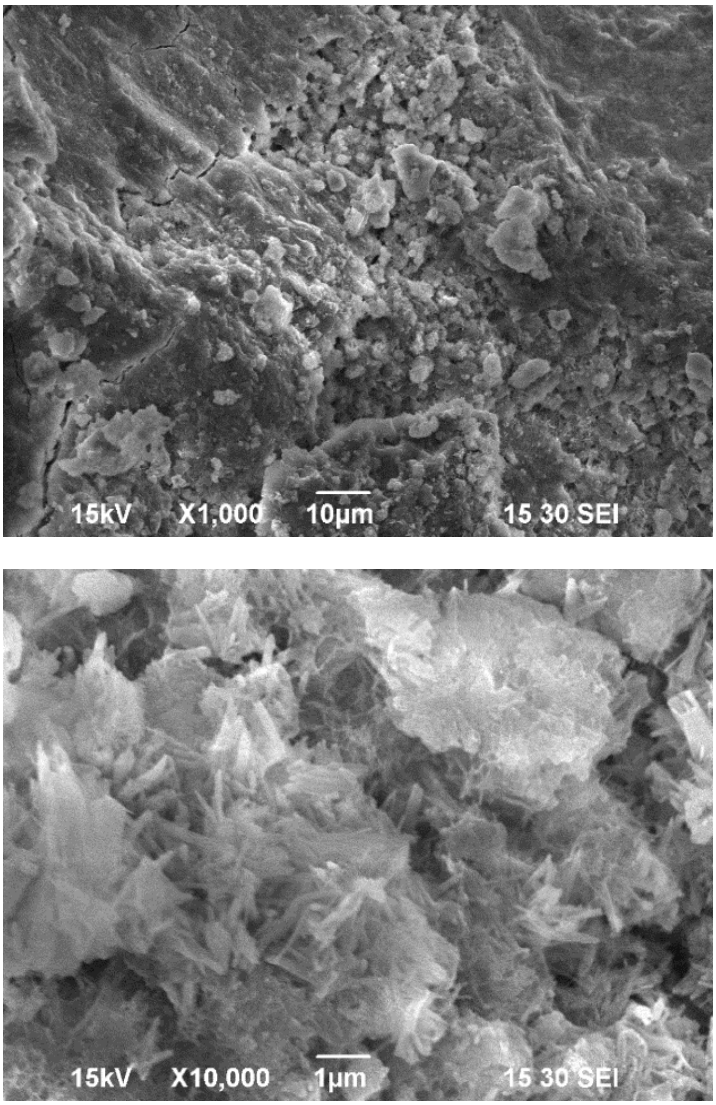

Figure 4.16 Scanning electron micrographs of 28 days samples: Mix 4.

5. COMPARISION BETWEEN NORMAL MIX AND BACTERIAL MIX

5.1. General

This chapter is a comparison of results between the properties of Normal mortar (Mix 1), *B Subtilus* added mortar cubes (Mix 2*), B Megaterium* added mortar cubes (Mix 3), and *B Subtilus + B Megtaerium* mortar cubes(Mix 4), based on the series of experiments conducted i.e. Compressive strength tests, Water absorption tests and SEM analysis.

5.2. Comparisons

5.2.1. Compressive strength

Figure 5.1 shows the graphical representation of trend of increase of compressive strength respective to the days and four different mixes and figure 5.2 shows variation in the strength with respect to the mix. Table 5.1 shows the increment of compressive strength in percentages in 1 day, 3 days, 7 days, 14 days and 28 days for Mix 2, Mix 3 and Mix 4, with respect to Mix 1. From, the data analysis, it has been demonstrated that compressive strength of mortar mix is enhanced with addition of bacteria.

- In 1-day testing, the Mix 2 cubes showed an increment of 4.42%, Mix 3 of 0.96% and Mix 4 showed an increment of 8.01 % respectively.
- In 3 days testing, the Mix 2 cubes showed an increment of 3.8%, Mix 3 of 2.5% and Mix 4 showed an increment of 8.9 % respectively.
- In 7-day testing, the Mix 2 cubes showed an increment of 7.86%, Mix 3 of 11.17% and Mix 4 showed an increment of 8.57% respectively.
- In 14 days testing, the Mix 2 cubes showed an increment of 7.34%, Mix 3 of 11.64% and Mix 4 showed an increment of 12.43 % respectively.

- In 28 days testing, the Mix 2 cubes showed an increment of 4.34%, Mix 3 of 12.15% and Mix 4 showed an increment of 18.09 % respectively.

(Fedko, 2012) also reported a lower compressive strength of 11 % at 7 days and 8% at 35 days comparable to the results obtained in this research. (Khaliq & Ehsan, 2016)concluded that *B Subtilus* resulted in slight increment of compressive strength, irrespective of incorporation technique. The improvement of compressive strength by inclusion of bacteria is probably due to deposition of calcite on the surface and within the pores of cement sand matrix which fills the pores (Ramachandran et al., 2001).The overall trend of an increase in compressive strength up to 28 days might be by inclusion of bacteria is probably due to deposition of calcite on the surface and within the pores of cement sand matrix which fills the pores. The decrease in compressive strength of Mix 2 i.e. *B Subtilus* could be because during the initial curing period, microbial cells obtained good nourishment because the cement mortar was still porous, but the growth was not proper as it was a new environment for microbes (V. Achal, Mukherjee, Basu, & Reddy, 2009)

Table 5.1

Increment in strength of the mortar cubes

	Curing Time (Days)				
Types of mix	1	3	7	14	28
Mix 1: Normal cubes (NC)	-	-	-	-	-
Mix 2*: B Subtilus* (BS)	4.42%	3.8%	7.86%	7.34%	4.34%
Mix 3*: B Megaterium* (BM)	0.96%	2.5%	11.17%	11.64%	12.15%
Mix 4: *B Subtilus +B Megaterium* (BS+BM)	8.01%	8.9%	8.57%	12.43%	18.09%

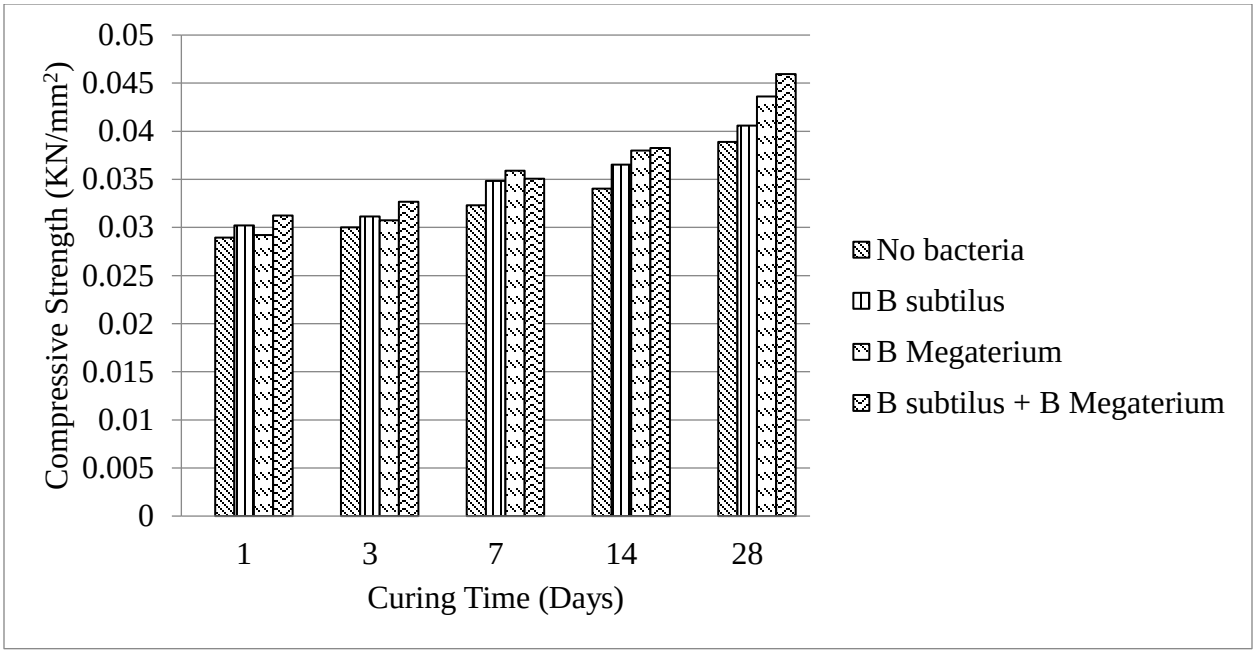

Figure 5.1. Graphical representation of compressive strength (All Mixes).

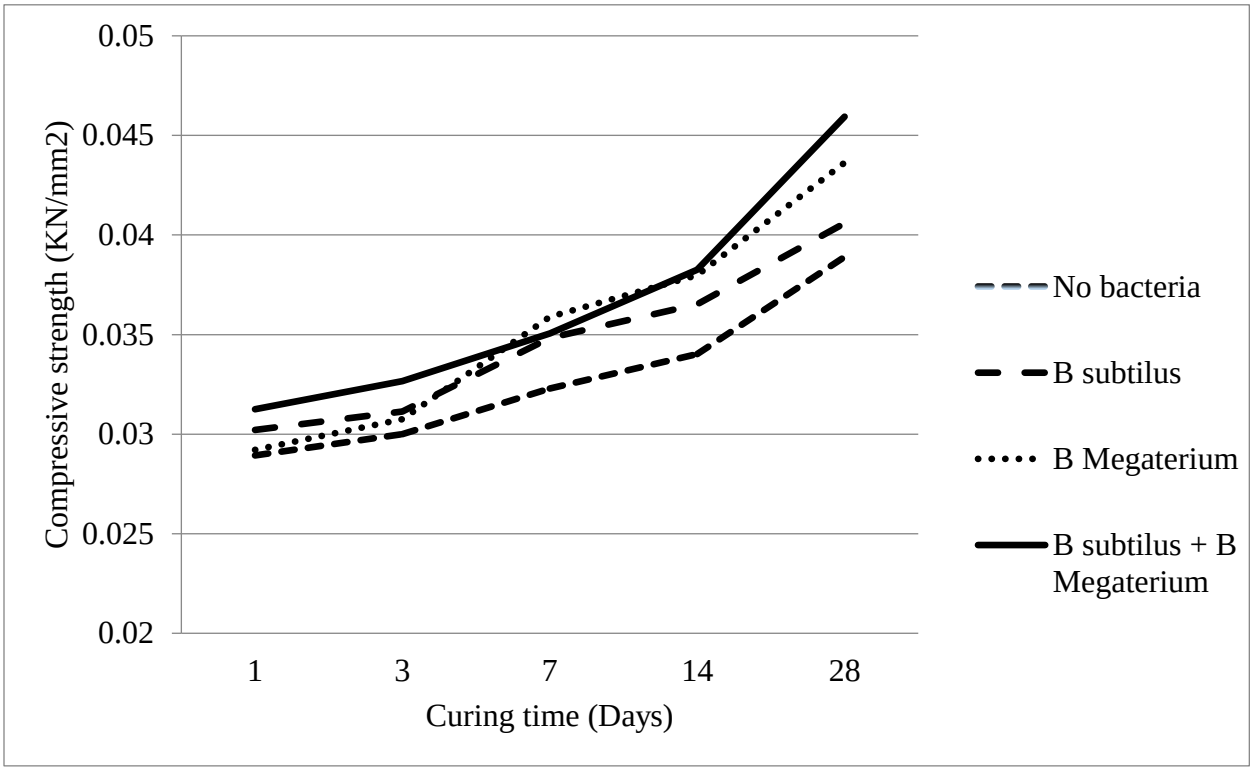

Figure 5.2. Variation of the compressive strength (All Mixes).

5.2.2. Water absorption

Figure 5.3 shows the graphical representation of trend of increase of water absorption rates of the mixes. From, the data analysis, it has been demonstrated that the addition of bacteria reduces the water absorption of the mortar. The test was carried out on the 28^{th} day, with addition of 10^8 cells per ml of live cells.

- The presence of bacteria absorbed less water compared to ones with no bacteria.
- The maximum decrease in water absorption was observed with Mix 3 mortar cubes with *Bacillus Megaterium* i.e. by 17.3%.
- Mix 2, mortar cubes had a reduction of 4.39%.
- Mix 4 mortar cubes had 16.26 % reduction in water absorption compared to Mix 1.

From previous studies, (Varenyam Achal et al., 2011)concluded how over a period of 168 hours (7 days), cubes embedded with fly ash 0 %, 10% and 20% with bacteria absorbed nearly 3.5 times less water than control cubes. (Chahal et al., 2012a)reported a similar result of a maximum reduction in water absorption for 10% silica fume with 10^5 cells/ml at 91 days. It was also reported that 5% silica fume gave 0.1% water absorption at 91 days and 0.3% at 28 days (Siddique et al., 2016)reported that addition of 10^5 cfu/ml played a significant role in decreasing water absorption of silica fume concrete. Hence, bacterial mortars uptakes less water than the normal mortar cubes. This is because of the deposition of a layer of Calcium carbonate on the surface and inside the pores of the concrete results in decrease of water absorption. Bacteria seals the pores, which will result in limitation of ingress of harmful substances. This bacterial action deposited can seal the pores, voids and micro cracks, which has the ability to improve the resistance of cementious material towards degradation. (Chahal & Siddique, 2013).

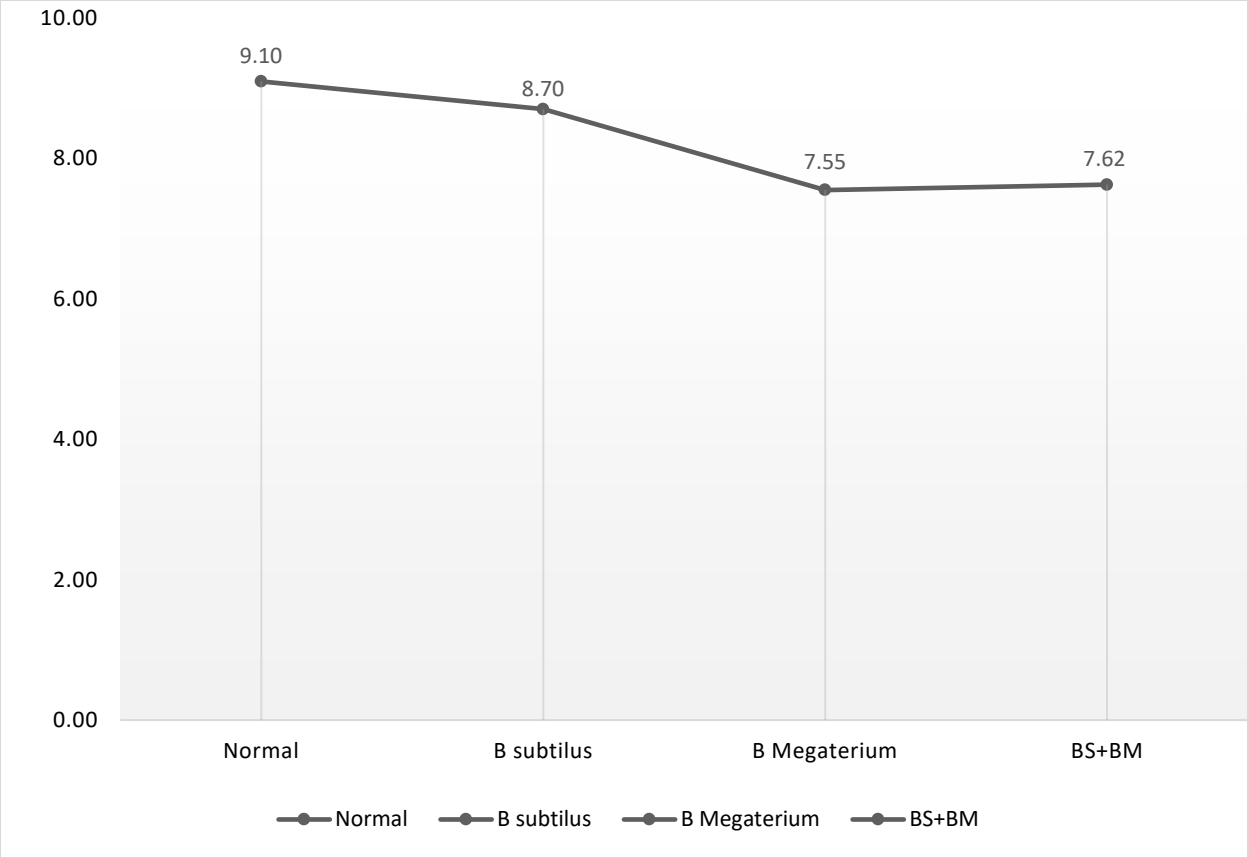

Figure 5.3. Variation of the compressive strength at 28 days (All Mixes).

5.2.3. SEM analysis

The SEM images analysis, with bacteria added cubes were seen to be densified and had negligible number of pores and spaces then the one with no bacteria. Mix 4 bacteria was seen less porous compared to the other three mixes. This is associated with presence of Calcium carbonate. The amount of calcium carbonate produced is very less and dissolves in water in the non-bacterial mix, whereas bacteria added cubes form calcium carbonate by converting calcium lactate directly to Calcium carbonate, which reacts to calcium hydroxide on the spot and doesn't wash away. Higher amount of Calcite is seen in Mix 4 cubes. The deposition of calcite also contributes by serving as barrier to harmful substances and thus improves impermeability.

Densification, and filling up of voids was clearly observed which made the matrix more compact, blocking the ingress of harmful materials inside the mix, and resulting in the better strength of the mix. Careful inspection of the SEM images reveals a denser microstructure with lower amounts of pores for the bacteria added specimens.

5.3. Comparison of properties of mortar cubes(normal vs bacterial mix)

From all the three tests carried out, it has been observed that bacterial mortar cubes worked better than the normal mortar cubes. It is a known fact that compressive strength is one of the contributing factors for improving the durability properties of mortar cubes. Compressive strength of bacteria added cubes were seen to be higher than normal mixes in all curing days. Therefore, higher compressive strength could potentially make concrete mortar stronger, durable and reduce the chances of developing major/minor cracks on concrete/mortar. It has been also noted that the bacterial mix absorbed less water than the normal mix. This could be attributed to the factor that there will be less chances of ingress of harmful materials into the mortar cubes which will ultimately prevent the degradation of cement, sand and other materials inside the mix, making it more durable. SEM analysis tests carried out showed densified structure with negligible number of pores and spaces in the bacterial mix which was due to calcite precipitation.

Therefore, from above comparisons we can observe that all the tests directly, indirectly contributes towards enhancement of mortar properties, and will ultimately make the mortar cubes stronger and durable. Even though the initial costs of bacterial mix are higher, it could save up a lot of money that would be spent on concrete/mortar repair in the future.

6. CONCLUSIONS AND FUTURE RECOMMENDATIONS

6.1. General

This research study was focused on determining the significance of mixing two bacteria's on properties of mortar cubes: on compressive strength and water absorption properties and if mixing of two bacteria's could be beneficial in the construction industry. A series of experiments were conducted to observe the compressive strength and water absorption of the mortar cubes. Meanwhile, the conclusions and future recommendations are summarized in this chapter.

6.2. Conclusion and benefits

According to the experimental study in Chapter three, the discussion in Chapter four and comparisons in Chapter five, some major conclusions are summarized below:

1. Addition of bacteria in the mix, does not alter the weight of the mortar cube in a significant way.
2. The compressive strength of bacteria added mortar cubes were higher than the normal ones. In 28 days testing, Mix 2 cubes showed an increment of 4.34%, Mix 3 of 12.15%, and the maximum increase in compressive strength was seen up to 18.09% at in Mix 4 cubes when compared to Mix 1(Normal mortar cubes).
3. It was also seen that Mix 3 mortar at 28^{th} day testing absorbed 17.3% less water, Mix 2 mortar cubes had a reduction of 4.39%, and mix 4 mortar cubes had 16.26 % reduction in water absorption compared to Mix 1.
4. Microstructure analysis using SEM conformed that bacteria added mixes (Mix 2, Mix 3 and Mix 4) had a denser structure and less bacteria which could be attributed to the calcite present in the samples.

5. Using of bacteria is recommended because the mineral precipitation which occurs is a completely natural process, is an environmental friendly method, and also cheaper in a long term.
6. The bacteria addition might be a costlier approach in the beginning, but improvement of major properties like compressive strength ,water absorption and microstructure of mortar cubes could make this approach worthwhile in a long term by potentially reducing all the repair and maintenance cost in the construction industry.

6.3. Limitations and recommendations for future research

Based on the above-mentioned limitations, some recommendations are suggested for future studies:

1. A continuation of research on use of bacteria in mortar is highly recommended.
2. Due to the limited time of this research, several other tests like flexural strength tests, rapid chloride permeability test, sulfate attack, freeze thaw, and other durability tests were not carried out.
3. The tests are designed to evaluate properties of mortar specimens with *B Subtilus and B Megaterium*, and more studies are recommended to investigate the influence of other bacteria, of Bacillus strains and their mixtures as well, that might have a profound effect on the mixture.
4. This research is focused on the test of 0.485 w/c ratio. Therefore, different water cement ratio could be experimented. Type II, Type III, Type IV cement can be used in the future research.
5. It is strongly recommended to conduct the experimental work to observe the performance of mortar or concrete specimens with different bacteria incorporation

technique. Also, addition of carrier compounds like Fly ash, LWAC, silica fume, is recommended.

6. Due to time limitations, the tests are carried out only up to the 28^{th} day. So, in future researches tests could be done at 56, 91 days as well.
7. Due to limited equipment's, it is hard to verify calcite crystals, and also the role of microorganism in mortar cubes. The images analysis by X ray diffraction, EDX and different magnifications in SEM is recommended.

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