BIO-INFLUENCED SELF-HEALING CONCRETE USING VARIOUS FIBROUS CARRIERS

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This is to certify that the

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Dedicated to

My parents and teachers

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Abstract

Microbial induced calcite precipitation for self-healing purpose in concrete is governed by several factors including type of bacteria and the carrier compound. In order to investigate the autonomous healing, this study presents a comparison between calcite precipitation activity of *B. subtilis* KCTC-3135^{T}, *B. cohnii* NCCP-666 and *B. sphaericus* NCCP-313 and explores the potential application of natural fibers as carriers for bacteria in concrete. For crack remediation, bacteria embedded in coir, flax and jute fibers were introduced in concrete. Calcium lactate pentahydrate and urea were incorporated as organic nutrients. For each formulation, specimens were prepared to evaluate mechanical, microstructural and visual properties of self-healing concrete. The results suggest that *B. sphaericus* NCCP-313 precipitated more calcium carbonate as healing agent which is reflected by higher compressive strength, ultrasonic pulse velocity and better healing characteristics. In terms of carriers, coir fibers resulted in highest compressive strength, while flax fibers were more efficient in regaining compressive strength and improving ultrasonic pulse velocity.

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CHAPTER 1

Introduction

1.1. General

Because of its low cost, durability, strength, and relatively simpler handling, concrete is highly used construction material. Remarkable developments and researches in concrete technology have made concrete design less porous, yet the hazard of occurrence of small cracks (<0.3mm) in concrete that may affect the strength of concrete has remained unaltered. Furthermore, in recently developed high performance concretes, the brittleness and early age cracking is more pronounced as compared to normal strength concrete. Although, such cracks do not necessarily put a structure at risk but they surely impair a structure's functionality, accelerate its deterioration, affect the sustainability and reduce the service life (De Belie et al., 2018). If cracks in concrete constitute a network, they significantly increase permeability. Thus, the structure becomes prone to aggressive substances from environment (Wiktor and Jonkers, 2011). The micro cracks provide paths for the inclusion of detrimental substances from environment which can cause steel reinforcement corrosion. Hence, the durability cracks which result from aging and shrinkage, are required to be healed to regain the mechanical as well as physical properties of concrete The need to repair cracks has led to the development of several passive crack treating techniques.

Concrete has the ability to heal cracks by itself. Self-healing concrete technologies are being globally used as remedial techniques to enhance the durability of concrete (Li and Yang, 2007). It is known for many years and several studies were conducted to study the phenomenon of crack healing (Huang et al., 2013; Jacobsen and Sellevold, 1996; Li et al., 1998; Li and Yang, 2007; Luo and Qian, 2016). Self-healing methodologies reduce the utilization of manual efforts for preservation and repair of structures. It reduces the consumption of environment unfriendly and expensive materials that are commonly used for repair of concrete and hence, it preserves environment and reduces overall structural cost. Self-healing of concrete is categorized into two types i.e., autogenous healing and autonomous healing. When cracks are produced, some of them are closed due to autogenous healing. The phenomenon of autogenous healing involves two procedures (i) formation of calcium carbonate crystals directly from calcium ions Ca^{2+} in cement and carbonate ions CO_3^{2-} present in water and (ii) continued hydration of anhydrous particles of cement or some pozzolan as cracks are exposed to humidity (De Belie et al., 2018; Luo and Qian, 2016). The governing equation for reaction is

$CaO + H_2O \rightarrow Ca(OH)_2$

$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2O$

The potential for autogenous or intrinsic healing in concrete depends on its composition. Amount of clinker governs the quantity of calcium ions which subsequently determine the calcite precipitation. The silicate content and its type effect the pozzolanic reactions and consumption of calcium hydroxide which affects the healing periods. Crack patterns are changed by alteration in type of aggregates. If concrete is high strength having low w/c ratio, unhydrated cement is present inside which forms new CSH gel at later stages. Age of concrete also contributes to autogenous healing in a way that early age concretes have more unhydrated cement as compared to late ages. Autogenous healing can repair cracks of widths lying in range of 0.01- 0.1 mm and seldom up to 0.2 mm only in humid environments but always remain less than 0.3 mm (De Belie et al., 2018; Shaheen and Khushnood, 2018). Autogenous healing is also endorsed by stimulating hydration or crystallization by use of mineral additives, crystalline admixtures, superabsorbent polymers and non-superabsorbent polymer additions. Thus, it is known as stimulated autogenous healing (De Belie et al., 2018). To repair wider cracks, autonomous healing is considered effective. It contains biological or non-biological substances which can fill the micro cracks. Various researches have been conducted on crack remediation by non-biological sources such as polymers and other engineered additions (De Belie et al., 2018; Minnebo et al., 2017). Biological sources for autonomous healing include various types of mineral producing bacteria. These mineral compounds such as calcium carbonate, calcium silicate hydrates act as fillers of cracks in concrete. The concept of biological self-healing concrete was first introduced by Jonkers and Schlangen (2007). When a crack is produced in bacteria embedded concrete, water and oxygen penetrate inside the crack activate dormant bacteria. The bacteria feed on substrate and produce calcium carbonate as a byproduct on the cracked region, thus sealing the crack after its solidification. In future, if any crack appears again, the bacteria activate again and fill the crack. This process is called microbial induced calcite precipitation (MICP). This technique has gained much importance as it is an environment friendly strategy for crack remediation and concrete protection (Xu et al., 2013).

According to , De Belie et al. (2018), calcium carbonate inside concrete provides a good bonding with concrete matrix. It can either be made part of concrete mix or can be produced chemically in concrete to achieve specific purposes. The self-healing bioconcrete has the ability of durable remediation of cracks. It precipitates calcium carbonate as a byproduct which provides efficient crack repair and can contribute to self-healing, reduction in permeability and recovery of compressive strength. The high alkaline nature of concrete does not provide a friendly environment to embedded self-healing bacteria. It is also deficient in nutrients necessary for bacterial growth. There are a few bacteria that can survive such an environment of concrete (Rao et al., 2013). The fundamental requirement for bio-based crack healing is that the microorganism should be able to transform soluble organic nutrients with inorganic calcium carbonate crystal (Rao et al., 2013). It should produce substantial amount of calcium carbonate when provided with a food source such as calcium lactate, calcium glutamate, calcium acetate, calcium chloride or urea and must not reduce the concrete compressive strength. Production of calcium carbonate depends upon a number of factors including alkalinity, nucleation sites, dissolved inorganic carbon and presence of calcium ions inside the mixture.

Many bacteria make calcium carbonate crystals in environment that is suitable for their survival. Previous studies have conducted research on ureolytic bacteria and non-ureolytic bacteria separately. The alkali tolerant urease positive bacteria have been commonly used for self-healing purpose. The basic mechanism lying behind activity of these bacteria is that they produce urease enzyme. During the urease activity of such bacteria, urease produced by bacteria acts as catalyst for hydrolysis of urea, which is food source for microbes to give carbon dioxide and ammonia products (De Belie et al., 2018; Dick et al., 2006; Xu et al., 2013). As a result of urea hydrolysis, pH is increased which in turn triggers Ca^{2+} and CO_3^{2-} ions to make $CaCO_3$ around bacterial cell according to following reaction. If adequate quantity of calcium ions are present, the carbonate ions and calcium ions combine together to form calcium carbonate (Anbu et al., 2016; Qian et al., 2010).

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

$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3$

$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O$

$Cell\text{-}Ca^{2+} + CO_3^{2-} \leftrightarrow Cell\text{-}CaCO_3$

An alternative strategy to achieve crack repair is utilization of non-ureolytic bacteria which form calcium carbonate as a result of their metabolic activity in presence of a calcium source. The governing equation for self-healing by non-ureolytic bacteria is given as follows (Wiktor and Jonkers, 2011).

$CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O$

In order to achieve better results in crack healing, an immobilizer for bacteria incorporation is necessary so that it can be potentially used for plugging the cracks in cementitious materials (Khaliq and Ehsan, 2016). Because of its size relative to cement paste pores, bacteria cannot sustain life when directly embedded in concrete (Stuckrath et al., 2014). Instead of direct incorporation of bacteria into the mix, it is better to immobilize the microbe using some carrier compound so that high efficiency and mineral forming ability can be maintained over longer time durations (Zhang et al., 2017). Studies show that generally, bacteria addition improves compressive strength of concrete even without a carrier compound as deposits of calcium carbonate form inside the pores (Andalib et al., 2016; Chahal et al., 2012; Ghosh et al., 2005). Studies also reveal that directly added bacteria in concrete is not as effective in crack remediation as compared to bacteria immobilized in some carrier (Khaliq and Ehsan, 2016). Carrier provides a protective medium to bacteria which assists in their survival in concrete by withstanding effect of mechanical forces during mixing of concrete. Direct mixing of bacteria in concrete is not endorsed as it kills significant number of bacteria. Thus, microbes can be incorporated via vascular healing, microencapsulation, macro encapsulation or absorption techniques (Khaliq and Ehsan, 2016).

Use of natural and synthetic fibers in cementitious materials have resulted in better mechanical properties. In particular, natural fibers depict several positive characteristics as follows (Femandez, 2002).

- Natural fibers are globally available. They are usually harvested either as primary or secondary material as a byproduct of some process.
- Natural fibers are biodegradable and their production does not have a bad environmental impact.
- They can retain carbon. The carbon dioxide which is released in environment during combustion returns to the environment from where it was initially expelled. Thus, these fibers do not deteriorate atmosphere.
- Natural fibers are globally known and can easily be cultivated. They are being used in several applications worldwide.
- They enclose less energy because they do not require excessive processing as in case of synthesis of artificial fibers.

The presented research compares different bacteria immobilized in natural fibers and used in concrete. Studies reveal that concrete reinforcement with natural fibers is a feasible and economical alternative to the conventional building materials (Yalley and Kwan, 2009). Natural fibers have been identified as promising components of composites used in construction materials and other applications (Bledzki and Gassan, 1999; Seber and Lloyd, 1996)

1.2. Objectives

This study is focused on achieving the following objectives

- To study the effectiveness of various bacteria as self-healing agents in concrete.
- To study the effectiveness of fibrous carriers as immobilizing technique.
- Comparing the efficiency of different bacteria species with various carriers.

Ureolytic and non-ureolytic bacteria species were used for comparison. Calcium lactate pentahydrate and urea were used as organic food source for bacteria. The authors believe that previously, adequate literature is not available on utilization of natural fibers as potential carriers for self-healing bacteria in concrete. Concrete was reinforced with coir, flax and jute fibers soaked with bacteria solution. These fibers have good sorption ability and they do not possess any anti-bacterial activity. The quantity of bacteria solution was fixed while respective fibers were added in amounts as per their sorption ability.

1.3. Organization of Dissertation

Introduction of the topic is described in Chapter 1 of this document. The following content covers Literature view in Chapter 2, Experimental Methodology in Chapter 3. The test setups, visual findings, microstructural analysis and analysis of mechanical properties are reported and explained in Chapter 4. Chapter 5 comprises conclusion and findings of this study. Recommendations to extend the study are also given at the end.

CHAPTER 2

Literature Review

2.1. Studies on use of microorganism in concrete

(Ramachandran et al., 2001) studied two kinds of cement mortar specimens, (i) prepared from mixing cement mortar with microorganisms and (ii) with simulated cracks on specimens filled with mixture of microorganisms. The concentration of cells was calculated by Ramachandran equation $Y=8.59 \times 10^{7} X^{1.3627}$, where X=reading at OD_{600} and Y= cells concentration per ml.

Bacillus pasteurii was suspended in saline and phosphate buffer. Two categories of biomass were prepared i.e. (i) pure culture of *B. pasteurii* and (ii) *B. pasteurii* and *Pseudomonas aeruginosa* in equal concentrations. Results showed that *B. pasteurii* increased compressive strength of mortar while dead microbial mass reduced compressive strength. The addition of *B. pasteurii* and *P. aeruginosa* in mortar increased strength due to adequate amount of organic materials due to biomass and not by calcite induced microbial growth. Overall, the microorganism helped in crack remediation however, insignificant effect was observed on strength of mortar specimens. Ghosh et al. (2005) achieved improved strength by mineral precipitation technique in mortar. An organism belonging to *B. sphaericus* species and *Escherichia colli* were selected to be incorporated in concrete by direct mixing. Different concentrations of the two types of bacteria were added in mixing water of cement mortar that ranged from 10 to 10^7 cells/ml of mixing water. W/C and C/S were taken as 1/3 and 0.4 respectively. *B. sphaericus*, an anaerobic microorganism increased compressive strength when used in mortar and the effect was maximum at concentration of 10^5 cells/mL of mixing water, while no significant strength improvement was observed by *E. colli*.

Jonkers (2011) used viable spores of bacteria as self-healing agents in concrete. The 2-4 mm LWA in concrete mix was replaced by clay particles of same size loaded with bacterial spores and calcium lactate. Immobilization in expanded clay provided protection and increased the life span of bacteria in concrete. The 2-4 mm LWA in concrete mix was replaced by clay particles of same size impregnated with bacterial spores and calcium lactate as organic mineral precursor. The concentration of bacterial spores is given as 1.7×10^5/g of expanded clay particles with 5×10^7 spores/dm^3 concrete. In 15 g/dm^3 of concrete, 5% w/w fraction of organic mineral precursor was added. The expanded clay particles reduced compressive strength of concrete specimens however, the healing efficiency was substantially improved. Rao et al. (2013) also studied biomineralization of bacteria in concrete by incorporating alkaliphilic *B. subtilis* JC3 into cement mortar with varying concentrations. Maximum compressive strength (25%) was observed at 10^5 cells/ml. The strength is improved due to growth of fillers within pores of cement-sand matrix.

Hydrogels are potential immobilizers in bacterial concrete as they can protect bacteria during hydration and mixing of concrete. They are biocompatible and have mass transport properties. Wang et al. (2014) encapsulated *B. sphaericus* spores in hydrogel capsules and observed the healing ratio. The concentration of spores in solution was 10^9 spores/mL and 1 mL spore suspension was encapsulated in one hydrogel sheet. Mortar specimens (prisms and cylinders) were prepared with w/c of 0.5 and a sand to cement ratio of 3 was fixed. Four series of specimens as control, with all nutrients, with hydrogels only and hydrogel and bacteria were designated as Group R, N, m-H and m-HS respectively. After cracking the prisms were placed in three different incubation conditions (i) 60% RH (ii) 95% RH and (iii) wet-dry cycles for 28 days. Crack repair was observed by light microscopy and final healing ratio was given as;

$$\mathbf{r = (C_{wi} - C_{wf}) \times 100 / C_{wf}}$$

where C_{wi} is initial crack and C_{wf} is final crack width measured after healing period of 28 days.

X-ray microtomography was carried out on cylindrical specimens to check the distribution of healing products inside the specimens. Results showed that wet-dry cycles were better incubating technique than 60% RH and 95% RH. 70%-100% healing was observed for cracks of upto 0.3 mm width in case of m-HS and it is 50% more than the m-H series. Most of the healing product was precipitated in surface layer of specimens. Only the specimens with bacteria had precipitation in subsurface layer to some extent as depicted by results of X-ray microtomography.

Wang et al. (2014) utilized dry paste of *B. sphaericus* that was produced in liquid minimal basal salts medium which was autoclaved at 120°C for 20 minutes. The bacteria culture was put in incubator for 28 days at 21°C after which it was pasteurized and harvested by centrifugation at 7000 rpm for 7 minutes. The paste was vacuum dried and ground in cement mill to get the powdered bacterial powder. The bacterial spores were encapsulated in 0.005 mm capsules by polycondensation reaction-based microencapsulation process. Each gram of microcapsule contained 10^9 bacterial cells. Mortar specimens with varying number of microcapsules were prepared. Based on dry weight content of cement, different amounts of microcapsule emulsion were added. The addition of microcapsules reduced the compressive and tensile strength of mortar. The pre-cracked samples were kept in five different incubation conditions for 56 days (i) 20℃, > 95% RH; (ii) water immersion; (iii) immersion in deposition medium; (iv) wet-dry cycles with water; (v) wet-dry cycles with deposition medium. Wet dry cycles gave the best healing for specimens with nutrients and 95% RH incubation condition was least effective. Healing efficiency of pre-cracked specimens was determined by light microscopy and water permeability test. Results showed that although microorganisms reduced the compressive strength, low water permeability and more healing was observed in bacterial specimens. Non-bacteria samples showed only 18% to 50% healing of cracked area while bacterial samples showed 48%-80% healing of crack area.

Nugroho et al. (2015) prepared mortar specimens at a fixed ratio of c/s as 1/3 and w/c as 0.485. Pulverized fly ash was added as 20% by weight of cement. Spores of *B. subtilis* were added in concentration of 10^4, 10^5 and 10^6 cells/ml in pulverized fly ash. The samples were provided with two types of immersion media. Submersion in water and in 5% urea and 1% calcium acetate solution. Results showed increased compressive strength in all bacteria containing series. In water submersed category, maximum compressive strength was achieved at 10^6 cells/ml concentration while for the other technique, highest strength was achieved at 10^5 cells/ml. The permeability co-efficient was decreased in specimens containing bacteria. Analysis of flexural loading depicted that bacterial mortar did not make significant improvement to flexural strength regain. Calcite precipitation was prominent in samples and better crystallinity was seen in X-Ray Diffraction (XRD) and Energy Dispersive Spectroscopy (EDS) of bacterial samples. Khaliq and Ehsan (2016) used various techniques for incorporating *B. subtilis* into concrete. Bacteria was introduced by direct mixing and through lightweight aggregate (LWA) and graphite nanoplatelets (GNP) as carrier compounds and used calcium lactate as organic precursor. Four different types of specimens Mix 1 without bacteria; Mix 2 with directly mixed bacteria; Mix 3 contained LWA as carrier and Mix 4 with GNP as carrier were used which were pre-cracked at 3,7, 14 and 28 days via compression till cracks were obtained. The specimens were allowed to heal for 28 days and scanning electron microscopy (SEM) and XRD tests were carried out. Samples containing LWA showed highest 28 days strength due to efficient microbial healing. In 28 days pre-cracked samples, healing of up to 0.52 mm was achieved.

A bacteria-based healing agent studied by Tziviloglou et al. (2016) consisted of a healing agent with bacillus spores (10^8 spores/L) which were incorporated in lightweight aggregates (LWA) using calcium lactate (200 g/L) and yeast extract (4g/L). The basic concept behind microbial self-healing of concrete is that the bacteria, in the presence of oxygen and water, converts calcium lactate present in healing agent, into calcium carbonate ($CaCO_3$). The healing agent was dried for few days which showed increased weight of impregnated LWA. A comparison was made between reference, non-impregnated LWA and impregnated LWA mortar samples. The samples were pre-cracked to 0.35 mm wide cracks by three-point bending test at 28 days and were cured by water submersion and wet-dry cycles. Impregnated LWA showed better crack sealing when healed in wet dry cycles. Flexural testing for non pre-cracked samples gave minimum strength in case of bacterial spores. SEM images depicted clustered rhombohedral formations of $CaCO_3$. Luo and Qian (2016) investigated the effects of two types of bacteria-based additives in cement paste matrix. Type 1 additive contained calcium lactate and bacteria spores powder while Type 2 consisted of calcium formate and bacteria spores powder. The specimens were cured at 90% RH and 20°C and were cracked at 3, 14 and 28 days. They were allowed to heal for 7 days. The area repair rate was calculated, carbonation depths and compressive strength tests were also conducted.

Choi et al. (2017) used technique of microbial induced calcite precipitation (MICP) in experimental research to repair cracks in mortar specimens. Φ50mm x 10mm cylindrical disks were cracked and subsequently treated with 21 cycles of MICP treatment in which each cracked sample was soaked in 60 mL bacteria solution for 2 hours. In each cycle, the specimen was left for 5 minutes to drain off bacteria solution and was then put in 4 L of urea-$CaCl_2$ solution for approximately 22 hours and then, the solution was allowed to drain off for 5 minutes. $CaCO_3$ was deposited on the cracked surfaces as a result. The tensile strengths of MICP treated specimens ranged from 32 kPa to 386 kPa, while the specimens undergone water treatment were too weak to be tested. Water permeability tests performed at 0, 7, 14 and 21 cycles of MICP treatment showed a significant reduction in permeability. Small cracks (< 0.52mm) were healed even at 7 MICP cycles, while 21 MICP cycles healed cracks widths of up to 1.62 mm. Vijay et al. (2017) reported that 35 mm deep cracks were healed by microencapsulation, 27.2 mm deep cracks by direct application and cracks of 0.97 mm width were healed by bacteria and encapsulation.

Self-healing behavior of *Sp. ureae* and *Sp. pasteurii* immobilized in zeolite was investigated. Normal and PVA fiber reinforced mortar mixes were studied for self-healing effect. The food source for bacteria comprised of yeast extract (0.2%), urea (2%) and calcium lactate (2%) with respect to mass of cement were also incorporated. The compressive strength of fiber reinforced samples was more than compressive strength of normal mortar specimens. In terms of bacteria, compressive strength was increased in normal and fiber reinforced bacteria mortar specimens.

Highest compressive strength was attained in fiber reinforced mortar specimens having zeolite immobilized *Sp. pasteurii*. Sorptivity and chloride permeability were also reduced due to presence of bacteria. The SEM images showed presence of $CaCO_3$ crystals in mortar mix due to bacterial activity (Bhaskar et al., 2017). Zhang et al. (2017) quantified the self-healing capability of concrete using non-ureolytic *B. cohnii*. Feasibility of expanded perlite as self-healing bacteria carrier was also investigated. Therefore, three incorporation techniques i.e., direct introduction of bacteria, use of expanded clay and use of expanded perlite were studied. OD_{600} value of bacteria suspension was maintained at 0.4 and concentration was reported at 3.6 x10^9 cells/mL. The crack widths were measured and healing percentage was noted after 28 days. The field emission scanning electron microscopic images show presence of $CaCO_3$ crystals which healed cracks up to maximum width of 0.79 mm. Figure 2.1 shows effect of some species of bacteria on compressive strength of cementitious materials.

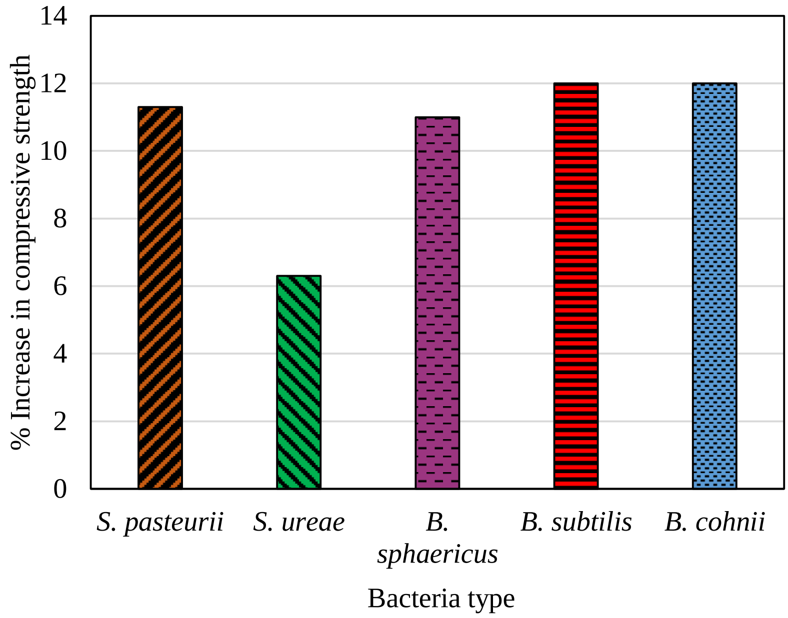


Figure 2.1: Comparison of bacteria in concrete (Bhaskar et al., 2017; Khaliq and Ehsan, 2016; Sierra-Beltran et al., 2014; Wang et al., 2014)

Gupta et al. (2018) immobilized biochar for immobilization of *B. sphaericus* in concrete for crack repair. Polypropylene fibers and superabsorbent polymers were also added in concrete. The specimens were pre-cracked at 50% and 70% of flexural strength. Biochar immobilized bacteria with polypropylene fibers gave best recovery of concrete compressive and flexural strength as compared to other combinations used.

B. pseudofirmus was immobilized in coated expanded perlite by Alazhari et al. (2018). The cells were routinely cultured on buffered lysogeny broth. Spores were incubated at 30°C for 3 days and harvested after being centrifuged at 10,000 rpm for 15 minutes. The spore pellet was freeze dried to a spore powder and stored for further utilization. Mondal and Ghosh (2018) investigated the effect of varying bacterial cell concentrations in mortar. Mortar cubes of dimensions 70.6 mm x 70.6mm x 70.6 mm were prepared with c/s of 1/3 with a w/c of 0.4. *B. subtilis* cells were added in varying concentrations of 10^3 cells/ml, 10^5 cells/ml and 10^7 cells/ml. Compressive strength, water absorption, self-healing efficiency, surface pore healing and water penetration depth were evaluated in mortar specimens with different concentration of microorganisms. Mortar specimens with 10^5 cells/ml was optimum in pertaining to strength enhancement while 10^7cells/ml showed maximum precipitation and thus, maximum crack healing and surface pore healing was observed in specimens with highest bacterial concentration. Figure 2.2 shows progress of crack healing by varying concentration of bacteria cells at different ages.

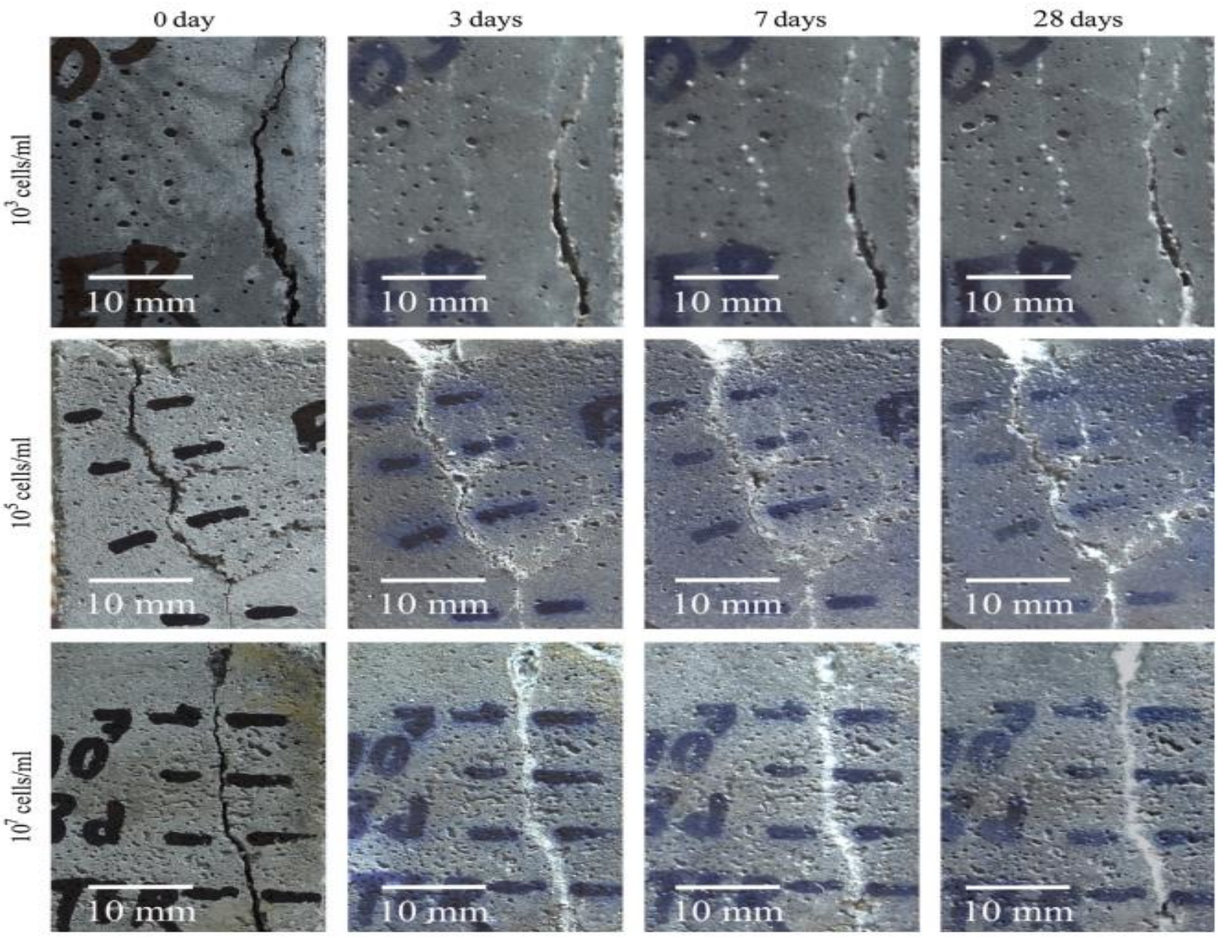


Figure 2.2: Progress of crack healing in microbial concrete by Mondal and Ghosh (2018)

Addition of bio-influenced self-healing agents not only induce self-healing properties, but also significantly affect mechanical properties of concrete mix. Bacterial treatment was performed on mortar specimens for improving bond between the substrate mortar and repair mortar surface as it deposited 0.05 mm thick $CaCO_3$ layer between the surfaces (Snoeck et al., 2018).

2.2. Studies on use of fibers in concrete

Several parameters affect self-healing capability of concrete. These include type of bacteria, type of immobilizer, quantity of bacteria solution and concentration of bacteria cells, pH of concrete and curing conditions etc. Although several carriers have been studied to check their effectiveness in self-healing, the literature on fibers is however, very limited. Fiber reinforced cementitious composites have been extensively studied in past researches (Elsaid et al., 2011; Sukumar and John, 2014; Zollo, 1997). It has proved itself to be an effective way of enhancing the strength and ductility of concrete. Various synthetic fibers such as polypropylene, nylon, glass, polyester, basalt, carbon and steel fibers can be utilized in concrete with appropriate sizes, types, aspect ratio and fraction with respect to volume (Sokolova et al., 2018; Sukumar and John, 2014). Nowadays, the potential of natural fibers is being studied for their use in cementitious materials.

Femandez (2002) optimized flax fiber reinforced concrete to encourage use of natural fibers in buildings. The study sought optimum length and optimum ratio of flax fibers in concrete in order to harvest its maximum benefits. Length of fiber was optimized at 3 cm. Tensile and compression strength tests were performed on cylindrical specimens and small beams were casted for three-point bending test. Results showed substantial improvement occurred in strength and toughness of flax fiber reinforced concrete. Another study was conducted by Yalley and Kwan (2009) on coir fiber for improving concrete properties. Coir fibers of diameter lying between 0.29 mm to 0.83 mm while length ranged from 6 mm to 24 mm was used in experiment which resulted in increase in angle of twist. Maximum compressive strength, tensile strength and torsion were achieved for concrete having 0.5 % fiber content.

Onuaguluchi and Banthia (2016) studied characteristics of bast, stalk, straw, leaf, seed and wood fibers and their effect on characteristics of cementitious materials. Fibers extracted from outer bark of plant stems are called bast fiber. These include jute (Corchorus olitorius /Corchorus capsularis), flax (linum usitatissimum), and abaca. These are long fibers with high tensile strength and are generally used in making yarn, fabric, ropes etc. Seed fibers also possess similar characteristics of strength. They are light weight and used in production of ropes, mats, geotextiles etc. Coir is a common example of seed fiber and is obtained from coconut husk. The chemical composition and mechanical properties of coir, flax and jute fibers are given in Table 2.1 & Table 2.

Table 2.1: Composition of Fibers (Dittenber and GangaRao, 2012; Ramakrishna and Sundararajan, 2005; Reddy and Yang, 2005)

Group	Fiber source	Cellulose	Hemicellulose	Lignin
Bast	Jute	33.4	22.7	28.0
	Flax	62-72	18.6-20.6	2-5
Seed	Coir	36-43	0.15-0.25	41-45

Table 2.2: Mechanical properties of fibers

Group	Fiber source	Tensile strength	Youngs' Modulus	Elongation	Density
		(MPa)	(GPa)	(%)	(g/cm^3)
Bast	Jute	393-773	26.5	1.5-1.8	1.3
	Flax	345-1035	27.6	2.7-3.2	1.5
Seed	Coir	175	4-6	30	1.2

Razmi and Mirsayar (2017) used jute fiber as reinforcement in concrete and investigated the fracture properties. Jute fiber was added as 0.1%, 0.3% and 0.5% by weight of concrete and length was maintained as 20 mm. Semicircular bend specimens of concrete were prepared. Results indicated significant improvement in compressive, flexural and split tensile strength of mix with 0.5% jute fiber. Fracture resistance was also increased however, in mixes with more than 0.3% jute fibers, significant improvement was not observed. Ferreira et al. (2018) conducted pull out tests on cement matrices containing sisal, curaua and jute fibers. Figure 2.3 shows SEM images of these fibers. The three types of fibers were washed in hot water (80°C-100°C) to remove fats, wax, mucilage. They were later heated in a container with warm water and dried. Metallic comb was used to brush the fibers and fibers were cut. Because of irregular morphology of fibers, the mechanical response of fibers presents a relatively higher scatter. The tensile strengths were found to be 484, 632 and 250 MPa for sisal, curaua and jute fibers respectively. The elastic moduli were found to be 19.5, 38.1 and 43.9 GPa. The results showed their potential for use in concrete and comparable properties of these natural fibers with other synthetic industrial fibers.

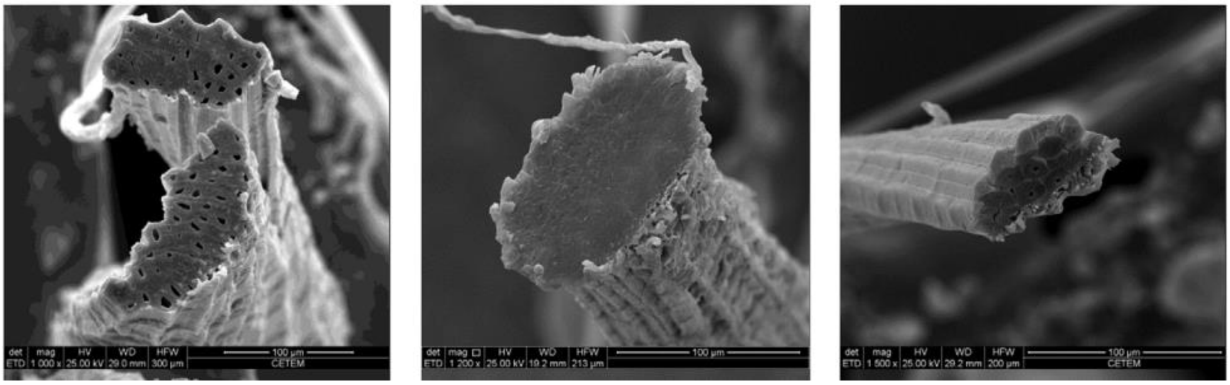


Figure 2.3: SEM Images of (a)Sisal (b) Curaua (c) Jute fibers

CHAPTER 3

Experimental Methodology

Several studies have been conducted on bio-influenced self-healing concrete. Most of the studies have focused on utilization of several kinds of bacteria in concrete to achieve self-healing. In addition to potential use of bacteria, some species of fungi are also being used. As sufficient efforts have already been put in finding out different types of crack-healing organisms, there is a need to find out effective bacteria carrying mechanism. So, this research work focusses on finding an efficient bacteria carrying technique while the bacteria used are those already used in past researches. For this purpose, three different types of natural fibers have been selected i.e. coir, flax and jute, as carrier compounds. *B. subtilis* KCTC-3135^T, *B. cohnii* NCCP-666 and *B. sphaericus* NCCP-313 obtained from National Culture Collection of Pakistan (NCCP) were used as crack healing bacteria. The specimen details matrix is shown in Table 3.1.

Table 3.1: Details of concrete specimens

Specimen	**Bacteria**	**Carrier**	**Compressive strength**	**Recovery of Compressive strength**	**Ultrasonic Pulse Velocity**	**Healing observation**	
						Pre-cracked at 7 d	**Pre-cracked at 28 d**
Control		-	3	3	1	1	1
BSC	*B. subtilis* KCTC-3135^T	Coir	3	3	1	1	1
BSF		Flax	3	3	1	1	1
BSJ		Jute	3	3	1	1	1
BCC	*B. cohnii* NCCP-666	Coir	3	3	1	1	1
BCF		Flax	3	3	1	1	1
BCJ		Jute	3	3	1	1	1
BSpC	*B. sphaericus* NCCP-313	Coir	3	3	1	1	1
BSpF		Flax	3	3	1	1	1
BSpJ		Jute	3	3	1	1	1

The nomenclature used for specimens indicates the bacteria and carrier used for bacteria incorporation. Control specimens do not contain any bacteria or carrier compound. *B. subtilis* KCTC-3135^T is denoted as BS, BC shows *B. cohnii* NCCP-666 and *B. sphaericus* NCCP-313 is denoted by “BSp”. The suffixes “C”, “F” and “J” represent the carriers coir, flax and jute respectively. A total of ten different formulations were made to carry out experimental procedure. The following standards were referred to during experimental procedure,

- ACI 211.1-91, “Standard practice for selecting proportions for normal heavyweight and mass concrete”.
- ASTM C 127-01, “Standard test method for density, relative density (specific gravity) and absorption of coarse aggregate.
- ASTM C 192/C 192M-02, “Standard practice for making and curing concrete test specimens in the laboratory.
- ASTM C 39/C39M-18, “Standard Test Method Compressive Strength of cylindrical concrete specimens.”
- ASTM C 597-16 “Standard Test Method for Pulse Velocity through Concrete”.
- ASTM C 1365-18, “Standard Test Method for Determination of the Proportion of Phases in Portland Cement and Portland-Cement Clinker Using X-Ray Powder Diffraction Analysis."
- ASTM C 1723-16, "Standard Guide for Examination of Hardened Concrete Using Scanning Electron Microscopy."

3.1. Materials

3.1.1. Microorganism

B. subtilis

It is also known as Hay bacillus or grass bacillus found in soil and gastrointestinal tract. The strain used was *B. subtilis* KCTC-3135^{T}. *B. subtilis* is a gram positive species having motile rods and form ellipsoidal or cylindrical spores. Length 2-3 µm and width ranges 0.7-0.8 µm. The morphological characteristics of colonies show their round to irregular shapes of diameter 2-4 mm. The colonies are opaque with dull surfaces that become wrinkled. The color is whitish, creamy or brown. According to Bonne et al. (2009), the urea hydrolysis ability of *B. subtilis* ranges from 0-15%. Few researches have also reported the limited urease forming ability of *B. subtilis* (Kim et al., 2005).

Figure 3.1: *B. subtilis* cells

B. cohnii

B. cohnii is a gram positive alkaliphilic bacteria, exists as motile rods and forms ellipsoidal spores. The colonies are creamy white in color and 1-2 mm in diameter after 2 days at 45 ^{O}C. The optimum temperature for growth is 10°C-47°C and pH 9.7. It does not hydrolyze urea (Bonne et al., 2009). However, it has the capability of forming calcite in presence of calcium lactate as a result of its metabolic activity. *Bacillus* sp. NCCP-666 (16S rRNA gene accession number: AB968095 have 100% sequence similarity of 16S rRNA gene with *B. cohnii*) was used in the study.

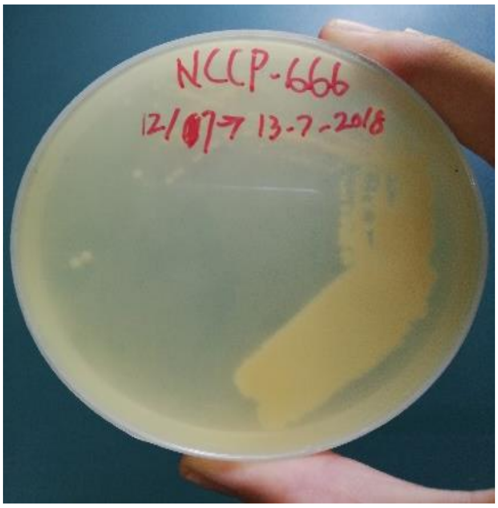


Figure 3.2: *B. cohnii* cells

B. sphaericus

It is aerobic gram positive bacteria, exists as motile rods and forms spherical spores. The cells are 1.0 by 1.5-5.0µm. Opaque, unpigmented colonies are often glossy and smooth. The growth temperature is 10°C-45°C. It grows at pH of 7.0-9.5. The urease forming ability of this species is variable and it is considered a urease positive bacteria (Bonne et al., 2009). *Bacillus* sp. NCCP-313 (16S rRNA gene accession number: AB734813 have 100% sequence similarity of 16S rRNA gene with *B. sphaericus*)

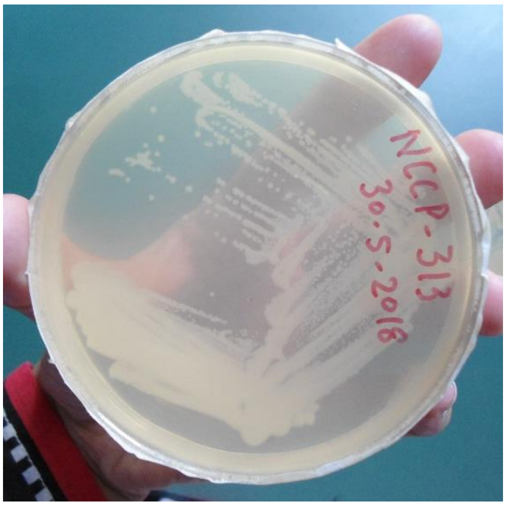


Figure 3.3: *B. sphaericus* cells

Preparation of Bacterial Culture

24g trypticase soya agar (TSA, Oxoid™) was added in 600 ml distilled water and mixed thoroughly. The solution was autoclaved at 121°C for 15 minutes. The solution was used to prepare 20 petri dishes that were left to dry up for 24 hours. Bacteria stocks stored at -80°C were streaked on the petri dish and left for 24 hours to give sufficient time for formation of colonies in incubator at 30°C.

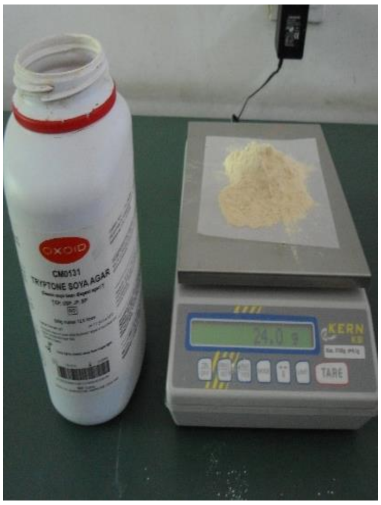


Figure 3.4: Trypticase Soya Agar

Figure 3.5: Tryptone Soya Agar Soln.

Figure 3.6: Autoclave

Figure 3.7: Incubator for controlled temperature

The bacterial culture was prepared in Trypticase Soy Broth (TSB, Oxoid^{TM}) media. The flasks were properly sealed, autoclaved at 121℃ for 15 minutes and allowed to cool down for some time. 24 hours were given before using the media for bacteria culturing, to confirm no growth of any contamination within media. Bacteria from the petri dish was inoculated in the TSB medium and the flasks were put on incubation shaker at 30℃ for 24 hours. The bacteria culture grown overnight was ready for sporulation.

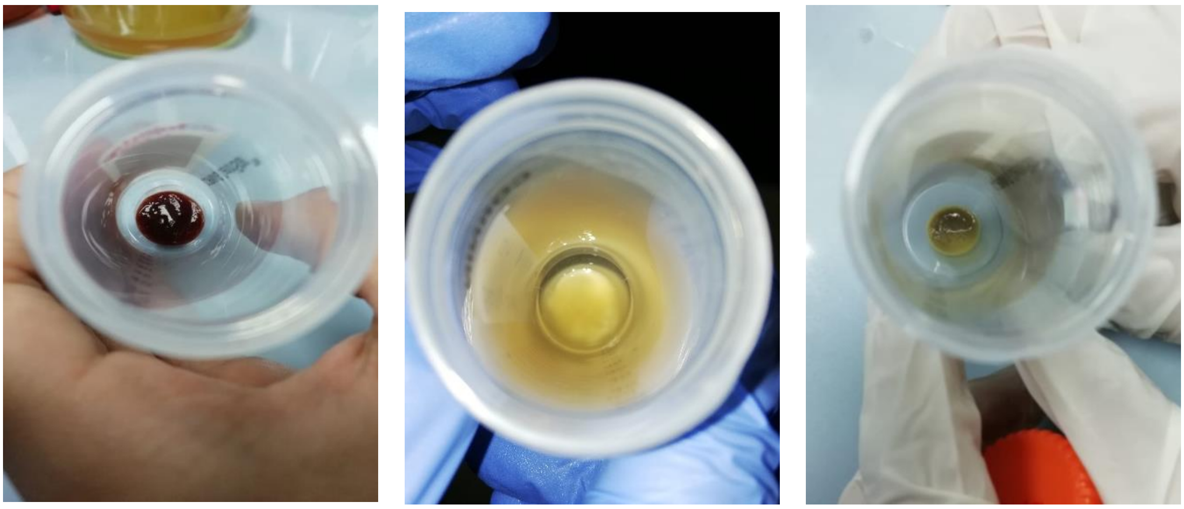
Figure 3.8: Sporulation salts

Figure 3.9: Spores in TSB medium

Figure 3.10: Centrifuge

Figure 3.11: Settled spores

For sporulation, the cells were grown in TSB with $MgSO_4.7H_2O$ (1.01 mM), KCl (13.4 mM), $FeSO_4$ (0.001 mM), $Ca(NO_3)_2$ (1.0 mM) and $MnCl_2$ (0.01 mM) at pH 7.0 for 6 days at 30℃ as described by (Ahmed et al., 2007). The spores were harvested by centrifugation (Centrifuge 5702, Eppendorf) at 4400 rpm for 15 minutes. The spores of each strain were suspended in distilled sterilized water to get desired cells concentration. OD_{600} equivalent to McFarland Standard 2 was maintained, which corresponds to approximately $6x10^8$ cells mL^{-1} bacterial cells. The suspension was kept at 4℃ until further use.



(a) (b) (c)

Figure 3.12: Spores (a) *B. subtilis*, (b) *B. cohnii*, (c) *B. sphaericus*

Figure 3.13: Spectrophotometer

Figure 3.14: Suspension of spores

McFarland Turbidity Standards

McFarland standard is a series of standards of different opacity which estimate density of bacterial suspension. It is commonly used as standard in various microbiological methods. There are designated tube numbers for suspension made up of barium sulphate. Various numbers are assigned to the suspensions of varying opacities. Each standard represents different opacity which is related to the opacity of bacterial suspensions for practical use. In order to find out bacterial concentration, the aforementioned standard numbers are related to concentrations and optical densities as tabulated in Table 3.2.

Table 3.2: McFarland Standard

Standard	Bacterial concentration (x 10^6 mL^{-1}	Theoretical Optical density at 550 nm
0.5	150	0.125
1	300	0.25
2	600	0.5
3	900	0.75
4	1200	1
5	1500	1.25

3.1.2. Fibers

The bacteria strains were incorporated in three different natural fibers as immobilizers to compare the effectiveness. Commonly available coir, flax and jute fibers were obtained. The coir and jute fibers were cut to 25±5 mm for their use in concrete. One important characteristic of these fibers is that they are extreme hydrophilic in nature, thus, they absorb water and form lumps. The sorption capacity and diameters were checked before utilizing them as bacteria carriers in experimental work (Table 3.3). Fourier Transformed Infrared Spectroscopy (FTIR) was carried out to characterize the fibers (Figure 3.16 & Table 3.4). The FTIR spectra of incorporated fibers resembled the previously reported results which confirmed that the selected fibers have same chemical constituents as coir (Ebele et al., 2016), flax (Titok et al., 2010) and jute (da Silva et al., 2016). The spectrum of coir fiber reveals a broad peak at 3427 cm^{-1} which suggests hydrogen bonded OH stretching vibration from cellulose and lignin structure whereas, peak at 1629 cm^{-1} represents amines compound class which indicates good mechanical properties of coir fiber (Ebele et al., 2016; Sari et al., 2015). The frequency band at 3398 cm^{-1} and 3418 cm^{-1} for flax and jute fibers respectively, indicate OH stretch which can be associated to water desorption thus, causing easy release of bacteria solution for self-healing in concrete (da Silva et al., 2016; Titok et al., 2010)

Table 3.3: Properties of natural fibers

	Coir	Flax	Jute
Sorption (%)	257%	985%	787%
Diameter (mm)	0.2-0.74	0.04	0.06
Length (mm)	25±5	25±5	25±5

(a) (b) (c)

Figure 3.15: Photographs of fibers (a) coir (b) flax (c) jute

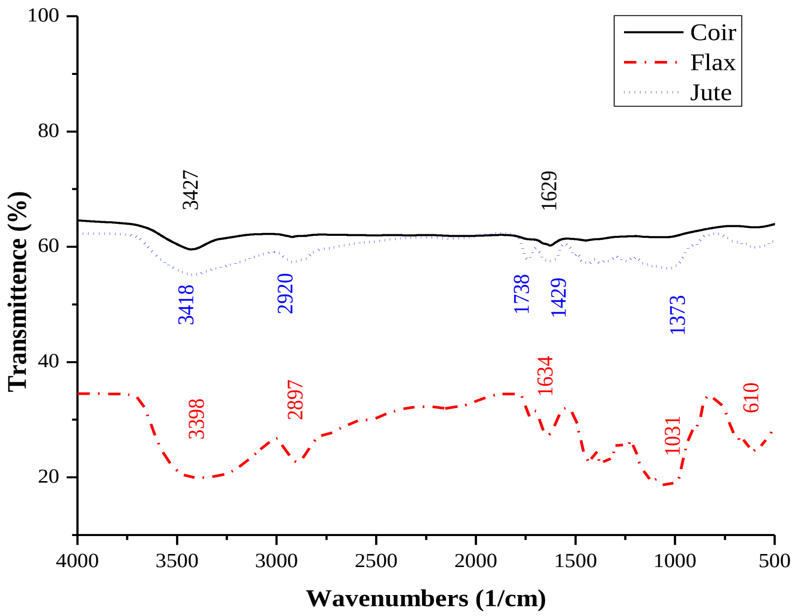


Figure 3.16: FTIR spectra of fibers

Table 3.4: FTIR analysis of fibers

Coir		**Flax**		**Jute**	
Frequency (cm^{-1})	**Assignment**	**Frequency (cm^{-1})**	**Assignment**	**Frequency (cm^{-1})**	**Assignment**
3427	OH stretch	3398	OH stretch	3418	OH stretch
1629	NH_2	2897	CH stretch	2920	CH_2 & CH_3
		1634	C=C stretch	1738	-CHO
		1430	CH	1631	NH_2
		1372	OH	1374	OH
		1032	C-O-C	1036	C-O-C
		610	OH	608	OH

3.2. Mix proportions

Ordinary Portland Cement Type-1 conforming to ASTM C-150 as 385 kg/m^3, 850 kg/m^3 naturally available sand as fine aggregate (FA) and 937 kg/m^3 coarse aggregate (CA) were used in preparation of concrete mix. Water to cement ratio was fixed as 0.45. Ultra Superplast-675 water reducing and plasticizing admixture was used for inducing workability to the mix. Ten series of samples were prepared with details in Table . Mix with no fiber and bacteria was named as control. The mineral precursors i.e. calcium lactate pentahydrate and urea were added during the dry mixing. As urea is relatively inexpensive than calcium lactate pentahydrate and species of *B. sphaericus* show efficient urease activity, therefore, it was added to *B. sphaericus* NCCP-313 formulations along with some amount of calcium lactate pentahydrate as bio-mineral precursor. On the other hand, urease enzyme is not produced by urease negative strains therefore, *B. subtilis* KCTC-3135^T and *B. cohnii* NCCP-666 formulations contained calcium lactate pentahydrate only to provide bacteria with sufficient Ca^{2+} ions. Previous literature shows that urea along with calcium source has been commonly used as food source for *B. sphaericus* and calcium lactate pentahydrate has been used for non-ureolytic bacterial species (Jonkers et al., 2010; Khaliq and Ehsan, 2016; Wang et al., 2014; Wang et al., 2012).

Fibrous carriers were soaked in bacteria solution for 15 minutes before their addition in concrete mix. Extra 10% solution was added to ensure complete soaking of fibers and to counter any losses. Cement, fine aggregate and coarse aggregate were dry mixed initially. In wet mixing process 80% of water was added at first and remaining 20% water consisting of mixed superplasticizer was added later. After that, the fibers were carefully added so that balling effect and clumps formation could be avoided. To ensure complete distribution of fibers throughout the mix, it is necessary to stop the mixer, manually apply a layer of fiber and activate the machine again (Yalley and Kwan, 2009). Cylinders of 100 mm diameter and 200 mm height and cubes of 100 mm x 100 mm x 100 mm were prepared. The specimens were demolded after 24h and water cured for 7 days and 28 days at 20±5℃.

Table 3.5: Composition of cement

Compound	w/w percentage (%)
SiO_2	20.57
Al_2O_3	4.72
Fe_2O_3	3.01
CaO	65.51
MgO	1.05
SO_3	2.42
Na_2O	0.11
K_2O	0.46

Table 3.6: Properties of aggregates

	Fine aggregate	Coarse aggregate
Bulk specific gravity	2.647	2.642
Absorption	1.426%	0.881%
Fineness Modulus	3.01	-

Table 3.7: Properties of superplasticizer

Appearance	Brown liquid
Specific gravity	1.150 at 20 ºC
Air entrainment	Less than 3% at normal dosage
Alkali content	Less than 72 g

Table 3.8: Mix design

	Control	***B. subtilis***			***B. cohnii***			***B. sphaericus***		
		Coir	**Flax**	**Jute**	**Coir**	**Flax**	**Jute**	**Coir**	**Flax**	**Jute**
Cement (kg/m^3)	385	385	385	385	385	385	385	385	385	385
FA (kg/m^3)	850	850	850	850	850	850	850	850	850	850
CA (kg/m^3)	937	937	937	937	937	937	937	937	937	937
W/C	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45	0.45
SP (% w/w cement)	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2
Bacteria soln. (L/m^3)	-	13	13	13	13	13	13	13	13	13
Calcium lactate (% w/w cement)	4	4	4	4	4	4	4	1.2	1.2	1.2
Urea (% w/w cement)	-	-	-	-	-	-	-	2.8	2.8	2.8
Fiber (kg/m^3)	-	4.6	1.2	1.5	4.6	1.2	1.5	4.6	1.2	1.5

3.3. Test Specimens

During pouring of concrete, the moulds were put on vibrating table to ensure proper compaction and elimination of voids from concrete. The specimens were removed from moulds after 24 hours of casting and were placed in curing tank for fully immersed water curing. Samples of two dimensions were prepared. For mechanical strength tests and visual observations of healing, cylindrical specimens of 100 mm diameter and 200 mm height were made, while for ultrasonic pulse velocity test, cubic specimens of 100 mm x100 mm x 100 mm were prepared. For mechanical tests, average of three specimens was taken. Figure 3.17 shows the specimens during casting procedure.

(a) (b) (c)

Figure 3.17: Casting, curing and prepared sample

3.4. Test Procedure

Compression strength tests were performed using “MCC- 08 Controls” compression testing machine. The specimens were cured for 28 days and were allowed to completely dry for 24 hours. Gypsum plaster was used for capping of cylindrical specimens to provide a smooth finish for their placement in compression testing machine. For each formulation average of three specimens was taken. The loading rate was set as 0.1 MPa/sec to adequately examine crack production and propagation (ASTM, 2018). For self-healing promise, the specimens were pre-cracked and exposed to fully immersed water cured healing condition. To check the regain in compressive strength, the specimens were carefully cracked till the point when small but visible cracks appeared on surface which was reached at 80-85% of compressive strength.

The specimens were then left for a healing period of 28 days to check regain in compressive strength. To measure compressive strength recovery, the following relation was used as described by (Shaheen and Khushnood, 2018)

$$\mathbf{CSR(\%)= [1-\frac{Cu-Cr}{Cu}] \times 100}$$

where CSR denotes compressive strength recovery, Cu = ultimate compressive strength at 28 days and Cr = strength of cracked specimen after healing.

Ultrasonic pulse velocity (UPV) test was performed on cubic specimens in which an electro-acoustical transducer was held in contact with concrete surface. Pulse was received on opposite side after traversing through a distance of 100 mm and its time of travel was recorded. Pulse velocity was obtained as ratio of length travelled to the time taken by ultrasonic wave in travelling the measured distance according to ASTM C597-16 (ASTM, 2016).

For visual observation of cracks, a crack width measuring microscope accurate up to 0.02 mm was used. Samples were pre-cracked till cracks of several sizes were produced. Samples were categorized as 7 days pre-cracked and 28 day pre-cracked corresponding to the age when cracks were produced. The initial widths of several cracks were marked and noted. Both types of samples were placed in fully submerged curing condition. The samples were taken out of curing after interval of 28 days and 56 days and widths of cracks were again measured. The difference in millimeters of initial widths and final widths was referred as “healing”.

Figure 3.18: Compression machine

Figure 3.19: Cracked samples

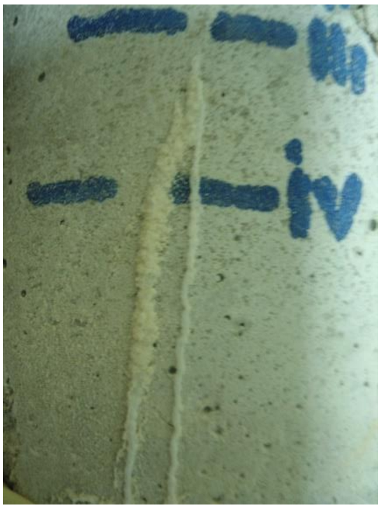


Figure 3.20: Crack after healing

Figure 3.21: Crack width measuring microscope

XRD test was carried out using D8 Advance DAVINCI (Bruker, Germany). The healing product obtained as a result of healing was subjected to X-ray diffraction analysis (ASTM, 2018). Four types of healing powders were obtained from (i) control specimen, (ii)*B. subtilis* KCTC-3135^{T} specimen, (iii) *B. cohnii* NCCP-666 specimens and (iv) *B. sphaericus* NCCP-313 specimens. SEM test was performed using TESCAN VEGA3 scanning electron microscope to study the microstructure and morphology of self-healing concrete (ASTM, 2016). Microstructural analysis was conducted on four specimens selected in such a way that all three types of bacteria and carriers were covered

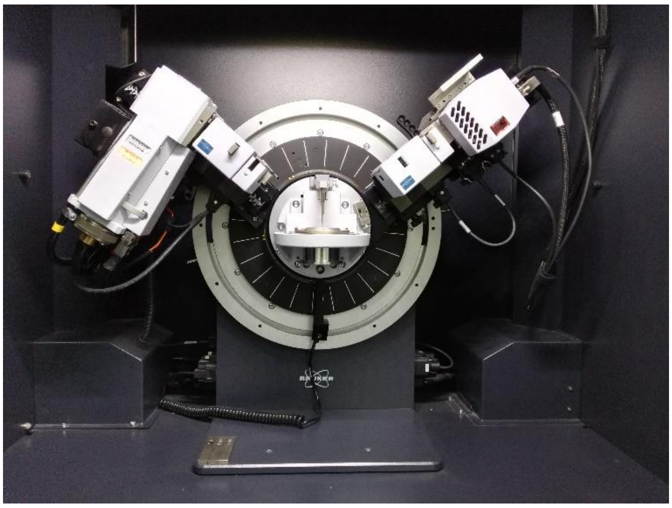


Figure 3.22: X-Ray Diffraction Machine

Figure 3.23: Scanning Electron Microscope

CHAPTER 4

Results and Discussion

4.1. Compressive strength

Figure 4.1 shows compressive strength of control and fiber reinforced microbial concrete at 28 days age. Irrespective of bacterial species and immobilizers, compressive strength values were significantly higher in all fiber reinforced microbial formulations as compared to control specimens which attained least compressive strength (29.3 MPa). It is attributed to fiber reinforcement as a proven technique to attain higher strengths and improved engineering properties in concrete (Femandez, 2002; Yalley and Kwan, 2009). Among immobilizers, coir fibers gave more pronounced values of compressive strength than flax and jute fibers which could be attributed to its better mechanical strength as evidenced via FTIR analysis (Table 3.4). Moreover, as diameter of coir fibers is much greater than flax and jute fibers and fiber radius is in direct correlation with axial compressive strength in composite according to Yerramalli and Waas (2004), therefore coir fiber reinforced microbial concrete resulted in better mechanical strength. With coir fiber immobilization, *B. subtilis* KCTC-3135^{T}, *B. cohnii* NCCP-666 and *B. sphaericus* NCCP-313 showed 36, 33 and 42% improvement in compressive strength respectively. The results of a previous study showed 27% increase in compressive strength of concrete by addition of coir fiber Krishna et al. (2018). For flax immobilized bacteria, improvement of 26, 32 and 39% was observed in BSF, BCF and BSpF respectively, while previously 2% improvement in flax fiber reinforced concrete was reported by Yaremko (2012). Razmi and Mirsayar (2017) reported increase of 24% for jute fiber (0.3% w/w cement) reinforced concrete which was lesser than 34, 32 and 33% in BSJ, BCJ and BSpJ respectively. Careful comparison with previous researches explained that although fiber reinforcement enhanced capacity of concrete in compression, the additional improvement in compressive strength observed in this research could be attributed to the deposition of $CaCO_3$ by bacteria. $CaCO_3$ product and organic nutrients fill the pores in concrete causing its densification thus enhancing its capacity in compression (Khaliq and Ehsan, 2016).

With respect to bacteria species, maximum increase in strength among fiber reinforced microbial specimens containing *B. subtilis* KCTC-3135^{T} and *B. cohnii* NCCP-666 was 36 and 33% respectively, while *B. sphaericus* NCCP-313 resulted in highest increase (42%) in compressive strength among all formulations. Previously, Khaliq and Ehsan (2016) and Sierra-Beltran et al. (2014) reported increase of about 12% when *B. subtilis* and *B. cohnii* were immobilized in LWA. A decrease of 17% was observed by Wang et al. (2014) when *B.*

sphaericus was immobilized in melamine microcapsules. Thus, it can be concluded that fiber immobilized bacteria did not adversely affect integrity of concrete rather they contributed more to compressive strength as compared to other immobilization techniques. Moreover, although urea is reported to cause reduction of split tensile strength (Jonkers and Schlangen, 2008), its effect on compressive strength was reported as negligible (Wang et al., 2014). Hence, compressive strength value of *B. sphaericus* NCCP-313 formulations which contained urea revealed that their higher strength attained could be attributed to the higher calcite forming ability of *B. sphaericus* NCCP-313 than *B. subtilis* KCTC-3135^T and *B. cohnii* NCCP-666. Even if urea contributed to loss of strength, the minimal effect was neutralized by abundant bacterial activity.

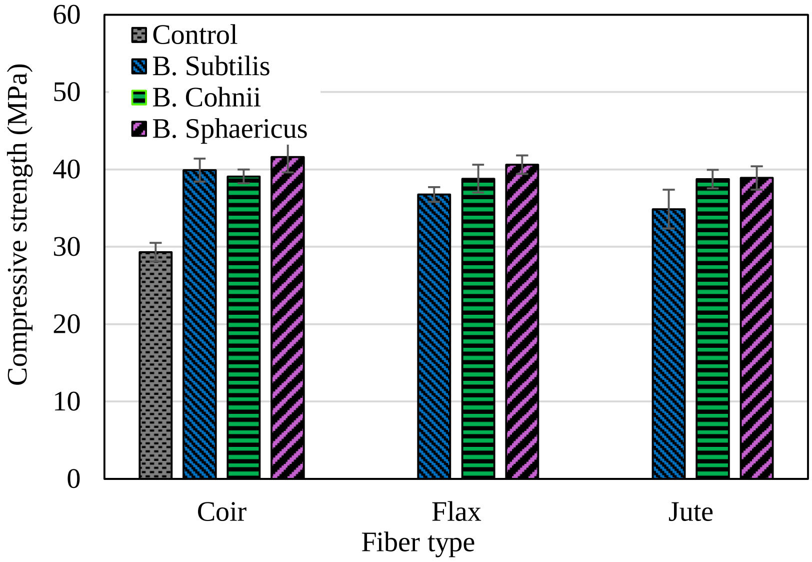


Figure 4.1: 28 days compressive strength

4.2. Compressive strength Recovery

Self-healing efficiency was also assessed via compressive strength regain after pre-cracking in compression. The recovery of compressive strength is plotted in Figure 4.2. Results reveal that coir fiber attained minimum regain in compressive strength in particular when used with *B. cohnii* NCCP-666 (only 60%). With *B. subtilis* KCTC-3135^T and *B. sphaericus* NCCP-313, coir fiber values reached up to 61 and 81% respectively, which was least among other carrier fibers. Contrary to that, flax and jute fibers achieved better compressive strength recovery after healing. The highest percentages were attained in BSpF and BSpJ with corresponding values of 95.1 and 98.4%. The relative ineffectiveness of coir fibers in regaining compressive strength indicates that it is not as efficient as flax and jute for carrying bacteria in self-healing concrete. The sorption capacity of coir fiber is much lesser as compared to other carriers (Table 3.3) which shows that the bacteria solution was not adequately penetrated inside coir fibers. Much of the healing agent was trapped on fibers surface only, therefore the spores could not be protected well from highly alkaline concrete environment and mechanical forces during mixing of concrete. Thereby, it can be inferred that, coir fiber did not provide an optimum protective medium for bacteria activity, while flax and jute fibers proved to be protective carriers for bacteria thus, resulting in recovery of higher percentage of ultimate strength.

The results indicate that, compressive strength recovery values in fiber reinforced microbial concrete ranged from 60-100% while Shaheen and Khushnood (2018) reported 50% regain in compressive strength when *B. subtilis* was carried in limestone powder which evidence higher efficiency of fibers as compared to limestone powder for immobilization purpose. Comparing compressive strength recovery of three bacteria species, Figure 4.2 also illustrates that in spite of lesser content of calcium source in *B. sphaericus* NCCP-313 (i.e. only 1.2% in comparison to 4% in other species), it contributed more to the regain in compressive strength than *B. subtilis* KCTC-3135^{T} and *B. cohnii* NCCP-666 which gave almost comparable values. Thus, it can be implied that calcite precipitation activity of *B. sphaericus* NCCP-313 is more efficient which was further triggered in presence of urea.

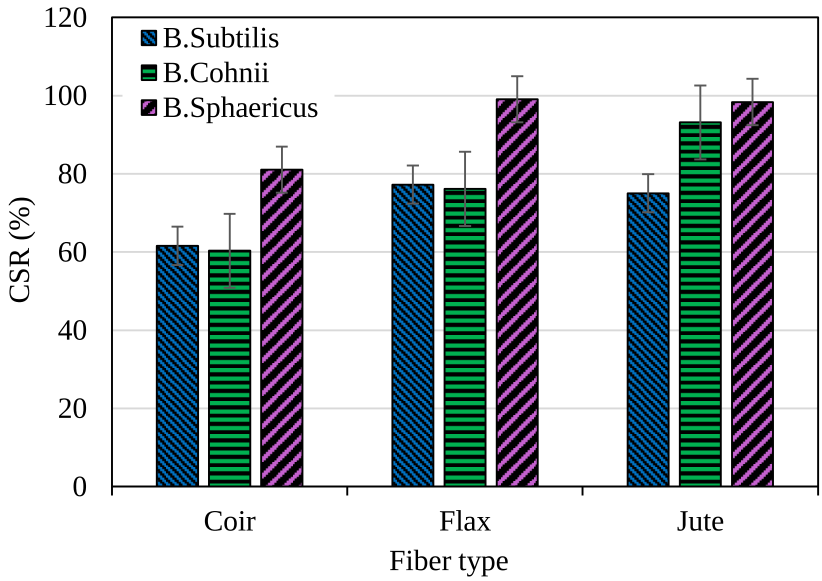


Figure 4.2: Regain in compressive strength

4.3. Ultrasonic Pulse velocity (UPV)

UPV was used to observe sealing of internal cracks in specimens after 28 and 56 days of water immersion. Speed of ultrasonic waves is more in concrete than in water. Upon production of crack, the time taken for an ultrasonic wave to travel a defined distance increases because the waves travel in surrounding of a crack. As a result of gradual healing and filling of pores with calcium carbonate precipitates, the transmission time gradually reduces and waves travel through shortest path (Williams et al., 2016). The percentage difference of final and initial transmission velocities was taken Figure 4.3. The percentage increase in UPV gave measure of filling of micro cracks and pores inside the specimens.

Figure 4.3 shows that the rate of healing in fiber reinforced microbial concrete was higher than control concrete and it gradually reduced with increasing age of concrete. Increase in UPV in control specimens was only up to 2.5 and 4.1%, corresponding to 28 and 56 days of healing respectively. At the time of occurrence of crack, transmission velocity was minimum. On exposure to healing conditions, the velocity steadily increased and maximum observed velocity was attained after 56 days of healing. Trend of improvement in UPV (%) also illustrates that among carriers, flax fiber depicted highest improvement of 21.4, 14.12 and 21.64% in BSF, BCF and BSpF respectively. It can be inferred that, due to extreme hydrophilic nature of flax fiber, the spores' solution penetrated well inside the fiber and spores remained protected when added in concrete matrix. Flax fiber also exhibited highest rate of improvement among all immobilizers during the initial healing period of 28 days, while in coir and jute fibers, the initial rate of healing was comparatively lower. It was observed that, coir immobilization did not give high improvement in UPV which rendered it as less suitable bacteria carrier than flax and jute. Cracks in coir reinforced specimens were not repaired adequately. This was presumably due to pull out of coir fibers loaded with bacteria cells as a result of stresses which generated transversely due to poisson's ratio effect. Some part of bacteria loaded fibers lost contact with concrete due to pull out thus, contributing lesser to crack healing. It can be deduced that, flax fiber provided better protection to bacteria cells which led to efficient carbonatogenesis and ureolytic activity in microbial specimens. Results also support that an immobilizer which gives higher healing at initial ages, also provides better overall healing characteristics. Careful comparison from the perspective of different bacteria species shows that best results were achieved by *B. sphaericus* NCCP-313 owing to its higher calcite forming ability. Crystals of calcium carbonate plugged the inner pores and cracks thus compacted the microstructure (Shaheen and Khushnood, 2018) and ultimately allowed easy transfer of ultrasonic waves.

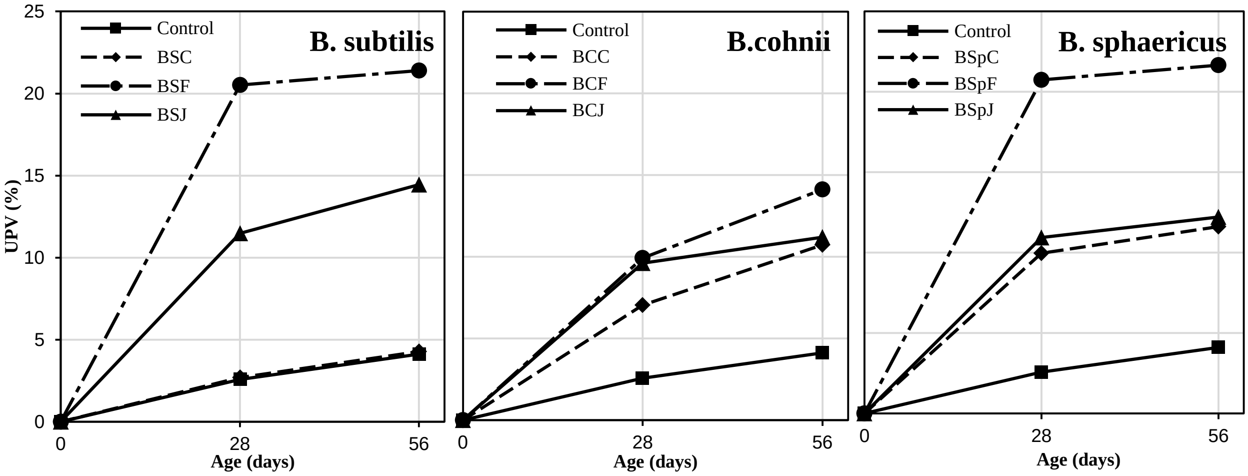


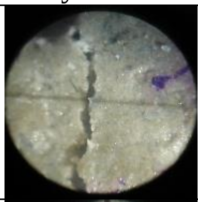
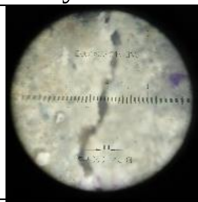
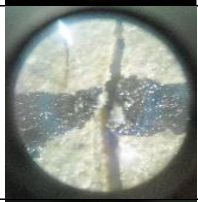
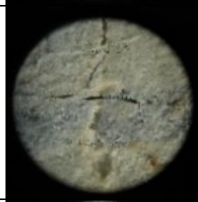
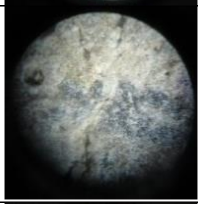
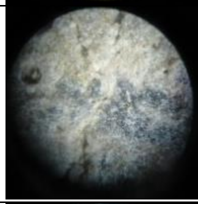
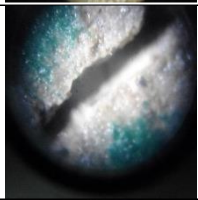
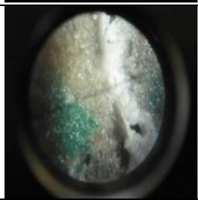
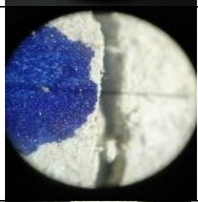
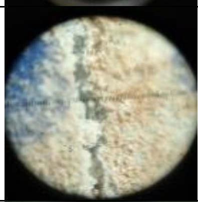
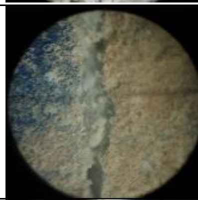
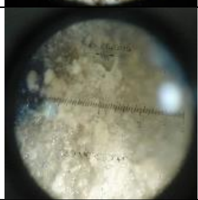
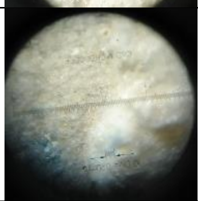
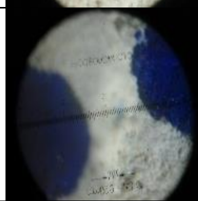
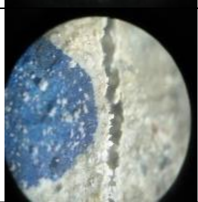
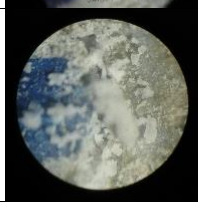
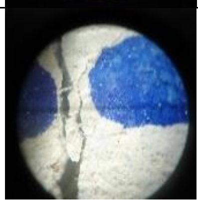
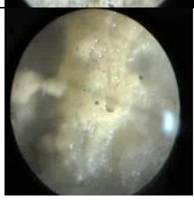
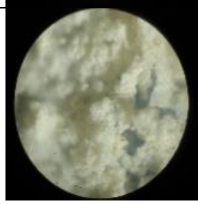
Figure 4.3: Improvement in UPV

4.4. Visual observation of self- healing

Specimens were collected after healing period of 28 days and 56 days from those previously cracked at 7 and 28 days age in order to visually determine the self-healing efficiency. They were later observed for crack healing at specific time intervals of 28 and 56 days of water immersion. Since cracking width could not be controlled during loading process, cracks of variable sizes were produced. Photographic images of specified cracks in ten types of specimens at different healing periods are illustrated in Figure 4.4. In general, most of the cracked regions were healed till 28 days of healing and healing up to 56 days further added to the $CaCO_3$ formation on surface of cracks. Crack in control specimen was not visibly healed although nutrient and autogenous healing caused some surface pore filling while in all the fiber reinforced microbial formulations, cracks were repaired as a result of ample mineral precipitation on cracked region which confirmed that bacteria immobilized in fibers are capable of producing copious amount of healing product. Furthermore, crack healing patterns were observed to be variable. Among bacteria groups, *B. sphaericus* NCCP-313 produced abundant amount of healing product with all three fiber carriers. Comparing the fiber carriers, it was witnessed that coir immobilized bacteria produced lesser precipitation than other carriers of their respective bacteria groups. After 28 days healing, *B. subtilis* KCTC-3135^T depicted partial healing of cracks in center while precipitates were not observed on edges. Moreover, it was observed that the filling of gaps initiated from cracked edges. On the other hand, cracks marked in *B. cohnii* NCCP-666 and *B. sphaericus* NCCP-313 formulations depicted uniform healing along cracked length. Deposition of $CaCO_3$ in control specimens is caused by carbonation of calcium hydroxide, contribution from nutrient and autogenous healing which results from secondary hydration of unhydrated particles of cement (Bhaskar et al., 2017; Shaheen et al., 2018).

Figure 4.5 demonstrates key observations in specimens pre-cracked at 7 days age. Under crack width measuring microscope, prominent healing was observed especially after 28 days exposure to healing conditions in all fiber reinforced microbial specimens. During initial days of healing, organic nutrient is abundantly present in concrete mix which is consumed by bacteria to form $CaCO_3$. The quantity of organic nutrient gradually reduces causing reduction in healing rate. Therefore, it was observed that the healing measurements did not change significantly on further exposure to water immersion (i.e. up to 56 days). Healing measurements were obtained as the difference between initial width of crack and final width of crack at the marked area in millimeters and results were plotted as a function of time in Figure 4.5 and Figure 4.6. Similar results were reported by Khaliq and Ehsan (2016). Figure 4.5 shows that control specimen attained least healing up to only 0.24 mm. It was evident that, *B. sphaericus* NCCP-313 provided more surface healing than *B. subtilis* KCTC-3135^T and *B. cohnii* NCCP-666, which achieved 0.6 mm and 0.65 mm crack closure respectively.

Figure 4.6 demonstrates results obtained after monitoring the samples pre-cracked at age of 28 days, it was confirmed that healing tendency reduces as aging progresses. At early ages, hydration is not completed therefore, some cracks were sealed due to secondary hydration of cement (Khaliq and Ehsan, 2016). Hence, the healing achieved in specimens pre-cracked at 28 days was relatively lesser than that of 7 days pre-cracked samples. About 0.4 mm average healing occurred in fiber reinforced microbial specimens when exposed to healing condition for 28 days. Khaliq and Ehsan (2016) reported maximum healing of 0.5 mm when samples were pre-cracked at 28 days and provided healing conditions for 28 days while in the presented study, healing widths increased to 0.6 mm and 0.5 mm in *B. subtilis* KCTC-3135^{T}and *B. sphaericus* NCCP-313 containing specimens respectively, on further provision of healing conditions for up to 56 days. In rest of the specimens' groups, the healing measurements did not alter significantly with passage of time. Crack closure attained in control specimen was only 0.2 mm. It is pertinent to mention that, findings of visual observations cannot be related to internal crack closure or pore filling as only few cracks were marked which could not be easily distinguished over the course of time. Therefore, variability of results was inevitable. However, authors have tried to make up the sense out of available results.

	0 days	28 days	56 days
Control			
BSC			
BSF			
BSJ			
BCC			
BCF			
BCJ			
BSpC			
BSpF			

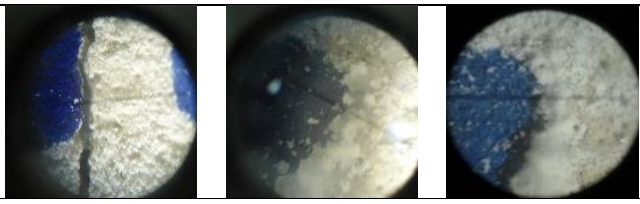
BSpJ	

Figure 4.4: Visual observations of self-healing

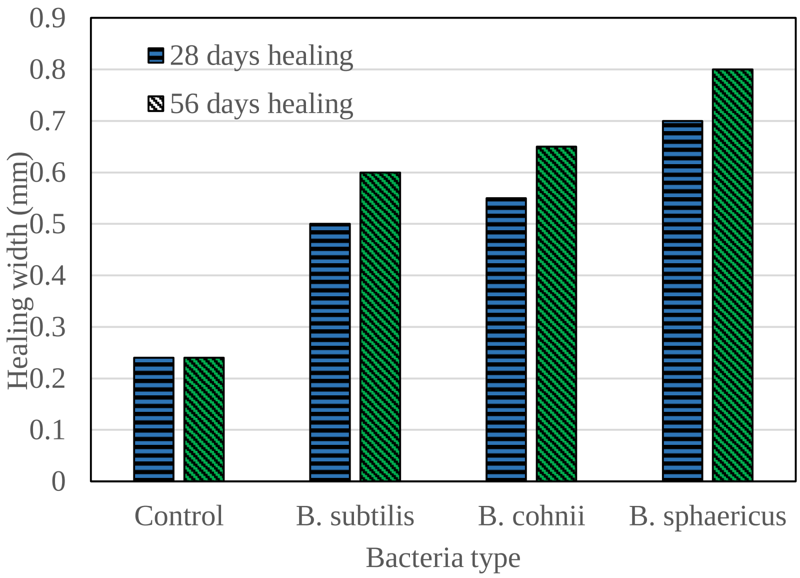


Figure 4.5: Healing in 7 day pre-cracked specimens

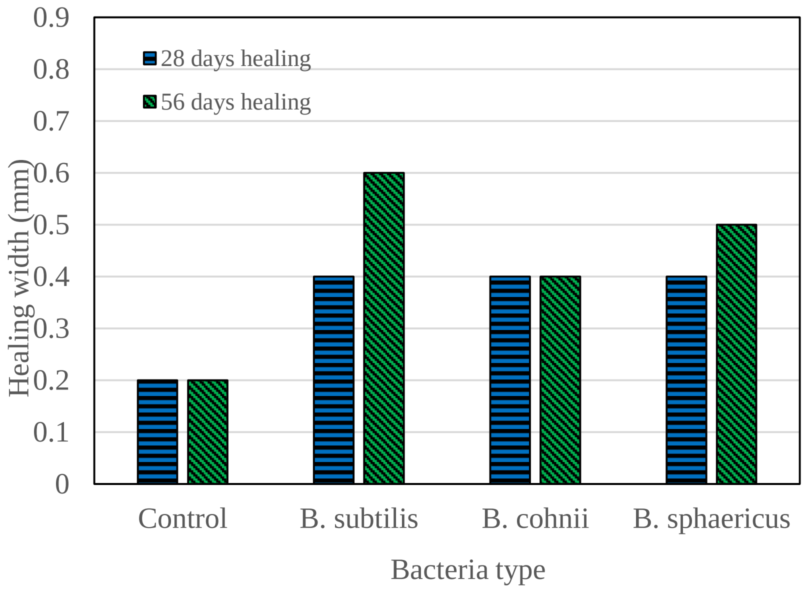
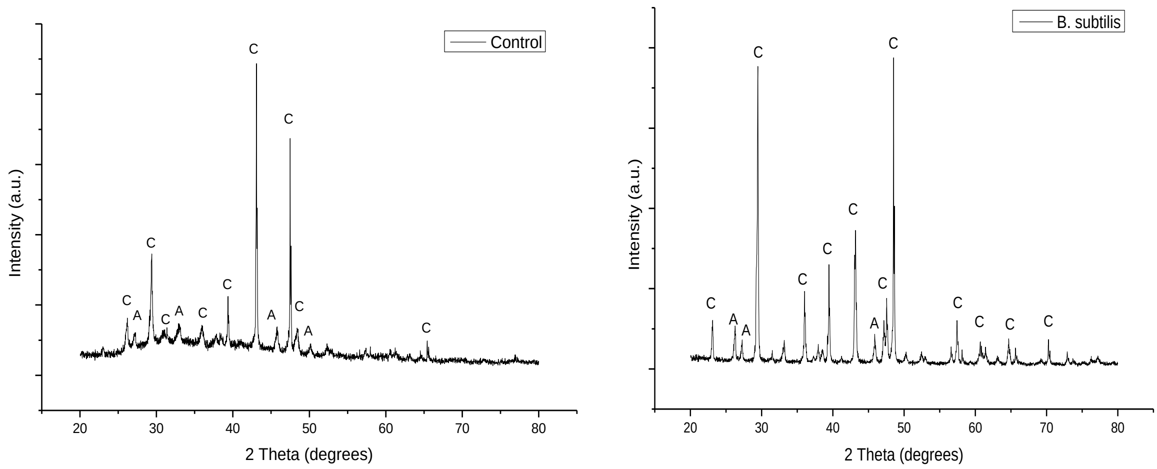


Figure 4.6: Healing in 28 day pre-cracked specimens

4.5. X-Ray Diffraction

The precipitation on cracks in control samples and bacterial samples was carefully scratched and tested by x-ray diffraction. Diffraction patterns were analyzed using X’Pert HighScore Plus and are reported in Figure 4.7. All the healing products were characterized as $CaCO_3$ which is produced as a result of autogenous and autonomous healing in concrete. In control specimen, the precipitation was solely due to autogenous healing, while in bacterial samples autogenous and autonomous healing occurred simultaneously. As a result, cracks were sealed due to precipitation of $CaCO_3$ on cracked region. XRD pattern for control sample showed a broad hump at $2\theta=30^o$ which indicated amorphous nature of calcium carbonate comprising of porous structure containing water and carbonate ions (Rodriguez-Blanco et al., 2011). Amorphous calcium carbonate is thermodynamically unstable and changes to crystalline polymorph of $CaCO_3$. Crystalline $CaCO_3$ exists in three polymorphs namely vaterite, aragonite and calcite listed in order of increasing stability (Ni and Ratner, 2008). Diffractogram of control specimen showed co-existence of amorphous and crystalline calcium carbonate. While, healing product obtained from bacterial specimens showed pure crystalline nature of $CaCO_3$. Crystalline nature is better that amorphous as it is rigid, incompressible and possess stronger intermolecular forces. It was also observed that calcite was the governing polymorph present in healing products while traces of aragonite were also present. More peaks of aragonite were obtained in control as compared to rest of the specimens. Previous studies also revealed the formation of vaterite and calcite as a result of self-healing (Bundur et al., 2017; Khaliq and Ehsan, 2016).



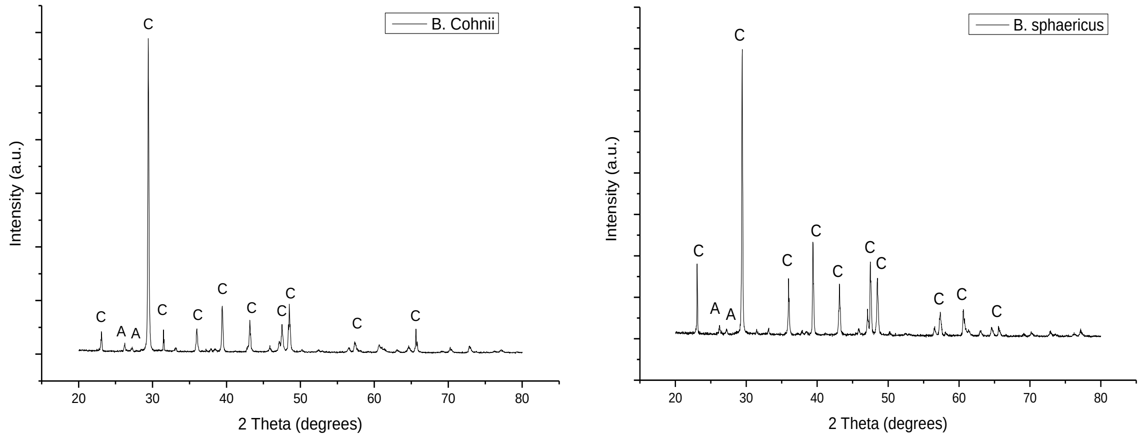
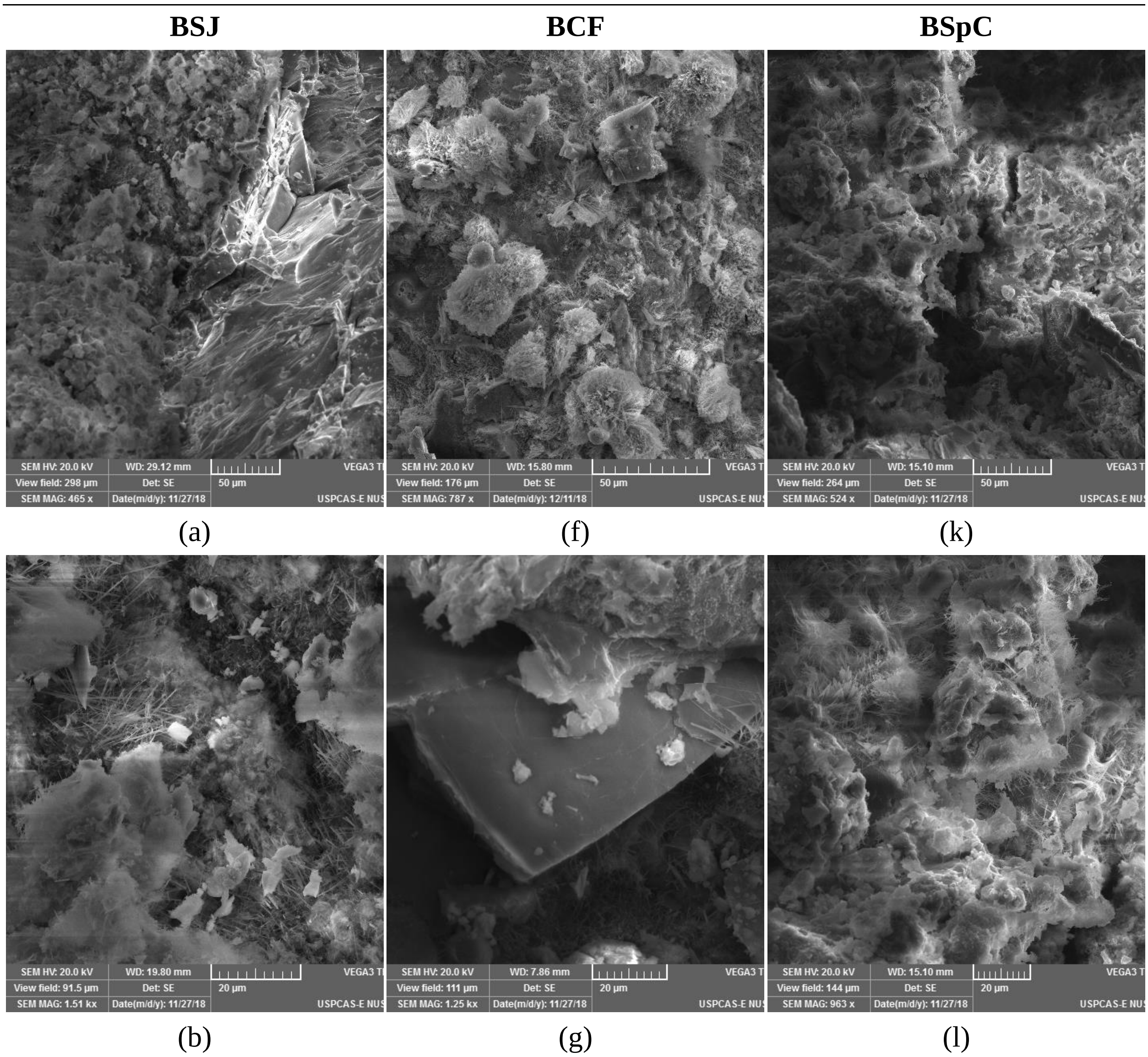


Figure 4.7: X-Ray diffraction analysis

4.6. Scanning Electron Microscopy

Specimens were subjected to microstructure analysis by scanning electron microscopy (SEM). Crack healing efficiency is proportional to production of $CaCO_3$ crystals so, presence of such crystals on cracked region and within microstructure of concrete is the main focus of this study. To visualize the microstructural changes in concrete as a result of self-healing effect, SEM analysis of fiber reinforced bacterial concrete was carried out. For SEM analysis, three specimens (BSJ, BCF and BSpC) were selected such that all bacteria and carrier types could be included and results could be extrapolated to all ten formulations. Figure 4.8 shows the morphology of self-healing concrete at various resolutions ranging from 5-50 µm. SEM images of concrete embedded with *B. subtilis* NCCP-3135^T are shown in Figure 4.8 (a-d). It can be seen that calcite crystals of size 50 µm existed in deformed lamellar form. Jonkers et al. (2010) reported that smaller sized calcite crystals lying between 1-5 µm range are formed by abiotic factors while larger sized calcite crystals are formed due to bio-mineral precipitation. Therefore, crystals produced by *B. subtilis* NCCP-3135^T can be attributed to bacterial conversion of organic substrate into calcium carbonate. Similarly, in case of *B. cohnii* NCCP-666 (Figure 4.8 (e-f)), large rhombohedral crystals of size greater than 20 µm are evident in Figure 4.8 (e) which confirm that *B. cohnii* NCCP-666 integrated with concrete mix also exhibited adequate bacterial activity to serve the purpose of crack healing. Figure 4.8 (i-l) shows microstructure of concrete in which *B. sphaericus* NCCP-313 was incorporated. In this type, perfectly shaped crystals of $CaCO_3$ were not visible rather the healing product existed in agglomerated form. Based on better performance of flax fibers in compressive strength recovery and UPV tests, it can be inferred that, BSpF has a more densified microstructure than BSpC. In Figure 4.9, microstructure of healing compound deposited on crack surface can be witnessed. Orthorhombic calcite crystals of 10 μm and larger size are clearly visible. Similar morphology was also obtained in research by Khaliq and Ehsan (2016). It is pertinent to mention that, apart from bacterial species, type of calcium source also affects the biomineralization, shape, size and morphology of crystals (Xu et al., 2013). The *B. sphaericus* NCCP-313 integrated concrete mix showed denser microstructure as compared to other two types due to better healing ability. Copious amount of $CaCO_3$ was found in BSpC mix (Figure 4.8 (i-l)). According to Mann (2001), growth of crystals takes place in steps which grow further and bunch up making a crystal irregular in shape. Past study has also confirmed the presence of agglomerates of calcite crystals in specimens containing plasticizers (Stuckrath et al., 2014). EDS analysis results of healing product show 38.8, 12.8 and 47.8% content of calcium, carbon and oxygen which further confirms presence of $CaCO_3$ due to microbial activity of bacteria (Figure 4.10).



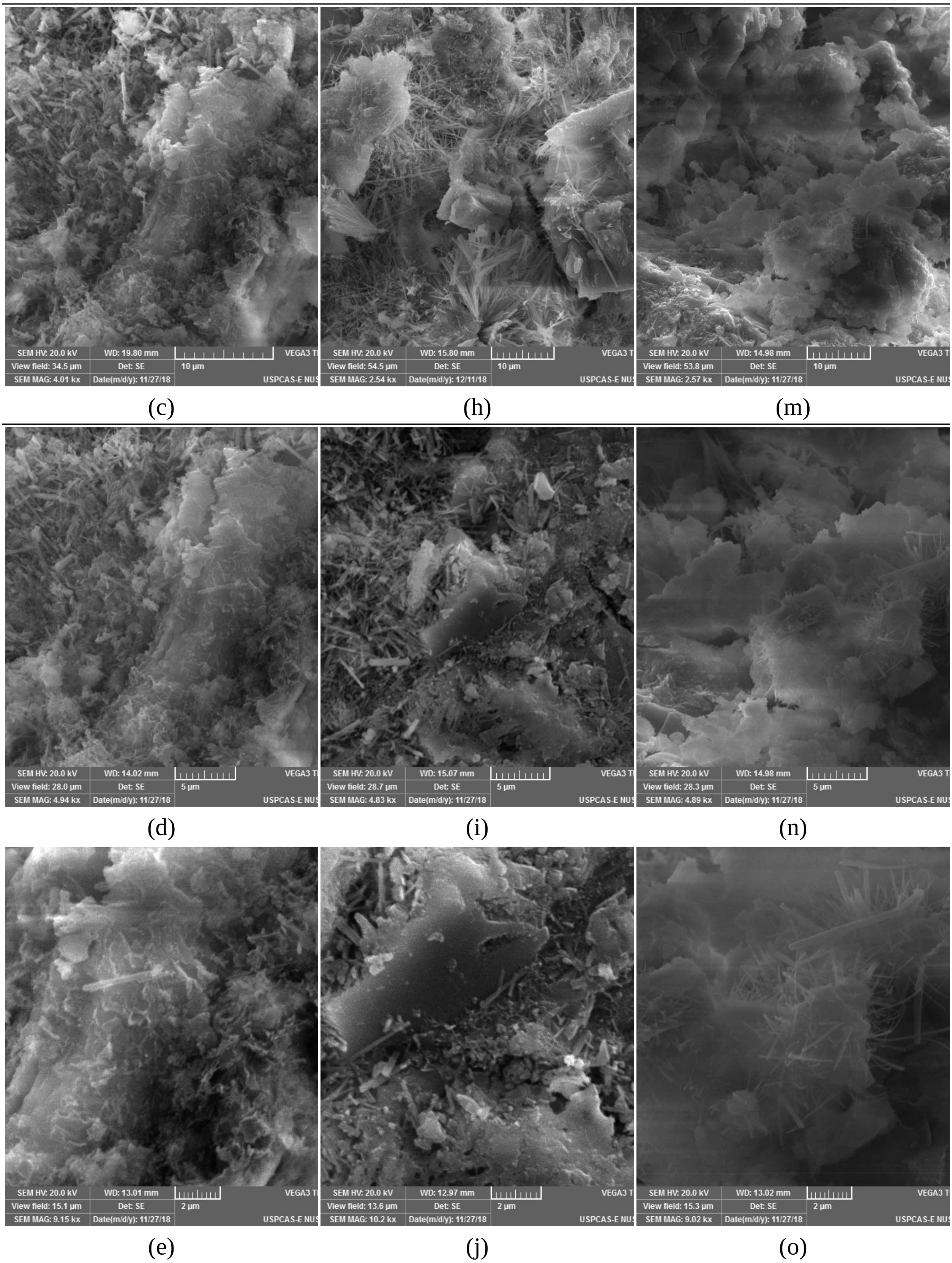


Figure 4.8: SEM photographs of healed specimens

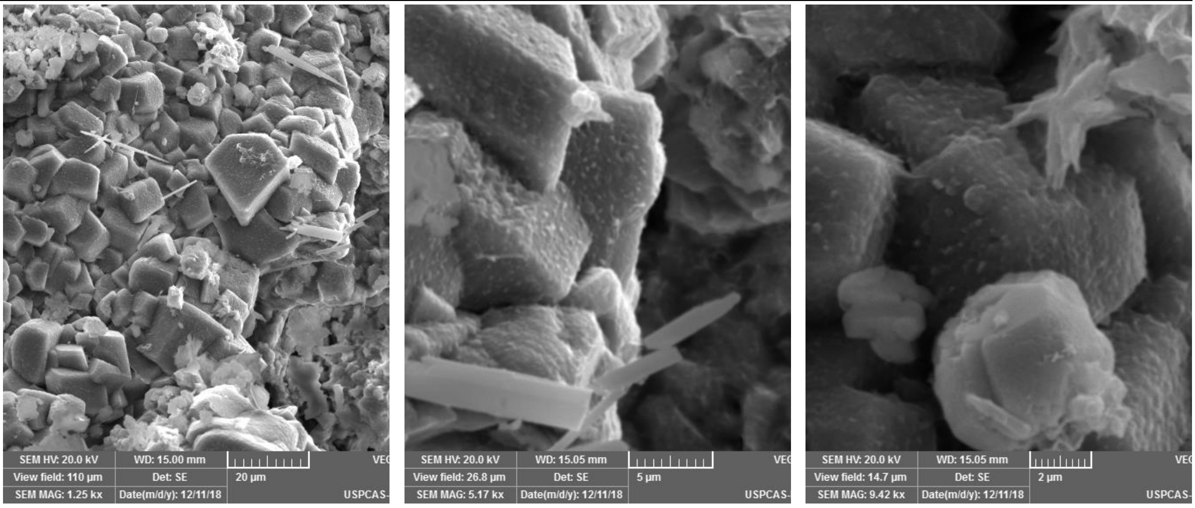
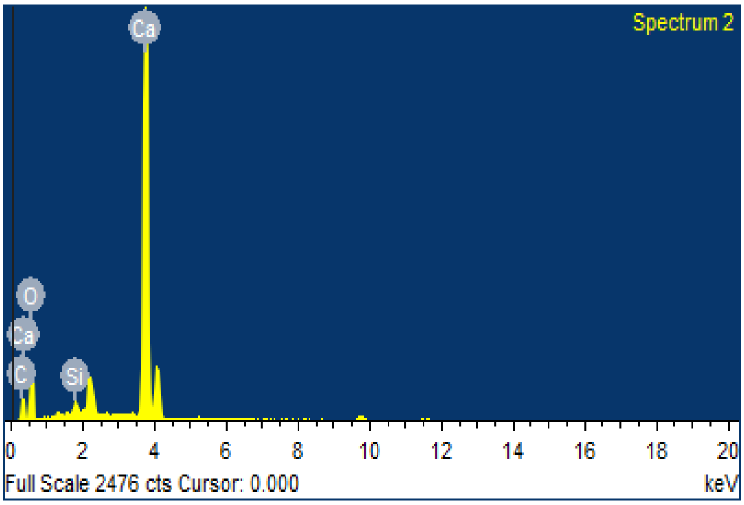


Figure 4.9: SEM photographs of healing product



Element	Weight (%)	σ
C	12.8	1.04
O	47.8	1.42
Si	0.7	0.12
Ca	38.8	1.10

Figure 4.10: EDS of healing product

CHAPTER 5

Conclusion and Recommendations

The study explored use of natural fibers as reinforcement and as protective carriers for immobilization of bacteria in concrete for crack healing purposes. Compressive strength, strength regain, ultrasonic pulse velocity and changes in crack widths were investigated after specified days of healing. Microstructure analysis was also carried out. Based on the results obtained and analyzed following conclusions were drawn from this study.

- Immobilization of bacterial spores in natural fibers successfully conserved urease and metabolic activity of bacteria which resulted in adequate production of CaCO3.
- Overall, 0.8 mm and 0.6 mm healing of cracks was achieved in 7 and 28 days pre-cracked samples respectively after 28 / 56 days healing time under controlled conditions. Agglomerated and lamellar crystals of calcium carbonate observed in microstructure of concrete deposited due to bacterial activity confirmed that fiber immobilized bacteria were capable of performing crack healing action in concrete.
- As carrier, flax fibers provided better protection to bacteria which was reflected by efficient crack-healing and pore-filling ability.
- Coir fibers attained highest compressive strength owing to high fiber stiffness as compared to flax and jute fibers.
- Bacteria, *B. sphaericus* NCCP-313 achieved better overall healing efficiency in concrete due to its higher ability of calcite formation than *B. subtilis* KCTC-3135^{T} and *B. cohnii* NCCP-666.

Following are the recommendations for further study.

- Quantity of organic nutrients should be optimized such that efficient strength and healing characteristics could be attained.
- Various concentrations of bacteria spores should be investigated in concrete and optimum concentration and quantity should be found that can give efficient strength and healing characteristics
- Mineral forming ability of different bacteria should be checked and bacteria with high mineral forming ability should be utilized for self-healing purposes.

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