BIOGENEIC TREATMENT FOR CRACK REMEDIATION AND SELF-HEALING IN CONCRETE STRUCTURES

A THESIS

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CERTIFICATE

This is to certify that Shelly Chauhan's thesis, Biogenic treatment for crack remediation and self-healing in concrete structures, which she is submitting with the roll number 302001016 in part fulfilment of the requirements for the award of a degree in Master of Science in Biotechnology from Thapar institute of Engineering and Technology in Patiala, Punjab, is a genuine work that was done with the guidance and inspiration of Dr M Sudhakara Reddy. .

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LIST OF ABBREVATIONS

SNO.	Abbreviation	Full form
1.	µL	Microliter
2.	µM	Micromolar
3.	mg	milligram
4.	mL	Milliliter
5.	min	Minute
6.	hrs	Hours
7.	pH	Potential of hydrogen
8.	mA	Milliampere
9.	SEM	Scanning electron microscopy
10.	XRD	X- Ray diffraction
11.	EDS	Energy-dispersive X-ray spectroscopy
12.	MPa	megapascal
13.	CPT	Control ponding treatment
14.	CST	Control spray treatment
15.	HPT	Hydrogel ponding treatment
16.	HST	Hydrogel spray treatment
17.	NB	Nutrient broth
18	UTC	Untreated concrete sample

ABSTRACT

Concrete is the most widely and extensively used material. Due to easy susceptibility of concrete to crack formation, it has become a challenge to protect concrete from pre-mature damage. It is a great task to identify microcracks at an early stage and then to cure them before they increase in size. Apart from using conventional techniques used for repair and maintenance of cracks this study focusses on using microbial based self-healing for curing and sealing cracks. For remediation in cracks, precipitation of calcium carbonate is one of the best-known techniques. The technique is not only used for healing the crack but improve durability and mechanical strength of concrete samples. The environmental changes e.g., fluctuations in temperature and pH can lead to hinderance in production of calcium carbonate produced by MICCP. These environmental factors directly affect the metabolism of bacteria in calcium carbonate production. Due to exposure of bacteria to such harsh environmental conditions MICCP could not take place effectively. This effects the viability of bacteria and its ability to of hydrolysing urea. So, to thrive through such environmental conditions the bacterial spores of *Bacillus paramycoides* were used instead of bacterial vegetative cells. In order to safe-guard bacteria inside high alkaline cementitious environment hydrogel was made. Hydrogel was used as the carrier material for immobilizing spores and then incorporating it to crack. Hydrogel properties were studied when it was exposed to unfavourable conditions. The study highly emphasizes on bacterial based self-healing approach for enhancing effectiveness of building materials. In order to achieve the aim of the study at best the potential of bacterial spores encapsulated in hydrogel has been explored.

CHAPTER-1

INTRODUCTION

Throughout human history concrete has been an extensively used material for construction. Concrete is presently the most widely used artificial man–made matter (Biernacki et al., 2017). Concrete has a great advantage that it does not corrodes and it is very economical (Bastidas et al., 2015). These two major properties make concrete more common for use in construction. It is used widely because of its indispensable use in construction of buildings, dams, roads, bridges, tunnels, subways and other infrastructures. (Aitcin et al., 2000). Concrete is made by mixing water, aggregates and cement. Cement binds with the aggregates and fills the cavities between the fine and coarse aggregate particles (Yaragal et al., 2019). Cement is easily available, prepared, durable, economical and most importantly casting concrete in suitable shapes and sizes make it the best choice for construction material out there. (Gambhir et al., 2013). Despite these advantages, service life of concrete is shortened by the presence of microcracks that initiate the formation of macrocracks that further lead to high proclivity to form more cracks, fissures and fractures in the constructed buildings. (Ahmad et al., 2022). Beginning of cracks in concrete structures is mainly due to following factors: (Golewaski et al., 2019)

(1) Faulty designs

(2) Relative humidity and alteration in temperature

(3) Shrinkage during drying process when concrete is restrained

Once the cracks start to appear, the ability of concrete to resist harmful, corrosive and aggressive substances reduces. (Ramli et al., 2013). Regularity in repair and maintenance is utmost important in order to protect concrete from further damage due to factors such as chemical attacks, earthquakes, floods etc. Quasi-brittle nature of concrete makes it an easy target to develop cracks due to tensile and sheer stress. (Bossio et al., 2015). The larger view of the issue is quite greater in terms of concrete structure early collapse that leads to consumption of high amount of raw material for rebuilding. The entry of harmful and penetrable chemicals in concrete structures increase the rate of crack formation. The larger the

pore size of concrete, the more it is vulnerable to penetrance of the aggressive chemicals inside concrete. (Achal et al., 2013).

Moreover, concrete has low ductility and low tensile strength so reinforcing it with steel comes into play and that is not usually an economical process. Furthermore, steel reinforcement cannot prevent crack formation. (Yuan et al., 2010). The brittle nature of concrete is the biggest reason of incorporation of reinforced steel inside it at the position of cross section of tension in order to bear tensile stress. Cracks in concrete play an important role to stimulate the role of steel bars. (Yuan et al., 2020). Crack formation above a certain rate leads reinforced bars being exposed to atmospheric air. The crack formation begins with the micro-cracks that are unavoidable and ordinary to the concrete and later it leads to formation of macro–cracks that further exposes reinforced bars to outer environment. (Wu et al., 2012). As the crack generation increases, concrete material becomes penetrable to hazardous agents and the reinforced steel inside the concrete becomes more vulnerable to the exposure of unmasked atmospheric air, and thus increases the risk of physical degradation of material. This whole procedure leads to weakening of the building structures. Therefore, preventive approaches to restrain and terminate crack formation at an early stage are crucial. Another big issue is with the high budget that is allocated for the repair of cementitious buildings. (Sarode et al., 2010). The cost of repairing crack and maintaining its intellectual properties has been estimated at \$147/ m^3 along with the fact that cost of production of concrete to be \$65-\$80/m^3 (Silva et al., 2015). The crack generation leads to significant reduction in concrete service life and has high replacement costs. (Cusson et al., 2010) Also, concrete producing companies are facing major problems due the high cost of production of concrete as well as the large amount of CO_2 emission that causes increase in overall global temperature (Khaliq at el., 2016). Since, it is unattainable to restrain crack formation there are various techniques that can be used to treat cracks and restore the concrete value and shelf life. It has been known that concrete healing and curing using injection of polymers and polymeric chemicals has hazardous effects on health, livelihood and environment and in addition, they have short term efficacy and hence, the need of the hour is to look for treatment techniques that sounds sustainable and environmentally friendly. Due to so many problems, microbial based self-healing approach is used for rapid crack curing and to increase shelf life of concrete. Practicing biomineralization of calcium carbonate with help of different microorganisms e.g., using *Bacillus* species is an innovative approach to reform defected buildings and cracks. (Achal et al,. 2011). It is an eco–friendly technique to repair cracks using MICCP (microbially induced calcium carbonate

precipitation) technique. The advantage of using MICCP is that it can heal the cracks through induced metabolic pathways of bacteria at places where it is not easy to locate cracks. This helps in decreasing the cost of spotting cracks and the difficulty in repairing, as well as increases the service life of the concrete structures. This whole technique is undertaken by the process of biomineralization using metabolic pathways of various bacterial species. MICCP helps in internal self-healing of concrete structures with the incorporation of microorganism carried by a carrier material along with the cement. The species used for self-healing in concrete is *Bacillus paramycoides.* The study is based upon infusion of healing agent into the penetrable cementitious material in order to fill up the voids and crack sealing in concrete structures. The process of healing was carried out by physical infusion of healing agent. The major aim of the present study is to design advanced framework of concrete for prolonged serviceable life by enhancing concrete and its composition that provides a remedy to conquer the problem. One adequate solution to this problem is to incorporate self-healing techniques present in the environment into cement structural components.

To overcome the drawback of crack formation and short-term efficacy of concrete structures, MICCP technology is studied in favour of the concept of self-healing. The technique also diminishes the cost of production of various chemicals and polymers, such as - epoxy and polyesters. If not properly polymerized these chemical substances show health and environmental risks and they have short–term efficacy so need of the hour is to look for eco-friendly treatment methods that are long lasting as well. The research approach revolves around microbial self-healing with potential for long lasting, rapid and active crack repair, while also being non–environmentally hazardous techniques. Concrete crack healing was studied by bacterial calcium carbonate precipitation which is a biomineralization technique. Biomineralization is a process by which living organisms produces minerals. In this technique, the extracellular components of microorganisms play an important role in order to facilitate calcium carbonate precipitation. A carrier material is used for bacterial infusion and is further mixed with the cement composition to undertake the process of crack healing. In the study described the carrier material for bacterial incorporation used is hydrogel. The major components of hydrogel used were poly–vinyl alcohol and chitosan. The cracks are repaired by immobilizing bacterial spores in a carrier material i.e., Hydrogel. Using bacterial spores for such purpose is one of the best techniques so far because spores are one of the most resistant forms of life. They can survive under high stress conditions like very high temperature, tolerant to various chemicals and can resist strident physical abrasion. Bacteria may not survive for a

longer time during cement mixing and casting because of physical and mechanical damage to the bacterial cell. Due to high sheer stress during mixing bacterial cell wall may get ruptured that may lead to cell death and loss of crack healing properties. (Zhu et al., 2022). Bacterial spores may resist such sheer stress and would do better job than the bacterial vegetative cells. Bacterial Spore immobilized specimens have shown sustainable crack-plugging, proving that using bacterial spores for healing is a promising application than using bare bacterial cells. The purpose of choosing *Bacillus* species is that these species produce calcite naturally whenever it comes in contact with calcium and carbon dioxide. Immobilizing these species into concrete helps to seal the crack as soon as it starts to form, which is a great way to obstruct any mechanical and structural damage. Encapsulated bacterial spores thus, help as an interior self-healing agent which actively and autonomously reduces the structural permeability during crack initiation and formation. An incorporated healing species helps in saving labouring inspection and repairing cost and plays a vital role in increasing structural strength, robustness and durability. Adding such agent to the cement mixture can save money and would require less man-power, additionally, it will protect the environment from unfriendly repair mechanisms and materials. In this research *Bacillus paramycoides* species is used that produces calcium carbonate in presence of few organic salts. The strain used is alkyl-halophilic i.e., it can strive in high salt conditions as well as high alkaline conditions. (Kim et al., 2022). In this study halophilic bacteria has been used for concrete healing, employing its property of biocalcification which is the process of microbially induced calcium carbonate precipitation.

RESEARCH GAP

The need of the hour is to look for building materials that shows effective endurance because building materials used presently does not provide long term efficacy. In order to overcome this problem microbially induced calcium carbonate precipitation (MICCP) which is a technique used for production of bio minerals is used. This process is based on the principle of using urea as an energy source and then production of ammonia creates an alkaline environment which is because of the increase in pH eventually leading to precipitation of Ca_2^+ and CO_3 and formation of $CaCO_3$.

Many bacterial species like, *Pseudomonas aeruginosa, Bacillus sphaericus* which undergoes the process of biomineralization which helps in durability enhancement of concrete structures. The pH of concrete structures in extensively high so researchers are working to isolate and identify microorganisms from higher alkaline environment. The reason behind is to identify

microbes which shows compatibility and can fulfil the purpose of self-healing in concrete structures. Exploitation of microbial species is done in order to exhibit calcium carbonate plugging in concrete specimens and to succeed in this goal identification and isolation of such microbes which are capable to survive in high alkaline environment is required. For these following reasons so many microbials species and natural resources have been exploited.

Another major problem with bacterial based self-healing is related to rate of survival of bacteria in harsh concrete environment. Viability of bacteria apparently decreases very quickly in such high alkaline environment. It may decrease even more quickly if bacteria are exposed to the other unwanted external alkaline conditions for example – if external surroundings are highly alkaline.

Another issue is with introduction of hydrogel into civil industries. Hydrogels are primarily used as medical based product. Till date hydrogel has been used in medical sector and commencement of it in civil sector is a task of queries and also cost of production for hydrogel would be quite expensive.

OBJECTIVES

1. To improve durability of building materials by using efficacy of microbially induced calcium carbonate precipitation. (MICCP)
2. To develop suitable bacterial carrier material for bacterial based self-healing in concrete
3. To facilitate development of autonomous self-healing and sustainable concrete

CHAPTER – 2

REVIEW OF LITERATURE

The approach of using concrete is increasing worldwide and thus a concrete that is environmentally friendly is the need of the hour. The most widely used material in construction is concrete and that too because of its high-ranking properties. Different techniques of using microorganisms for domestic cement based healing and curing has been studied. (Luo et al., 2015). The study has been carried out with the help of various biomineralization characteristics of specific strains that produces calcite that sticks tightly to concrete and protect it from further damage and ongoing crack and fissures formation. (Siddique et al., 2011)

2.1 Biomineralization It is a natural phenomenon that is analogous to number of bacterial species. It is based upon the technique of microbially induced calcium carbonate precipitation. (Seifan et al., 2018). During this whole procedure the production of inorganic substances by microorganisms as a step of their metabolism is used in biomineralization. (Dhami et al., 2012) in other words biomineralization can be defined as the process by virtue of which microorganisms stimulate the production of inorganic salts, and these salts and minerals are eventually used to enhance the plus properties concrete materials. Biomineralization can develop an amazing increment in the cohesion and hardness of soil. Undoubtedly, the best way to attain biomineralization can be by using ureolytic bacteria. (Dhami et al., 2012) Few bacteria produces urease enzyme as a part of their secondary metabolism which initiate the hydrolysis of urea and turns a series of reactions that activates the process of microbial-induced calcium carbonate precipitation (MICCP). (Ali et al., 2022). Hydrolysis of urea assembles ammonia and carbon dioxide, leading the calcium carbonate to precipitate in dissolved calcium presence. The larger the amount of dissolved calcium present the higher the MICCP takes place because it generates the pores inside cement that are abundant in calcium. (Dhami et al., 2013). The major task is of bacterial activation once the crack is generated. But most of the time bacterial vegetative cells failed the process of activation, ie, they died before the crack generation during the process of mixing and casting. *Bacillus cohnii* spores (10^9 spores/cm^3 present in the native inoculum were reduced to (1.15×10^6 spores/cm^3) only after 10 days.ie. Only 0.12 % vegetative cells could survive. (Williams et al., 2017)

Vast decrease in viability of spores of bacillus species. If we undertake the case with vegetative cells there are chances of very few vegetative cells to be alive. So, the ideal choice for encapsulation is bacterial spores itself and bio cement attain unbreakable property by the process of biomineralization.

A special group of spores producing bacteria is used for the process of biomineralization. Bacteria for genus bacillus is used ie. Alkyl halophilic bacteria for that shows resistance to high temperature and high alkaline environment.

Healing concrete specimens with the bio active calcium carbonate precipitation has been indeed the best way to cure cracks in the cementitious environment. (Wiktor et al., 2011). High pH of cement might cause hindrance for the bacteria to survive for a longer time, also decrease in nutrients may lead to early arrival of death phase. (Stanaszek et al., 2022). Bacteria that are not thermophilus may not survive in a higher level of temperature. So due to all these above-mentioned reasons the further choice is to rely on bacteria spores that can live up to thousands of years. Urease enzyme is produced as a part of metabolism by bacterial species, Urease is further catalysed into carbonate and ammonia. Both the carbonate and ammonia is hydrolysed into ammonia and carbonic acid that is further converted into bicarbonate. (Henze et al., 2018) This is the basic principle of by biocalcification or MICCP. The harsh conditions present in cement reduces the viability of bacterial cells that contribute to reduction in urea hydrolysis. (Williams et al., 2017). Viability and hydrolysis of urea play utmost important role in biocalcification but the factors like high temperature > 55 °C and pH increases the rate of cell rupture and cell death. (Luhar et al., 2022)

Ureolysis by bacteria takes place in a sequential manner. In the very first step, (Eq. 1) decomposition of urea into carbamate and ammonia takes place intracellularly. This step is initiated with the help of urease enzyme, which act as a catalyst. (Eq.1). In the second step Carbamate gets hydrolysed into more amount of ammonia and carbonic acid. (Eq.2). Formation of bicarbonate takes place when ammonia and carbamate are released from bacterial cells. (Eq.3) Along with bicarbonate hydroxide and ammonium ions are also formed which are responsible for an increase in pH, (Eq. 4). Carbonate and bicarbonate ions will start to follow an equilibrium. (Eq.5) Positively charged calcium ions will be attracted to negatively charged bacterial cell wall and at this particular point of time carbonate ions along with calcium ions will begin to react that would further lead to production of calcium carbonate. (Eq. 6). Bacterial cell serves as the nucleation site for this process. (Eq.7) (Intarasoontron et al., 2021)

2.2 Biomineralization via urea hydrolysis

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

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

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

(4) $2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-$

(5) $HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \rightarrow CO3^{2-} + 2NH_4^+ + 2H^+$

(6) $Ca_2^+ + Cell \rightarrow Cell\text{-}Ca_2^+$

(7) $Cell\text{-}Ca2^+ + CO_3^{2-} \rightarrow Cell\text{-}CaCO_3$

Biomineralization takes place when negatively charged cell wall comes in contact with the positively charged calcium ions and leads to formation of calcite. (Skinner et al., 2014). The process is explained in Fig. 2.1.

Fig (a). Bacterial cell

Fig (b). Negatively charged cell wall and the positive charged Calcium ions

Fig (c). Biomineralization due to binding of Calcium ions to the cell wall

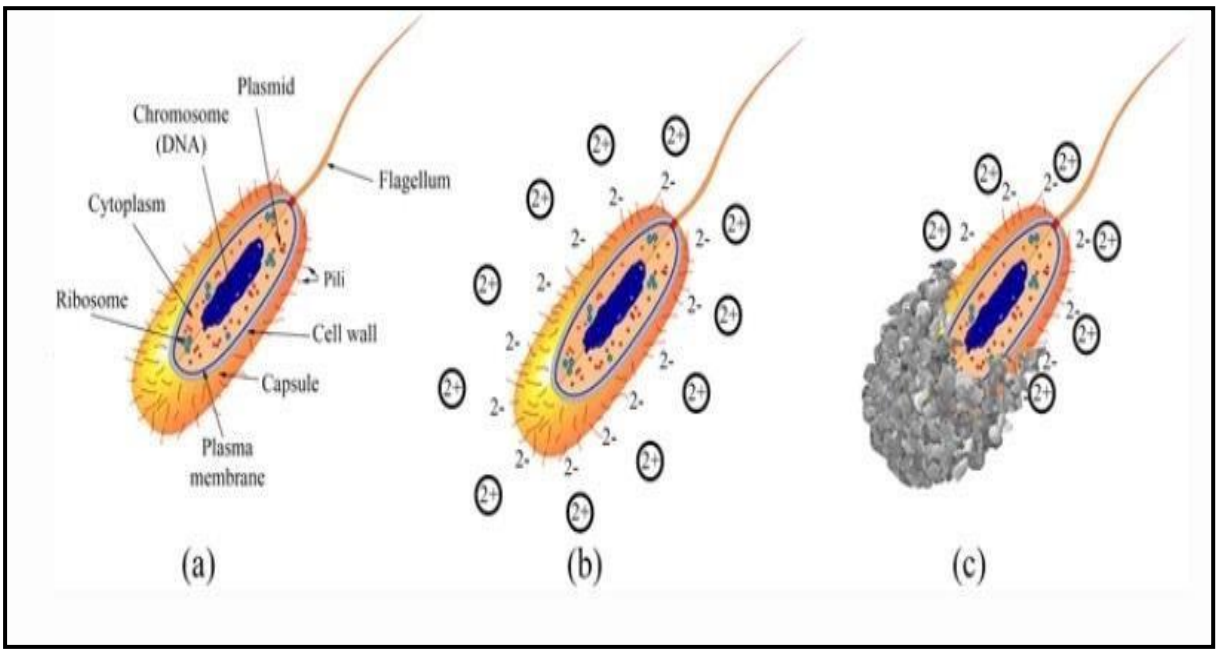


Fig.2.1 Biomineralization in bacteria (Skinner et al., 2014)

2.3 Microbially induced calcium carbonate precipitation mediated by urea hydrolysis in saline environment

Carbonate precipitation depends majorly upon the following factors: (Bansal et al., 2016)

(1) Amount of calcium present
(2) Amount of carbonate. carbonate present
(3) Environmental pH
(4) Presence and activation of nucleation sites

There are number of mechanisms that offers carbonate precipitation in alkaline environmental conditions which has high amount of Ca^{2+} ions. calcium carbonate precipitation mediated with the help of ureolytic bacteria is known to be common technique. In this technique, hydrolyzation of urea takes place into bicarbonate and ammonia. Ca^{2+} ions when comes in contact with CO_3^{2-} ions, The reaction involved leads to the $CaCO_3$ precipitation. All of these reactions take place at the surface of the cell, which also acts as the nucleation site for urea hydrolysis. (Bansal et al., 2016). The basic mechanism of biomineralization as predicted by (Bansal et al., 2016) can be diagrammatically explained in Fig. 2.2. Urease hydrolysis reaction taking place during biomineralization is explained with the help of Fig. 2.3.

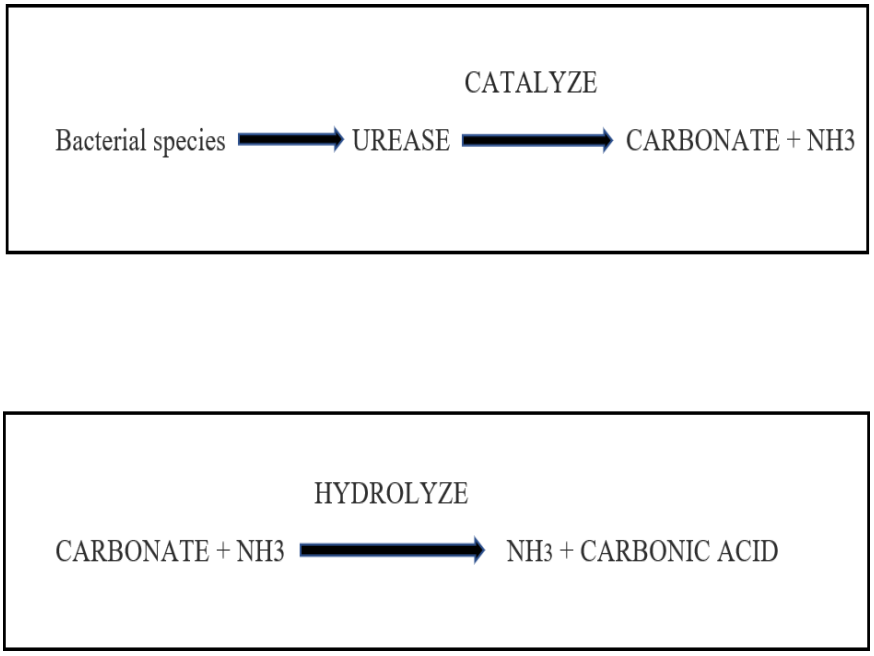


Fig 2.2 Basic mechanism of biomineralization

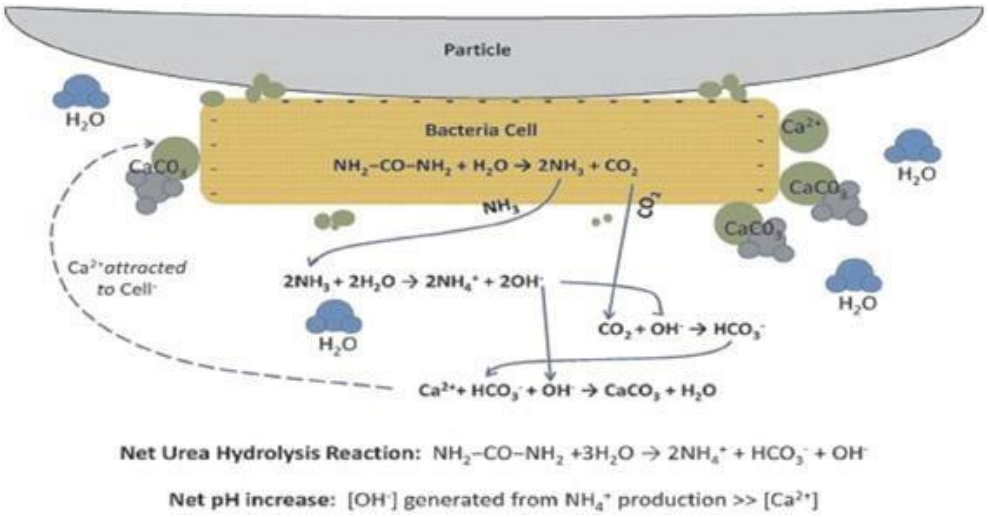


Fig 2.3 Bacterial nucleation site for CaCO3 precipitation (Dejong et al., 2010)

2.4 Carbonate precipitation mediated via carbonic anhydrase

Carbonate precipitation via carbonic anhydrase (CA) is also a process apart from carbonate precipitation via urea hydrolysis. The enzyme carbonic anhydrase has shown promising results for being a biocatalyst that is used for carbon dioxide hydration that aids in $CaCO_3$ formation whenever a calcium source is present. (Bansal et al. ,2016)

HCO_3^- acts as an origin for dissolved inorganic carbon (DIC), CA is catalysed to convert into CO_2.

$HCO{-}3 + H^+ \rightarrow H2O + CO_2$

$HCO^{-3} + H^+ \rightarrow H2O + CO2$

In case if carbon dioxide acts as the origin of DIC, conversion of CA into HCO_3^- is catalysed.

$CO_2 + H_2O \rightarrow HCO^{-3} + H^+$

$CO_2 + H_2O \rightarrow HCO^{-3} + H^+$

Bio-concrete Can be made by assimilating ureolytic bacteria in normal concrete with the help of two methods:

(1) Required nutrients are blended with the bacteria and then they are mixed with the concrete material.

(2) Bacteria can be incorporated or encapsulated in a carrier material that drives the bacterial vegetative cells, or bacterial spores to the concrete mixture. (Trenson et al., 2017)

Both the above-mentioned techniques are reliable for self-healing in damaged concrete structures but the second technique has valid advantages over the another one.

During the process of mixing and casting the bacterial cells gets ruptured and loses its shape and integrity Due to cell wall damage. (Souradeep et al., 2016). So to compensate the loss of bacterial cell death, the bacterial cells or bacterial spores are encapsulated in a carrier material that acts as both protective covering layer for bacterial cells, as well as a carrier material that can act as a driving force of bacterial cells to the cementitious environment. (Wang et al., 2016)

Immobilization is the process of encapsulation of bacteria in a carrier material before it is left to mix with the concrete. (Feng et al., 2022). Bacterial cell has high risk of death when mixed in high alkaline environment of concrete because bacteria is unable to tolerate such high alkalinity. (Gupta et al., 2017). Ureolytic bacteria cannot grow inside concrete without any in encapsulation technique. Without any immobilizing or encapsulation techniques the survival rate of ureolytic bacteria decreases considerable times. (Ersan et al., 2015) The present need of the hour is to look for such techniques that allows bacterial cells or bacterial spores to live in such extreme conditions. Once the bacterial cells are mixed with the concrete material, they should have the property of striving high temperatures, high humid environment and should have the property of surviving under high alkaline conditions. Gram positive ureolytic bacteria that produces spores is our choice of interest. These are the dormant form of cells that can resist harsh environmental conditions till a significant level. (Pungrasmi et al., 2019) They can survive in extremely high moisture conditions as well as in conditions where there is moisture exhaustion along with that, they can tolerate ultraviolet radiation exposure, also they can show resistance to many toxic chemicals. Technique of encapsulation of bacterial spores till date is an ideal technique. Bacterial spores can be produced by introducing the normal vegetative cells to harsh conditions for example: Introducing vegetative cells to high temperature and by exposing them to high mechanical shear (centrifugation for a longer time and at rpm more than 8000). Bacteria cannot survive in pH greater than 12 and also most of the bacteria dies during hydration process for casting concrete because the pore diameter of cement is quite smaller than the pore diameter of bacteria itself. (Paine et al., 2016). Therefore, for immobilizing bacteria a suitable choice of carrier material is required.

In order to reduce repair and maintenance cost at later stage autogenous healing techniques with the help of encapsulation of healing force have been a great choice to replace polymers and its composites. (Joseph et al., 2011). The technique of encapsulating bacteria and then

using it for self-healing purposes has been developed recently. Modern researchers prefer encapsulated bacterial spores or mineral cargos such as colloidal silica and sodium silicate for self-healing in concrete specimens. (Belie et al., 2018)

2.5 Commercial encapsulation techniques are as follows

1. Melamine based microcapsules.

2. Gelatin / acacia gum microcapsules using complex coactivation. (Awasthy et al., 2020)

In the cases mentioned above microcapsules that are formed are certainly and initially really soft and flexible but after a span of they get transitioned in to harder state which is brittle and all of this takes place after the process of drying.

The environment inside the concrete is extremely harsh so immobilizing or encapsulating bacteria in a carrier material that acts as both carrier material as well as a protective layer for the bacterial spores. (Tang et al., 2021). Scientists have used different carrier material for example. Clay that has the property of expansion as well is porous in nature. These healing agents that are biologically active showed healing result of 0.46 mm in 40 days. Other carrying material that can be used are polyurethane, calcium chloride beads and silica gel. (Zheng et al., 2022)

(Wang et al., 2012) studied the effect of polyurethane and silica gel as a carrier material for bacterial immobilization. Silica sols were used as dispersions. Silica gel is used as a carrier material because of the following properties:

(1) It is biologically inert. ie. It does not act as a source of food for bacteria

(2) Thermostability

(3) Photochemical stability

In the following study, bacteria are encapsulated inside the tubes made up of glass, the nutrients required for bacterial growth are immobilized, along with the bacteria. (Rauf et al., 2020). They are incorporated in concrete specimens during the process of casting. Whenever the cracks get initiated breaking up of glass tubes will release the healing agents. The healing agents will approach to the crack region. When calcium comes in contact with silica sol in situ formation of silica gel takes place. At this specific point of time immobilization of bacterial cells into silica gel is done and whenever bacterial cell reacts with urea and calcium ions precipitation of calcium carbonate takes place.

(Bang et al., 2001) Ever since PU is used for crack healing, it was firstly used for repairing cracks manually. PU foam was incorporated with bacterial cells and it was cut into pieces of small equal sizes. Later, these strips were placed into crack specimens. Incubation of these motor specimens in urea and calcium chloride medium at room temperature was done. With the help of $CaCO_3$ precipitation compressive strength of specimens which had simulated cracks was remediated by 12%. In this study. PU foam was applied manually and after initiation of crack.

Another study involves preformed PU polymers where bacteria was incorporated in PU polymers Add the mixture was applied internally. (Bang et al., 2001). This led to successful healing of the crack internally. The aim of successful investigation of healing in cracked structures was fulfilled by both PU and silica gel carrier material for self- healing.

This study involves the use of spores for healing purpose in cracks. Spores were derived from alkaliphilic bacteria and few organic mineral-based compounds. Species used is *Bacillus cohnii*. Bacteria was allowed to grow in normal nutrient broth media and OD_{600} value after 24 hours of the same suspension of bacteria was found to be 0.4. The bacterial spore concentration was obtained with the help of microscope and was found to be 3.6×10^9 cell/ml. The spores were collected industrial manner and satisfactory repeated washing was done with the help of distilled water and stored at 4 °C. Bacteria was immobilised and packed with EP and EC particles. The particles were subjected to surface spray technique with the medium of yeast extract (1 g/L) and calcium lactate ($CaC_6H_{10}O_6$, 8 g/L).

EP and EC particles were allowed to dry in hot air oven for next two days in order to reduce the water absorption capacity of the particles. Coating of geopolymer was used on EP and EC particles for us to create high vacuum pressure. Geopolymer used for coating was composed of and sodium silicate and metakaolin with a fixed composition of (15% by weight). (Jonkers et al., 2007).

2.6 Self-healing in concrete specimens mediated by hydrogel as bacterial carrier material

Hydrogel is a network made up of 3 dimensional structures consisting of chain of polymers, which are hydrophilic in nature. In the presence of hypotonic environment hydrogel shows swelling properties and can hold on to great amount of water without losing its structural integrity. (Rasidzadeh et al., 2014). Cross linking between its physical and chemical polymers

helps in maintaining mechanical structure of hydrogel. Due to large amount of water content present inside hydrogel, it shows high flexible degree as well as water content signifies hydrophilicity of hydrogel. Polymeric chains of hydrogel show hydrophilic nature because of the presence of chemical groups like: -SO_3H, NH_2, -CONH -, -COOH, -$CONH_2$, -OH. (Chuang et al., 2018).

Whenever hydrogel comes in contact with any external physical or chemical stimulus it changes its confirmation. (Buenger et al., 2012). Hydrogel gets back to its normal state whenever the external trigger is stopped, In other words, hydrogel transitions are reversible. The amount of transition response produced by hydrogel is directly proportional to the amount of external trigger given. Hydrogels are responsive to external stimulus, depending upon density of charge, type of monomer and degree of cross linking between two monomers etc. (Lorenzo et al., 2020) Hydrogels are polymers of hydrophilic nature that can cross link to absorb water, saline liquids and few other solutions. (Lftah et al., 2011). Network of hydrogels is uniquely three-dimensional and its attachment with different functional groups have made the use of hydrogel in synthesizing newly launched polymeric substances. (Dou et al., 2017). Also, hydrogels have wide application in agriculture, medical sector, civil. It is used as a tool for water retention in agricultural fields, e.g., in barren land hydrogel helps in increasing capacity of land to hold water. (Nascimento et al., 2018) So, learning about water retention properties and hydrogel swelling properties is important because on holding water hydrogel swells. So, apparently the larger the volume of hydrogel holding water the more is the swelling ability of hydrogel. (Kaith et al., 2013).

The physical trigger includes the electric fields and magnetic fields, pressure, temperature, high pressure, intensity of light and composition of solvent, etc.

Biochemical and chemical stimulus that triggers the conformational changes in hydrogel can be as follows: Change in pH, movement of ions and composition of chemicals from which hydrogel is prepared. (Hasan et al., 2022)

Bacterial mediated crack self-healing has been a favourable answer for concrete durability and sustainable maintenance. The following study is based upon the germination of bacterial spores from bacterial vegetative cells and then immobilising those bacterial cells in hydrogel and then transmission of bacterial spores, along with the carrier material, hydrogel was done into the motor specimens. Spores were successful in generating $CaCO_3$ and the production of $CaCO_3$ was identified with the help of thermogravimetric analysis (TGA). The demonstration

of superior self-healing was observed in the crack. The crack was healed till the maximum width of 0.5 mm and 68% average water permeability decreased was observed. (Zamani et al., 2020). Physiological and structural properties of hydrogel are explained in Fig.2.4

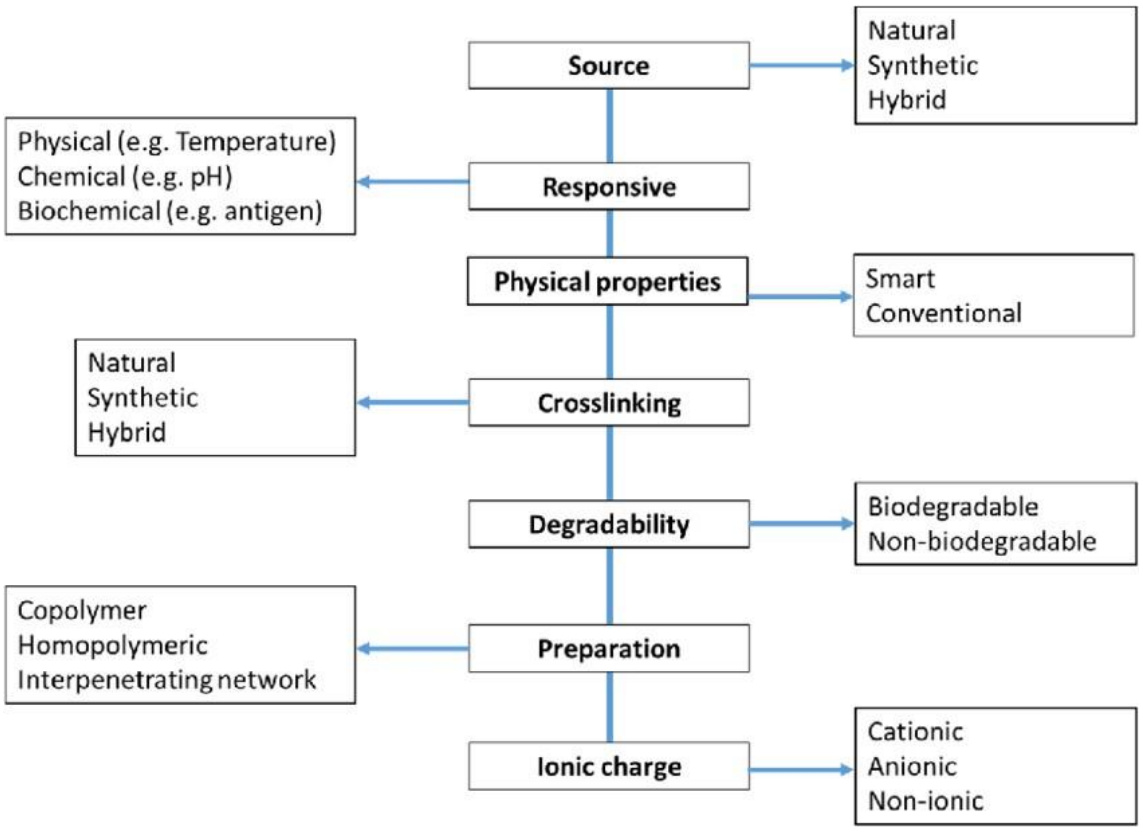


Fig 2.4. Properties of hydrogel (Karoya et al., 2021)

2.7 Hydrogel synthesising methods

Hydrogels can be synthesized using physical and chemical cross - linking properties. In the presented study we opted for production of chemically crosslinked hydrogels because physically crosslinked hydrogel is used for medical purposes, for example, wound dressing. Synthesising hydrogel chemically is not an appropriate technique for medical based treatment because it involves the presence of different chemicals that might interfere with the body's ease of recovering at the region of discomfort or injury.

2.8 Hydrogels produced by chemical cross–linking

Rising interest for cross linking of hydrogel via chemical methods is because of high productivity to generate satisfactory mechanical strength. There are different methods used for different types of hydrogel production via chemical-cross linking agents that have been explained in Table. 2.1

Table 2.1 Cross-linkage of different hydrogels via chemical bonds

Sr. no.	Polymer	Method type	Reference
1	Chitosan-PVA	Crosslinking with aldehyde	(Zu et al., 2012)
2	Gelatin	Crosslinking with aldehyde	(Yamamoto et al., 2000)
3	Albumin	Crosslinking with aldehyde	(Willmott et al., 1984)
4	Chitosan	Crosslinking with aldehyde	(Jameela et al., 1995)
5	Dextran	Addition reaction	(Brondsted et al., 1995)
6	PVA	Condensation reaction	(Ray et al., 2010)

CHAPTER 3

MATERIALS AND METHODS

Isolation of bacteria

Bacillus paramycoides SL-1 was isolated from Sambhar Lake, Rajasthan, India by researchers at TIFAC-CORE lab. Sambhar lake is India's largest lake located in Jaipur, Rajasthan.

Bacterial subculturing

In order to preserve the bacterial culture subculturing was done after every 15 days from glycerol stock at -80 °C. Subculturing was done on freshly prepared agar plates

Urease assay

Bacterial suspension was exposed to different salt concentration (at 3.5 %,5 %,7.5 %,10 %,15 %) and urease assay was evaluated at each salt concentration to determine urease activity i.e., Urease activity was calculated by estimating total amount of ammonia produced via urea hydrolysis.

Method used: Phenol-hypochlorite assay technique at regular intervals. (Natarajan, 1995).

Type of assay: Colorimetric assay

Standard used: Ammonium chloride of (50-1000 μM).

Bacillus paramycoides SL-1 were grown in nutrient broth for 12-16 hours. 1ml of overnight grown culture was taken and reinoculated in normal NB media after setting the OD at 0.5. The culture was allowed to grow at shaking conditions at 37 °C in a shaker incubator at 130-140 rpm. Centrifuged the culture at 8000 rpm for 5 minutes and assay was performed at regular time intervals of 24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours and 168 hours.

After centrifugation pellet was discarded and supernatant was restored out of which 250 μl of filtrate was collected. To the collected filtrate 1ml of 0.1 M potassium phosphate buffer with a set pH of 8.0 was added. Simultaneously, 2.5 ml of urea (0.1 M) was added. The mixture of the following reagents was vortexed for few seconds and incubated for 5 minutes at 37 °C.

After incubation period is over 1ml alkaline hypochlorite is added along with 1ml phenol nitroprusside after which the mixture was incubated at 37 °C for 25 min. the mixture of the optical density was measured at 626 nm.

Urease unit: Amount of urease enzyme required to hydrolyse one mole of urea in one minute is known as one unit of Urease.

Estimation of urease activity was done with help of reference of a calibrated graph.

Estimation of Calcium carbonate

This test was done in order to estimate the total amount of dissolved calcium present in bacterial suspension. Culture was grown in nutrient broth media. Media was grown with same concentrations in two flasks consecutively. One set of culture media flask is required to check an increase in pH during calcium carbonate production. Second set of culture, media flask is required to check calcium carbonate precipitation. Calcium carbonate was precipitated from each set and the amount of calcium carbonate present was measured with the help of EDTA titration. The test was done at fifth day from the day of inoculation for growth. Took 5 ml of culture from each set and was mixed with hydrochloric acid (3 N) followed by addition of 3-4 ml of sodium hydroxide (5 N) for precipitation order to check final pH at 12-13. Hydroxyl naphthol blue used as an indicator. Added 3-4 drops of indicator. Against 0.05 M EDTA the complete mixture was titrated. (Jian et al., 2012)

Calcium carbonate precipitation

Calcium carbonate is the sole product which is going to participate in self-healing by concrete structures. The bacterial culture was inoculated in NB with constant O.D of 0.5 at media supplemented with filter sterilized 2% urea and 25 Mm calcium chloride. Both urea and $CaCl_2$ cannot be autoclaved, so they were filter sterilized using syringe and filter 0.2 µm filter. Sliced a small piece of hydrogel incorporated with bacterial spores and subjected to this whole media. The flask containing media and hydrogel was left undisturbed at shaking conditions at 37 °C. Calcium carbonate was catalysed by urea hydrolysis mediated via bacterial cells. After 168 hours, calcium carbonate was precipitated were collected on filter paper (pore size 0.45 µm) by connecting the membrane filter to a vaccum pump. The precipitated powder was dried using different concentrations of ethanol and was left undisturbed at room temperature for 6 hours. After drying completely calcium carbonate precipitates were weighed. (Koutsoukous et al., 1998)

Germination of bacterial spores from bacterial vegetative cells

Bacterial spores were prepared with the help of two medias. (Media A and B). Bacteria was cultured in medium A for 48 hours (37 ˚C) under shaking conditions. (100 ml).

After incubation of 48 hours the culture media was transferred to medium B which was cultured for 48 hours at 200 rpm at 30 °C. Spores were collected after centrifugation done at 8000 rpm for 5 minutes. (Silva et al., 2015)

Method used: Nutrient depletion.

Table. 3.1 Media composition for bacterial spore germination

Ingredients	Medium A (100ml)	Medium B (100ml)
Calcium acetate.	0.71g	0.51g
Sodium citrate.	0.2 g	-
Yeast extract.	0.22 g	0.10 g
Dextrose.	-	0.10 g
Magnesium chloride.	0.02 g	-
Sodium chloride.	0.02 g	-
Manganese sulphate.	0.01 g	-
Potassium phosphate.	0.01 g	-

Synthesis of hydrogel:

Materials required: Chitosan (99 % pure) / 85 % deacetylated with molecular weight of (100-300 KD), polyvinyl alcohol with molecular weight of (85-124 KD), pellets of sodium hydroxide, glutaraldehyde (25 % v/v) / (98 % pure), acetic acid, ethanol, magnetic stirrer, magnetic rod, Aqua regia, beakers (100 ml), syringe.

Preparation of Aqua regia: To 90 ml hydrochloric acid 30 ml nitric acid was added.

Procedure: Washing of beakers was with aqua regia and last washing was done with distilled water. Beakers were allowed to dry completely. In 2% v/v acetic acid solution 2% w/v chitosan suspension was made. The suspension was left for overnight shaking at 40 °C. Acetic acid was used as a solvent for dissolving chitosan. Chitosan was dissolved completely in acetic acid and clear and transparent solution was obtained. Chitosan should not be left undissolved. In case, it is not dissolved by means of centrifugation or filtration the insoluble impurities are removed

(Li et al., 2015). Prepared 4 % PVA by heating at 90 °C for around 4 – 5 hours under vigorous stirring at magnetic stirrer. The PVA used for making hydrogel was hot water soluble so water was pre heated a in order to make PVA get dissolved easily in water.

Both PVA and chitosan solution were mixed in equal volume (1:1) and were mixed for next 2 hours at 60-65 °C by vigorous shaking and later it was allowed to dissolve completely at 25 °C for approximately 12 hours. This whole step was done in order to obtain complete homogeneous blend mixture of PVA and chitosan as transparent solution.

To this homogeneously blended solution of PVA and chitosan 100 µl of 25% v/v glutaraldehyde was added. Glutaraldehyde acts as crosslinker between PVA and chitosan which helps in gelation to form hydrogel. Glutaraldehyde was used carefully as it can cause nasal and lung irritation. (Takigawa et al., 2006) It can cause breathlessness and trigger asthma. Along with the glutaraldehyde spore culture 1 ml was added in the hydrogel.

Syringes were cut in the shape of required moulds. After adding glutaraldehyde hydrogel formation starts and it was moulded in the moulds for gelation. Later it was left for gelation and curing at 45-55 °C in hot air oven over overnight.

Next day, the formed hydrogel was precipitated with the help of 12% w/v sodium hydroxide solution for 8 hours at room temperature.

Demoulded the hydrogels from the moulds and sodium hydroxide was completely washed off by repeated washing under distilled water. Hydrogel freeze - thaw cycle was run. Freezing was done at -4 °C for 16 hours and thawing was done room temperature for 4 hours. Freeze thaw done in order to increase crosslinking between PVA and chitosan. (Dhiman et al., 2020)

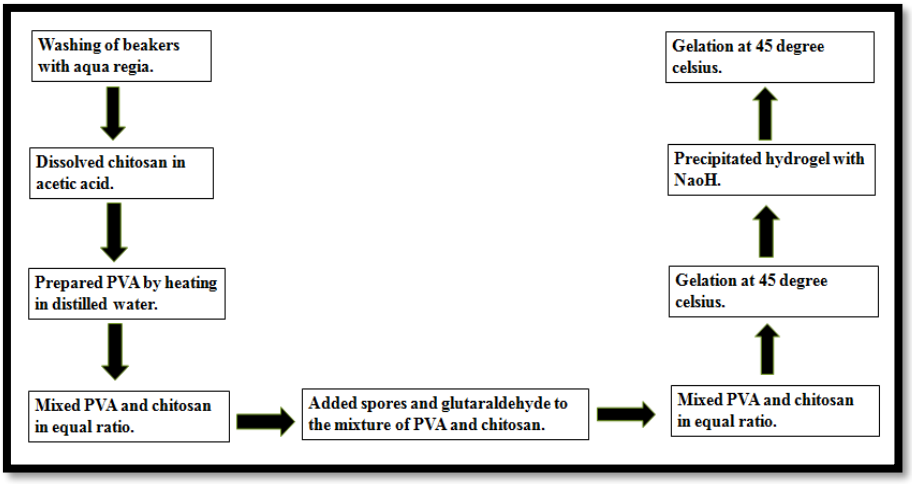


Fig. 3.1 Procedure of synthesising hydrogel

Testing viability of bacterial spores immobilized hydrogel

Plate count method for determination of cell numbers, plate count method was used. Serial dilution of spores immobilized with spores was done from 10^{-1} to 10^{-5} in NB media. For determination of number of viable bacterial cells count nutrient agar plates were used. The plates were incubated at 37 °C for 24 h for counting the number of colonies. (Peters et al., 2010)

Principle Calculating bacterial ability to grow in liquid media or in media for solid culture and is determined by counting the number of visible colonies is known as bacterial viability testing. (Robertson et al., 2019)

Procedure Small pieces of hydrogel containing bacterial spores were cut with the help of hands itself after wearing gloves. These small hydrogel pieces were immersed in NB media (1.3 g NB in 100 ml water) with 5% salt stress. Agar plates were prepared simultaneously. Spores were provided with this media for 24 hours. Later, after 24 hours, spreading of the spores on agar plate was done. Culture was spreaded on each agar plate (100 µl). Dilutions of culture were made till 10^1 - 10^5. Spreading of these aliquots taken from each dilution was done at agar plates which were incubated in B.O.D for 24 hours at 37 °C temperature.

After 24 hours colony count was done with help of colony counter. Plates with colony number from 100-300 were considered as appropriate plates for testing viability. (Gonzalez et al., 2020)

Viability testing of bacterial spores after exposing hydrogel to UV rays

A segment of hydrogel containing bacterial spores was sliced and exposed to UV light under laminar air flow for 15 minutes. Small pieces of hydrogel which were previously exposed to UV light containing bacterial spores was cut with the help of hands itself after wearing gloves. These small hydrogel pieces were immersed in NB media (1.3 g NB in 100 ml water) with 5% salt stress. Agar plates were prepared simultaneously. Spores were provided with this media for 24 hours. Later, after 24 hours, spreading of the spore hydrogel culture was done on agar plate. Culture was spreaded on each agar plate (100 µl). Dilutions of culture were made till 10^1 - 10^5. Spreading of these dilutions was done at agar plates which were incubated in B.O.D for 24 hours at 37 °C temperature.

After 24 hours colony count was done with help of colony counter. Plates with colony number from 100-300 cfu/g were considered as appropriate plates for testing viability

HYDROGEL SWELLING TEST

For checking hydrogel swelling properties small pieces of prepared hydrogel were cut and weighed and these pieces were dried completely under desiccator machine by generating appropriate amount of vaccum. After desiccator was allowed to run for around 4 hours, prepared hydrogel was completely dried and it lost almost all the water it had. Swelling test was done for control hydrogel, hydrogel incorporated with bacterial cells, hydrogel incorporated with bacterial spores. All three sets were run in triplicates. After drying all three samples (in triplicates) completely.

Dried weight for all three sets of hydrogels was noted and hydrogels water retention capacity were checked in different solutions as described below.

Hydrogel swelling test in distilled water

The swelling property of hydrogel specimens was carried out at room temperature in distilled water. Swelling capacity was calculated for control sample of hydrogel, hydrogel containing bacterial culture and hydrogel containing hydrogel spores at different intervals of time 1 min, 5 min, 10 mins, 15 mins, 30 mins, 60 mins, 120 mins, 180 mins, 240 mins,

Calculating swelling capacity of hydrogel

Hydrogels were subjected to different pH and salt stress in distilled water. Also, hydrogel was subjected to normal distilled water and water retention capacity was checked in it as well. Water retention capacity was checked at 5 min, 10 mins, 15 mins, 30 mins, 1 hour, 2-hour, 3-hour, 4-hour, 5 hour, 6 hours. After noting down the readings till 6 hours hydrogel reaches its saturation point. (Rashidzadeh et al., 2014)

Hydrogel swelling test at different pH with 5 % salt stress

The swelling property of hydrogel specimens was carried out at room temperature at pH range from 7.0 to 13.0. HCl (1.0 M) was used to drop pH down in case high pH not required and NaOH (1.0 M) was used to achieve appropriate pH.

Hydrogels were subjected to 5 % salt stress, which was dissolved in distilled water and test was carried out at salt stress with different pH. (Rashidzadeh et al., 2014)

Equilibrium swelling ratio = $W_S - W_D / W_D$ Eq.1

W_S = weight of swollen hydrogel

W_D = weight of initial hydrogel

Degradation test of hydrogel

Degradation of hydrogel in cementitious environment was observed for two months to test hydrogel degradation. (Nie et al., 2004) Hydrogel which was previously freezed thaw for 7 days was subjected to cementitious environment for degradation analysis. The initial weight of hydrogel was noted and the loss of weight after each week was observed for a time period of 8 weeks.

Casting of concrete samples

For casting of concrete samples ordinary Portland Cement (OPC) of grade 43 was used in the presented study. (IS:8112-2013,2013). Took natural sand from local river making sure the sand is sterile. Value of specific gravity used for fine aggregates was 2.70. Value for water absorption for specific gravity of fine aggregates was 1.8%. For coarse aggregate gravels which were crushed and has particle size of 10-20 mm were used. Specific gravity for 20 mm particles was 2.63 and 2.65 for 20 mm particles. Water absorption for 20 mm particles was 1.4% and water absorption for 10 mm particles was 1.38%. Confirming to IS: 383-1970 standard coarse and fine aggregates were used for preparing concrete specimens.

Generation of artificial crack in concrete

Concrete cubes specimen size: (150 mm × 150 mm × 150 mm), were prepared for both bacteria containing hydrogel and hydrogel for control. Concrete cylinder disc size: (100 mm diameter × 50 mm height) were also prepared with standard ratio of cement: sand: aggregate (coarse) in ratio of 1:1.82:3.24 (w/w) and cement ratio to water ratio (c/w) of 0.5. To the casting mixture 5 % salt was also added. With the help of a steel plate placed inside the mix, artificial crack was generated. The concrete mix at this stage is very fresh (before maturing), concrete mix was transferred to casting steel moulds. Up to 20 mm depth a 0.8 mm width steel plate was inserted to the concrete mixture. Steel plates were withdrawn from the concrete mix with utmost care so as to maintain integrity of concrete formed around the crack. Once the samples were casted, all the specimens were allowed for moulding in the iron moulding chambers. The specimens were allowed to cast at room temperature 27 ± 2 °C for 24 hours. Specimens were demoulded from the iron casting chambers and were left for curing. (Joshi et al., 2021)

Crack repair mediated via hydrogel encapsulated spore and crack repair mediated via control hydrogel

For repairing crack spores were incorporated in hydrogel which was completely lyophilised in order to make hydrogel containing spores completely dry. Hydrogel (2g) was taken for each (spore containing hydrogel and control hydrogel), were mixed with 35 g sand (26 g- 600 µm sand and 9 g- 300 µm sand) and 15 g cement. The whole mixture of sand, cement, hydrogel was mixed properly with water and supplemented by injecting (20 ml syringe capacity) into the artificial crack generated. In the following study, crack has been repaired in two different sets of specimens. 12 cubes were casted and 12-cylinder discs were casted. 6 cube samples were filled with 35 g sand (26 g- 600 µm sand and 9 g- 300 µm sand) and 15 g cement and 2 g hydrogel containing spores. Other 6 cubes were supplemented with 35 g sand (26 g- 600 µm sand and 9 g- 300 µm sand) and control hydrogel with no spores. 12-cylinder discs were also casted and out of which 6 cylinders were supplemented with 35 g sand (26 g- 600 µm sand and 9 g- 300 µm sand) and 15 g cement and 2 g hydrogel containing spores. Other 6-cylinder discs were supplemented with 35 g sand (26 g- 600 µm sand and 9 g- 300 µm sand) and control hydrogel with no spores. Out of 12-cylinder discs samples 6 were used for control hydrogel treatment and 6 were used for bio-hydrogel treatment and from them each three samples from the 6 samples set were used for ponding and spray treatment. Likewise, in the control hydrogel Out of 12-cylinder discs samples 6 were used for control hydrogel treatment and 6 were used for bio-hydrogel treatment and from them each three samples from the 6 samples set were used for ponding and spray treatment. Later, all the specimens of cylinders were used for testing water sorptivity and cubes were used for testing compressive strength of the specimens. For following standard 28 days of healing the artificially generated cracks in both cylindrical discs and cubes of bio-hydrogel were treated with 25 mM $CaCl_2$ solution (w/v) and 2% urea. For the ponding, Pond like structures were made above the concretes from where the nutrients were supplied and in spraying technique spray bottle filled with nutrients were used for supplementing nutrients to the spores inside the crack. (Sangadji et al., 2017). Ponding and spray treatment used for curing crack has been schematically described in Fig. 3.2.

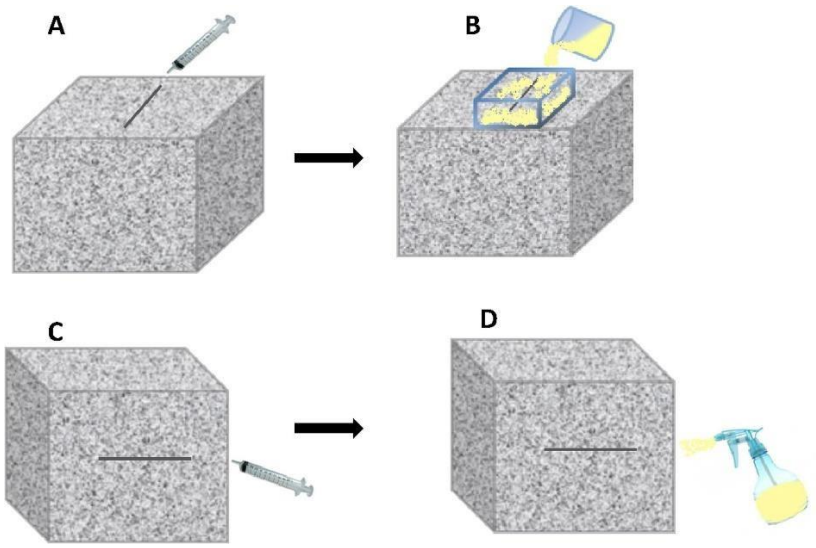


Fig. 3.2 Schematic diagram representing crack remediation via ponding and spray treatment. (Joshi et.al, 2020)

Compressive strength test of cube specimens

The cubes (12) specimens were subjected to compute compressive strength with compressive testing machine (CTM) that were repaired using bacterial and control hydrogel. Control samples containing only hydrogel with no encapsulated bacterial spore by spray as well as ponding treatment were used to evaluate compressive strength. Cubes comprising of bacterial spore immobilized in hydrogel were also used to estimate compressive strength test. The repaired crack through ponding and spray treatment was studied for 14 days according to Indian standard (IS 516: 1959) by applying compressive strength testing machine COMPTEST 3000 (Joshi et al., 2017).

Water Sorptivity test

Source of water sorptivity test was ASTMC1585 (Zhimin et al., 2012). The basic principle of the test is based upon total increase in mass of cylinder specimens. The rise in total mass was checked at regular intervals of time for 72 hours. The cylinder specimens were allowed to dry in an oven 2-3 days prior to testing (50 ± 5 °C). Water was filled in trays such that only one surface of cylinder specimens was exposed to water. Samples were only allowed to be in contact with water up to 3-5 mm depth. Apart from the side exposed to water all other edges were covered with epoxy coating so as to assure unidirectional flow of water through specimens. (Zhigang et al., 2014). Absorption rate is well-defined as mass(g) variation in specimens after water contact divided to the area of cross section of cylinder specimens (mm^2)

and water density at noted temperature (g/mm^3). The graph was plotted in contrary to time square root Water movement inside concrete is designated by relation of square- root time ($min^{1/2}$). Specimen sorptivity was recorded at 1 min, 5 min, 10 min, 15 min, 30 min, 1 hour, 2-hour, 3-hour, 4-hour, 5-hour, 6-hour, 24-hour, 48-hour, 72 hours.

Micro structural analysis

Analysis of calcium carbonate crystals formed during healing of crack after 28 days was done (FE-SEM) field emission scanning electron microscopy (SEM), via sigma – ZEISS Field emission SEM. Identification of composition of micro structural minerals and crystals was done via (EDX) X-ray spectroscopy which works on the principle of dispersive X-ray spectroscopy. For performing both SEM and EDX analysis samples were cut into small pieces and finely coated with gold coating with spitter coating.

For XRD analysis of concrete samples. The samples were finely powdered along with the region of calcium carbonate production. Coated a thin carbon coating on polished surface in order scatter excessive charge from the sample. The powder form concrete sample was obtained through 90 μm sieve. This powdered sample was used for XRD analysis. Scanning was performed from 10° to 80° 2θ with Cu anode of (40 kV and 30 mA). (Joshi et al., 2021).

Statistical analysis

All the experiments were performed in triplicates. The data was recorded by analysis of variance and significant differences among the means were compared by Tukey test $p < 0.05$. All the analyses were performed using GraphPad prism 5 software.

CHAPTER – 4

RESULTS

UREASE ASSAY

Alkaline hypochlorite method was used for confirming the production of ammonium carbonate. The appearance of blue color assured the presence of ammonium carbonate. The urease activity of bacterial strain was observed at different salt concentrations and bacteria was successful in showing effective presence of enzyme urease confirming the ureolytic activity. Urease activity was carried out in nutrient broth media at different salt concentrations (0 %, 3.5 %, 5 %, 7.5 %, 10 %, 15 %). The assay was determined at regular intervals of time (24 hours, 48 hours, 72 hours, 96 hours, 120 hours, 144 hours, 168 hours) by following protocol of indophenol assay (Natrajan, 1995). Bacteria at 5 % salt concentration exhibited maximum urease activity. Bacteria at 96 hours at 5 % salt concentration displayed maximum urease activity. After 5% salt stress bacteria, was succesful in displaying urease activity at 3.5 % salt stress followed by 7.5 %, 10 %, 15 % salt stress. Out of all salt stress concentrations 15 % displayed least urease activity. Urease activity exhibited by bacteria at 15 % salt stress was found to be negligible. In all the salt concentrations bacterial urease activity from 96 hours started to decreases. At 96 hours maximum urease activity was observed. After 96 hours onwards gradual decrease in urease activity of all salt concentrations was observed. It can be observed from Fig 4.1 the higher the intensity of blue color produced the higher is the urease concentration present. Intensity of blue color produced is directly proportional to urease concentration. Deepest color intensity determines highest urease concentration in (U/ml).

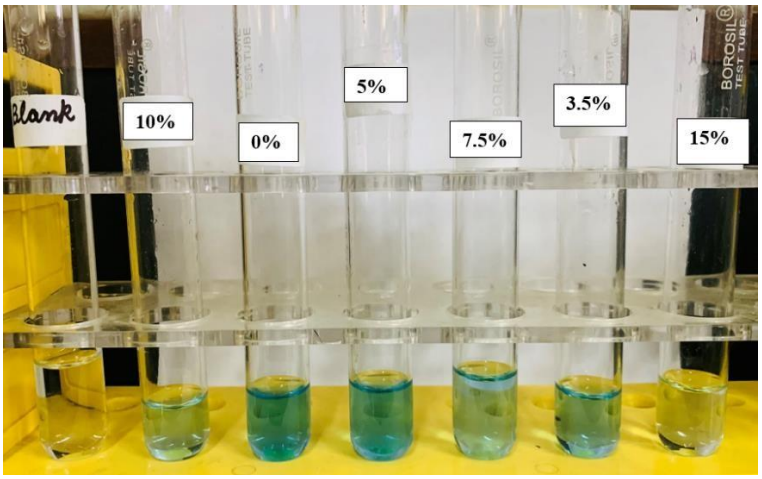


Fig 4.1 Appearance of blue color due to ammonia production in urease assay

Out of all the salt concentrations, 5 % salt concentration displayed highest urease activity which can be predicted from Fig. 4.2. This urease activity (at 5 % salt concentration) was used as standard for other experiments in this project. Also, the same concentration urease activity exhibited the maximum amount of precipitation of calcium carbonate.

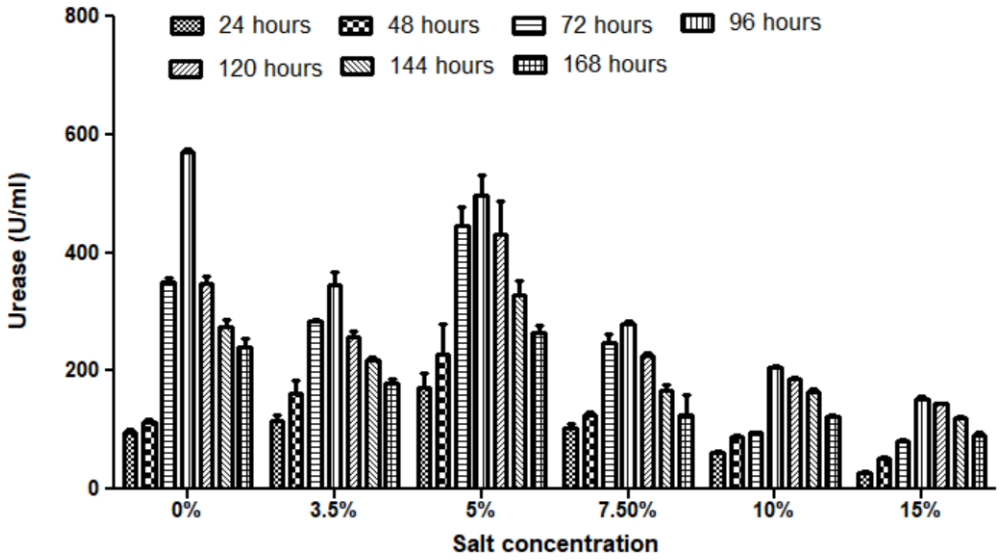


Fig. 4.2 Bacterial urease activity expressed in terms of concentration (U/ml) at different salt concentration for 24 - 168 hours.

Calcium carbonate precipitation

Calcium carbonate was precipitated at 5 % salt concentration. Bacteria was inoculated with nutrient broth media 90 ml, 2 % Urea (5 ml) out of 40 % urea stock and 25 mM (5 ml) $CaCl_2$. After a week of inoculation calcium carbonate precipitates were seen inside the flask. The calcium carbonate precipitates were clearly seen as shown in Fig. 4.3 and collected in cellulose membrane filter of pore size (0.45 µm).

Fig. 4.3 Calcium carbonate precipitates

Effect of different salt concentrations on calcium carbonate production

Calcite carbonate production was experimentally observed in nutrient broth media at different salt concentrations (3.6 %, 5 %, 7.5 %, 10 % and 15 %) which was supplemented with 2 % urea (5 ml) from 40 % urea stock and 5ml $CaCl_2$ (25mM). Maximum calcite precipitation was perceived in nutrient broth media of the 5 %. Lesser amount of calcium carbonate precipitates was observed in 10 % and 15 % salt concentrations as shown in Fig.4.4 due to the fact that the increase in salt concentration cause hinderance in calcite precipitation.

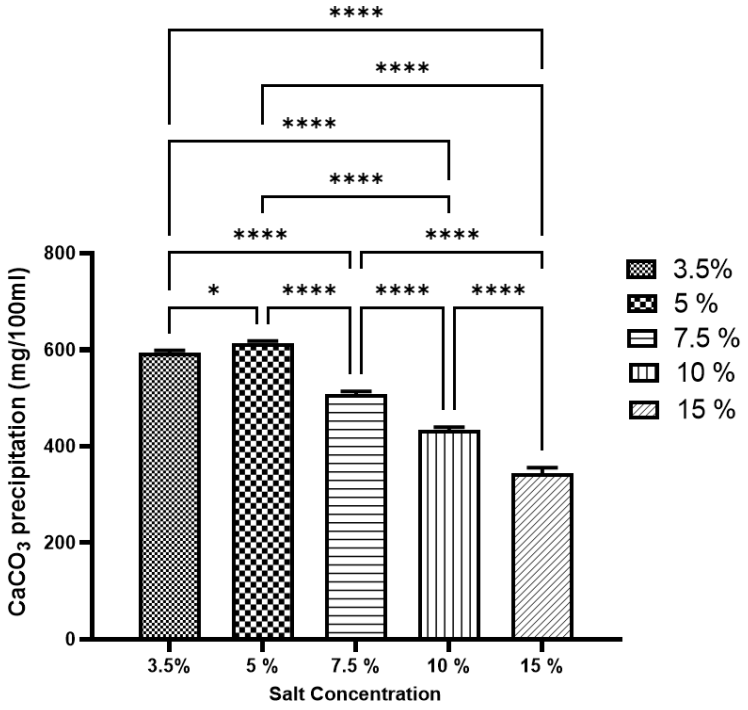


Fig. 4.4 Calcium carbonate precipitation in 100 ml NB supplemented with different concentration of salt. Error bars represents standard deviation (n=3). The factional ANOVA results indicating the salt concentration and time period are highly significant (P values <0.001)

Calcium carbonate precipitated from different salt concentration is expressed in figure 4.5. Recorded amount of calcium carbonate produced after 24 hours and amount of calcium carbonate produced after 168 hours. On that basis it was concluded that calcium carbonate produced at 5 % was maximum following 3.5%. As, we achieved maximum calcite precipitates at 5 % the other experiments of the study were performed at 5 % salt stress.

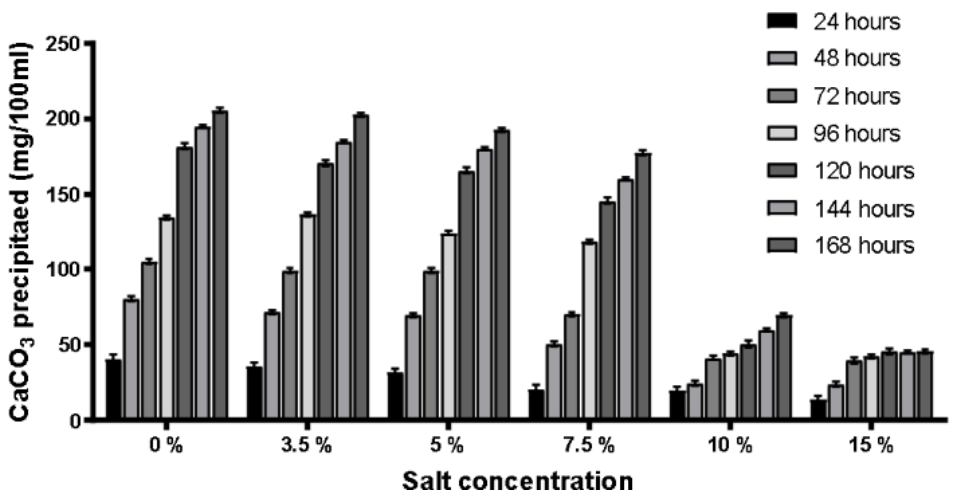


Fig. 4.5 Calcium carbonate precipitation in nutrient broth supplemented with different salt concentration at regular intervals of time (24 – 168 hours). Error bars represents standard deviation (n =3). The factional ANOVA results indicating the salt concentration and time period are highly significant (P values <0.001)

Germination of bacterial spores

Bacterial spores possess the ability to survive for hundreds of years. Bacterial cells lost their viable count when introduced into such high alkaline environment because they are not compatible to the environment. So, bacterial cells from the compatible environment were isolated. Bacterial vegetative cells die during cement mixing and due to mechanical sheer provided by coarse and rough structure of cements. In order to overcome this problem, bacterial spores are produced which can withstand tough environmental conditions. Bacterial spores were produced by the method of nutrient depletion. Full turbid growth was seen when bacteria was grown in media 'A' which was composed of all essential nutrients required for bacterial growth and after when these bacterial cells were introduced to Media 'B' which comprises of limited nutrients. Nutrient depletion led to production of bacterial spores. The obtained suspensions of bacterial spores were analysed microscopically as shown in Fig. 4.6. The produced bacterial spores were stored in refrigerator at (4 °C) until they were further used for mixing with cement mixture.

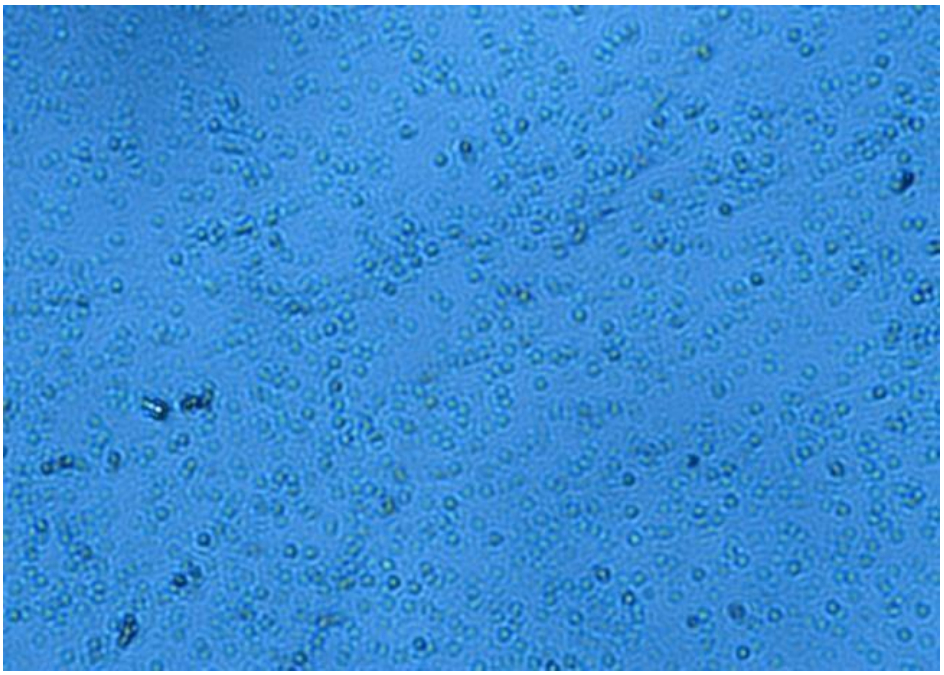


Fig. 4.6 Spores produced by *bacillus paramycoides*

Synthesis of hydrogel

Hydrogel was produced by using equal volume ratio of PVA/CS. This selective ratio of PVA/CS displayed finest mechanical, physical as well as chemical properties. Furthermore, a stable hydrogel with no toxicity was synthesized.

After partial polymerization and gelation of PVA/CS hydrogel with the help of glutaraldehyde hydrogel was formed and the formed hydrogel was subjected to moulds made by cutting the front side of injection and sealing it with the tape, making sure it does not leak.

PVA/CS in moulds got polymerized when kept in oven at 55 °C. After, this step, the hydrogels were completely formed and then these polymerized hydrogels were precipitated with 12% w/v sodium hydroxide (NaOH) solution for minimum 8 hours at room temperature. Sometimes, it takes longer for hydrogels to get precipitated easily, in that case time for precipitation is increased to almost 16 hours. Better the precipitation easier it is to demould the hydrogels from injection moulds. Pictorial representation of hydrogel production is explained in Fig. 4.7

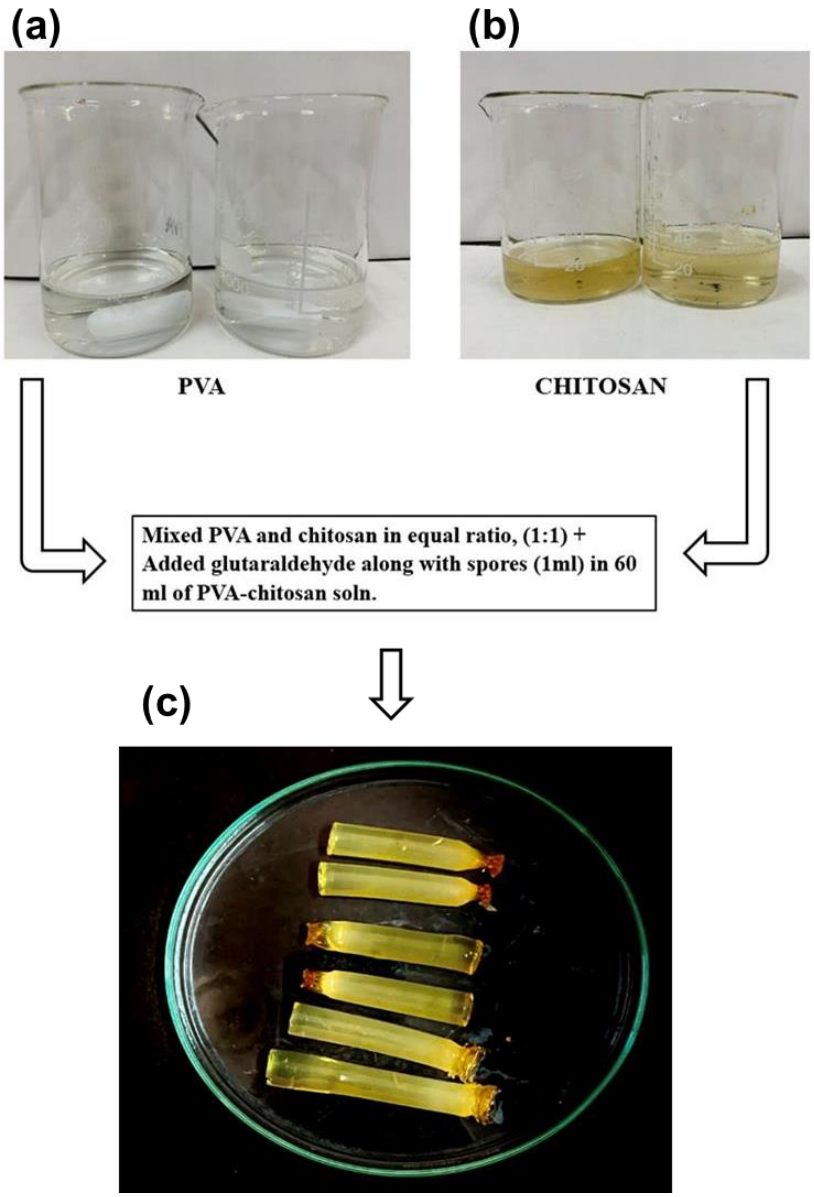


Fig 4.7 (a) PVA solution (b) chitosan suspension (c) bio-hydrogel immobilized with bacterial spores.

In order to achieve better cross linkage between PVA/CS hydrogel freeze-thaw cycles were run. Freezing at -4 °C for 16 hours was done and thawing at done room temperature for 4 hours. Once, the freeze – thaw cycles were completed for 7 days, hydrogels were cut in to small pieces with hands. These, circular small hydrogel pieces were subjected to lyophilizer machine in order to completely lyophilized hydrogels, and to remove the excess water present to make them entirely dry. Pictorial representation of hydrogel after freeze thaw is depicted in Fig. 4.8. Due to water loss from these circular pieces of hydrogels, the size of these pieces reduced to almost half of their initial size. After drying process, the dried hydrogel pieces were crushed with pestle and mortar and fine powder like structures were formed which was stored in LDPE

bags at room temperature for further experiments. This powder, was mixed with sand and cement and incorporated into concrete specimens for crack remediation.

Fig. 4.8 Hydrogel after freeze-thaw cycle

Viability of spores encapsulated in hydrogel

Cell viability was studied by plate count method. The viability of spores was checked for 12 weeks. It was seen that the spores were able to break their dormancy and grow when growth enrichment media was provided. (NB). Fig.4.9 shows bacterial colony count at week 4.

Fig. 4.9 Bacterial colonies (cfu-296) representing cell viability at week 4

The viability of spores was checked for 12 week to investigate survival and growth of spores.The bacterial cell viability was plotted as log/cfu as shown in Fig. 4.10. It was observed that the spores were viable at a constant rate and not much difference was seen in the decrease of viability.The log cfu/g was maintained almost at a constant rate duribg storage. The initial cell viability was found to be 2.96 log cfu/g which declined to 2.92 log cfu/g. Therefore, not much difference was observed in cell viability of spore immobilized hydrogel. Fig. 4.10 depicts viability of spores for 12 weeks.

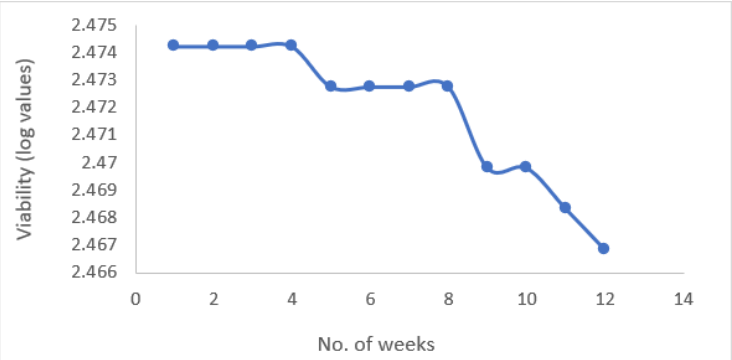


Fig. 4.10 Viability of spores immobilized in hydrogel

Viability of spores immobilized in hydrogel exposed to UV rays

Along with viability of spores immobilized in hydrogel it was important to study the spore viability when the surrounding environment is not favourable. In this case, not much difference was seen in cell viable count as shown in Fig. 4.11. Viability till week 4 was 2.96 log/cfu which declined to 2.91 log cfu/g. Decline in viability was not seen because spores were encapsulated in a hydrogel which acted as a spore guard as well as the carrier material for spores.

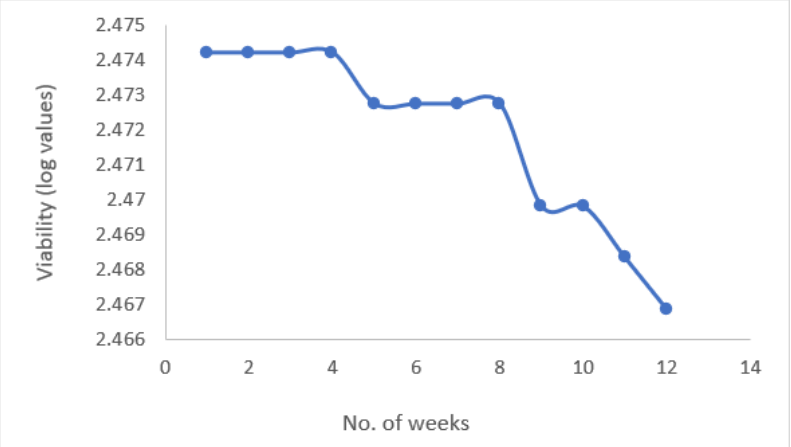


Fig. 4.11 Viability of spores immobilized in hydrogel exposed to UV rays

Swelling studies of hydrogel

Apart from self-healing in concrete structure other main aim of the present research was to study the behaviour of hydrogel when exposed to different environment. One such crucial aim has been to study swelling property of the hydrogel. In other words, swelling properties of hydrogel was studied after measuring the capacity of hydrogels to captivate water (water absorbance ability).

Swelling kinetics of hydrogel in distilled water

The swelling behaviour of hydrogel was observed until the swelling point reaches to a saturation point. Primarily, water intake by hydrogel increases sharply followed by lower water absorbance rate till a point when equilibrium is achieved. It was observed that hydrogel swelling standards reached an equilibrium point after 240 - 360 minutes. As time passes by swelling capacity of hydrogel decreases. It was also observed that saturation point of swelling was observed quite earlier for hydrogel containing spores and bacterial culture than the saturation points for control hydrogel as shown in Fig. 4.12. Hydrogel which was immobilized with spore and culture displayed almost same swelling rate. Hydrogel which acted as control showed maximum water absorbance, comparison can be viewed from Fig.4.13 and Fig. 4.14 because its interstitial space was not occupied as shown in Fig.4.13, whereas the interstitial space (cross-linking region of hydrogel) of spore and culture hydrogel was occupied by spores and culture which led to less water absorbance capacity of hydrogel.

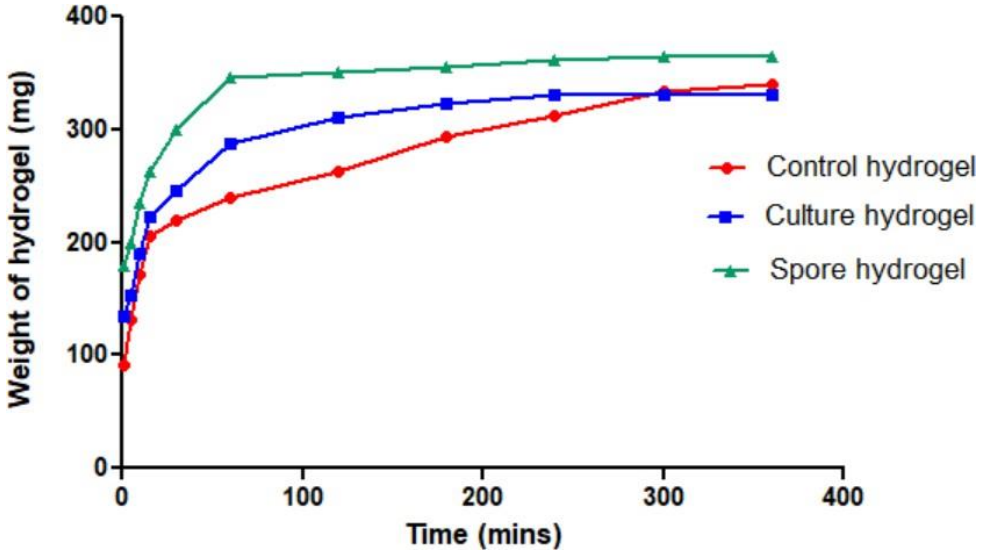


Fig .4.12 Swelling dynamics of hydrogel in distilled water.

Fig. 4.13 Hydrogel after complete drying in desiccator.

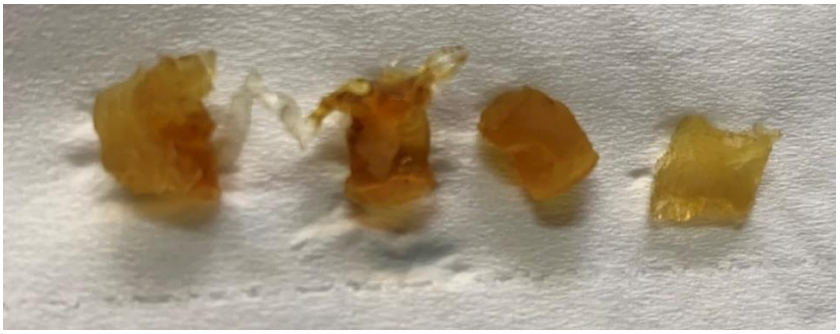


Fig. 4.14 Hydrogel after swelling period of 15 minutes.

Effect of salt stress on swelling kinetics of hydrogel in 5 % salt concentration

The swelling kinetics of the hydrogel specimens were studied at 5% salt stress and at room temperature. Studying swelling kinetics of hydrogel with salt stress is an important aspect because it is vital in many applications. For example, due to natural salinity in agricultural lands it is important to study swelling behaviour of hydrogel provided with salt stress. 5% NaCl was used for studying swelling behaviour of hydrogel. It was clearly observed that swelling of hydrogel was significantly lower in salt stress than compared to hydrogel swelling behaviour in distilled water. In control hydrogel swelling was maximum in spite the same salt conc. as provided to culture and spore hydrogel because spores and culture interstitial space inside culture and spore hydrogel were filled with bacteria and spores which led to less water ingression inside the hydrogel membrane structure (Feng et al.,20201) as shown in Fig. 4.15. Also, another reason behind it can be described by the hydrogel elasticity produced differently in distilled water and in salt stress. Another important reason can be determined by change in osmotic pressure due to presence of salt stress. Concentration of salt ions entering matrix of hydrogel causes change in osmotic pressure that creates a difference in swelling behaviour of hydrogel in distilled water and salt stress water. It was observed that in saline water hydrogel swelling takes place at a lower rate as compared to swelling of hydrogel in distilled water.

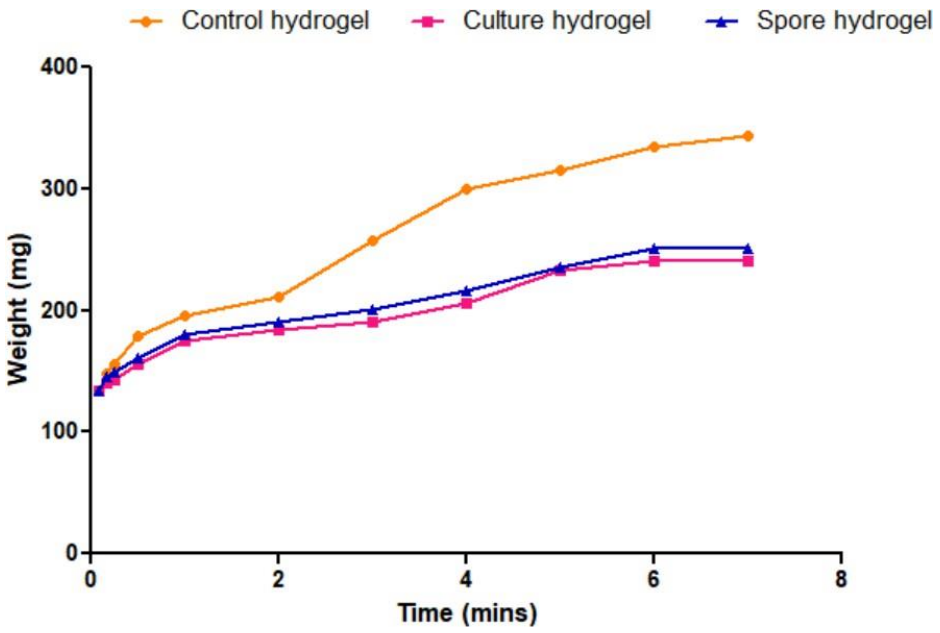


Fig. 4.15 Effect of 5% salt stress on swelling kinetics of hydrogel

Effect of pH on swelling kinetics of hydrogel

Hydrogel swelling kinetics of control was studied at different pH ranging between 7.0 - 13.0 and at room temperature. It was observed that at pH 7, the rate of swelling of hydrogel was constant and quite stable. Maximum swelling of hydrogel was observed at pH 10 as shown in Fig. 4.16. The reason of maximum water absorbance capacity at pH 10 is the conversion of –COOH group to –COO– group. Due to this higher rate of anion-anion repulsion was seen which resulted in higher swelling capacity. Whereas least water absorbance capacity of hydrogel was seen in pH 13 because high concentration of H^+ are present and protonation of most of the carboxylate ions occurs. Due to which electrostatic repulsion of anion–anion is prevented. This led to least water absorbance capacity of hydrogel in pH 13 as shown in Fig. 4.16

Apparently, when the outer environmental pH is increased, carboxylic ions accumulate inside nanocomposite network of hydrogel. Due to this gathering of carboxylic ions hydrogel bonds present between carboxylic groups and water molecules weakens. And this weakening of hydrogen bonds leads to increase in water discharge from hydrogel network. And thus, weight of hydrogel reduces as shown in Fig. 4.16.

In, control hydrogel, culture hydrogel, spore hydrogel pH swelling rate was observed to be maximum at pH 10 followed by pH 7 at was observed to be least in pH 13.

Similarly, spore hydrogel pH swelling rate was observed to be maximum at pH 10 followed by pH 7. At pH 13 instead of swelling it was observed that control hydrogel shrinks.

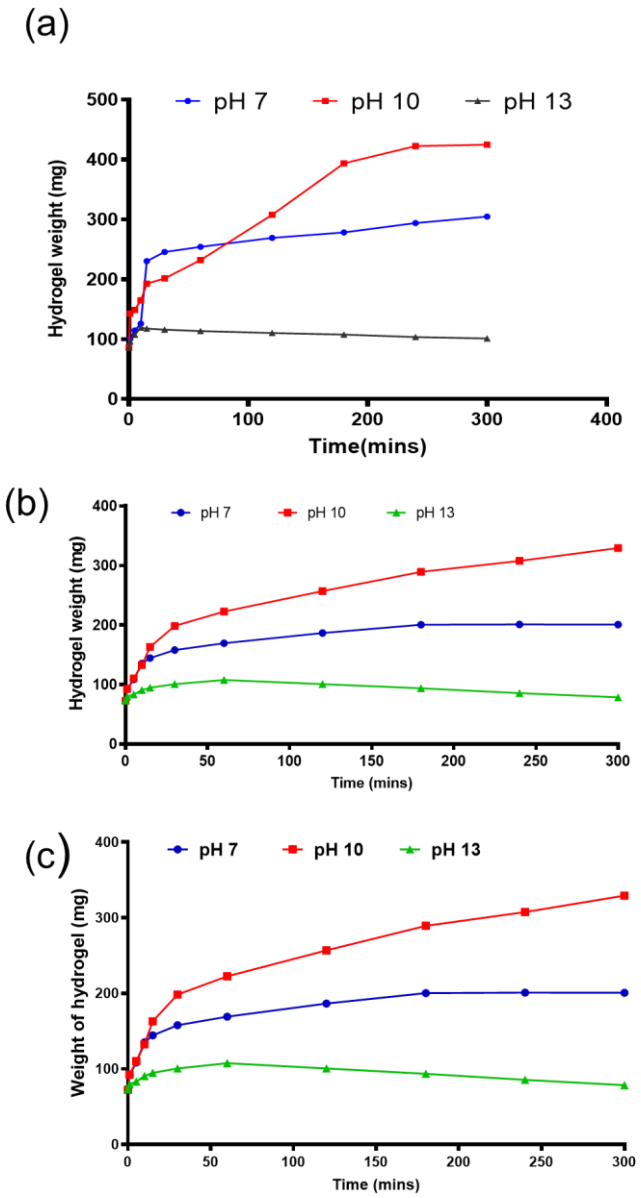


Fig 4.16 (a) Swelling kinetics of control hydrogel in different pH, (b) Swelling kinetics of culture in different pH hydrogel, (c) Swelling kinetics of spore hydrogel in different pH.

HYDROGEL DEGRADATION

Degradation of hydrogel depends upon environment of the surrounding tissues. Hydrogel immobilized with spores when introduced to cementitious environment started to degrade at a very high rate but later got stable. Hydrogel was subjected to a mixture of cement and sand. It was observed that the percentage water loss of hydrogel at the beginning was quite higher and

at later stages it attained an equilibrium. It is because the water loss from hydrogel during the initial stages was higher and later water loss after a certain period of time made hydrogel harder. This intactness of hydrogel made it sustainable in the cementitious environment. Hydrogel consisting of bacterial culture and spore lost its water at a lower rate than control hydrogel. Weakening of hydrogen bonds leads to higher increase in water discharge from hydrogel network. And thus, hydrogel shrinks in the initial stages and then the weight of hydrogel becomes stable. At initial stages water loss from control hydrogel was maximum as shown in Fig. 4.17. It was noted that in week 1 the water loss from control hydrogel was 24.3 %. Whereas, water loss from control hydrogel after week 1 was least i.e., 11.33 %. Water loss from spore immobilized hydrogel after week 1 was 17.33 %. The rate of degradation of hydrogel was quite slow in other weeks as compared to week 1. The final rate of degradation of control hydrogel at week 8 was 26%. There was not much difference seen in hydrogel degradation after week 1 till week 8 in all the three cases. Rate of culture hydrogel degradation at week 8 was 13.33%. Rate of hydrogel degradation of spore hydrogel at week 8 was 20.33%.

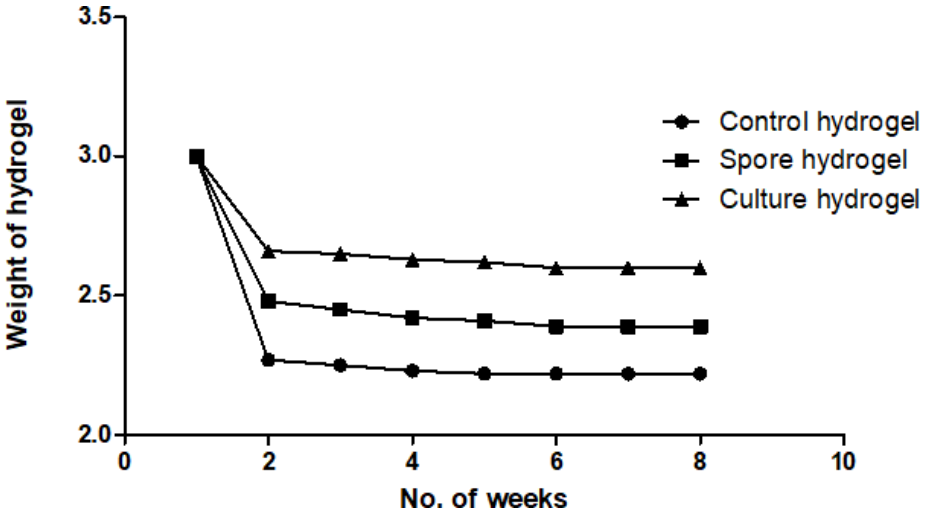


Fig. 4.17 Degradation of hydrogel

Crack repair mediated via hydrogel immobilized spore

Hydrogel immobilized with bacterial spores was sliced in small and thin pieces with hand after wearing gloves. These pieces lyophilised for 2 days, till the water from the hydrogel pieces completely evaporates. These dried hydrogel pieces were crushed with mortar and pestle to form a fine powder like consistency as shown in Fig. 4.18

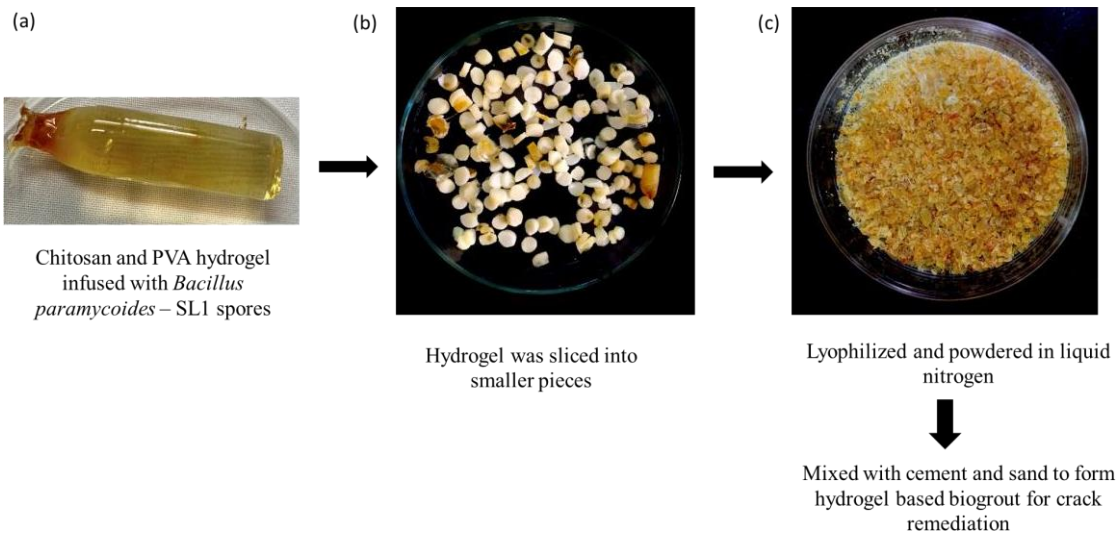


Fig. 4.18 Pictorial representation of process of introducing hydrogel in concrete specimen.

After preparing powder of hydrogel pieces, it was mixed with bio-grout as shown in Fig.4.19 (a) and injected the hydrogel-based bio-grout in 0.5 mm cracks. As shown in Fig. 4.20 (b)

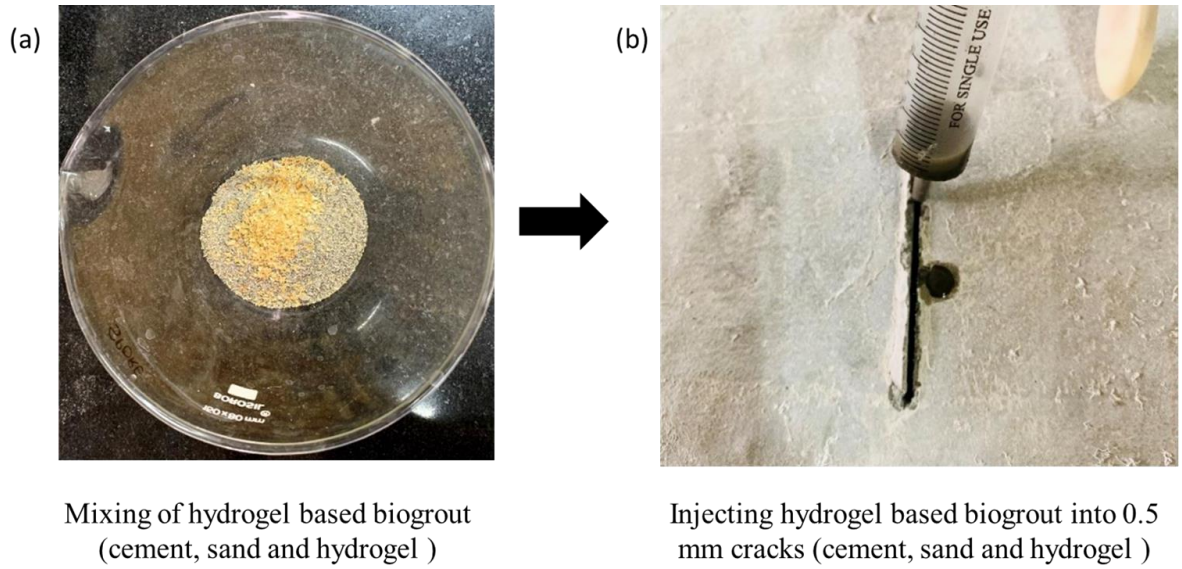


Fig. 4.19 (a) Mixing hydrogel-based bio-grout (b) Injecting hydrogel-based bio-grout in 0.5 mm crack.

Compressive strength

Compressive strength was checked for specimens of concrete cured with spore hydrogel and sample with control hydrogel in COMPTEST 3000 machine as shown in figure 4.20

Fig. 4.20 Compressive strength test

For different specimens of concrete compressive strength is expressed by- CPT- Control ponding treatment, CST- Control spray treatment, HPT- Hydrogel ponding treatment, HST- Hydrogel spray treatment and UTC- Untreated concrete. The observed compressive strength for UTC without any repair method was found to be- 22.7 MPa. Compressive strength of control sample with hydrogel containing no spores was observed to be- 28.5 MPa in CPT and 27.46 in CST. On the other hand, compressive strength for HPT which is a specimen containing hydrogel with spores and treated with ponding treatment was found to be 37.23. Compressive strength for HST which is a specimen containing hydrogel with spores but treated with spray treatment was found to be 31.433. In comparison to UTC, crack healed with hydrogel ponding treatment showed 14.53 MPa increase in compressive strength. In comparison to UTC crack cured with hydrogel spray treatment method (HST) showed 8.733 MPa higher compressive strength. Compressive strength of CPT and CST was experimentally found to be 28.5 and 27.46. The highest recovery of mechanical strength was observed in cracks which were cured with HPT (hydrogel ponding treatment) as shown in Fig. 4.21

Fig. 4.21 Compressive strength of UTC: untreated concrete; CST: plain (control) grout spray treatment; CPT: Plain (control) grout ponding treatment, HST: hydrogel bio-grout with spray treatment; HPT: hydrogel bio-grout with ponding treatment at 28 days of curing. Individual data points are shown in each bar. Error bars represents standard deviation (n = 3). The star sign (*) indicates the statistical analysis at P < 0.05.

Water sorptivity test

After repairing crack with hydrogel immobilized with bacterial spores was absorption test was done which was based upon the principle of absorption by capillary action. For all the concrete specimens test was conducted after 28 days of curing time period. The surface where artificial crack was generated was exposed to water for water sorptivity analysis as shown in Fig. 4.22

Fig. 4.22 Surface of epoxed crack exposed to water for water sorptivity analysis

Out of all the concrete specimens, UTC displayed the maximum sorptivity coefficient. (0.04872 ± 0.003685). In comparison to UTC sorptivity coefficient for HPT was observed to be 0.008897 ± 0.0004097. Among all the concrete specimens, UTC registered the highest sorptivity coefficient. As compared to UTC, sorptivity coefficient for HST was recorded to be 0.02492 ± 0.001169. However, the least water absorption was observed in HPT specimen followed by HST specimen. Sorptivity coefficient for CPT was found to be 0.0338 ± 0.002877, and CST displayed 0.03735 ± 0.001398. As, compared to HPT minor increase in sorptivity coefficient of 0.016 was observed in HST as shown in Fig. 4.23 Significant water restriction in HPT specimen showed the efficiency of curing crack via ponding treatment with spore immobilized inside hydrogel.

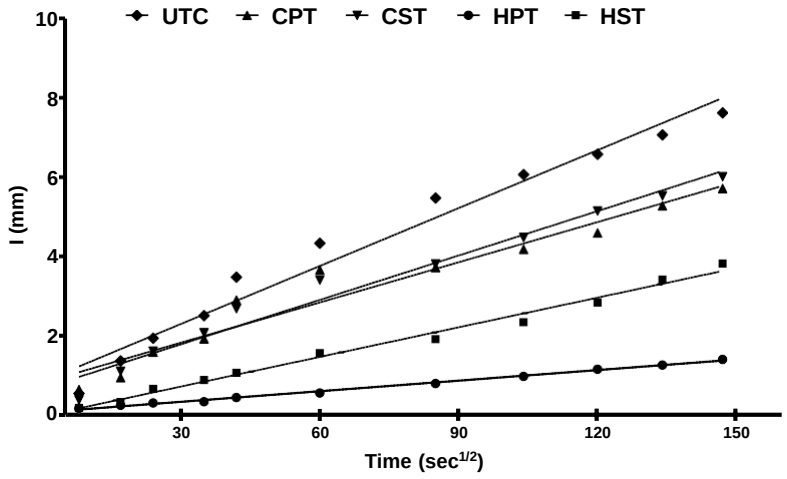


Fig. 4.23 Sorptivity analysis of UTC (untreated concrete), HPT (Hydrogel ponding treatment), HST (Hydrogel spray treatment), CPT (Control ponding treatment), CST (Control spray treatment.

FE-SEM and EDX analysis

Field emission SEM was performed in order to study microstructural analysis of control hydrogel and spore hydrogel, as shown in Fig. 4.24. Spores immobilized in cavities of hydrogel were seen. EDX was performed to study elemental composition of hydrogel as shown in Fig. Fig 4.25. Carbon, nitrogen and oxygen are the natural components found in hydrogel. Composition of carbon was evaluated as 50.09%, composition of oxygen was observed to be 36.48% and nitrogen was evaluated as 0.38%.

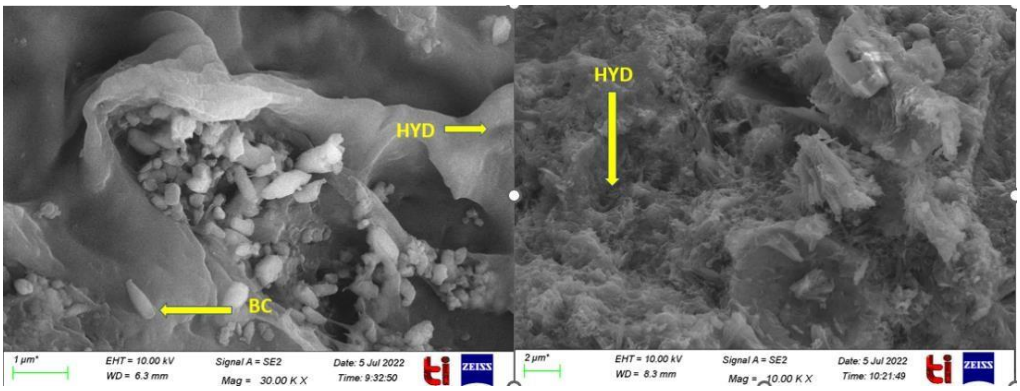
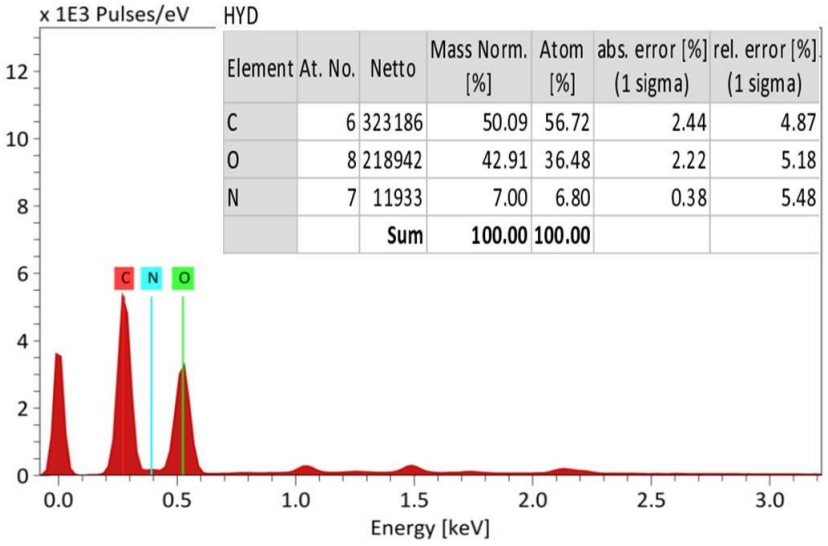


Fig 4.24 SEM- analysis of bio-hydrogel v/s control hydrogel.



Element	At. No.	Netto	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)	rel. error [%] (1 sigma)
C	6	323186	50.09	56.72	2.44	4.87
O	8	218942	42.91	36.48	2.22	5.18
N	7	11933	7.00	6.80	0.38	5.48
		Sum	**100.00**	**100.00**		

Fig 4.25. EDX- analysis of hydrogel

Crystalline mineral structure was seen in SEM – microstructural analysis of bio-concrete along with bacterial spores and hydrogel structures as shown in Fig.4.26. Crystal morphology of calcite production during crack remediation was visibly observed along with the bacterial cells used for healing.

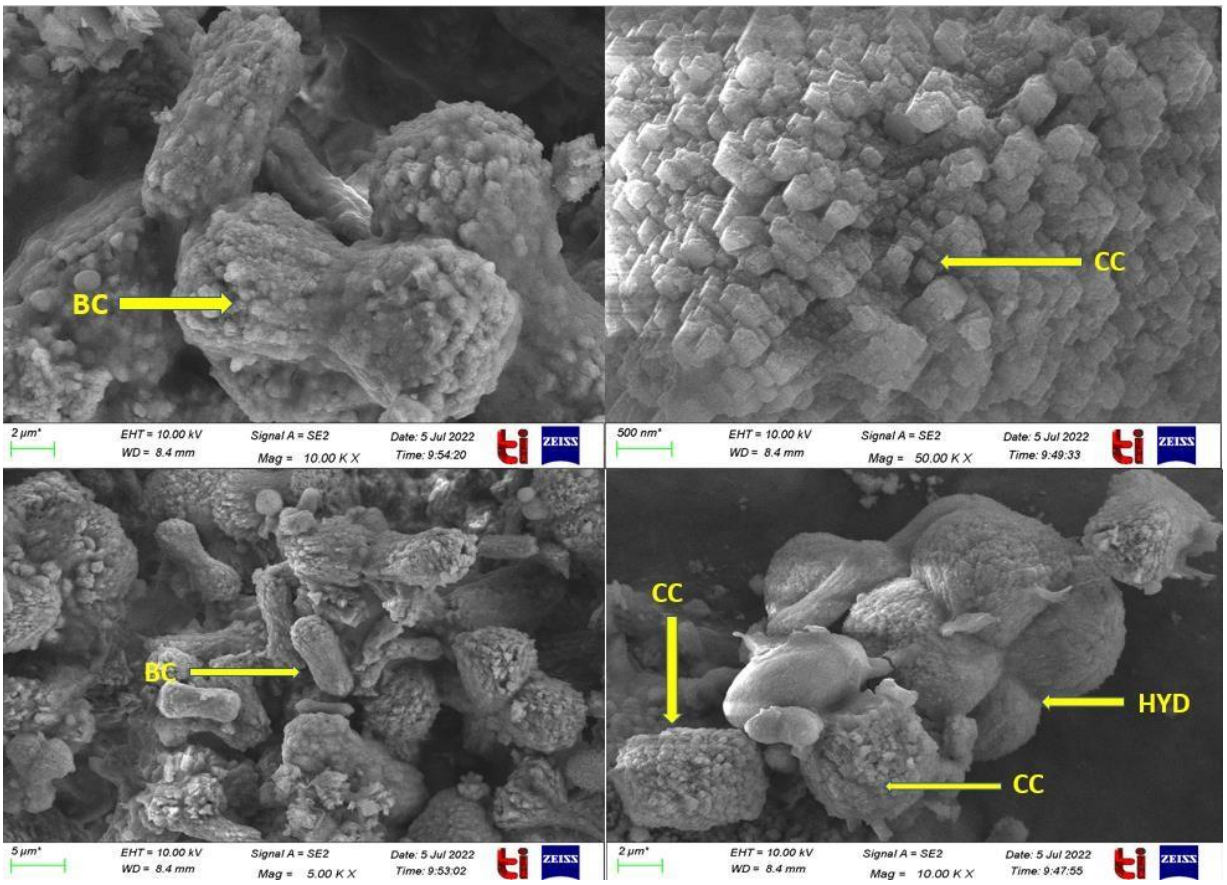
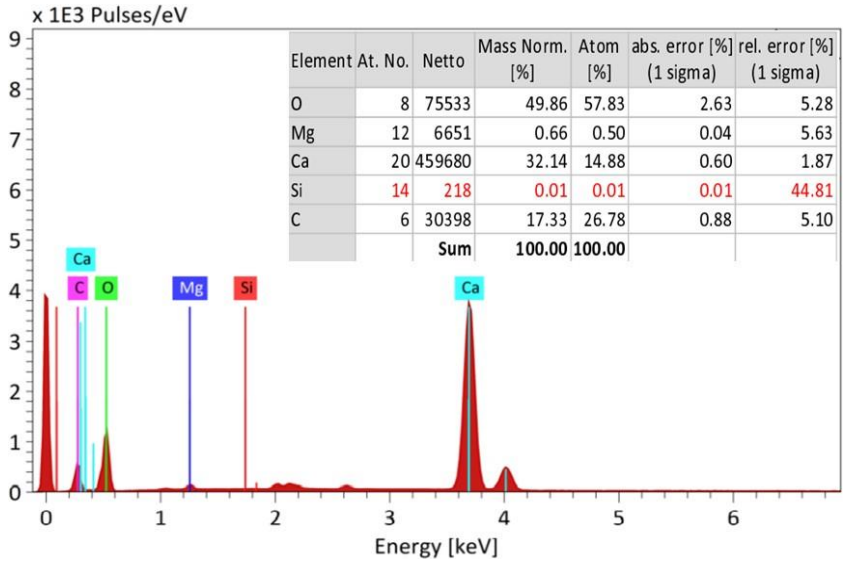


Fig. 4.26 SEM – microstructural analysis of bio-concrete incorporated with hydrogel.

From EDX results of bio-concrete it was clearly observed that calcium with mass normality of (32.14 %) was generated along with magnesium (0.66%), silicon (0.01%), carbon (17.33%) and oxygen (49.86%) as shown in Fig .4.27



Element	At. No.	Netto	Mass Norm. [%]	Atom [%]	abs. error [%] (1 sigma)	rel. error [%] (1 sigma)
O	8	75533	49.86	57.83	2.63	5.28
Mg	12	6651	0.66	0.50	0.04	5.63
Ca	20	459680	32.14	14.88	0.60	1.87
Si	14	218	0.01	0.01	0.01	44.81
C	6	30398	17.33	26.78	0.88	5.10
		Sum	100.00	100.00		

Fig. 4 .27. EDX- analysis of bio-concrete.

XRD analysis

XRD analysis of HPT and CPT was performed to identify constitution of minerals present.

The XRD spectrum of HPT specimen the highest peak was observed at 2 theta value of 29.77 which is very close to peak of pure calcite. Also, the corresponding peaks displayed results nearly equal to pure calcite.

Mineral composition found was as follows- Q: Quartz, CSH: Calcium silicate hydrate, C: Calcite, CH: Calcium hydroxide as shown in Fig.4.28 and Fig 4.29.

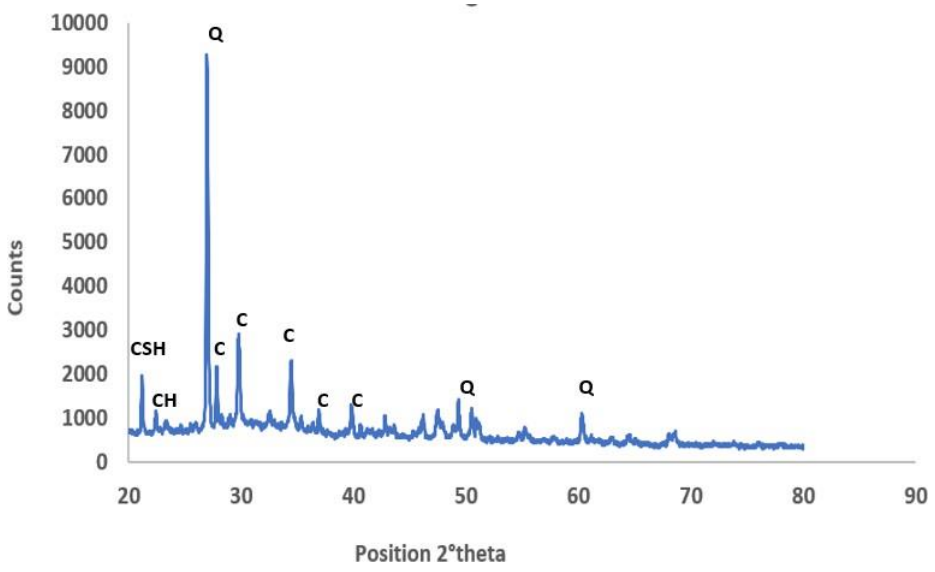


Fig 4.28. XRD pattern of HPT sample (hydrogel ponding treatment)

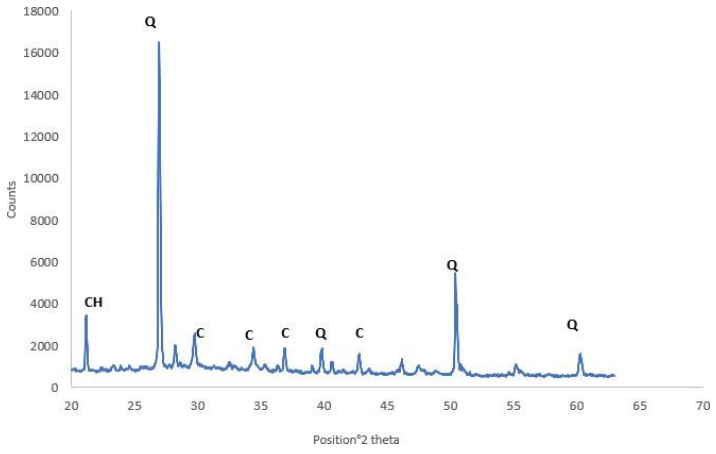


Fig 4.29. XRD pattern of CPT sample (Control ponding treatment)

CHAPTER 5

DISCUSSION

Cement has been extensively used to repair cracks generated in concrete structures. For curing and repairing of these cracks cement mixed with a proportion of hydrogel immobilized bacterial spores were injected for filling the cracks. Cement grouting which is a technique that involves mixing of cement with fluids (water) and other additive mixtures was used for crack curing and healing. The research approaches towards producing sustainable concrete which displays autogenous self-healing properties, environmentally safe and ecologically balanced.

Using MICCP (microbially induced calcium carbonate precipitation) technique significant healing in concrete is observed. (Wiktor et al., 2017).

Using bacterial spores to fulfil the purpose of curing cracks is the foremost choice bacteria shows remarkable resistance to adverse and hostile environmental conditions. (Silva et al., 2015)

In the presented study, mixing of cement with additives like hydrogel immobilized bacterial spores were used for sealing cracks. Compressive strength test was investigated after complete hardening of cement grout mixture with bacterial spores. It was clearly observed that maximum compressive strength was gained by concrete sample which was treated with grout comprising of bacterial spores. The results clearly indicates that samples treated with hydrogel immobilized bacterial spores improved the strength and mechanical properties when compared to UTC (untreated concrete samples).

Ingression of harmful chemicals and hostile agents inside cracks increases the rate of degradation of concrete structures. In order to study rate of ingression sorptivity of the concrete control specimens, hydrogel immobilized bacterial samples were studied for both spray treated and ponding treated samples along with UTC (untreated concrete samples).

According to results, it was observed that maximum water ingression was seen in UTC samples and minimum water penetration was observed in samples treated with immobilized bacterial spores i.e., hydrogel ponding treated (HPT) samples displayed least water sorptivity.

By performing sorptivity experiment on different samples we can conclude that treatment of cracks with bacterial spores in grout is an efficient technique to seal cracks. Due to achievement of high mechanical strength in HPT sample due to calcium carbonate precipitation induced by bacteria a higher rate of reduction in water transport was observed.

After 28 days of curing period SEM-EDX analysis of HPT samples were done. It was clearly observed that production of crystals of calcite helped in increasing strength of sample.
Lower rate of porosity in treated sample was seen. High oxygen and calcium composition in EDX expresses the presence of morphological crystals of calcium and oxygen. XRD analysis displayed presence of calcium carbonate crystalline structures in HPT samples.

CHAPTER 6

CONCLUSION

Investigation of efficiency of crack healing via bacterial spores embedded in grout mixture was done in this study. Among different samples the maximum compressive strength and maximum hardening property was observed in samples treated with hydrogel immobilized with bacteria.

Non-toxic hydrogel was prepared whose properties were studied at different physical and chemical levels. Bacterial viability inside these hydrogel networks was studied and it was clearly concluded that bacterial spores which are present in dormant state inside hydrogel shows negligible decrease in viability along with that mixing of the powdered hydrogel with concrete was quite compatible.

Cracks treated with injectable cement grout with ponding treatment displayed maximum rate of mechanical strength and lowest rate of water permeability. Mechanical strength was also observed in samples treated with spray treatment but at a less rate than ponding treated samples. Overall enhancement of mechanical strength was achieved in samples treated with ponding treatment with bacterial cement grout. In the self-healing approach significant achievement in curing cracks was achieved. When compared to untreated samples, treated sample with hydrogel immobilized with bacterial showed 8.733 MPa higher compressive strength.

Water permeability recorded inside the HPT (hydrogel ponding treatment) specimen showed 0.016 decrease in sorptivity coefficient.

Developing a grout mixture which is environmentally safe as well as effective will help us to remediate cracks.

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