# HIGH STRENGTH CONCRETE WITH SELF-HEALING PROPERTIES

A THESIS REPORT

*Submitted in partial fulfillment of the requirements for the award of the degree*

*of*

**MASTER OF TECHNOLOGY**

**in**

**CIVIL ENGINEERING**

*With specialization in*

**CONSTRUCTION MANGAMENT**

Under the supervision of

**Mr. Abhilash Shukla**

**(Assistant Professor)**

*by*

**Rishab Attri (182601)**

to


**JAYPEE UNIVERSITY OF INFORMATION TECHNOLOGY**
**WAKNAGHAT, SOLAN – 173234**
**HIMACHAL PRADESH, INDIA**
**May - 2020**

# STUDENT'S DECLARATION

I hereby declare that the work presented in the Project report entitled **"High Strength Concrete With Self-Healing Properties"** submitted for partial fulfillment of the requirements for the degree of Master of Technology in Civil Engineering at **Jaypee University of Information Technology, Waknaghat** is an authentic record of my work carried out under the supervision of **Mr. Abhilash Shukla**, **,** Assistant Professor, Civil Engineering Department, Jaypee University of Information Technology, Waknaghat.**.** This work has not been submitted elsewhere for the reward of any other degree/diploma. I am fully responsible for the contents of my project report.

Signature

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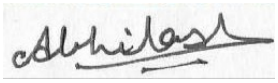
Date: 27/05/2020

# CERTIFICATE

This is to certify that the work which is being presented in the project title **" HIGH STRENGTH CONCRETE WITH SELF-HEALING PROPERTIES"** in partial fulfillment of the requirements for the award of the degree of Master of Technology submitted in Civil Engineering Department, **Jaypee University of Information Technology, Waknaghat** is an authentic record of work carried out by **Rishab Attri (182601)** during a period from January 2020 to May 2020 under the supervision of **Mr. Abhilash Shukla,** Assistant Professor, Civil Engineering Department, Jaypee University of Information Technology, Waknaghat.

The above statement made is correct to the best of my knowledge.

Date: 27/05/2020



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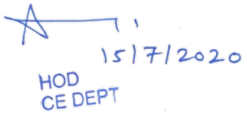


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# ABSTRACT

High-strength concrete is indeed a new progression in concrete technology. It groups compressive strength of 40 MPa or above. Since HSC is another kind of solid, it has not been broadly utilized by the designers. Because of absence of research, it has just been utilized as a part of some reinforced concrete members and few large and precise structures.

In our study, we will try to discover the ideal extent of mineral admixture with cement to accomplish most extreme packing density and make a mix design based on the obtained results. We will be utilized five mineral admixtures as a pozzolanic material in cement. The mineral admixtures utilized were Quartz powder, Fly ash, Metakaolin, Ultra-fine slag and Rice-husk ash. A third-generation superplasticizer will also be additionally used to set up the mix design with a specific end goal to minimize the water necessity for cement hydration.

In this study we will also like to address two challenges that are commonly faced with concrete. One is the negative impact produced on environment due to huge Carbon Dioxide emission during cement manufacturing. We will try to in cooperate some greener materials partially replacing the cement in order to reduce harsh effect on environment.

Crack development is also another bigger challenge that is faced by concrete. Crack development in high strength concrete is not a common phenomenon due to higher pore refinement and interface refinement. But with later ages if cracks developed it will deteriorate the structure. We can also do its reaping by filling those cracks, but it is not a sustainable technique. In this study we will try to make a concrete that will be able to heal its crack with the help of calcite precipitating bacteria. So that it can provide us a more sustainable structure.

The scope of this study is huge as in recent times due to limited space we need to design a structure that may occupy space as less as possible meanwhile provide a great strength. So, HSC is key player for this situation. The Self-Healing concrete will help us to make a durable and sustainable structure. It will also help inn structure where crack development is common phenomenon and the repairing is that is also tough e.g.: Dams Bridges etc.

The trial work will be done in four stages. The main stage incorporates finding of different properties of materials like specific gravity and water retention. The second stage incorporates optimization of binary blend by accomplishing greatest packing density utilizing strategy given by Puntke. After the analyses utilizing diverse materials, graphical portrayal was done to acquire the advanced extent the third stage which can go simultaneously with other stages is the development of calcite precipitating bacteria colonies. In the last stage we will make an optimum mix design which we will find from the above three stages. We will conduct experimental work for it and find the suitability of this concrete.

*Keywords: Bacteria, Self-healing concrete, High strength concrete, Crack, Mineral precipitation, Biomineralization*

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# LIST OF ACRONYMS

| | |
|---|---|
| BM | Bacillus megaterium |
| BOD | Biochemical Oxygen Demand |
| CSL | Corn Steep Liquor |
| CTM | Compression Testing Machine |
| DNA | Deoxyribonucleic Acid |
| EDS | Energy Dispersive Spectroscopy |
| IST | Initial Setting Time |
| FST | Final Setting Time |
| LB | Luria Broth |
| MICP | Microbiologically Induced Calcite Precipitation |
| MTCC | Microbial Type Cell Culture |
| SEM | Standard Electron Microscope |
| UA | Urea Agar |
| UB | Urea Broth |
| OD | Optical Density |

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# CHAPTER-1
# INTRODUCTION

## 1.1 GENERAL

Nowadays, Self-healing concrete has come out as the material of choice as a repair construction material which makes concrete more durable. In this report, self-healing is done through biological processes as a repair material is completely reviewed. This report represents a new research in the field for repair of unexpected cracking of concrete. In this study we attempted to make a High Strength Concrete which will also have self-healing properties, which will give this concrete some extra durability.

## 1.2 Biomineralization in High Strength Concrete

In recent times concrete has become the second most consumes material on the planet after water. In past concrete mixes of low grades or strength were enough to meet our requirements. But due to recent innovations and big structures it was found that past methodologies were not enough. So, researchers decided to find new methods and materials that can meet our requirements. In this series researches came up with a new term known as High Strength Concrete. High Strength cement is a rising innovation that gives another measurement to the expression "High performance concrete".[1–4] It has a lot of potential in construction development industry. It has great mechanical properties and durability properties when contrasted with the traditional cement. It can likewise substitute basic steel in a few applications by joining fiber support. It can also substitute structural steel in some applications by combining fiber reinforcement.

Standards like packing density, micro structural improvement can be used to accomplish HSC. The advantage like water resistance and strength are likewise given by HSC. Different examination of the HSC has been performed for assurance of mechanical and durability properties. The outcomes demonstrate that HSC have more prominent compressive and flexural strength and a decreased water penetrability. The most extreme compressive strength is between 120-150 MPa. [5-7]

Occasionally strength may likewise reach up to 200MPa.[8] At such a high compressive strength the coarse aggregates are the weakest part in concrete. The concrete is liable to fail from coarse aggregates.

To accomplish a compressive quality, we can remove the coarse totals and accomplish consistency and homogeneity in the blend. The pozzolanic properties of materials like silica fume, fly ash and so forth are utilized to accomplish high density and strength. HSC incorporates bond of higher grade (for the most part OPC 53), quartz powder, quartz sand, steel fibres and silica fume, steel aggregates and a superplasticizer (III generation). We likewise utilize superplasticizers so as to decline water-cement ratio with extra advantage of getting great workability. [9],

Here comes up the two new challenge. One is the extensive production of cement and concrete give rise to some hazardous environmental effects to concrete leads to negative environmental effects. The second one is durability of concrete. In concrete the cracks are the major shortcoming in concrete structures, Cracks are responsible for deterioration of concrete ne it Micro or Macro cracks. We need to overcome these two challenges. We know that key constituent of concrete is cement and aggregates. Some facts related to concrete is that the key constituents of concrete are cement and aggregates.[10], [11] The making of cement only leads to 7% $CO_2$ emission by human's activities, which is a huge number. By knowing these facts, it is difficult to say concrete is a sustainable material[12]–[14]. To avoid these phenomenon and make a eco-friendly concrete, concrete was replaced partially with come greener materials we replace concrete partially with greener choices like fly ash, blast furnace slag, or rice husk ash which are by results of iron, coal and agrarian materials or businesses and so on.

Cracks in concrete make a major impact over durability and serviceable life of concrete. Cracks makes it easy for moisture, Carbon dioxide ($CO_2$), Sulphate, gases and other liquids for trans-pass concrete effectively up to its centre and fortification which brings about rot of support and decrease the quality and sturdiness of cement. Therefore, it makes cracks themselves undesirable in concrete structures. The micro cracks can rehabilitate by concrete itself. This healing process is known as "Autogenic healing" which is also known as "Self-healing". [15]–[18]Therefore, cracks can be healed by mixing specific healing material in the concrete matrix. In this we will try to make HSC which will be capable to heal its cracks by itself if occurs to increase the life of concrete and give a concrete structure serviceable for more time. Bacteria plays a major role in making a self-healing

concrete. A type of bacteria that can must precipitate with calcite ($CaCO_3$) to form crystalline layer over cracked surface. The bacteria also should be alkali-resistant (alkaliphilic) in nature because concrete is extremely alkaline. [19]–[23]

## 1.3 Bacteria

Bacteria are the single cell microbes. There is no nucleus and any other membrane in them therefore, they have simple cell structure. DNA contains generic information of bacteria in a single loop., all this present in the control centre of the bacteria. Plasmid is also one of many circles of different genetic materials. It contains genes, which give advantages to bacterium over bacteria.[17], [21], [24]

### 1.3.1 Classifications of Bacteria

Classification based on shapes: According to their basic shapes, bacteria can be classified into 5 groups.

i) Spherical (Cocci)

ii) Comma (vibrios)

iii) Spiral (spirilla)

iv) Rod (Bacilli) &

v) Corkscrew (spirochaetes).

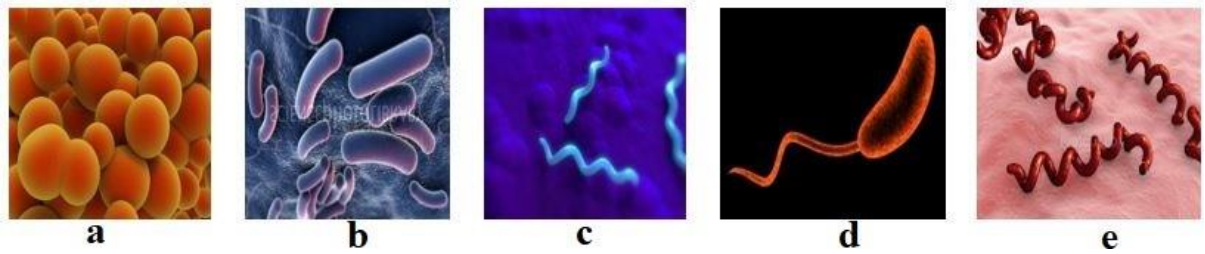



Figure 1.1 Classification of bacteria based on shapes. (a. Spherical (Cocci); b Rod (Bacilli); c. Spiral (Spirilla); d Comma (Vibrios); e Corkscrew (Spirochaetes). “Source: www.microbiologyonline.org

**Classification based on Gram strain**

According to gram strain, bacteria can be classified into2 groups.

i) Gram Positive (gives positive results in gram strain test) &

ii) Gram Negative (gives negative results in gram strain test).

**Classification based on Oxygen requirement**

According to oxygen, required by bacteria can be classified into 2 groups.

i) Aerobic (tomic oxygen is required as terminal electron acceptor) &

ii) Anaerobic (does not require atomic oxygen as terminal electron acceptor).

## 1.3 Bacteria Used in Concrete

Concrete is extremely alkaline; its pH is about 11 to 13 and it mixed under high mechanical stresses. Therefore, immobilized bacteria must be alkaliphilic (alkali-resistant) and must have propensity to endure against the mechanical stresses. The key point against crack repairing is that the bacteria must precipitate with calcite ($CaCO_3$) to form crystalline layer over cracked surface. Bacillus spores show this kind of properties. The crack-filling phenomenon is due to the urease activities due to the alkaliphilic bacteria, which form calcite.[17], [21], [25], [26]

In bio-concrete following *Bacillus* species can be used:

i) Bacillus pasteurii.

ii) Bacillus subtilis.

iii) Bacillus megaterium.

iv) Bacillus cohnii.

v) Bacillus halodurans.

vi) Bacillus pseudofirmus.

And other similar species.

## 1.4 Reproduction and growth of bacteria

Practically all microorganisms multiply by two-fold split technique. A solitary cell of bacteria, the "parent," copies its own DNA and becomes larger in size by expanding the contents of cell by multiple times. This multiplied substance is sent to each divided body of the cell. At that point, little opening rise in focal point of parent cell, at last parting it into two comparable "girl" cells which appeared in Figure 1.2 couple of bacterial animal groups like firmicutes and cyanobacteria increase through maturing. At maturing stage, little girl cell develops a posterity as

the posterity of its parent . It begins as minuscule stub, develops till the size of its parent lastly separates.

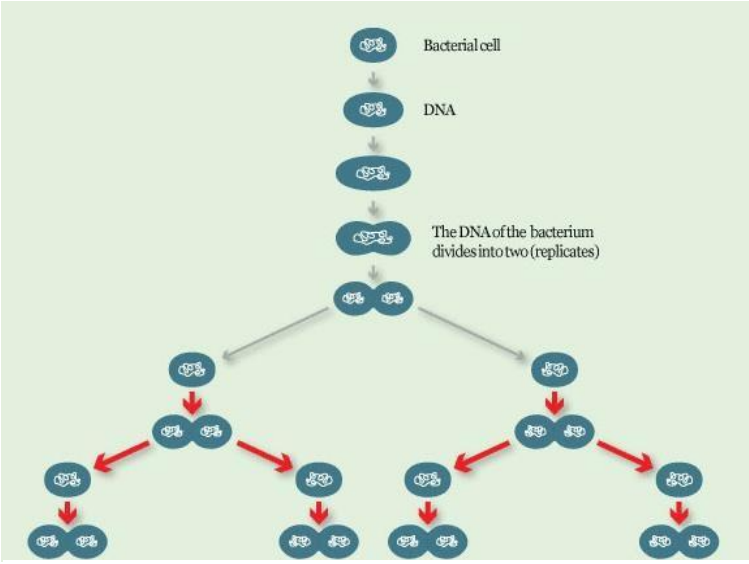



Figure 1.2 Bacteria Reproduction through Binary Fission
"Source: www. Microbiologyonline.org

## 1.5 How will HSC prove useful to construction industry?

The concrete possessing mighty compressive strength of over 150MPa can find various uses in construction industry such as.:

1. Because of high compressive strength, the structural members cast from HSC will be slender as compared to the structural members cast using ordinary concrete for taking up the same amount of load. The material prerequisite will be significantly lessened which will at last make the concrete economical. Likewise, because of decline in size of basic individuals, the dead load will be incredibly decreased. [27]
2. Casting of HSC uses various mineral and chemical admixtures. These admixtures fill up the voids of cement and decrease the permeability of concrete. Because of this, the penetrability of outside material, for example, chloride and moisture is diminished. This makes the solid impervious to different corrosive assaults and warmth. In this manner, the durability of concrete is increment[28]

3. HSC can locate its noteworthy application in pre-cast industry. This concrete can be used to cast bridge girders and various structural members of very high strength. Accelerated curing of this concrete can give very high early strength within 3 days of curing. Researchers have presumed that around 200MPa of compressive quality can be accomplished via autoclave curing of cement at 90°C for 3 days. These structural members can be utilized when the work must be done in less time. [29]
4. HSC discovers its real application when it is utilized with fibers. Different sort of fibers can be added to HSC to enhance its ductility and toughness. Incorporation of fiber reinforcement in HSC can eliminate the structural steel completely. Various foot-over bridges constructed using fiber reinforcement and HSC have indicated great serviceability and durability. High compressive strength of HSC makes it a reasonable material for establishment of tall structures, underground shelters and different other auxiliary portions. [1]

## 1.6 Advantages of High Strength Concrete

1. The structures made will be sooner available for us.
2. The cross section of columns and beams of large structures are reduced.
3. Ultra-high strength concrete can give a highly durable structure.
4. Concrete with any desirable mechanical properties can be made.

## 1.7 Limitations of High Strength Concrete

1. Extra care is required while placing High Strength Concrete which may not be required while using conventional concrete.
2. For making Ultra-high strength concrete good quality control is required.
3. The cost will be increased because in use of HSC we need to do test at site as well.
4. The mix of concrete may require some special materials and skilled labor as well

## 1.8 Advantages of Self-Healing Concrete

1. Cracks in concrete is a major reason of structure failure as it will corrode the reinforcement, with this new technology bacteria will be able to utilize calcite and form less cracks, which will give structure a new stability.
2. It is claimed to increase the structural integrity for many years.
3. By doing more research its scope can be expanded to dams, bridges, swimming pools, sewer and construction exposed to harsh conditions.

# CHAPTER-2

# LITERARUTE REVIEW

## 2.1 GENERAL

Several research papers are available on the topic of high strength concrete and self-healing concrete. Some of the most important research papers in the context of HSC and self-healing concrete have been reviewed here in the present study.

## 2.2 Literature Review

*Lee et.al (2009)* pointed discover the suitability of HSC with (RPC) to be utilized as another repair material and they additionally assessed its bond soundness and durability with existing cement. They attempted to test the solid utilizing quickened maturing condition test in which constant freezing–thawing cycles were made. It is one of the Important tests toinvestigate the durability of repair materials of concrete. Compressive strength of concrete sections was tried prior and then afterward the solidifying defrosting activity and the distinction between the quality decided the appropriateness of RPC as repairing material. [29]

In this trial concentrate Reactive Powder Concrete showed great outcomes which makes it suitable to utilize it as a repairing and retrofitting material as it upgraded the compressive and flexural quality of old tried examples. The impacts of flexural and compressive strength with holding RPC having thickness of 10-mm was about 150% and 200% increase than that of ordinary quality cement. Compressive strength tests after 1000 freeze–defrost cycles demonstrate that RPC is profoundly solid.

The authors *Lee et. al (2012)* assessed to decide if UHSC and RPC can oppose terrorist attacks or impact loading due to accidental damage such as bomb blasting. In their investigation they did the accompanying tests which are compressive strength, elastic modulus, slump flow and various strength tests. What's more, to simulate genuine blasting conditions, ANFO blasts were carried out on RPC panels and UHPC panels reinforced with steel bars and short steel fibres. From their review, they reasoned that reinforcing the UHSC and RPC with meta fibres and structural reinforcement provides enough ductility to the structural members and they increase the ductility

of concrete which ultimately increases the toughness. So, the reinforcement arrests the crack propagation and reduce the brittleness of concrete. [30]

The authors *Chanand et. al (2004)* did analysis to check the impact on bond characteristics between the matrix of RPC and steel fibre due to silica fume, which incorporates pull-out energy and bond strength to break the bond between the two materials. A typical and most popular test named Fibber pull-out tests was received to test the bond between RPC matrix and steel fibres. After the results, it was seen that on silica fume addition addition of silica fume as a cementitious material can increase and enhance the facial properties of fiber-matrix like bond strength and pull-out energy, significant increment in fiber pullout energy was observed. [31]

From the results of the bond strength and pullout energy test, the perfect silica fume concrete extent was seen to be in 20% and 30% under the given conditions of the test program. At this silica fume dose, the bond quality and the fiber pullout energy were the most elevated among all cases.

The authors *Liu et. al (2009)* attempted to discover the resistance of various types of concrete. after conducting several fire resistances tests on HPC, RPC and OC. The samples of different concrete were side by side put in a heavy oil burning furnace. A typical and most popular test named Fibber pull-out tests was received to test the bond between RPC matrix and steel fibres At this silica fume dose, the bond quality and the fiber pullout energy were the most elevated among all cases From the experiment results it was concluded and initially expected that remaining compressive strength of RPC specimens seteriorates showed increasee in fire timig in the furnace. In comparison to HPC and OC, they studied RPC show better results as it not only has a larger residual compressive strength but also more after subjecting to fire for the same duration.[32]

The authors *Yanzhou Peng et. al (2010)* investigated the effect of different pozzolans rich in silica. They used steel slag, silica fume and ultra-fine fly ash in different proportions with cement to obtain packing density of cementitious materials in binary, ternary and quaternary manner.

After obtaining the results they concluded that the incorporation of rich minerals like silica can improve the packing density of the binary mixture and also the packing effect as these admixtures fills up the voids of cement. They performed test under wet condition, both in cooperating superplasticizer and being of the cement mix being applied. They performed test under wet condition, both in cooperating superplasticizer and being of the cement mix being applied A further

increase in composite's packing density was obtained with mixture of different mineral admixtures because of greater packing effect.[28]

The authors *Kwan et. al (2009)* tried to compare the density of the mix obtained by dry mixing and wet mixing. For measuring the packing density, they discovered new method to assess the packing densities using different variations. packing density is fundamentally higher and more sensitive when compacted under dry condition than under wet condition .They performed test under wet condition, both in cooperating superplasticizer and being of the cement mix being applied. They achieved the expected results by this new method as packing densities was achieved having a very less absolute mean error which is 2.1% for blended fine aggregates and only 1.1% for the mortars.

The outcomes revealed that the fine aggregates packing density is fundamentally higher and more sensitive when compacted under dry condition than under wet condition. However, they did not find significant benefit because of addition of superplasticizer on the packing density. Hence, they observed that for measuring packing density wet packing density method is more reliable. wet packing method is more reliable for finding out the packing density of fine aggregates. A typical and most popular test named Fibber pull-out tests was received to test the bond between RPC matrix and steel fibres At this silica fume dose, the bond quality and the fiber pullout energy were the most elevated among all cases The dry packing method is inclined towards underestimating the packing density. The wet method of packing density additionally gauge the of mortar blends in with various proportions of fine aggregates. Results revealed that extent of fine aggregates in mortar affect the packing.

Bulk density $\frac{M}{V}$, the concentration of the mortar might be resolved as mentioned in (Eq. 1)

$$\phi = \frac{M\ /V}{q_{\mathrm{w}}\mathrm{u}_{\mathrm{w}} + q_a R_a + q_b R_b} \tag{1}$$

Where, $q_w$ is the water density , $q_a$ & $q_b$ are, respectively, the cementitious material density of material a and b, $q_s$ is fine aggregates density, $u_w$ is the ratio of $\frac{W}{S}$, and $R_a$, $R_b$ and $R_s$ are,

respectively, the volumetric ratios of fine aggregates and total solid content of materials a, b and c.

The authors *Jonkers et. al (2010)* tried to identify suitable bacteria for bacterial concrete. The main objective was to get bacteria, when incorporated in concrete for long time survive concrete incorporation for long time, but it can also become a better self-healing agent. As we know that concrete is highly alkaline in nature i.e. its pH value lies in between 11 and 13) the alkaliphilic bacteria is most suitable for such conditions. The concrete matrix is also toxic because of ingression of oxygen (matrix capillaries diffusion) so, that the bacteria must be oxygen resistant. Therefore, aerobic alkaliphilic spore forming bacteria is required for self-healing concrete. This type of bacteria occurred in genes Bacillus.

Few bacillus bacteria tested for compatibility. Two species, which were cultivated from soils samples alkaline in nature, *Bacillus cohnii* and *Bacillus pseudofirmus* were acquired from the German Collection of Microorganisms. Cells were cultured in liquid medium with 3g meat extract, 5g peptone, 0.42g $NaHCO_3$ and 0.53g $Na_2CO_3$ per litre distilled water and insoluble mineral enhanced with manganese for better sporulation. The pH of the solution was about to 10. To examine suitability of incorporated microorganisms test with and without joined microscopic organisms (mixture of bacterial cells and vegetative cells) are prepared.

OPC was used to prepare the samples. Water cement ($\frac{w}{c}$)ratio of 0.4 or 0.5 and bacterial specimens of 1-10 x 108 spore's cm-3 cement stone were used. Testing moulds of 4cm x 4 cm x 4 cm were used. On concrete incorporated with bacteria different viability tests has been done by Most Probable Number (MPN) technique. The bacteria spore's survival rate was controlled by estimating number number of practical microscopic organisms present in cement stone specimens at the age of 9, 22, 42 and 153 days. The MPN numbers were calculated by computer program. For this technique, microscopic organisms ought to be liberated from a concrete matrix and must be acquired a single cell suspension.

In this study, they crushed and pulverised the sample using high mechanical. It was obtained that according to age the number of bacteria spores decreased. It was found that the 9 days cured sample had 1.8 x 106 of the incorporated 2.4 x 108 spore's cm-3 concrete was obtained. The number of bacteria cells reduced with increase in age. The number of usablecells from 135 days cured sample were below the MPN detection limit (i.e., < 0.5 x 103 cm-3). The mineral producing capacity was

analysed by ESEM analysis (Philips XL30 Series ESEM). It was seen that in case of control sample cured for 7 & 28 days 1-5 μm sized particles on crack surface. While in case of bacteria treated concrete Copious amount of minerals having 20-80 μm were seen on the 7 days crack surface and not on 28 days cured sample. This is due to viability of bacteria cells decreases with age. It was observed that genus *Bacillus* which is alkali resisting spore forming bacteria showed very good results as self-healing agent. It was observed from this study that alkaliphilic Bacillus spore forming bacteria play important role in, precipitation by such bacteria is high for early age and it could be used as self-healing agent.[33]

The authors *Wang et. al (2013)* conducted a study. The primary objective of this investigation was to get self-healing concrete using microencapsulated bacterial spores. Microcapsules used to encapsulate the bacteria spores for SHC. This encapsulation technique used to increase the bacterial life in concrete. The viability of encapsulated bacteria was investigated. This was a great research by author, which helped to attain more life for incorporated bacteria. Bacteria spores were encapsulated in a size of 5 μm. The capsule contains inert substances to protect the spores of bacteria. The concentration of spores of bacteria in the microcapsule was about $10^9$ cells/g microcapsule (dry weight). These microcapsules are broken down under high tensile force (crack generates). These broken capsules liberate bacteria in the matrix of concrete and precipitation takes place. Series of tests were performed for different concentration.

Viability of the spores can be calculated by using the amount of decomposed urea. The spores in the capsules only germinate when the capsules were broken. Then they reached the nutrients. They need time to transform from dormant state to active state. It was found that spores remained viable after immobilization into the microcapsules. The micro capsulation does not affect the volume of the sample, but this process show decrease in tensile and compressive strength. In addition to higher than 3% microcapsules dosage there was a significant loss in tensile strength while in case of 1% to 5% microcapsules dosage there was a huge loss of about 15% to 34% in compressive strength was recorded by testing specimens.

In case of water absorption there was a lower water absorption in case of nutrients and microencapsulated bacteria. The distribution of pore size and porosity is obtained with the Mercury intrusion porosimetry (MIP) test. they observed that microencapsulated technique increased the viability of the bacterial spores, but it certainly decreases the mechanical properties of the concrete

like decrease in tensile and compressive strength. This type of technique could be useful in further scopes of bacterial concrete.[34]

The authors *Mian et. al (2015)* conducted a study with main objective of this study was to get proficiency of crack healing in concrete is dependent on bacterial carbonation precipitation. In this study, the author observed different aspects of crack healing by bacteria. The microstructural analysis was done by using SEM images and SEM observation of carbonation precipitation and by X-Ray diffraction (XRD). The compressive strength and water permeability along with visual inspection were also observed. The tests specimens and testing methods were used according to the Chinese standards. They observed slight rise in the compressive strength of concrete. Water permeability of bacterial concrete was relatively decreased by 84% and 96% for 7- and 28-days immersion in water, which was greater than that of control concrete.

The microstructural analysis by (SEM) Scanning Electron Microscope shown a complete precipitation and mineral formation, visually the healing capacity of bacteria was observed clearly. The crack with width of 0.48 mm was completely healed within 80 days. This study concludes, using biological healing agent helps in proper crack repairing and give eco-friendly concrete, which helps in green construction.[35]

The authors *Krishnapriya et. al. (2015)* conducted a study. The main objective of this study was identify and isolate of bacteria which can improves the concrete strength. It was India based research. The Bacteria culture (Bacillus megaterium) collected from MTCC located in Chandigarhm An alternate substrate for growth of bacteria wheat bran was used in this study for maintaining economical sporulation. OPC 53 grade was used according to India standards. Concrete grade of M25 was prepared according to the Indian standards.

The compressive strength was obtained over the specimen prepared. Its seen strength of bacteria incorporated concrete was greater than strength of control concrete specimen. The microstructural analysis was done using SEM micrographs using Jeol JSM – 6390 apparatus. The SEM analysis shown positive results towards precipitation of calcium carbonate ($CaCO_3$) by bacteria and visual inspection also shown crack healing capacity of bacteria. It was observed that the use of Bacillus genus spore forming bacteria as self-healing agent is possible and it could help in increasing life of structural concrete.[36]

The authors *Jonkers et. al. (2016)* attempted a study, recovery of water tightness (RWT) was analysed along with different tests performed against water ingression. The healing agent was used of Bacillus genus having alkaliphilic properties including other organic mineral compounds. Healing agent introduced in OPC (CEM I 42.5 N, ENCI, The Netherlands) along with normal weight aggregates and lightweight aggregates (LWA) were mixed to get desirable mortar mix. The efficiency of crack healing of bacterial-based healing agent was analyzed by using stereomicroscopic images. The test was performed, and it was observed that lightweight mortar having bacteria-based healing agent can show improved crack sealing than other samples. The better liquid tightness was also observed in this sample. It was observed that oxygen was only consumed by the bacteria-based healing agent samples. This study concludes that the lightweight mortar shows better liquid sealing along with better crack sealing. The lightweight aggregates could perform better role in self-healing bacterial concrete.[37]

The authors *Kim et. al. (2018)* isolated three strains for Bacillus genes (JH7, JH3 and HYO08), from two different samples of concrete. This study suggests that $_{CaCO3}$ crystals having differentt properties which can be produced by different calcium carbonate precipitation (CCP)-capable strains.

In their study they finally concluded that self-healing ability of concrete depends upon many factors, type of bacteria, availability of calcium carbonate, pH and others. Therefore, it is very important to cultivate and induce the bacteria as per the nature available and requirement

The capacity to existing in such a basic, high pH environment is significant for $CaCO_3$ precipitating microbes since solid itself speaks to such situations. In this manner, the proposed that three strains, two of Bacillus species JH3 an Jh3 and one Sporosarcina sp. HYO08, could be promising possibility for eco-accommodating mechanical applications. Each of three strains were brooded under similar conditions, the distinctive structure (state) of the subsequent accelerated precious stones could be because of contrasts in their intrinsic systems of use of calcium. Along these lines, strain likely have a ideal micro-environment to initiate CCP, for example, a particular pH level.

Development of the calcium carbonate precious stones required a very high grouping of calcium and carbonate resources.

In an environment plentiful with calcium, for example, cavern, soil, and limestone use and creation of carbonates through metabolic exercises, for example, hydrolysis driven by urease might become the foundation of precipitation of $CaCO_3$. Every one of the three strains showed urease movement droven naturally impacted and organically instigated CCP under ideal antacid ecological conditions

In their study they finally concluded that self-healing ability of concrete depends upon many factors, type of bacteria, availability of calcium carbonate, pH and others. Therefore, it is very important to cultivate and induce the bacteria as per the nature available and requirement.[38]

The authors *Varenyam Achal et. al. (2013)* studies the effect of inducing bacteria on the durability of concrete, in their study they used Bacillus. sp. CT-5 which was isolated from cement. Cubes of 70.6 mm is utilized in the study, according to IS 4031-1988. Cement and sand are altogether blended, When pores in the matrix get filled, it will scarce the availability of nutrients, and the bacteria may end up dying or endospore formation. They inferred that the compressive strength had significantly increased by almost about 36% for mortar solid shapes which contained microbial cells.

The enhancement by Bacillus sp. CT-5 in compressive strength is brought by testimony of CaCO3 on exposed. They likewise reasoned that in the underlying time frame microbial cells acquired great nourishment because the matrix was still porous. There might be another possibility because of high pH of cement the initial growth might be slow till the bacteria accumulate itself the environment.

The matrix with bacteria in it will produce a less porous structure and which will also be less porous. When pores in the matrix get filled, it will scarce the availability of nutrients, and the bacteria may end up dying or endospore formation. They also concluded that with different types of bacteria there will be different increase in strength. In Bacillus sp. CT-5 increase was 36 %, in Sporosarcina pasteurii the increase was 18% and in Shewanella sp. Increase was 25%.[22]

The authors *Ramchandran et. al (2001)* in their other study, studied the effect of using micro-biologically-induced mineral precipitation for concrete remediation. The concluded that microbial-stopping was influenced by the porosity of medium, the quantity of cells present, and the total volume of supplement included. They made Portland concrete mortar light emissions 25.4 x 25.4 x 152 mm. The examples were relieved in water 28 days and afterward left presented to air. The kept up the cut-width consistent at 3.175 mm while the profundity of slices ran from 3.175 mm to 9.525 mm. For each mix of break width, they threw a sum of 10 examples. The initial five examples were utilized as a standard without filling the breaks.

Different examples with splits were loaded up with blend od sand and B. pasteurii. The sand utilized went through the U.S. standard strainer No. 16 yet was held on the U.S standard strainer No. 4. The microbes suspension was blended in with sand to a last convergence of 3.8 x 109 cells for every cm3. The bars with and without microorganisms were tried for their solidness following 28 days utilizing a three-point stacking technique with a range of 127 mm. They made Portland concrete mortar Cube of measurements 50.8mm x 50.8mm x 50.8mm. The examples were restored in water 28 days and afterward left presented to air. The kept up the cut-width steady at 3.175 mm while the profundity of slices went from 3.175 mm to 9.525 mm.

For each blend of split width, they threw a sum of 10 examples. The initial five examples were utilized as a standard without filling the breaks. Different examples with breaks were loaded up with blend of sand and B. pasteurii. The sand utilized went through the U.S. standard sifter No. 16 yet was held on the U.S standard strainer No. 4. The microscopic organisms suspension was blended in with sand to a last convergence of $3.8 \times 10^9$ cells per $cm^3$. The bacteria B. pasteurii reduced the porosity by upto 50% and permeability up to 90%.[24]

**Summary of Literature Review**

Concrete is a heterogeneous material mainly composed of sand, cement, aggregates and water. These materials are subjected to various weathering action due to many physical, chemical and biological factors which deteriorates its strength and quality with time. Other factors like shrinkage, creep, freeze-thaw also makes concrete weak. Once cracks occur in concrete, they may grow wider leading to the exposure of reinforcement to external environment. So, there is always a need to identify cracks and repair them as soon as possible. There are many crack repairing

techniques available, but they are time-consuming and expensive. Self-healing of cement is characterized as the capacity of cement to fix its micro cracks automatically.

Biological self-healing technique needs the bacteria's in repairing the cracks in concrete. Bacteria's capable to precipitate calcium carbonate in presence of calcium nutrients are used in biological self-healing technique. The bacteria is added during the mixing of concrete. Once the crack occurs these bacteria's precipitate calcium carbonate and form a layer over the crack which seals the crack. There are large number of communities of bacteria which can precipitate calcium carbonate like Bacillus Bacillus Cohnii, Bacillus subtilis, Bacillus Pseudofirmus, Pseudomonas aeruginosa, Bacillus subtilis, Diaphorobacter nitroreducens etc.

It is also marked in a study that self-healing of concrete depends upon various factors like the type of bacteria, availability of calcium nutrient source, pH of concrete, temperature.. Around 36 % compressive strength increase was observed with Bacillus sp. CT-5, 25% with Shewanella sp. And 18% with Sporosarcina pasteurii. It is difficult to repair crack size more than 0.8mm with calcite precipitation . Once cracks occur in concrete, they may grow wider leading to the exposure of reinforcement to external environment. So, there is always a need to identify cracks and repair them as soon as possible. There are many crack repairing techniques available, but they are time-consuming and expensive.

When optimization of bacterial cells was done to find the optimum quantity of cells, researchers observed that $10^5$ cells/ml optimizes the microstructure. The compressive strength of bacterial concrete was found 10.63% more than the control concrete. The porosity was found to be 2.44% of bacterial concrete as compared 2.8% of control specimen. They concluded that when dead cells were added the strength came about 35 MPa in comparison to 30 MPa of control specimen.

**OBJECTIVES OF STUDY**

After an exhaustive literature review, we decided to make a concrete having strength 40MPa or above. To develop this concrete, we will partially replace cement with Metakaolin, Quartz powder for binary and ternary mixes. By adding the bacteria at $10^5$ cells/ml concentration in mixing water. Which will induce the self-healing properties in concrete.

# CHAPTER-3

# METHODOLOGY

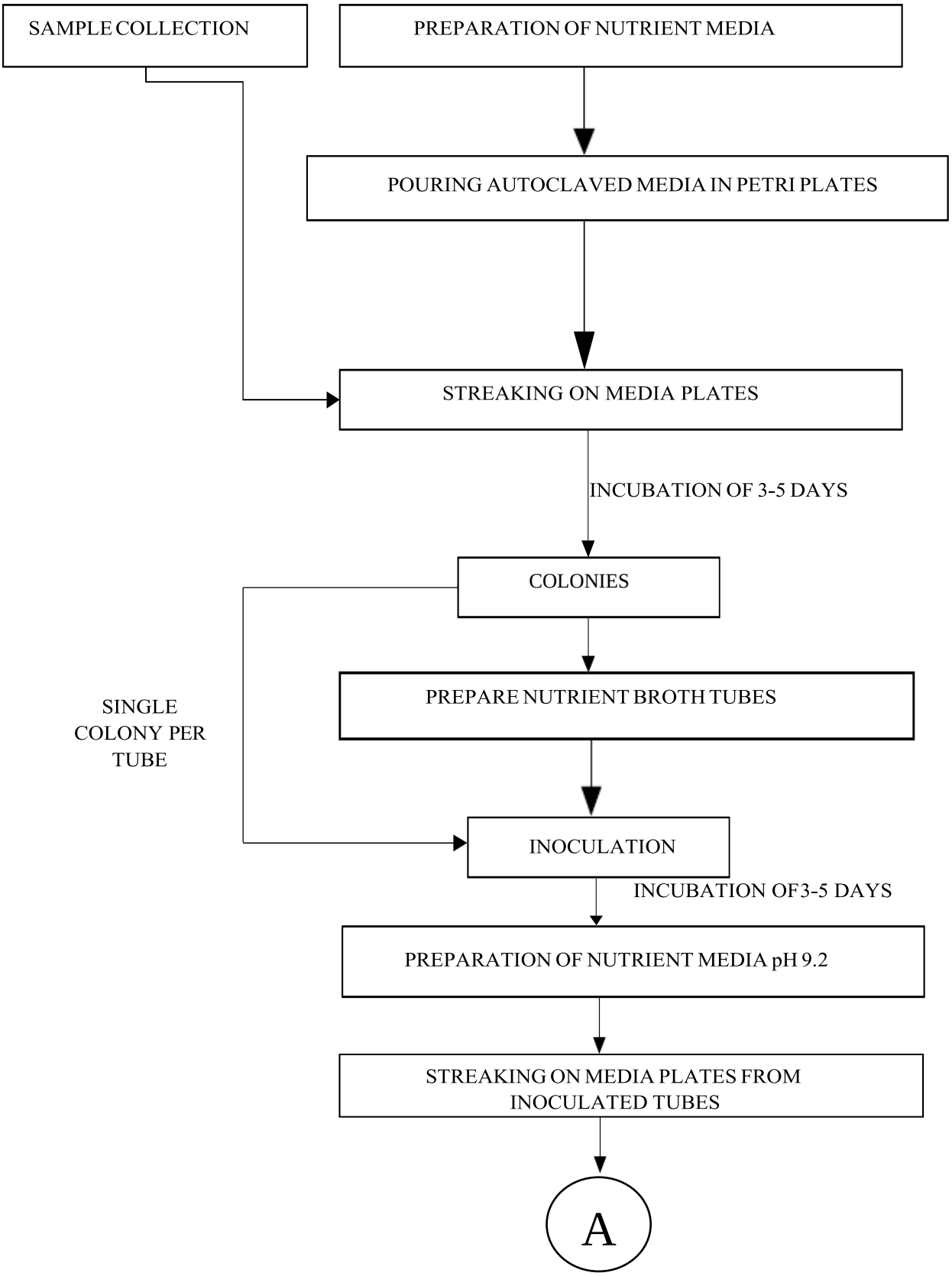
## 3.1 GENERAL

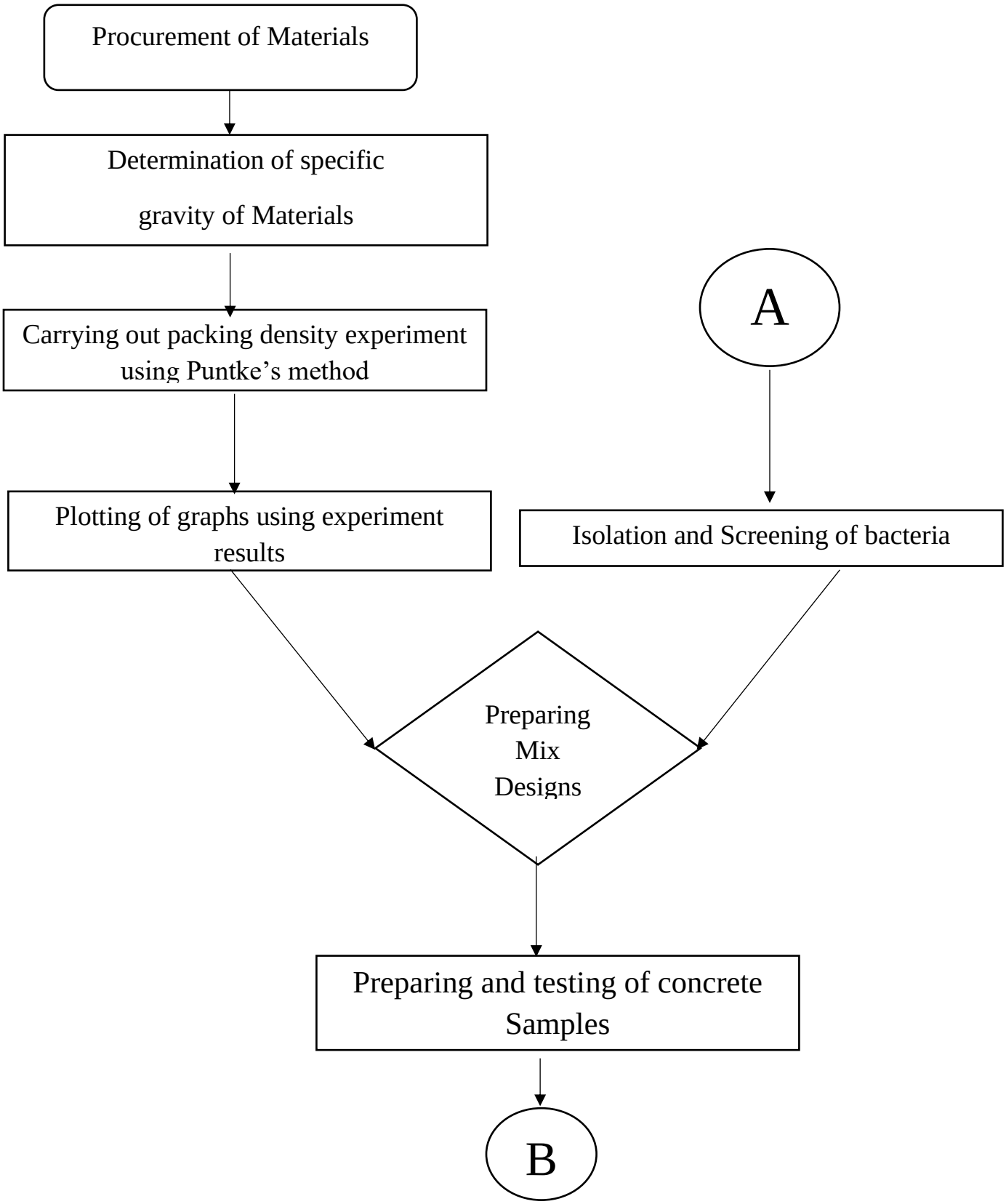
In order to complete the study, we need to go through various steps in a systematic manner. These steps include the procurement of raw materials and then determining their properties.

We need to carry out the Optimum packing density optimization of the materials in Binary, Tertiary, quaternary models in order to achieve Highest packing density. Side by side we will get the samples for Bacteria isolation and to all the necessary steps to isolate and screen Calcium precipitating bacteria for our Study. Once the samples are made, they will further be tested for their mechanical properties as well as water absorbing capacity, The voids within the Concrete Cubes. In order to get the idea of crack healing capacity of concrete we also need to do continuous Microscopic and SEM Analysis. The step by Step Flow methodology adopted for the study is given below.

## 3.2 Experimental programme

In the First stage we will isolate the Bacteria from the samples and side by side we will do the optimization process. Meanwhile, we will test the materials for their properties. In the second stage after isolation of bacteria, we will prepare a mix design. The mix of concrete will be based on the results obtained from Puntke Method. In stage 3 we will test the concrete specimens for compressive strength, Flexural Strength and Tensile Strength. Crack quantification will also be done in stage 3


SAMPLE COLLECTION
PREPARATION OF NUTRIENT MEDIA
POURING AUTOCLAVED MEDIA IN PETRI PLATES
STREAKING ON MEDIA PLATES
INCUBATION OF 3-5 DAYS
COLONIES
SINGLE COLONY PER TUBE
PREPARE NUTRIENT BROTH TUBES
INOCULATION
INCUBATION OF3-5 DAYS
PREPARATION OF NUTRIENT MEDIA pH 9.2
STREAKING ON MEDIA PLATES FROM INOCULATED TUBES
A


Procurement of Materials
Determination of specific gravity of Materials
Carrying out packing density experiment using Puntke's method
Plotting of graphs using experiment results
A
Isolation and Screening of bacteria
Preparing Mix Designs
Preparing and testing of concrete Samples
B

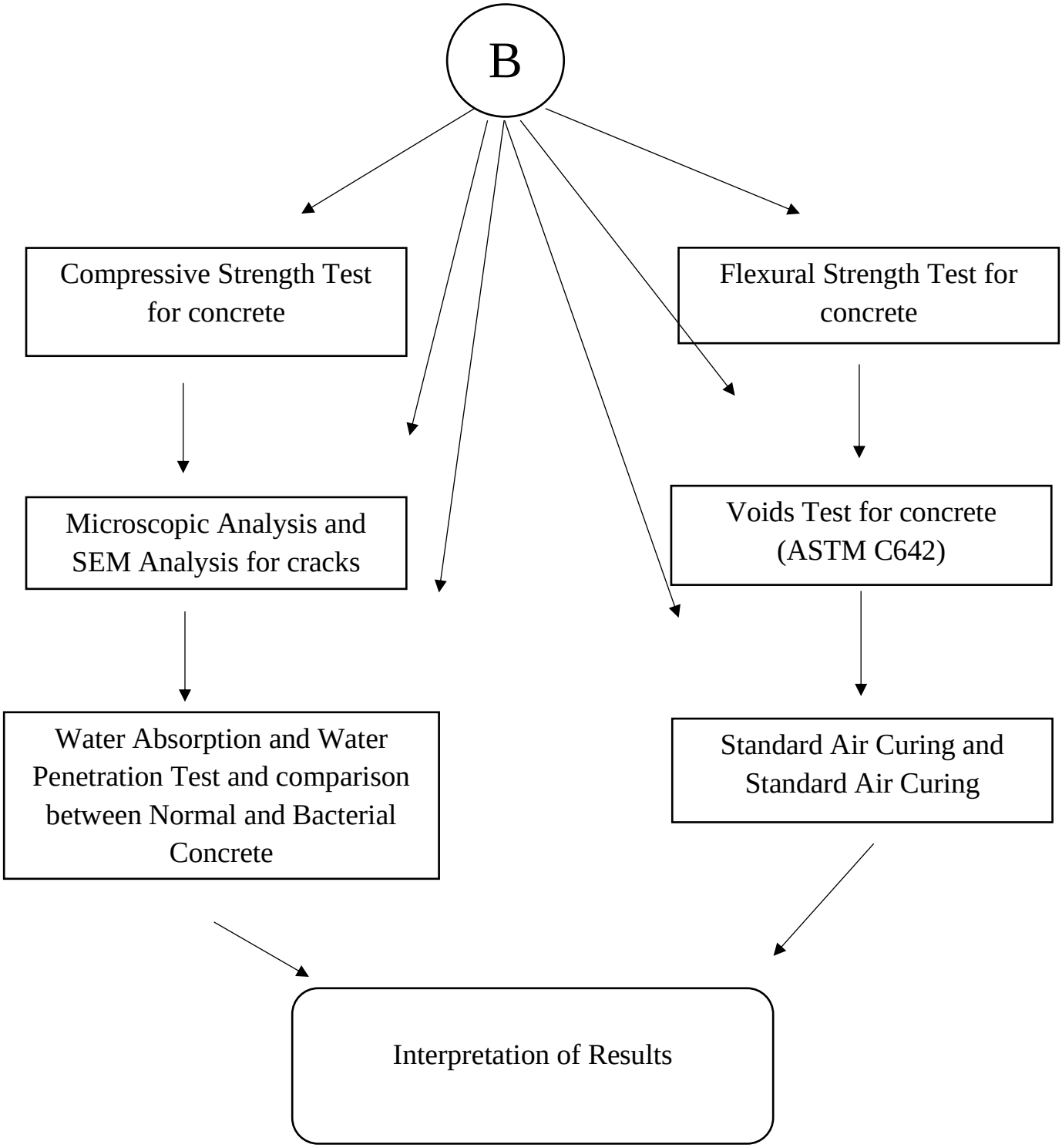



Figure 3.1 Step by Step representation of methodology adopted in a flowchart

**3.3 Puntke Method**

The fundamental guideline of the test is when water is added in dry materials, it will fill up the voids which are present in middle of the particles. It acts as an lubricating material and let concrete compact efficiently. Once the voids are filled, the water left in excess comes up the surface and form glittering surface on the top indicating the saturation limit.

Initially, mass of the total dry cementitious materials is required for optimization is placed in a beaker. If the optimization is to be done by using another admixture along with cement, the cement to admixture ratio must be fixed a. All dry materials are required to mix rigorousy to make a homogeneous dry mix before the addition of water to the. Refined water is included bit by bit mixing with stirrer till it gains a glossy structure after continuing tapping of the container. In the following stage, water included drop by drop, blending cautiously, till immersion point is reached. Now, surface mitigates itself after continued tapping the measuring beaker seems glossy.

Complete time taken for every trial is roughly 15 minutes. The investigation is repeated multiple times to get minimum water to achieve full saturation. Volume of water utilized, in packing density is dictated through utilizing Eq. 2.

$$Packing\ Density = 1 - \frac{(Vw)}{(Vp + Vw)} \tag{2}$$

where,

$V_w$ = Water volume ($cm^3$)

Vp = particle volume ($cm^3$)

Specific gravity ("Sp.G.") of a liquid tells us about the heaviness of the liquid with respect to water. Standard specific gravity of of wtaer is taken as 1.000 (at 4°C). In the event that a fluid is more thick than water, at that point its particular gravity is more noteworthy than 1. In the event that it is less thick than water, at that point positively the particular gravity under 1. To calculate the specific gravity of any liquid, it is necessary for us to know its density. After getting the density simply calculate specific gravity by taking the ratio of density of the liquid to the density of water (1 $gm/cm^3$). The Formula for Calculating Specific Gravity is given in (Eq. 3)

$$Specific\ Gravity = \frac{Desnity\ of\ liquid}{1\ gm/cm3} \tag{3}$$

## 3.4 Experimental Work

### 3.4.1 Determination of material specific gravity

**Table 3.1** Material required for Specific Gravity test

| | |
|---|---|
| Le-Chatelier Flask | 250ml, the neck graduated 0-1ml and 15-24ml |
| Dispersing medium | Kerosene |
| Wash bottle | Plastic having250 ml capacity |
| Spatula | 150 mm blade length |
| Funnel | Glass, narrow mouth |
| Thermometer | Glass having range 0-50°C |
| Pipette | Glass having 10ml capacity |

The specific gravity of materials described as proportion of the mass of a volume of solids to water. Specific Gravity relies on the chemical composition of the material. The distinction between the initial and final value speaks about the volume of liquid displaced by the mass of concrete utilized in the test. The density is determined according to the beneath referenced equation to the second spot of decimal as explained in (Eq. 4).

$$Density = \frac{mass\ of\ cement, g}{Displaced\ volume, cm^3} \quad (4)$$

**Procedure :** Firstly, fill up the flask with kerosene up to the mark below the bulb. Now, Take 55-65 grams of the material. The material is gradually poured into the flask through funnel as the kerosene rise the lowest point of graduation, material is cautiously poured. As the first gradation if achieved, stop the material to be poured. Note down the volume of the material. Calculate the weight of material used

## 3.5 Packing density optimization using Puntke method

Plactic container of 500ml capacity with uniform diameter and flat bottom surface is required. A metal spatula for mixing, wash bottle. For measurement of weight we need a

weighing scale with an accuracy of 0.1 grams. We require cement and the mineral admixture which is to be partially replaced. In our study we took the admixture/cement ratio was taken from 2.5% to 30%.

**Procedure:**

Initially, dry empty weight of the container is calculated using weighing machine. Then cement admixture is weighed on the weighing machine and added to the plastic container. Dry mixing of cement and admixture is done using metal spatula. Once the homogeneous dry mix is obtaines , the water is added drop by drop into the mixture it is well mixed by the spoon keeping in mind that no lumps are formed. When the mix appears to have sufficient water content, start tamping the container to check whether the glossy surface is appearing or not. After the appearance of the glittering surface, weigh the container again (say $W_m$).

$$Wt.of\ water = Wm - (wt.of\ empty\ container + wt.of\ cement + wt.of\ admixture$$

After the determination of water content, the packing density is calculated using the formula given by Puntke which is described in (Eq. 3)

$$Packing\ Density = 1 - \frac{(Vw)}{(Vp + Vw)} \tag{3}$$

The results are plotted in graphical form and the optimum proportion of mineral admixture w.r.t. cement is obtained.

## 3.5 Isolation and confirmation of bacteria:

### 3.5.1 Materials and Chemicals required

The required chemicals and materials for isolation of bacillus (mainly Calcite precipitating bacteria) isolation of bacteria along with material required for optimizing mortar samples and casting them are discussed on this section. Table 3.2 summarizes the culture media for the microbes of interest.

**Table 3.2** Chemical required for isolation of bacteria

| Sr. No. | Material & Chemical Name | Purpose |
|---|---|---|
| 1. | Urea ($CH_4N_2O$) | Nutrient Media |
| 2. | Sodium Bicarbonate ($NaHCO_3$) | Nutrient Media |
| 3. | Ammonia Chloride ($NH_4Cl$) | Nutrient Media |
| 4. | Nutrient Broth | Nutrient Media |
| 5. | Calcium Chloride two Hydrate ($CaCl_2.2H_2O$) | Nutrient Media |
| 6. | Agar ($C_{14}H_{24}O_9$) | Solidification of Nutrient Media |
| 7. | Soil and water sample from different locations | Isolation of bacteria |

Table 3.2 gives the list of materials and chemicals required for the nutrient media composition. The purpose of each material mentioned along with the material. These materials used to grow the alkaliphilic bacteria

Table 3.3 consists of various samples taken for the isolation of bacteria(alkaliphilic). This type of bacteria is mostly found and can be isolated mostly from the alkaline soils, sewage and from water. For this, a total of twelve samples comprising six soil (alkaline in nature and rich in Iron oxide and lime) samples and six sewage samples were composed from the different locations of district Bilaspur & Solan (H.P).

**Table 3.3** Samples collected till date for the isolation of bacteria.

| S. No. | Sample Type | Location |
|---|---|---|
| 1. | Soil Sample 1 | Ambuja Cement Factory Dadlaghat, Solan (H.P.) |
| 2. | Soil Sample 2 | ACC Cement Factory Barmana, Bilaspur (H.P.) |
| 3. | Soil Sample 3 | Jhamakhdi Pul, Solan (H.P.) |
| 4. | Soil Sample 4 | Ghumarwin, Bilaspur (H.P.) |
| 5. | Soil Sample 6 | Luhnu Ground, Bilaspur (H.P.) |
| 6. | Soil Sample 6 | Brahampukhar, Bilaspur (H.P.) |
| 7. | Bore well Water 1 | Ambuja Cement Factory Dadlaghat, Solan (H.P.) |
| 8. | Bore well Water 2 | ACC Cement Factory Barmana, Bilaspur (H.P.) |
| 9. | River Water Sample | Jhamakhdi Pul, Solan (H.P.) |

| | | |
|---|---|---|
| 10. | Bore well Water 3 | Ghumarwin, Bilaspur (H.P.) |
| 11. | Lake Water Sample | Gobind Sagar Lake, Bilaspur (H.P.) |
| 12. | Bore well Water 4 | Brahampukhar, Bilaspur (H.P.) |

The list of materials required for testing of cement, mortar and concrete is given in Table 3.4

**Table 3.4** Material required for cement, mortar and concrete testing.

| S. No. | Material |
|---|---|
| 1. | Cement (PPC-Fly Ash Based) for plastering |
| 2. | Cement OPC (43/53 Grade) |
| 3. | Kerosene or Benzene |
| 4. | Bricks For making Wall. |
| 5. | River Sand for Plastering |
| 6. | Metakoilin |
| 7. | Fine Aggregates |
| 8. | Coarse aggregates. |

The chemicals required for checking urease activity of bacteria are listed in Table 3.5

**Table 3.5** Chemicals used for checking urease activity tests

| S. No. | Material |
|---|---|
| 1. | Phenol Red ($C_{19}H_{14}O_5S$) |
| 2. | Urea ($CH_4N_2O$) |
| 3. | Deionised Water |

### 3.5.2 Equipment required for isolation and growth of bacteria

For isolation and growth of bacteria the required equipment along with their purpose are enlisted in Table 3.6 and Table 3.7

**Table 3.6** Equipment used for the isolation and growth of bacteria

| S. No. | Equipment | Purpose |
|---|---|---|
| 1. | Digital Weighing Balance (1-220 gm) | Weighing Materials |

| | | |
|---|---|---|
| 2. | Inoculating Loop | For Inoculating Bacteria |
| 3. | Laminar Airflow | Provides Sterilised Environment |
| 4. | Autoclave | To Sterilised Media & Glass Plates |
| 5. | BOD Incubator (@ 37°C) | For Growth of Bacteria |
| 6. | Freezer (@ 4°C) | Prevent culture against over growth and contamination |
| 7. | pH Meter | To measure pH |
| 8. | Conductivity Meter | To measure bacterial activities |
| 9. | Microwave Oven | For melting Agar |

**Table 3.7** Glassware required for the preparation of bacteria culture.

| **S. No.** | **Equipment** | **Purpose** |
|---|---|---|
| 1. | Petri Dish (90 ∗ 15 mm) | Culturing Bacteria |
| 2. | Conical Flask | Mixing Media |
| 3. | Test Tube | For making Slants & Growing Bacteria |
| 4. | Measuring Cylinder | Measuring Media & Distilled Water |
| 5. | Centrifuge Tubes (2 ml, 15 ml and 50 ml) | For Centrifuge Cells. |

For material testing, the required equipment's along with their purpose are enlisted in Table 3.8

**Table 3.8** Equipment and apparatus that are required for testing of Cement, concrete and Mortar samples

| S. No. | Equipment | Purpose |
|---|---|---|
| 1. | Vicat Apparatus "IS:4031(4)-1988" & "IS:4031(5)-1988" | Consistency, Initial and Final Setting Time of Cement |
| 2. | 90 micron Sieve "IS:4031(1)-1996" | Fineness of Cement |
| 3. | Le-Chatelier Mould "IS:4031(3)-1988" | Soundness of Cement |
| 4. | Le-Chatelier Flask "IS:4031(11)-1988" | Specific Gravity of Cement |
| 5. | Pycnometer Bottle | Specific Gravity of Sand |
| 6. | Digital weighing Machine | For weighing Samples |

## 3.6 Methodology adopted to obtain calcite precipitating bacteria

The methodology of culturing bacteria explained stepwise step in this section. The first stage is to isolate the bacteria of purpose and consists of preparing nutrient media get a standard bacterial species Bacillus megaterium species for the growth of bacteria to getting pure colonies. Different tests that will be performed for selecting the best isolates this whole process termed as the screening of the bacteria. We will get a standard bacterial species Bacillus megaterium species for performing MIC. All the screenings results will be compared with this culture. The steps of isolation and screening procedure explained below:

**Step 1**

**Sample Collection** A total of twelve samples comprising six soil (alkaline in nature and rich in Iron oxide and lime) samples and six water samples were collected from the different locations of district Bilaspur & Solan (H.P). The soil samples will be diluted in the distilled water for the purpose of getting isolates. These details of samples are mentioned with their location in Table 2

**Step 2**

**Preparation of Nutrient Media** The chemicals used in the preparation of urea broth already discussed in Table 3.10 200 ml of urea broth will be required to be prepared and autoclaved. The composition of media chemicals is given below. Agar powder will be used to solidify the media to prepare urea broth plates. Media will be prepared in the conical flask and tightly plugged with

the help of cotton plugs. We will do the sterilization with the help of Autoclave. The nutrient media (Urea Agar and broth) prepared for bacterial culture given in Table 3.9. The photo of nutrient broth is given in Figure 3.1.

**Table 3.9** Composition of nutrient media

| SR. NO. | CHEMICAL | COMPOSITION *(gm/l) |
|---|---|---|
| 1. | Urea | 20 |
| 2. | Sodium Bicarbonate | 2.12 |
| 3. | Ammonia Chloride | 10 |
| 4. | Nutrient Broth | 3 |
| 5. | Calcium Chloride two hydrate | 25 |
| 6. | Agar | 17 |

* The composition here shown is for per litre of nutrient media

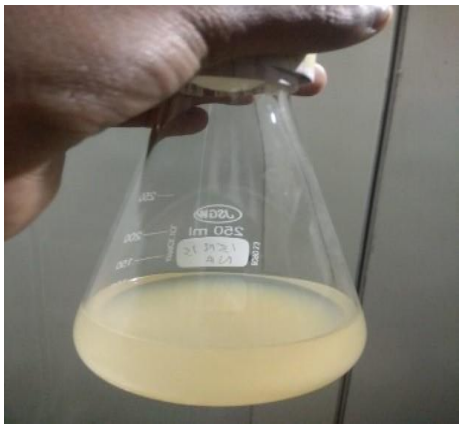


Figure 3.2 Nutrient Broth

**Step 3**

**Autoclave:** An autoclave is used for the sterilization of media, glass wares and plastic wares (tools and materials). Autoclaving effectively destroys potential viral or bacterial contamination through exposure to extreme heat. It works under the principle of steam under pressure.

**Step 4**

**Pouring** We will pour the autoclaved media in the Petri plates inside the Laminar Airflow and incubated at 37°C.

**Step 5**

**Streaking** We will then dilute Soil and sewage samples in distilled water and then streaked. Figure 3.2 shows streaking done on Petri-dish with isolated colonies.

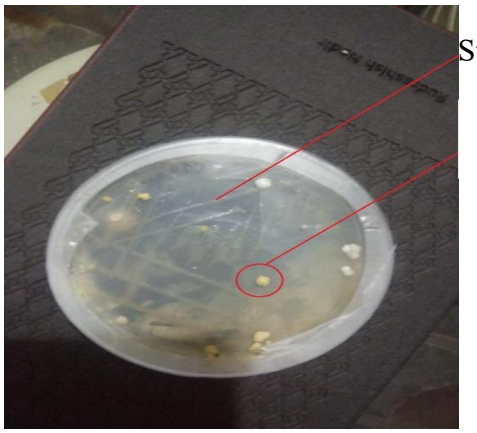



Figure 3.3 Petri Dish with streaking and isolated colonies

**Step 6**

**Incubation** Streaked plates will be incubated at 37°C for 3 to 4 days.

After that we will get the Appearance of Colonies on nutrient media (precipitated and non-precipitated)

**Step 7**

**Inoculation** We will take the Single colony developed from the petri plate and then inoculate it in the nutrient broth tubes. Tubes will be incubated at 37°C for 2-3 days

**Step 8**

**Streaking** We will do Streaking on the plates having media of pH 9.2 from the tubes and then again incubate it at 37°C for 3-4 days. Figure 3.4 shows bacterial growth in culture media in a petri-dish

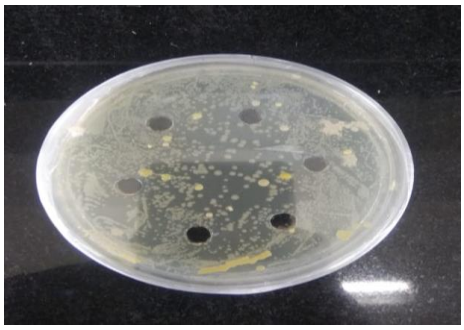


Figure 3.4 Petri-dish with bacterial growth

## 3.7 Tests for urease activities

Test required to check the urease activities are these:

1) Qualitative Test (Phenol Red Test).
2) Quantitative Test (Electrical Conductivity Test)

The methodology of these tests is explained below.

### 3.7.1 Qualitative Test (Phenol Red Test)

In this, we can measure the activity rate of bacteria precipitation and urease assay. Using phenol red as the indicator, which turned to yellow color if such activities takes place. First, the media will be prepared by adding indicator phenol red in it then the media composition and microwaved for the melting of agar powder. After microwaving, the media is poured in test tubes. These test tubes then autoclaved. After autoclaving these tubes are placed in slanted positions so that media solidifies in slanted position. This procedure is known as slants making. The colour of these slants must be pink. Further, the slants are streaked using isolated bacterial strains. After streaking the test tubes were incubated for 3-4 days to get results. The slants which turned to yellow colour are urease positive (+) and those remained pink are urease negative (-).

### 3.7.2 Quantitative Test (Electrical Conductivity Test)

For getting measurable, extant of isolated bacterial species activities, the electrical conductivity test is performed. A solution of 1M Urea along with deionised water is prepared. 2 ml of culture inoculating to this solution by adjusting the culture OD (Optical Density) up-to 1.The conductivity is measured by using conductivity meter. The conductivity meter is first calibrated with distilled water. Distilled water was used as the cell constant. After calibration the media of urea is checked for its conductivity. By using this method, the rate of activity can be measured.

Its unit is Mhos/cm.

### 3.7.3 $CaCO_3$ Precipitation test

This test is performed to check the efficiency of isolates towards calcite precipitation. In order to perform this test we need to make two solution of nutrient broth and calcium chloride two hydrates. The solution is prepared in the centrifuged tubes of 15 ml. The incubation of these tubes after inoculating isolated bacterial strains was done for 4 days at 37°C. The centrifuged tubes then centrifuged @ 7000 rpm for 10 – 15 minutes to get the cell pellet. The supernatant was discarded, and the wet and dry pellet weight was compared. The isolates that shown greater results then further selected for other screening processes

### 3.7.4 FT-IR Spectroscopy

FT-IR (Fourier Transform Infrared Spectroscopy) in order to get the growth rate and getting the wavelength at which bacteria is stabilized. A dry sample of $CaCO_3$ precipitated pellet is used for the performance of this test. The results obtained in the form of graphical peaks compared to the standard wavelengths of the different compounds groups and the confirmation of the compound group was done.

### 3.7.5 Gram Straining

The cells were stressed on the minute glass slide with precious stone violet color and the gram iodine arrangement was added to shape a complex between gem violet and iodine. A decolorizer, e.g.$CH_3CO_2$ and ethyl liquor was added in sample, which can dry out the layer of peptidoglycan. A counter strain of feeble water-solvent safranin was included. Since, safranin is lighter than gem violet color; in gram positive cells it doesn't intrude the purple tinge.

### 3.7.6 Growth Curve

This experiment is required to perform in order to obtain the growth parameters of the bacteria. The selected isolates along with standard culture will be grown at the same time with same inoculum. The OD (Optical Density) of the sample cells will be checked by Spectrophotometer The graphs will be obtained by getting the results. These results will help us to form a mathematical model of the growth rate of the bacteria.

### 3.7.5 Endospore Straining

This test is required to perform in order to obtain the results of isolated bacteria, whether they form spores or not. The spore forming bacteria are generally Bacillus species. Spores are resistant to heat, desiccation, chemicals and radiations. This strategy chips away at Schaeffer-Fulton technique, which used to recognize vegetative cells and the endospores. An essential strain "malachite green" is utilized to strain the endospores. The malachite green penetrates the spore divider by warming. In this strategy warming goes about as a stringent. There is no need of utilizing decolorizer in this spore stressing. Just water is sufficient for the decolorizer. Vegetative cells have been disturbed by heat. As the endospores are impervious to stressing, the endospores are similarly impervious to de-stressing and will hold the essential color while vegetative cells will lose the strain. Safranin was utilized as the counter strain. The vegetative cells ought to seem pink/red in shading. The vegetative cells that contains endospores should strain pink while the spores ought to be green circles inside the cells. While free endospores ought not be related with the vegetative microorganisms and ought to be green circles.

## 3.8 Testing of Concrete Samples

Test for compressive strength long with split tensile strength was conducted in this part of study. Test was conducted along with split tensile test of all samples whether they were heaving bacteria or lacking the bacterial cells, whether having bacterial cells or not. In this study Compression testing machine (CTM) was utilized for conducting the tests. By conducting the tests properties of both bacterial and nonbacterial concrete was investigated.

### 3.8.1 Consistency test

IS 4031 (Part 4): 1988. was the standard used for conducting this test. In a concrete paste, when Vicat’s plunger (Diameter and length is 10 mm and 50mm respectively and 50mm length) penetrates through 5-mm in depth in fresh concrete is known as standard consistency test. Standard consistency test is valuable for deciding the content of water for different tests, for example, IST and FST etc.

### 3.8.2 Initial Setting Time and Final Setting Time Test

IS 4031 (PART 5): 1988. was the standard used for conducting this test. from the second water is added to the cement till the time cement begin to lose its plasticity and FST is the time

upto when plunger can't penetrate the paste. Water should be added at 0.85P to the cement weight weight of cement..

### 3.8.4 Soundness test

IS 4031 (Part 3): 1988. was the standard used for conducting this test The capacity of cement to oppose volume change is known as soundness test. Constituents like unburnt lime, calcium sulphate, magnesia make the concrete unsound unsoundness happens in concrete. Water in this test should be 0.78P where p is consistency of cement.

## 3.9 Test on aggregates

### 3.9.1 Specific gravity test

IS 2720 (Part 3): 1980 . was the standard used for conducting this test. Pycnometer bottle was used for conducting the test.

### 3.9.2 Fineness modulus test

Fine aggregate's fineness modulus is determined after conducting sieve analysis. Fineness modulus test to know the gradation of zone of sand. It is a factor gotten by including the combined rates of aggregates held by every sieve varying from size 10 mm to 150 µ,

## 3.10 Preparation of concrete specimens

Concrete Samples of 3 different categories were prepared, as portrayed in Table 3.10

**Table 3.10** Types of concrete specimens prepared

| S. No. | Specimen | Description |
|---|---|---|
| 1 | Control Concrete | Without bacterial cells |
| 2 | Standard Concrete | Incorporated with standard culture (*Bacillus megaterium*) |
| 3 | I2 Concrete | Incorporated with isolate 3 bacterial cells |

In concrete the bacterial cells re incorporated at 105cells/ml concentration. The cells were mixed in concrete along with mixing water. In this study water cement ratio was kept 0.24. To determine the cell concentration OD of 0.7 to 1.0 was utilized. OPC (Grade 43) and water to cement ratio

0.24 was kept making concrete samples. This design mix was set up according to IS 10262: 2019 and the following calculations were made:

**1) Target Strength:**

Target Compressive Strength (Mean) = 60 + 1.65 (5) = 68.25 MPa

**2) Selection of Cementitious Content:**

According to trial mixes done by Puntke method maximum packing density achieved was at 70% cement, 20% fly ash and 10% silica fume.

First trials were made with following contents

Cement = 400 kg/$m^3$

Fly Ash = 80 kg/$m^3$

Silica Fume = 40 kg/$m^3$

Total Cementitious Content = 520 kg/$m^3$

**3) Selection of water-cement ratio:**

For making HSC, w/c ratio is needed to be kept low we need to have a very low w/cm ratio

Here water is restricted to 0.24

Therefore, Water content = 0.24x520 = 124.8 = 125 kg/$m^3$ (approx.)

**4) Dosage of HRWRA**

Dosage of HRWRA was fixed at 1% by weight of total cementitious content.

Content of HRWRA = 0.01 x 520 = 5.2 kg/$m^3$

**5) Water correction due to water present in HRWRA:**

As per the specification given by supplier, Solid content of HRWRA was 42 % and water present in it eas 56%

Water contributed by HRWRA = 0.56 x 5.2 = 2.9 kg (approx.)

Corrected Water content = 125-2.9 = 122.1 = 122 kg/$m^3$ (approx.)

**6) Proportion of Volume of C.A and F.A Content:**

From Table 3 of IS 10262:2019, Volume of C.A corresponding to 20 mm size and F.A (Zone 1) =0.60

**7) Estimation of Concrete mix calculations:**

a) Concrete volume = $1m^3$

b) Cement volume = $\frac{Mass\ of\ Cement}{Specific\ Gravity\ of\ Cement}$ x $\frac{1}{1000} = \frac{400}{3.15}$ x $\frac{1}{1000}$ = 0.127 $m^3$

c) Volume of Fly Ash = $\frac{Mass\ of\ Fly\ Ash}{Specific\ Gravity\ of\ Fly\ Ash}$ x $\frac{1}{1000} = \frac{80}{2.2}$ x $\frac{1}{1000}$ = 0.036 $m^3$

d) ) Volume of Silica Fume = $\frac{Mass\ of\ Silica\ Fume}{Specific\ Gravity\ of\ Silica\ Fume}$ x $\frac{1}{1000} = \frac{40}{2.2}$ x $\frac{1}{1000}$ = 0.018 $m^3$

e) Volume of HRWRA = $\frac{Mass\ of\ HRWRA}{Specific\ Gravity\ of\ HRWRA}$ x $\frac{1}{1000} = \frac{5.2}{1.1}$ x $\frac{1}{1000}$ = 0.005$m^3$

f) Volume of water = $\frac{Mass\ of\ water}{Specific\ Gravity\ of\ water}$ x $\frac{1}{1000} = \frac{122}{1.0}$ x $\frac{1}{1000}$ = 0.122$m^3$

g) Total aggregates volume = 1-(b+c+d+e) = 1- (0.036+0.127+0.005+0.018+0.122) = 0.692 $kg/m^3$

h) Coarse aggregate mass = Total aggregate volume x Coarse aggregate specific gravity x 1000 = 0.692 x 0.60x 2.8 x 1000 = 1162.56 = 1163 $kg/m^3$ (approx.)

i) Fine aggregate mass = Total aggregate volume x Fly ash Specific gravity x 1000 = 0.692 x 0.40x 2.8 x 1000 = 1162.56 = 775 $kg/m^3$ (approx.)

**8) Final Concrete Mix Proportions:**

Cement = 400 $kg/m^3$

Fly Ash = 80 $kg/m^3$

Silica Fume = 40 $kg/m^3$

HRWRA = 5.2 $kg/m^3$

Water = 122 $kg/m^3$

Fina Aggregate = 775 kg/$m^3$

Coarse Aggregate = 1163 kg/$m^3$

Water-Cementitious Ratio = 0.24

## 3.11 Testing of concrete Samples

Test for compressive strength long with split tensile strength was conducted in this part of study. Test was conducted along with split tensile test of all samples whether they were heaving bacteria or lacking the bacterial cells, whether having bacterial cells or not. In this study Compression testing machine (CTM) was utilized for conducting the tests. By conducting the tests properties of both bacterial and nonbacterial concrete was investigated.

### 3.11.1 Compression test

IS 516: 1959. was the standard used to conduct this test. The capacity of materials to withstand against load applied on its surface without getting splitted or cracked is known as the compressive strength of concrete. Compressive strength is the capacity of material to bear the loads on its surface with no split or deflection. This test was performed by making concrete cubes having each side of 150 mm. Cubes were kept for curing. The compression test is conducted t a interval of 7 days, 14 days and 28 days. The load is applied at a steady rate of 140 kg/$cm^2$/minute (0.22 MPa/s) till the failure occurs. Compressive test was performed on the concrete cubes as shown in Figure 3.4

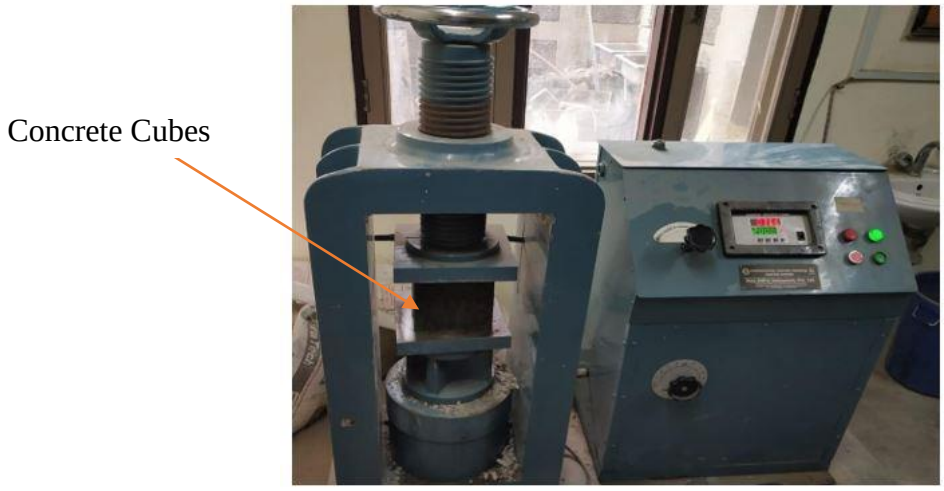



Figure 3.5 Compressive strength test under CTM

The formula for calculating compressive strength is as given in (Eq. 4):

$$\text{Compressive Strength (N/mm}^2\text{)} = \frac{P}{A} \quad (4)$$

Here, P = Load at Failure (N)

A = Specimen Area ($mm^2$)

### 3.11.2 Split tensile strength test

IS 5816: 1999 was the standard used for conducting this test. Due to brittle nature of concrete it becomes extremely weak in tension compared to compressive strength. Hence, it is basic to decide the concrete tensile strength to at which it my break. CTM is used for performing this test and concrete cylinders (diameter and length is 100mm and 200mm respectively). The load is applied at a consistent rate of 1.2 to 2.4 MPa/min. The split tensile test is conducted on concrete cylinders as shown in at Figure 3.5

Figure 3.6 Split tensile strength test under CTM

For calculating Split tensile strength following equation is used (Eq. 5):

Split Tensile Strength Test ($N/mm^2$) = $\frac{2P}{\Pi L D}$ (5)

Here, P = Load at failure (N)

L = Length of cylinder (mm)

D = Diameter of cylinder (mm)

## 3.12 Development of Crack healing

In this stage concrete cracks occurred are investigated on both the samples with or without bacteria. For inducing the cracks in the concrete cubes copper plates of thickness 0.3 mm to 0.5 mm were put in cubes up to depth of 5-10 mm. After 24 hours these plates were taken off., after demolding. A comparative technique for inducing cracks was used by the investigators. For further evaluating the process crack healing, a visual review of samples, at regular intervals is done. Figure 3.7 shows the cracks prompted in a new concrete block example.

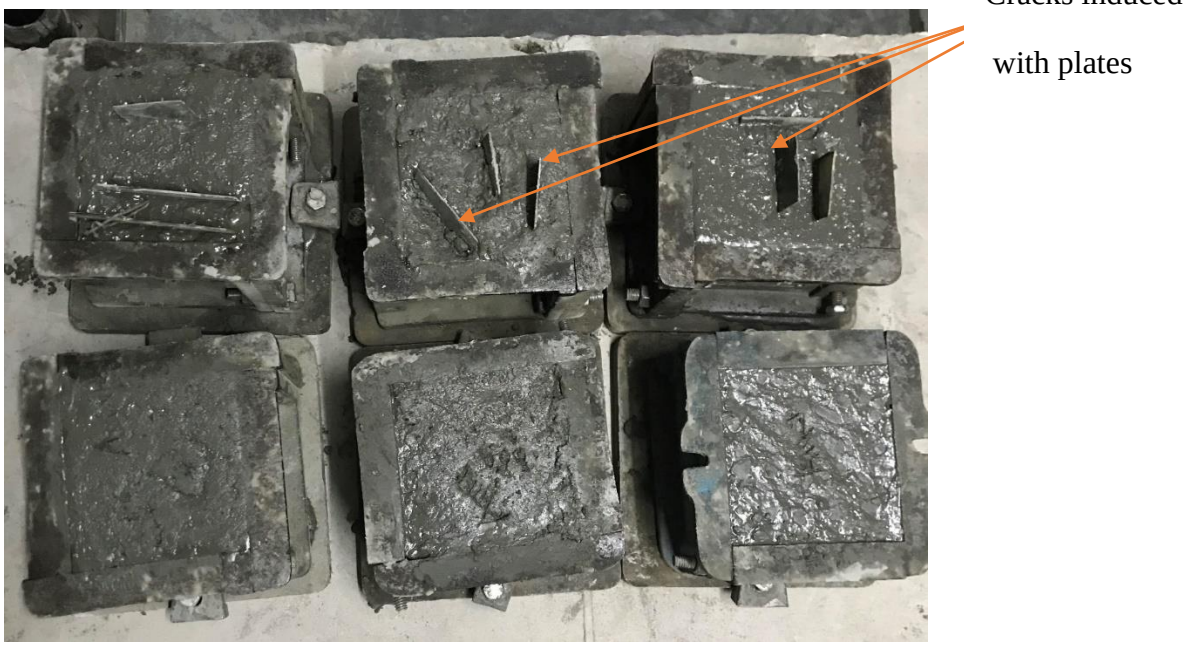



Figure 3.7 Concrete cube specimens with cracks induced (top) and without cracks (bottom)

# CHAPTER-4

# RESULTS

## 4.1 GENERAL

In this Chapter results of all the experimental work conducted till date are given in respective tables. In this discussion of the results obtained after conducting various tests is donechapter all the results obtained from various tests are discussed

## 4.2 Results obtained during identification and isolating bacteria with calcite precipitating properties of calcite precipitating bacteria

The data of the size of colonies obtained, and time for obtaining those colonies are given in Table 4.1

**Table 4.1** Time taken for colonies appearance along with their size

| Sample No. | Colonies Size | Time taken to obtain colonies |
|---|---|---|
| 1 | Small | 3 days |
| 2 | Medium | 4 days |
| 3 | Small | 4 days |
| 4 | Very Small | 4 days |
| 5 | Medium – Large | 4 days |
| 6 | Very Small | 5 days |
| 7 | Small | 3 days |
| 8 | Small | 5 days |
| 9 | No colony | - |
| 10 | Medium | 5 days |

From these outcomes, sub culturing was done of some selected isolates with purpose to get the

best bacterial isolate of all..11 particular isolates of bacteria, including the isolate obtained from MTCC located in Chandigarh were acquired on petri dishes subsequent to developing urea stock cylinders. The morphology of chosen isolates is shown in Table 4.2

**Table 4.2** Morphology of Selected Isolates.

| Isolate No. | Sample No. | Colony Size | Shape | Colour |
|---|---|---|---|---|
| 1 | 1 | Small | Round | Greyish white |
| 2 | 1 | Small | Perfectly Round | Greyish white |
| 3 | 1 | Small | Round | White |
| 4 | 1 | Very Small | Round | White |
| 5 | 1 | Small | Round | Pale White |
| 6 | 1 | Small | Irregular Round | Pale White |
| 7 | 1 | Small | Round | White |
| 8 | 1 | Small | Round | Pale White |
| 9 | 1 | Very Small | Perfectly Round | Greyish White |
| 10 | 1 | Very Small | Round | White turned safranin |
| 11 | 1 | Small | Irregular Round | White turned green |

### 4.2.1 Calcite Precipitation Results

From the test it was concluded that the isolated 1, 3, 4, 5, 6, are heavy calcite precipitating bacteria. Table 4.4 shows the difference in the weight isolates on dry filter paper having calcite and empty filter paper.

**Table 4.3** Calcite Precipitation Results

| Isolate No. | Weight of Calcite Precipitated (mg) | Isolate No. | Weight of Calcite Precipitated (mg) |
|---|---|---|---|
| 1 | 79.6 | 7 | 45.1 |
| 2 | 68.9 | 8 | 39 |
| 3 | 117.5 | 9 | 16 |
| 4 | 86.4 | 10 | 47.6 |
| 5 | 68.9 | 11 | 51.1 |
| 6 | 92 | | |

### 4.2.2 Urease Assay Results

Isolates 5 & 9 showed negative urease activity. It means that when the test was conducted the isolates did not show any colour change towards the test.

**Table 4.4** Results of urease activity.

| Isolate No. | Colour Change | Urease Activity | Isolate No. | Colour Change | Urease Activity |
|---|---|---|---|---|---|
| 1 | Yes | + | 7 | Yes | + |
| 2 | Yes | + | 8 | Yes | + |
| 3 | Yes | + | 9 | Yes | - |
| 4 | Yes | + | 10 | Yes | + |
| 5 | Yes | - | 11 | Yes | + |
| 6 | Yes | + | | | |

### 4.2.3 Growth Curve Results:

For Isolate 2 and 7 Growth curve tests was conducted for 64 hours. Urease assay activity and amount of calcite precipitated was the basis for the selection of these 2 isolates. Growth curve of isolate 2 is shown in Figure 4.1. Similarly, growth curve of isolate 7 is shown in Figure 4.2. It was observed that Isolate 2 show quicker and higher growth than isolate 7

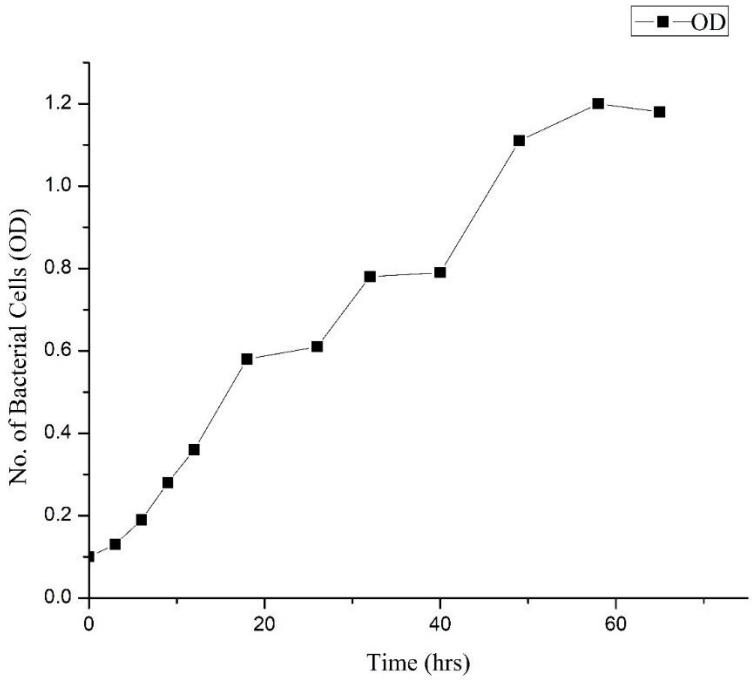



Figure 4.1 Growth curve for isolate 2 (isolate culture)

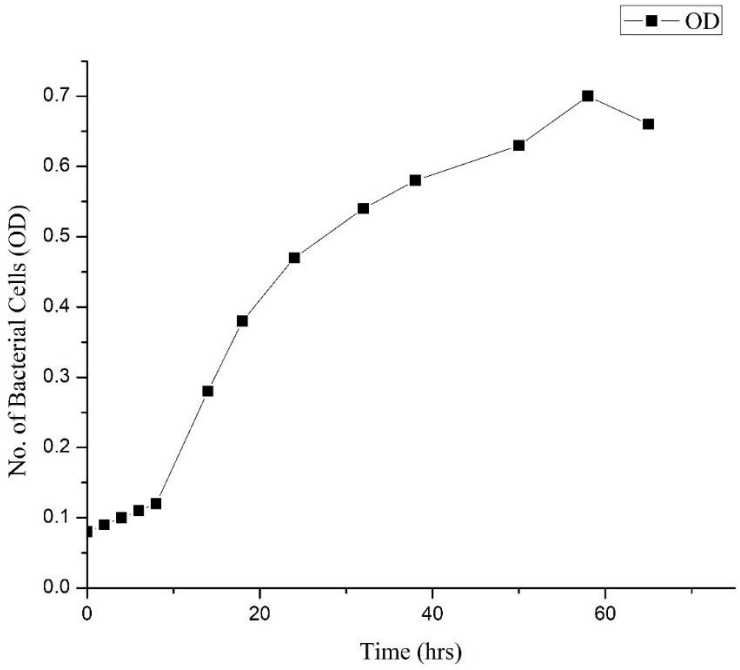



Figure 4.2 Growth Curve of isolate 7 (standard Culture)

## 4.2 Materials Testing Results

The results obtained from conducting various test on materials like cement, sand, aggregate were compared with the standard values specified in Indian Standards. It was concluded that all the materials satisfy the conditions given in Indian Standards and hence can be used in our study. Table 4.6 gives the data of the test performed along with the value obtained from the tests.

**Table 4.5** Material Testing Results

| S. No. | Test performed | Value Obtained |
|---|---|---|
| 1 | Standard consistency of cement | 30% |
| 2 | Initial Setting Time of cement | 40 min |
| 3 | Final Setting Time of cement | 230 min |
| 4 | Soundness of cement | 2.43 mm |
| 5 | Fineness of cement | 2.18% |
| 6 | Specific Gravity of cement | 3.15 |
| 7 | Fineness Modulus of Fine Aggregate | Conforming to Zone 1 of Table 4 IS 383 |
| 8 | Specific Gravity of Fine Aggregate | 2.70 |
| 9 | Specific Gravity of Coarse Aggregate | 2.80 |
| 10 | Specific Gravity of Fly Ash | 2.2 |
| 11 | Specific Gravity of Silica Fume | 2.2 |
| 12 | Specific Gravity of HRWRA | 1.1, Solid, Content 42% |

### 4.2.1 Compressive Strength Test Results

Compressive strength test results performed on concrete specimen at interval of 7, 14 and 27 days respectively are shown in Figure 4.3

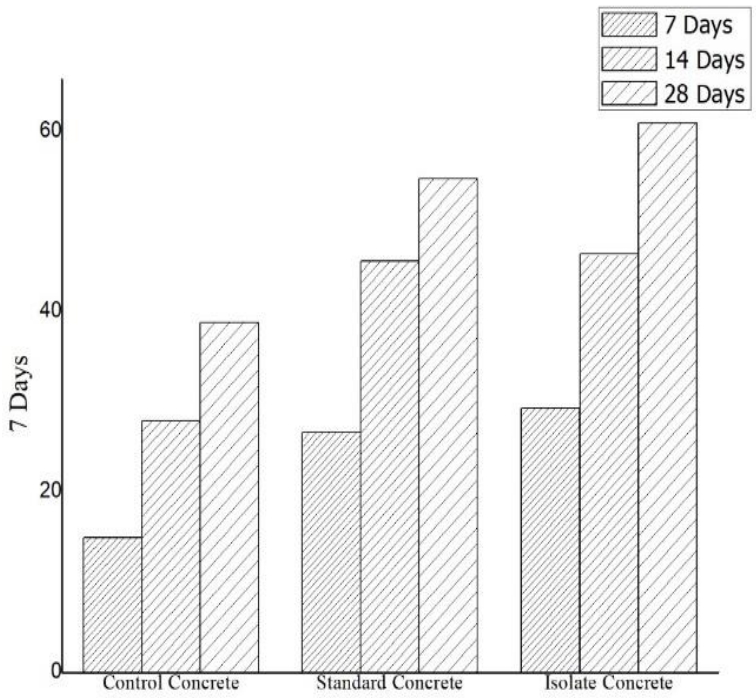



Figure 4.2 Compressive strength of control, isolate and standard concrete at 7, 14, 28 days respectively

# CONCLUSION

Results from this investigation revealed that, in concrete microbial cells can be utilized for purpose of crack healing of both Macro and Micro sizes. From the isolation stage, 11 bacterial cultures having potential are isolated, and when the further screening proceeded, the number reduced to just 2. It is because of the concrete's high alkaline harsh environment. The survival of major group of bacterial genus becomes difficult in such a high pH environment. In this way it can be said that only those isolates which can survive in high pH environment needs to be isolate, separated for use in concrete. Test conducted on concrete revealed that the performance of concrete with microorganisms in it showed higher strength and better characteristics when compared to control concrete (without bacteria). This happened due to the presence of calcite precipitating bacteria in concrete which filled the pores inside matrix and the cracks appeared on the surface with thin calcium carbonate crystals. Bacteria is only able to precipitate Calcium carbonate when it gets nutrition i.e rich calcium course and moisture. However the moisture requirement and food requirement of the colony of bacteria is so less that it can be fulfilled with the moisture present in air and minute food particles travelling in air .Whitish-yellow colored crystals were observed near the crack surfaces when visual inspection of the crack was done at 7 days of concrete casted. As the investigation further continued for 28 days it was observed that the highest crack healed in comparison to both isolate and control concrete was found in Standard concrete system. According from this investigation following conclusion were drawn.

I. It is better to use soil which is rich in lime and magnesia in order to obtain calcite precipitating bacteria. As the chances of getting one in such soil is quite higher
II. For developing bacterial cells, it was observed that rather than using direct plate technique we should use enrichment culture technique. With the help of this technique we can limit the growth of other bacteria which are not required
III. The concrete with Standard culture of bacteria showed highest compressive strength 60.92 (MPa) when compared to compressive strength of Isolate concrete 54.74 (MPa) and control concrete specimen 38.80 (MPa).
IV. Only those bacterial isolates can be used in crack healing of concrete which show positive urease activity and endospore formation. It is a fact that the microscopic organisms which are unable to frame endospore can't survive in an exceptionally highly alkaline environment of new concrete.

V. The scope of this examination was to make a concrete which has high strength and if cracks occurred it will be able to heal its crack autonomously. In further research the long ter durability, its cost effectiveness and its behavior in normal world needs to be explored. It is also needed to be checked that how this type of concrete will behave in marine conditions.

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# APPENDIX – 1

## A-1.1 COMPOSITION OF NUTRIENT MEDIA

The nutrient media (Urea Broth and Urea Agar) prepared for the culturing of bacteria given in Table A.1.1.

**Table A.1.1. Nutrient media composition per litre.**

| SR. NO. | CHEMICAL | COMPOSITION (gm/l) |
|---|---|---|
| 1. | Urea | 20 |
| 2. | Sodium Bicarbonate | 2.12 |
| 3. | Ammonia Chloride | 10 |
| 4. | Nutrient Broth | 3 |
| 5. | Calcium Chloride two hydrate | 25 |
| 6. | Agar | 17 |

### A-1.2 INCREASING pH OF NUTRIENT MEDIA COMPOSITION

The pH of nutrient media was increased by adding 4 pellets of Sodium Hydroxide.

## A-1.3 PHENOL RED ADDITION FOR MAKING SLANTS

Phenol red was used as indicator for getting urease activities. 0.018gm/l phenol red was added to the nutrient media composition.

# APPENDIX – 2

## A-2.1 IMAGES OF PRECIPITATED AND NON-PRECIPITATED COLONIES


**Figure A.2.1. Mixed colonies precipitated and non-precipitated.**

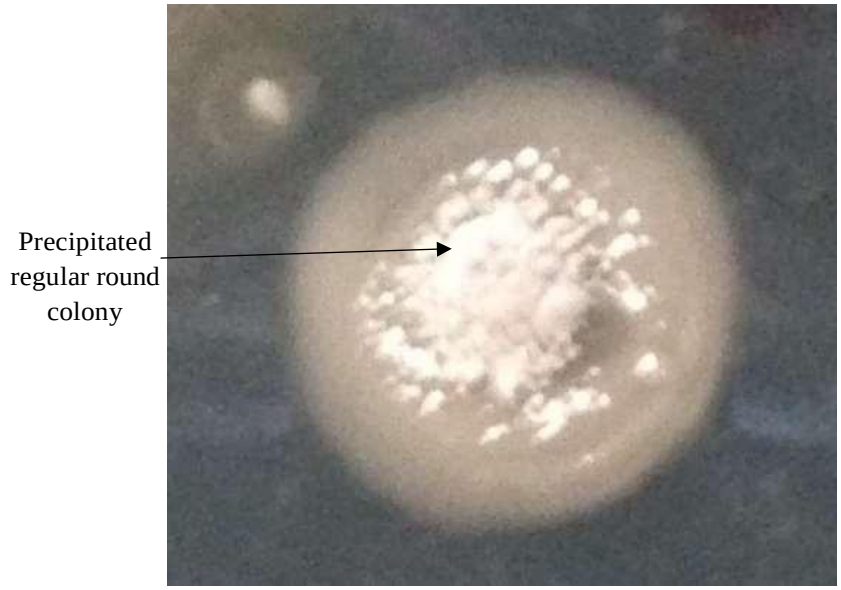


**Figure A.2.2. Precipitated colony.**

## A-2.2 IMAGES OF PURE COLONIES

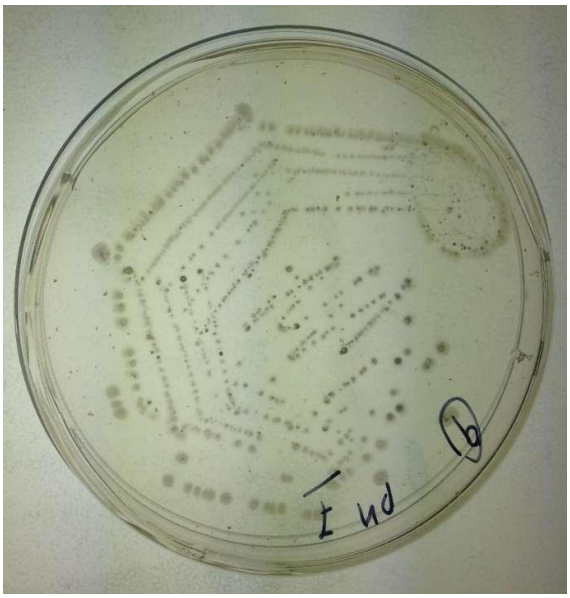

**Figure A.2.3. Pure colonies.**

## A-2.3 IMAGES OF SLANTS FOR UREASE ASSAY

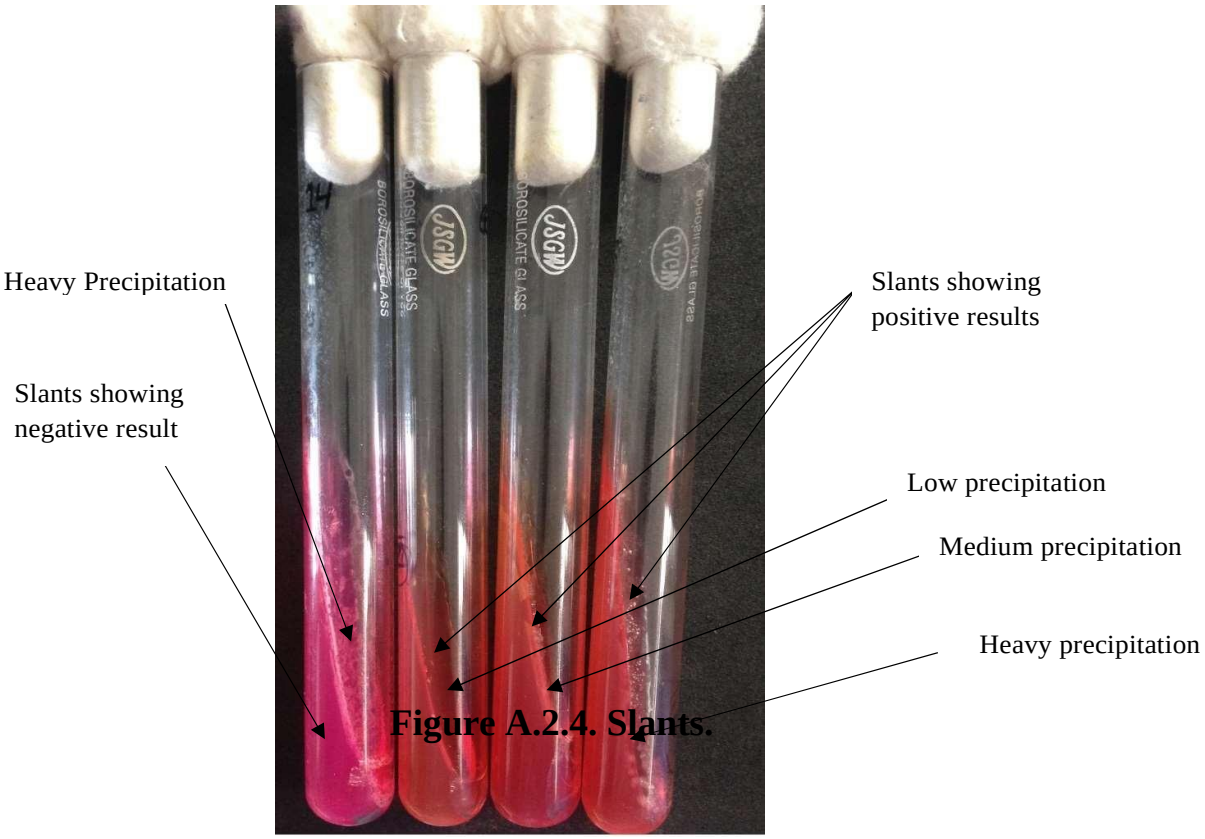


**Figure A.2.4. Slants.**

# APPENDIX – 4

## A-4.1 IMAGES OF CRACK HEALING BY BACTERIA

The visual inspection of crack healing by bacteria shown in the following images and the comparison made with control concrete sample.

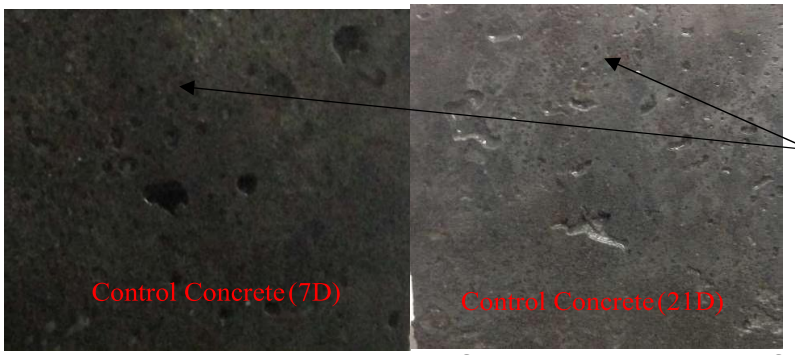


**Figure A.4.1. Control concrete sample after 7 and 21 days of concrete cubes curing.**


**Figure A.4.2. Potential of crack healing by standard culture seen after 7 days and 21 days of concrete cubes curing.**

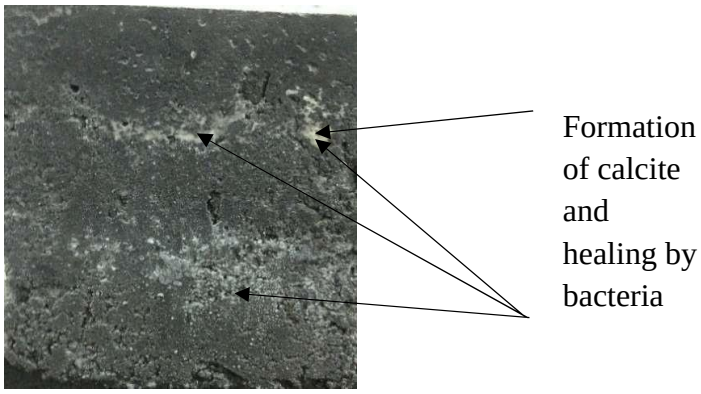


**Figure A.4.3. Heavy calcite formation in concrete by isolate 13 after 21 days of concrete cubes curing.**

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