Self-Healing of Concrete using Bacteria

Project Report submitted in partial fulfillment of the requirement for the degree of

Bachelor of Technology

In

Civil Engineering

under the Supervision of

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By

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Certificate

This is to certify that project report entitled “Self-Healing of Concrete using bacteria”, submitted by Jashan Deep Singh in partial fulfillment for the award of degree of Bachelor of Technology in Civil Engineering to Jaypee University of Information Technology, Waknaghat, Solanhas been carried out under my supervision and guidance.

This work has not been submitted partially or fully to any other University or Institute for the award of this or any other degree or diploma.

Date: **Mrs. PoonamDhiman**

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DECLARATION

I hereby declare that the research work presented in this Project entitled "*Self-Healing of Concrete using Bacteria*" submitted for the award of the degree of Bachelor of technologyinthe Department of Civil Engineering,Jaypee University of Information and TechnologyWaknaghat, is original and my own account of research. This research work is independent and its main content work has not previously been submitted for degree at any university in India or Abroad.

(Jashan Deep Singh)

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Jashan Deep Singh

ABSTRACT

The concrete structures deteriorate in contact with the surroundings which lead to an irreversible damage and ultimately reducing the strength of the structure. The characteristics of pore structure of concrete have a direct influence on its durability. The durability and strength of concrete can be enhanced by using a novel technique which involves bacterial-induced calcite precipitation. Bacteria are capable of precipitating calcium carbonate by providing heterogeneous crystal nucleation sites in super-saturated $CaCO_3$solution.Bacteria are believed to affect carbonate precipitation both through affecting local geochemical conditions and by serving as potential, nucleation sites for mineral formation. A novel technique for the remediation of damaged structural formations has been developed by employing a selective microbial plugging process, in which metabolic activities promote precipitation of calcium carbonate in the form of calcite. Recently, microbial mineral precipitation resulting from metabolic activities of some specific microorganisms in concrete to improve the overall behavior of concrete has become an important area of research. It has been hypothesized that almost all bacteria are capable of CaCO3 production because precipitation occurs as a byproduct of common metabolic processes such as photosynthesis, sulfate reduction, and urea hydrolysis.

The significant objective of the research work further involved the use of ureolytic bacteria (*Bacillus sphaericus)* in concrete which would make it, self-healing. The bacteria present in the concrete rapidly sealed freshly formed cracks through calcite production. The bacterial concentrations were optimized to 10^3 and 10^5 cells/ml. In concrete mix, cement was replaced with fly ash, and silica fume. The percentage replacement of fly ash and silica fume was by weight of cement. The percentage use of fly ash was 0 &10%, and that of silica fume was 0 & 10%. The experiments were carried out to evaluate the effect of *Bacillus sphaericus* on the compressive strength of concrete made with fly ash and silica fume up to the age of 91 days. The test results indicated that inclusion of *Bacillus sphaericus*enhanced the compressive strength, reduced the porosity and permeability of the concrete with fly ash and silica fume. The improvement in compressive strength was due to deposition on the bacteria cell surfaces which revealed calcium carbonate precipitation.The bacteria improve the impermeability of concrete by improving its pore structure and thereby enhancing the life of concrete structures.

TABLE OF CONTENTS

List of Figures

List of Tables

Abbreviations

Abbreviation		Word (s)
ASTM	-	American Standard for Testing and Materials
BLAST	-	Basic Local Alignment Search Tool
CAH	-	Calcium Aluminate
CFA	-	Coal Fly Ash
CSH	-	Calcium Silicate
CSL	-	Corn Steep Liquor
DNA	-	Deoxyribonucleic Acid
EDS	-	Energy Dispersive X-ray Spectrocopy
EDX	-	Energy Dispersive X-ray
EPS	-	Extracellular Polymeric Substance
FA	-	Fly Ash
HPC	-	High Performance Concrete
HVFA	-	High Volume Fly Ash
IAP	-	Ion Activity Product
LML	-	Lactose Mother Liquor
MCP	-	Microbial Calcite Precipitation
MEGA	-	Molecular Evolutionary Genetics Analysis
MICP	-	Microbial Induced Calcite Precipitation
MPa	-	Mega Pascal
NCBI	-	National Centre for Biotechnology Information
OPC	-	Ordinary Portland Cement
RCPT	-	Rapid Chloride Permeability Test
SCM	-	Supplementary Cementing Material
SD	-	Standard Deviation
SEM	-	Scanning Electron Microscope
SF	-	Silica Fume
TASC	-	Tubular Aerosol Suspension Chamber
XRD	-	X-Ray Diffraction

Chapter 1

INTRODUCTION

GENERAL

Microorganisms also called microbes are microscopic, minute living things that are very small to be seen with the unaided eye. These microorganisms are incredibly diverse in nature. There are numerous diverse microbial species which participate in the precipitation of mineral carbonates in various natural environments. The environment may include soils, geological formations, freshwater biofilms, oceans and saline lakes. Microorganisms can affect the carbonate precipitation both through affecting local geochemical conditions and by serving as potential, nucleation sites for mineral formation. A selective microbial plugging process has been developed and employed as a novel technique for the remediation of damaged structural in which metabolic activities results in precipitation of calcium carbonate in the form of calcite.

1.1 CONCRETE

Concrete is a composite building material comprised of aggregate and a binder (cement). Concrete finds good use in all types of building construction. Fly ash and silica fume can be used in concrete mix because of its lightweight and high thermal insulation. More recently, new types of building materials are being used. These include metals (for the structural framework of larger buildings), plastics, asbestos and fabrics. Tar-based waterproof materials, paper linoleum, polyvinyl chloride clay and solvent coatings for inner wall are other building materials. Cement, bricks and tiles are the main building materials used in the construction of buildings. Today, increase in the demand for various building materials have led to many building material manufacturing companies. Many new building materials are environmental hazards, which have become a big concern to all.

1.1.1 Durability of Concrete

Durability is defined as the capability of concrete to resist weathering action, chemical attack and abrasion while maintaining its desired engineering properties. It normally refers to the duration of trouble-free performance. Concrete require different degrees of durability depending on the exposure environment and properties desired.Concrete will remain durable if:

- The cement paste structure is dense and of low permeability
- Under extreme condition, it has entrained air to resist freeze-thaw cycle.
- It is made with graded aggregate that are strong and inert.
- The ingredients in the mix contain minimum impurities such as alkalis, chlorides, sulphates and silt.

1.1.1.1 Factors affecting durability of concrete

Cement content

Mix must be designed to ensure cohesion and prevent segregation and bleeding. If cement is reduced, then at fixed w/c ratio the workability will be reduced leading to inadequate compaction. However, if water is added to improve workability, water / cement ratio increases and resulting in highly permeable material.

Compaction

The concrete as a whole contain voids can be caused by inadequate compaction. Usually it is being governed by the compaction equipments used, type of form works and density of the steel work.

Curing

It is very important to permit proper strength development aid moisture retention and to ensure hydration process occur completely.

Permeability

It is considered the most important factor for durability. It can be noticed that higher permeability is usually caused by higher porosity .Therefore, a proper curing, sufficient cement, proper compaction and suitable concrete cover could provide a low permeability concrete.

1.1.1.2 Different methods to improve concrete durability

- **Chemical methods:** By applying epoxy coating which thereby reduces steelcontact with water and oxygen. Also penetrating sealer siloxane can be used, as these materials combine with siliceous portions of cement and aggregates.
- **Physical methods:** Use of pozzolans like silica fume, fly ash can improve theconcrete durability by enhancing impermeability and chemical durability. Sulfate resistance in concrete can be improved by incorporating supplementary cementing materials.
- **Development of Self-healing bacterial concrete**: A novel technique for theremediation of damaged structural formations has been developed by employing a selective bacterial plugging process, in which metabolic activities promote precipitation of calcium carbonate in the form of calcite. Biomineralisation of calcium carbonate is one of the strategies to remediate cracks in building materials.

1.2 SUPPLEMENTARY CEMENTING MATERIALS

Supplementary cementing materials (SCM) are often used in concrete mixes to reduce cement contents, improve workability, increase strength and enhance durability through hydraulic or pozzolanic activity. Utilization of these byproducts in cement/concrete not only prevents them from being land-filled but also enhances the properties of concrete in the fresh and hardened states. In addition, the use of SCMs conserves energy and has environmental benefits because of reduction in carbon dioxide emission as a result of reduction in manufacture of portland cement. Strict air pollution controls and regulations have produced an abundance of industrial byproducts that can be used as supplementary cementitious materials. Typical examples are fly ash, silica fume, ground granulated blast furnace slag, metakaolin, rice husk ash and natural pozzolans which can be used incorporated in concrete addition or as partial cement replacement.

1.2.1 Fly Ash

Fly ash is the residue generated in the combustion of coal. Fly ash is generally captured from the chimneys of coal-fired power plants. It is removed by the dust collectionsystems from the exhaust gases of fossil fuel power plants as very fine, predominantly spherical glassy particles from the combustion gases before they are discharged into atmosphere. The size of particles is largely dependent on the type of dust collection equipment. Diameter of fly ash particles ranges from less than 1 µm to 150 µm. It is generally finer than Portland cement. The chemical composition of fly ash is determined by the types and relative amounts of incombustible material in the coal used. Fly ash is a fine, glass-like powder recovered from gases created by coal-fired electric power generation.

Fly ash accounts for 75 to 85% of the total coal ash, and the remainder is collected as bottom ash or boiler slag. Fly ash because of its mineralogical composition, fine particle size and amorphous character is generally pozzolanic and in some cases also self cementitious whereas bottom ash and boiler slag are much coarser and are not pozzolanic in nature. Total fly ash generation in India from Thermal Power Plants is estimated at about 100 million tonnes per year. India utilizes approximately 20% of the fly ash.

1.2.1.1 Properties of fly ash

Size, shape and colour

Fly ash particle size is finer than ordinary portland cement. Fly ash consists of silt-sized particles which are generally spherical in nature and their size typically ranges between 10 and 100 micron. These small glass spheres improve the fluidity and workability of fresh concrete. Fineness is one of the important property contributing to the pozzolanic reactivity of fly ash. Fly ash colour depends upon its chemical and mineral constituents. It can be tan to dark gray. Tan and light colours are generally associated with higher lime content, and brownish colour with the iron content. A dark gray to black color is attributed to elevated unburned carbon (LOI) content. Fly ash color is usually very consistent for each power plant and coal source.

Fineness

Fineness of fly ash is most closely related to the operating condition of the coal crushers and the grindability of the coal itself. Fineness of fly ash is related to its pozzolanic activity. For fly ash use in concrete applications, fineness is defined as the percent by weight of the material retained on the 5 µm (#325) sieve. ASTM C618 (1993) limits the maximum amount of fly ash retained on the 45 µm (#325) mesh sieve on wet sieving as 34%. Generally, a large fraction of ash particle is smaller than 3µm in size. In bituminous ashes, the particle sizes range from less than 1 to over 100 µm. Joshi (1970) reported that average size lies between 7 to 12 µm. A coarser gradation can result in a less reactive ash and could contain higher carbon content.

Specific gravity

The specific gravity of fly ash is related to shape, color and chemical composition of fly ash particle. In general, specific gravity of fly ash may vary from 1.3 to 4.8 (Joshi, 1970). Canadian fly ashes have specific gravity ranging between 1.94 and 2.94, whereas American ashes have specific gravity ranging between 2.14 and 2.69.

Pozzolanic activity

A pozzolan is a siliceous or siliceous and aluminous material which, in itself has little or no cementitious value but which will in finely divided form and in presence of water, reacts with calcium hydroxide to form compound possessing cementitious properties. The pozzolancic activity is the measure of degree of reaction over time between pozzolanandcalcium hydroxide in presence of water (Wikipedia). The silicates present in self-cementitious fly ash react with calcium ions in the presence of moisture to form water insoluble calcium-alumino-silicate hydrates. The pozzolanic activity of a fly ash depends upon its (i) fineness; (ii) calcium content; (iii) structure; (iv) specific surface; (v) particle size distribution; and (vi) and LOI content (Joshi, 1979).

1.2.1.2 Classification of fly ash

ASTM C 618-93 categorizes fly ash into three types

- Class C
- Class F
- Class N

Class C fly ash

This class of Fly ash normally produced from lignite or sub-bituminous coal and has both pozzolanic and varying degree of self-cementitious properties. Most Class C fly ashes contain more than 12-15% Calcium oxide.

Class F fly ash

This class of Fly ash produced from burning anthracite or bituminous coal falls in this category. This class of fly ash exhibits pozzolanic property but rarely, if any, self hardening property. It generally contains high percentage of Fe_2O_3. Their crystalline minerals are generally composed of quartz, hematite, mullite, magnetite (Roy et al., 1984).

Class N fly ash

This class of Fly ash is produced from Raw or calcined natural pozzolans such as opalinechert, shale, volcanic ashes and pumice are included in this category. Chemical composition of fly ash as per ASTM C 618-93 is given in Table 1.1.

Table 1.1: Chemical Composition of Fly Ash as per ASTM C 618-93

Chemical Requirements	Class C	Class F	Class N
$SiO_2 + Al_2O_3 + Fe_2O_3$ - Min %	50.0	70.0	70.0
SO_3 – Max %	5.0	5.0	4.0
Moisture content –Max %	3.0	3.0	3.0
Loss on ignition – Max %	6.0	10.0	10.0

1.2.1.3 Reaction mechanism of fly ash

It can be basically explained as Pozzolanic Reaction Mechanism. Setting or hardening of OPC concretes occurs due to the hydration reaction between water and cementitious compounds in cement which give rise to several types of hydrates of calcium silicate (CSH), calcium aluminate (CAH) besides calcium hydroxide (CH).These hydrates are generally called as “Tobermorite gel”. The adhesive and cohesive properties of the gel bind the aggregate particles. Calcium hydroxide is a by-product of cement hydration. When fly ash is incorporated in concrete, the calcium hydroxide liberated during hydration of Ordinary Portland Cement reacts slowly with the amorphous alumino-silicates, the pozzolanic compounds, present in the fly ash. The products of these reactions, termed as pozzolanic reaction products, are time dependent but are basically of the same type and characteristics as the products of the cement hydration. Thus additional cementitious products become available which impart additional strength to concrete.

1.2.1.4 Uses of fly ash in cement/concrete

Even though fly ash is a byproduct of thermal power plants it is now being widely used in the construction industry. Earlier, the use was restricted for mixing in bricks only but now uses have become diverse.

1.2.1.4.1 High volume uses

- As structural fills in embankments, dams, dikes and levees, and
- As sub-base and base courses in road way construction.

1.2.1.4.2 Medium volume uses

- as raw material in cement production
- as an admixture in blended cements
- as partial replacement of cement or as a mineral admixture in concrete
- in addition coal ash including fly ash may be used as partial replacement of fine aggregate in concrete

1.2.1.4.3 Low volume uses

- In high value added applications such as metal extractions. High value metal recovery of Aluminum (Al), Gold (Au), Silver (Ag), Vanadium (Va) and Strontium (Sr) fall in this category.
- Fly ash has potential uses for producing light weight refractory material and exotic high temperature resistant tiles.
- Fly ash is used as special refractory material and also as additives in forging to produce high strength alloys.

1.2.1.4.4 Miscellaneous uses

- As land fill for land reclamations for residential, commercial and recreational development projects.
- As filler in asphalt, plastics, paints and rubber products.
- In water treatment and as absorbent for oil and chemical spills.

1.2.1.5 Benefits of using fly ash in cement / concrete

Inclusion of fly in cement or concrete has several benefits. Benefits to concrete vary depending on the type of fly ash, proportion used, other mix ingredients, mixing procedure, field conditions and placement. Some of the benefits of fly ash in concrete are:

Improved workability.

The spherical shape and glassy surface of fly ash particles permit greater workability for equal w/c ratio. In other words, w/c ratio may be reduced for equal workability.

Reduced heat of hydration

Hydration of cement paste is accompanied by liberation of heat that raises the temperature of concrete. Because of the slower pozzolanic reactions, partial replacement of cement by fly ash results in release of heat over a longer period of time, and the concrete temperature remains lower slowly. This is of immense importance in mass concrete where cooling, following a large temperature rise, can lead to cracking. Low-calcium Class F fly ashes generally tend to reduce the rate of temperature rise more as compared to high-calcium Class C fly ashes.

Higher ultimate strength

The additional binder produced by the fly ash reaction with available lime allows fly ash concrete to continue to gain strength over time. Mixtures designed to produce equivalent strength at early ages (less than 90 days) will ultimately exceed the strength of straight cement concrete mixes.

Reduced permeability

The decrease in water content combined with the production of additional cementitious compounds reduces the pore interconnectivity due to refinement of pore structure of concrete resulting in reduced permeability. The reduced permeability results in improved long-term durability and resistance to various forms of deterioration.

Increased resistance to sulfate attack

Fly ash in concrete increases the sulphate resistance and potentially corrosive salts that penetrate into concrete and cause steel corrosion with accompanying cracking and spalling of concrete. Fly ash induces three phenomena that improve sulfate resistance (i) consumes the free lime making it unavailable to react with sulfate; (ii) reduced permeability prevents sulfate penetration into the concrete; and (iii) replacement of cement reduces the amount of reactive aluminates available.

Improved resistance to corrosion

Fly ash addition to concrete improves the long term corrosion resistance of concrete. The reaction of fly ash with Ca $(OH)_2$ produces a denser concrete and thus inhibits the ingress of chloride ions takes place at a slower rate.

Increased resistance to alkali-silica reactivity

Fly ash reacts with available alkali in the concrete, which makes them less available to react with certain silica minerals contained in the aggregates.

1.2.2 Silica Fume

Silica fume, also known as micro-silica, is a byproduct of the reduction of high-purity quartz with coal in electric furnaces in the production of silicon and ferrosilicon alloys. Silica Fume is also collected as a byproduct in the production of other silicon alloys such as ferrochromium, ferromanganese, Ferro magnesium, and calcium silicon (ACI Comm.226, 1987b). It contains large proportions of extremely fine amorphous particles of silicon dioxide (SiO_2) which usually makes up more than 90% of silica fume constituents. The fineness of silica fume in terms of specific area can range around 20000m^2/kg. A typical silica fume exhibits most particles smaller than 1 micron and they have an average diameter of about 0.1 micron. Because of its extreme fineness and high silica content, silica fume is a highly effective pozzolanic material. Standard specifications for silica fume used in cementitious mixtures are ASTM C1240.

1.2.2.1 Properties of silica fume

1.2.2.1.1 Physical properties

Silica fume particles are extremely small, with more than 95% of the particles finer than 1 µm.

1.2.2.1.2 Chemical composition

Silica fume is composed primarily of pure silica in non-crystalline form. Silica fume has a very high content of amorphous silicon dioxide and consists of very fine spherical particles. Small amounts of iron, magnesium, and alkali oxides are also found.

1.2.2.2 Importance of silica fume in concrete

The importance of using silica fume in concrete is to obtain high strength, reduced permeability and bleeding, reducing the cement content to reduce costs, reduced heat of

Hydration. Silica fume has a pronounced effect on the concrete properties. It has been estimated that for a 15% silica fume replacement of cement there are approximately 200,000 particles of silica fume for each grain of portland cement in a concrete mix. Silica fume in concrete can be studied basically under three roles:

(i) Pore-size refinement and matrix densification

The presence of silica fume in the portland cement concrete mixes causes considerable reduction in the volume of large pores at all ages. It basically acts as filler due to its fineness and because of which it fits into spaces between grains in the same way that sand fills the spaces between particles of coarse aggregates and cement grains fill the spaces between fine aggregates grains.

(ii) Reaction with free- lime (from hydration of cement)

Calcium Hydroxide (CH) crystals in portland cement pastes are a source of weakness because cracks can easily propagate through or within these crystals without any significant resistance affecting the strength durability and other properties of concrete. Silica fume which is siliceous and aluminious material reacts with Calcium Hydroxide resulting reduction in content of Calcium Hydroxide in addition to forming strength contributing cementitious products which in other words can be termed as “ Pozzolanic Reaction”.

(iii) Cement paste-aggregate interfacial refinement

In concrete the characteristics of the transition zone between the aggregate particles and cement paste plays a significant role in the cement-aggregate bond. Silica fume addition influences the thickness of transition phase in mortars and the degree of the orientation of the Calcium Hydroxide crystals in it. The thickness compared with mortar containing only Ordinary Portland Cement decreases and reduction in degree of orientation of Calcium Hydroxide crystals in transition phase with the addition of silica fume. Hence mechanical properties and durability are improved because of the enhancement in interfacial or bond strength.

Applications of silica fume

(i) High Performance Concrete (HPC) containing silica fume –for highway bridges, parking decks, marine structures and bridge deck overlays which are subjected to constant deterioration caused by rebar corrosion current, abrasion and chemical attack;

(ii) High-strength concrete enhanced with silica fume – provides architects and engineers with greater design flexibility;

(iii) Silica-fume concrete – delivers greater economy, greater time savings and more efficient use of sprayed concrete. Silica fume produces superior shotcrete for use in rock stabilization; mine tunnel linings, and rehabilitation of deteriorating bridge and marine columns and piles;

(iv) Oil Well Grouting **-** Whether used for primary (placement of grout as a hydraulic seal in the well-bore) or secondary applications (remedial operations including leak repairs, splits, closing of depleted zones); the addition of silica fume enables a well to achieve full production potential;

(v) Repair Products- Silica fume is used in a variety of cementitious repair products. Mortars modified with silica fume can be tailored to perform in many different applications—overhead and vertical mortars benefit from silica fume’s ability to increase surface adhesion;

(vi) Refractory & Ceramics- The use of silica fume in refractory castables provides better particle packing. It allows for less water to be used while maintaining the same flow characteristics. It also promotes low temperature sintering and the formation of mullite in the matrix of the castable.

1.2.2.4 Advantages of using silica fume

Use of silica fume in concrete gives following advantages:

(i) High early compressive strength; (ii) high tensile, flexural strength, and modulus of elasticity; (iii) very low permeability to chloride and water intrusion; (iv) enhanced durability; (v) increased toughness; (vi) increased abrasion resistance on decks, floors, overlays and marine structures; (vii) superior resistance to chemical attack from chlorides, acids, nitrates and sulfates and life-cycle cost efficiencies; (viii) higher bond strength; and (ix) high electrical resistivity and low permeability.

1.3 BIOREMEDIATION

A variety of ions can non-specifically get deposited on bacterial cell surface at the nucleation site. It has been studied that the bacteria have the largest surface area to volume ratio of any life form (Schultze et al., 1996) and have a net electronegative charge (Beveridge, 1988). The combination of the large surface area and net negative charge results in the binding of dissolved metal ions on the surface of the Bacteria. The common contaminants play an important role in the amount of calcium carbonate precipitation under extreme conditions. The conditions may be such as harsh environments, pH and high temperatures and certain bacteria are capable of surviving in these extremes. Since the mineral formation can occur in extreme or toxic conditions, biomineralization can be viewed as a possible mechanism for cleaning up hazardous environments.

It was studied and proved that there are ureolytic bacteria capable of precipitation inherent in the contaminated aquifers (Fujita et al., 2000). The transportation of harmful chemicals in the subsurface is slowed down by adding urea which would accelerate the precipitation process. An easier and efficient method for removing the excess calcium in the water could be by ureolysis which would be induced with the addition of urea, and calcium carbonate would precipitate. In this regard an experiment was performed by (Hammes et al., 2003), which showed that up to 85% of calcium was removed from industrial wastewater using this method.

A concrete product prepared by mixing a cement paste containing bacterial cells in specific ratio is known as bacterial concrete. The use of bacteria in bioremediationprocesses can be more environmentally friendly, efficient, and cost effective as the use of costly reagents would be eliminated. Due to major property of crack remediation of bacterial concrete it is also known as self healing concrete.

1.3.1 Applications of Bacteria in Concrete

1.3.1.1 Bacterial concrete as an alternative surface treatment

An important measure to protect concrete against damage is diminishing the uptake of water (Basheer et al., 2001). Many of the physical and chemical deterioration mechanisms of concrete are related to aggressive substances present in aqueous solution. Surface treatments play an important role in limiting the infiltration of water and consequently of detrimental components into concrete. Broad arrays of organic and inorganic products are available in the market for the protection of concrete surfaces, such as a variety of coatings, water repellents and pore blockers.

But these means of protection beside their favorable influences even show disadvantageous aspects such as:

- Degradation over time
- Need for constant maintenance
- Different thermal expansion co-efficient of the treated layers
- Use of certain solvents contributes to environmental pollution as well

Bacterial induced carbonate mineralization is a novel and eco-friendly strategy for the protection and remediation of stone and mortar.

1.3.1.2 Bacterial concrete as concrete crack remediation/healing

When cracks appear in the concrete, the possibility for corrosion of the embedded steel arises which could eventually ruin the integrity of the structure. Without immediate attention, the cracks can expand and cause extensive damage. Current forms of concrete crack remediation are structural epoxy, resins, epoxy mortar, and other synthetic filler agents.

These synthetic solutions often need to be applied more than once as the cracks expand. Clearly there is a need for an effective, long-term, environmentally safe method to repaircracks in concrete structures. Several research groups have investigated the possibility of biomineralization as an effective method to remediate cracks and fissures in concrete structures.

Cracks filled with a mixture of *Bacillus pasteurii* (now reclassified as *Bacillus sphaericus)* and sand showed a significant increase in compressive strength and stiffness,compared to cracks without cells. Microscopy confirmed the presence of calcite crystals and cells near the surface of the cracks (Ramachandran et al., 2001). Other groups have noted that biomineralization can be used in the conservation of ornamental limestone statues or carvings, similar to its use in concrete remediation. *Myxococcusxanthus* is capable of precipitating calcium carbonate. The $CaCO_3$ cements pre-existing calcite grains on the pore walls of the limestone without completely plugging the pore. The resulting crystals are strongly attached and more resistant to stress than were the pre-existing calcite grains (Rodriguez et al., 2003).

1.3.1.3 Bacterial concrete as water purifier

Concrete and steel are arguably the most widely used construction materials in the world today. Steel bars are embedded in concrete to produce stronger building structures and the concrete provides the added benefit of protecting the steel bars from the elements. Bacteria which have been used to create excellent water purification effect comprises:

1. *Bacillus subtilis*
2. *Bacillus thuringiensis*
3. *Bacillus sphaericus.*

These bacterial cells have sufficient resistance against strong alkalinity even after they are mixed in the cement paste and against high temperature during production process. Microbial concrete as water purifier has the following advantages:

- Useable as water purifier tank walls.
- Floor lining of a water purifying facility in homes, industrial plants.
- The cement containing microbial cells can be effectively used for purifying water such as river water or lake water and in particular can be effectively used at a location where water flows at a low rate with stagnation. Various bacteria used in concrete are shown in Table 1.2.

Table 1.2: Bacteria used in concrete

Applications	**Types of Bacteria**
Bacterial concrete as crack healer	*Bacillus pasteurii* *Myxococcusxanthus Bacillus megaterium*
Bacterial concrete as surface treatment	*Bacillus sphaericus*
Bacterial concrete as water purifier	*Bacillus sphaericus* *Thiobacillus*

1.4BACTERIA

Bacteria are unicellular organisms. The genetic material of bacteria is not enclosed in a nuclear membrane. The bacteria have wide range of shapes and are a few micrometers in length. Bacteria are abundantly found in soil, water, as well as in organic matter and the live bodies of plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a milliliter of fresh water and in all, there are approximately five nonillion (5×10^{30}) bacteria on Earth, forming much of the world's biomass. The weight of the cells is 30.2×10^{-6} gm in 1gm of wet soil while the weight in 1 gm of dry soil is 40×10^{-6} gm. Cells cover a volume of 42.3×10^{-6} ml per 1ml of the soil. Normally, 1ml of concrete has 1.95×10^{5} cells which cover a volume of 13.35×10^{-8} ml. The weight of the cells in a gram of concrete is around 8.4×10^{-8} gm. Bacteria help in recycling nutrients by process of fixation of nitrogen from the atmosphere. Recently, it has been found that bacteria are used in concrete as a self healing agent.

1.4.1 Morphology of Bacteria

Morphology of bacteria refer to the shapes and sizes which are exhibited by bacteria. Spherical bacterial bacterial species are called cocci (*sing*. coccus, from Greek *kókkos*, grain, and seed) and the ones which are rod-shaped are called bacilli (*sing*. bacillus, from Latin *baculus*, stick). Some rod-shaped bacteria are called vibrio and are slightly curved or comma-shaped; while the others can be spiral-shaped which are called spirilla, or tightly coiled, called spirochaetes. Tetrahedral or cuboidal shapes are also seen in some bacterial species.

1.4.2 Growth and Reproduction of Bacteria

Bacteria reproduce through binary fission which is a form of asexual reproduction. Usually bacteria are grown in solid or liquid media in the laboratory. Pure cultures of bacterial strain can be isolated using solid growth media such as agar plates. When measurement of growth or large volumes of cells are required liquid growth media are used. With the use of selective media is helpful in identifying specific organisms.

There are three phases for bacterial growth. The cells get adapted to the new environment which may be a high-nutrient environment which allows growth. A period of slow growth when the cells are adapting to this high-nutrient environment and preparing for fast growth is the lag phase. The second phase of growth is the logarithmic phase (log phase) which is also known as the exponential phase. The log phase is a rapid exponential phase. The *growth rate* (*k*) is the rate at which cells grow during this phase, and the time taken by the cells to double is known as the *generation time* (*g*). In the log phase, nutrients are utilized at a high speed till the time is reached when one of the nutrient depletes and the limits the growth. The depleted nutrients cause the final phase of growth which is the *stationary phase*. The metabolic activity of the cells gets reduced and consumption ofnon-essential cellular proteins starts.

1.4.3 Factors Affecting Bacterial Activity

The factors which affect the growth of bacteria and the production of calcite include nutrients, water, pH, temperature, presentation of the organic contaminants and heavy metals, space of solids, the concentration of dissolved organic carbon, concentration of calcium ions, presence of nucleolus sites (Mitchell and Ferris, 2005; DeJong et al., 2006).
To sustain life the nutrients are energy source for the bacteria and the growth of bacteria is effected by the available type and amount of nutrient in system. CO_2, N, P, K, Mg are some of the nutrients helpful in the growth of bacteria (Mitchell and Ferris, 2005). The activity of bacteria also depends upon water which has different type and amount of soluble materials, different pH, aeration control and thermal stability. It was studied that calcium carbonate precipitation reached peak at pH level of 8 (Stocks et al., 1999).The production of $CaCO_3$ was improved with lower concentration of enzyme (0.03 g/1) and an increase of temperature from 20 to 50°C (Nemati et al., 2005).

1.5 UREOLYTIC AND CARBONATE BIOMINERALIZATION

A biologically induced precipitation in which an organism creates a local micro-environment with conditions allowing optimal extracellular chemical precipitation of mineral phases is called biomineralization (Hamilton, 2003). Various natural environments have number of diverse microbial species which participate in the precipitation of carbonates. While the precise role of the microbes in the carbonate precipitation process is still not clear but almost all bacteria are capable of calcium carbonate precipitation *(*Boquet et al., 1973) and precipitation occurs as a byproduct of common metabolic processes such as photosynthesis, sulfate reduction and urea hydrolysis (Hammes et al., 2003).

The hydrolysis of urea generates carbonate ions without production of protons. When hydrolysis occurs in calcium rich environment, calcite precipitates are formed. The rate of carbonate formation has an important role to play in the strength of precipitated crystals and under suitable conditions it is possible to control the reaction to generate hard binding Biocement.

The urease enzyme (e.g. urea amidohydrolase; EC 3.5.1.5) is common in many microorganisms and ureolysis can be induced in a lab setting by adding urea. One mol of urea is hydrolyzed intracellularly to 1 mol of ammonia and 1 mol of carbamate (equation 1), which spontaneously hydrolyzes to form an additional 1 mol of ammonia and carbonic acid (equation 2). These products subsequently equilibrate in water to form bicarbonate, 2 mol of ammonium, and 2 mol of hydroxide ions equations (3) and (4). The latter giverise to a pH increase, which in turn can shift the bicarbonate equilibrium, resulting in the formation of carbonate ions (equation 5), which in the presence of soluble calcium ions precipitate as $CaCO_3$ equation (6) & (8). Equation (7) is an overall reaction for the system, showing that urea and calcium are added to the system, and ammonium and calcium carbonate are products.

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

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

$$H_2CO_3 \rightarrow HCO^{3-} + H^+ \qquad (3)$$

$$2NH_3 + 2H_2O \rightarrow 2NH^{4+} + 2OH^- \text{ (pH increase)} \qquad (4)$$

$$HCO_{3-} + H^+ + 2OH^- \rightarrow CO_3^{2-} + 2H_2O \qquad (5)$$

$$CO_3^{2-} + Ca^{2+} \rightarrow CaCO_3 \text{ (carbonate precipitation)} \qquad (6)$$

$$CO\,(NH_2)_2 + 2H_2O + Ca^{2+} \rightarrow 2NH^{4+} + CaCO_3 \text{ (overall reaction)} \qquad (7)$$

Calcium carbonate is an appropriate mineral to use for the reduction of porosity of underground formations for many reasons. Ca^{2+} is one of the most abundant cations and carbonate ions (HCO^{3-} and CO_3^{2-}) are some of the most abundant anions in most subsurface waters. In order to produce the most mineral mass, utilizing elements already present in the subsurface is a more efficient method than adding another chemical. Injection of supercritical CO_2 into the underground formations will also make more carbonate ions by the dissolution and disassociation of CO_2, which in turn will be used to precipitate more mineral.

Bacterial calcium carbonate precipitation results from both passive and active nucleation. Passive carbonate nucleation occurs from metabolically driven changes in the bulk fluid environment surrounding the bacterial cells. This increases the mineral saturation and induces nucleation (Schultze et al., 1996). In the ureolysis driven system, this occurs from an increase in pH due to ammonification (Stocks et al., 1999).Active carbonate nucleation occurs when the bacterial cell surface is utilized as the nucleation site. The cell clusters exhibit a net electronegative charge which favors the adsorption of Ca^{2+} ions. The Ca^{2+} ions attract CO_3^{2-} and HCO^{3-} ions, which will eventually form calcium carbonate precipitates (Hammes et al., 2003; Mitchell and Ferric, 2005). Although it is known that there are many different types of bacteria capable of calcium carbonate precipitation, it has been hypothesized that there are specific attributes of certain bacteria that promote and affect$CaCO_3$ precipitation more than others (Hammes et al., 2003). It has already been noted that cell walls have an inherent electronegative charge that affect the binding of certain ions (Beveridge, 1988), but the extracellular polymeric substance (EPS) associated with biofilms may also be involved. Biofilm cells are contained in the EPS matrix and may use it as an attachment device, for structural support, and/or protection (Ghannoum and O’Toole, 2004). The EPS matrix is composed primarily of polysaccharides and, depending on the side chains attached to the polysaccharides (e.g. carboxyl groups, pyruvate, phosphate, or sulfate), the matrix can exhibit an overall negative charge. This negative charge is important in trapping metal ions within the EPS matrix (Kawaguchi and Decho, 1999).

One of the primary applications of biomineralization is the plugging of porous media with applications leaning toward bioremediation (Mitchell and Ferris, 2005). Because plugging of porous media can occur in many different environmental locations and involve many different factors, such as soil alkalinity, temperature, and pressure, it is important to monitor the effectiveness of the bacteria’s ability to precipitate out calcium carbonate in each different environmental situation. Research done by (Ferris et al., 2003) showed that the hydrolysis of urea by *Bacillus pasteurii* (now reclassified as *Bacillus sphaericus* (Yoon et al., 2001) is temperature dependent and that the highest calcite precipitation rates occurred near the point of critical saturation (Mitchell and Ferris, 2005). It also highlighted the fact that calcite precipitation is kinetically dependent on saturation state and independent of temperature. This research by (Ferris et al., 2003) emphasized the impact of environmental conditions on calcite precipitation that were previously noticed.

Members of the genus *Bacillus* are Gram-positive, rod-shaped, endosporeforming bacteria commonly found in soil (Todar and Kenneth, 2005). *Bacillus pasteurii*, a member of this genus, converts urea to ammonium carbonate more actively than any other known bacterium. Therefore, *B. pasteurii* and other members of the *Bacillus* genus are incorporated into studies to determine their influence on calcium carbonate precipitation in various environments. Experiments performed indicated that urease activity at high pH in *B. pasteurii* favored calcium carbonate precipitation (Stocks et al., 1999). Upon examination of the sand grains from columns used in the experiment, bacterial cells were shown encased in calcite crystals, which indicated that the bacteria acted as a nucleation site for the mineralization process, an example of active nucleation.Another study conducted by (Hammes et al., 2003) looked at strain specific $CaCO_3$ precipitation. Isolates collected from various soil locations in Belgium yielded some crystal growth and urease activity and, when sequenced, showed that all the isolates were closely related to one another and the group *Bacillus sphaericus*. Other close relatives of the group are *Bacillus pasteurii*, *Bacillus psychrophilus*, *Bacillus globisporus*, *Planococcusokeanokoites*, and*Filibacterlimicola*.

Microbiologically induced (also called “bacteriogenic”) calcium carbonate precipitation is comprised of a series of complex biochemical reactions, including concomitant participations of a bacterium like *Bacillus pasteurii*, urease (urea amidohydrolase; EC 3.5.1.5), and high pH. In this process, an alkalophilic soil microorganism, like *Bacilluspasteurii*, plays a key role by producing urease that hydrolyzes urea to ammonia andcarbon dioxide. The ammonia increases the pH in surroundings, which in turn induces precipitation of $CaCO_3$, mainly as a form of calcite. In aqueous environments, the overall chemical equilibrium reaction of calcite precipitation can be described as (Stumm and Morgan, 1981):

$$Ca^{2+} + CO_3^{2-} \longrightarrow CaCO_3\downarrow$$

The solubility of $CaCO_3$ is a function of pH and affected by ionic strength in the aqueous medium. Generally in a medium, say provided with Urea-$CaCl_2$ medium that supports microbial growth, additional ions including NH_4^+, Cl^-, Na^+, OH^-, and H^+, may affect chemically induced $CaCO_3$ precipitation at different pHs. Microbiologically induced calcium carbonate precipitation occurs via more complicated processes than chemically induced precipitation.

The bacterial cell surface with a variety of ions can nonspecifically induce mineral deposition by providing a nucleation site. Ca^{2+} is not likely utilized by microbial metabolic processes; rather it accumulates outside the cell. In medium, it is possible that individual microorganisms produce ammonia as a result of enzymatic urea hydrolysis to create an alkaline microenvironment around the cell. The high pH of these localized areas, without an initial increase in pH in the entire medium, commences the growth of$CaCO_3$ crystals around the cell. Possible biochemical reactions in Urea-$CaCl_2$ medium to precipitate $CaCO_3$ at the cell surface can be summarized as follows (Stocks *et al.*, 1999):

$$Ca^{2+} + \text{Cell} \longrightarrow \text{Cell-}Ca^{2+}$$

$$Cl^{-} + HCO_3^{-} + NH_3 \longrightarrow NH_4Cl + CO_3^{2-}$$

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

1.6 OBJECTIVES OF PRESENT WORK

- Isolation of bacteria fromsoil.Screening and its identification on the basis of calcite formation and Gram character.
- Addition and Optimization of the bacterial inoculums on the basis of calcite formation.
- Preparation of concrete mixtures by partial replacement of cement with 0 and 10% fly ash, and also the addition of silica fume (10%) by weight of cement in M20 grade of concrete with varying bacterial inoculums.
- Determination of influence of bacteria on the compressive strength of concrete containing fly ash and silica fume.

Chapter 2

REVIEW OF LITERATURE

2.1 BACTERIAL CALCIUM CARBONATE PRECIPITATION

Bacterially induced calcium carbonate precipitation is an environmentally friendly method to protect decayed stones and concrete. The carbonate cement is highly coherent (Le Metayer-Levrel et al., 1999). Calcium carbonate precipitation adopts two different mechanisms which involves both biological and controlled or induced (Lowenstan and Weiner, 1988). In biologically controlled mechanism, the nucleation and growth of the mineral particles is controlled by the organism which is independent of environmental conditions wherein no specialized specific molecular mechanism is involved (Sarda et al., 2009; Morita, 1980) whereas positively charged metal ions can be bound on bacterial surfaces, at a neutral pH (Douglas and Beveridge, 1998; Ehrlich, 1998). In bacterially induced carbonate precipitation the essential role in the morphology and mineralogy is played by exopolysaccharides and amino acids (Braissant et al.,2003; Ercole et al., 2007). The examples of controlled mechanism includes magnetite formation in magnetotactic bacteria (Bazylinski et al., 2007) and silica deposition in the unicellular algae respectively (Barabesi et al., 2007). Even this technique has been involved for the improvement of the durability of cementitious materials (Ramachandran et al., 2001; Ramakrishnan et al., 2001; De Muynck et al., 2008a, b).

Microbial involvement in the process of carbonate precipitation has led to the exploration of this technique in a variety of fields. The field of bioremediation includes a series of applications which include removal of metal ions, the treatment of groundwater contaminated with heavy metals, radionucleotides and the removal of calcium from wastewater while conventional bioremediation strategies mainly rely on the biodegradation of organic pollutants (Chaturvedi et al., 2006; Warren et al., 2001;Fujita et al., 2000;Hammes et al., 2003b). Whereas some authors believe that carbonate precipitation is a specific process with ecological benefits for the precipitating organisms (Ehrlich, 1996; McConnaughey and Whelan, 1997) while others have different viewpoint with consideration that it is an unwanted and accidental by-product of the metabolism (Knorre and Krumbein, 2000). (Gupta et al., 2007) also suggested that production andrecovery of an alkaline exo-polygalacturonase from *Bacillus subtilis* can be done under solid-state fermentation using statistical approach. Microorganisms are ideal crystal nucleation site because microbial cell walls favours the binding of divalent cations such as Ca^{2+} and Mg^{2+} (Rivadeneyra et al., 1998). Biological extracellular polymeric substances cause the formation of different calcium carbonate polymorphs due to the presence of specific proteins in it (Kawaguchi and Decho, 1999).

Microbial cells are encapsulated in polymers through the immobilization technique which further enhances the strength by calcium carbonate precipitation (Bang et al., 2001). Calcium carbonate precipitation occurs as a by-product of common microbial metabolic process which increase the alkalinity and produce microbial calcite precipitation (Knorre and Krumbein, 2000). Microbial mineral precipitation using ureolytic bacteria is able to influence the precipitaton of calcium carbonate by the production of urease enzyme. An increase of the pH and carbonate concentration in the bacterial environment catalyzes the hydrolysis of urea to CO_2 and ammonia (Stocks et al., 1999).

Calcium carbonate precipitation can be induced extracellularly by some bacteria through a variety of processes that include ammonification, photosynthesis, denitrification, sulfate reduction and even through anaerobic sulphide oxidation. (Castanier et al. 2000; Riding, 2000). Under appropriate conditions calcium carbonate precipitation is a general process performed by bacteria (Boquet et al., 1973).

Dick et al., (2006) concluded that bacillus strains were capable of depositing calcium carbonate but different in amount. For this five different strains of the *Bacillus sphaericus* group and one strain of *Bacillus lentus* were monitored for checking their bio-deposition activity. Seven different parameters were examined which included: urea degrading capacity, calcite deposition on limestone cubes, pH increase, extracellular polymeric substances (EPS)-production, biofilm formation and deposition of dense crystal layers. Bacteria capable of high ureolytic efficiency were regarded as best. Other characteristics which were also considered included homogeneous calcite deposition on limestone cubes.

Douglas and Beveridge (1998) also suggested a new approach in which microbes deposited minerals constantly and concluded that primary role of bacteria is their ability to create an alkaline environment through various physiological activities. Further it wasobserved that microorganisms occur in natural environments and include bacteria, fungi, archaea and some microscopic plants and animals such as plankton.

Sanghi et al. (2009) reported a cost effective substrate under submerged fermentation by alkalophilic bacteria named *Bacillus subtilis* and concluded that high level production of a cellulose free xylanase can be recovered using wheat bran. Further Sanghi et al. (2010) reported a potentially effective alternative for industrial applications for this characterization of extracellular cellulose-free xylanase was done from a newly alkalophilic and moderately thermophilic strain of *Bacillus subtilis*.

2.2 OPTIMUM CONDITIONS FOR BACTERIAL CONCRETE

Biological mortar consists of a mixture of bacteria, limestone and a nutritional medium containing a calcium salt. The term biological refers to the microbial origin of the binder, i.e. microbiologically produced calcium carbonate. Lian (2006) suggested that the process of cementation occurs at the contact areas between the surface of the aggregates due to nucleation and growth of carbonate crystals. Soil bacterium *Bacillus megaterium* induced carbonate biomineralization by producing a long term effect on calcium carbonate precipitation.

Mansch and Bock (1998) suggested that the presence of large amounts of ammonium salts should be avoided in order to avoid nitrification. The hydrolysis of urea produces higher concentrations of ammonium therefore use of paste is an effective solution. It was concluded that colonization is controlled by the pH of the pore solution. The optimal pH for growth is 9. The pH will drop to a value of about seven because the carbonate producing bacteria uses more oxygen to complete the process of precipitation. The paste was used as an alternative method to remove salts from the materials used in buildings (Woolfitt and Abrey, 2008; Carretero et al., 2006). It was further observed that usage of paste would also help in protecting the bacteria from drying (May, 2005). When application of paste was carried in wet form, it helps in the dissolution of salts within stones but upon drying it can be easily washed or removed off. Even removal of black crusts on stone artworks is possible by applying combinations of paper pulp, clay materials or cellulose derivatives. (Ranalli et al., 1997; Cappitelli et al., 2006, 2007). Factors such as salinity and composition of the medium influence the calcium carbonateprecipitation by bacteria. Different types of bacteria can perform well in alternative environments (Knorre and Krumbein, 2000; Rivadeneyra et al., 1998).

Calcium carbonate precipitation is controlled by four factors mainly by (1) the concentration of calcium, (2) the dissolved inorganic carbon (DIC), (3) the pH and the last factor being (4) the nucleation sites (Hammes and Verstraete, 2002). Bacteria through various physiological activities has an ability to create an alkaline environment. To create the alkaline environment the pathways involved include both autotrophic and heterotrophic (Castanier et al., 1999). Calcium carbonate precipitation occurs due to the action of bacteria in heterotrophic environment and also helps in enrichment of organic matter. Khanna et al., (2003) studied the process optimization and scale-up production and further concluded that bacteria namely *Bacillus thuringiensis* helps in the process of fermentation by producing delta endotoxin production. The concentration of dissolved inorganic carbon and pH helps the microbes and microbial process for the utilization of organic acids (Braissant et al., 2002). Ion exchange through cell membrane also helps in producing calcium carbonate particles (Rivadeneyra et al., 1994; Castanier et al., 1999). Bacteria helps in inducing calcium carbonate precipitation under optimum conditions (Boquet et al., 1973).

De Muynck et al. (2009) carried out the biodeposition experiments and concluded that when pH of the solution reached to 7 ammonium was the major compound which was formed. In ureolyticbiodeposition the production of ammonium was lowered when compared to conventional sources of nitrogen pollution example includes production of 4.7 g of nitrogen with 1 litre of a biodeposition medium containing 10 g/l urea, results in the production (DeCuyper and Loutz, 1992).

2.3 EFFECT OF BACTERIA ON CONCRETE PROPERTIES

2.3.1 Compressive Strength

The property of a material to withstand axially directed pushing force is called compressive strength. This property and durability depend on the microstructure of the concrete. For the fastest production of carbonate ions, the hydrolysis of urea is the best possible option, as it is very rapid process and depends on only one enzyme. Problems related to chemical and physical incompatibilities can best be avoided with the usage ofbiological mortar. This type of mortar usually repairs the brittle materials (Castanier, 1995; Orial et al., 2002).

Ramachandran et al., (2001) studied the effect of the buffer solution, type and amount of microorganisms on compressive strength of portland cement mortar cubes. To determine this both living and dead cells of *S. pasteurii* and *P. aeruginosa* were investigated. After demolding, the mortar specimens were stored in a solution containing urea and calcium chloride for 7 days. At lower concentrations, the presence of *S. pasteurii* was shown to increase the compressive strength of mortar cubes while the contribution of *P. aeruginosa* to the strength was found to be insignificant.

Intracellular urease constitutes close to 1 % of the cell dry weight as produced by *Bacillus pasteurii*(Bachmeier et al., 2002). An organism creates a local environment withconditions that allow optimal extracellular chemical precipitation of mineral phases (Hamilton, 2003). De Muynck et al. (2008a) reported that the durability of mortar specimens increased due to bacterial carbonate precipitation. Baert et al., (2009) concluded that fly ash decreased the acceleration period. For this the study was carried through thermogravimetry and isothermal calorimetry on the reactivity of fly ash.

Calcite producing bacteria has a major applicability value for the restoration of deteriorated calcareous monuments due to its high purity and coherency (Lee, 2003). De Belie et al., (2005) concluded that when weathered concrete samples were treated with Thiobacillus bacteria a dense layer of calcite and vaterite crystals were observed.

Calcium carbonate biomineralisation is a technique which helps to remediate cracks in building materials. The durability of concrete structure is influenced by cracks as cracks are harmful for the structure safety (Zhong and Yao, 2008).

2.4 ECONOMIC ADVANTAGES OF BACTERIAL CONCRETE

Due to environmental conditions and the properties of the material there may be decay of the surface. This decay may ultimately lead to increase in unwanted properties like water porosity, water absorption and permeability. This may also accelerate the decrease in mechanical properties like compressive strength (Tiano et al., 1999). In order to minimize the decay, conservation treatments as studied by De Muynck et al., (2010) need to be applied which would modify the characteristics as required. For this purpose, the water repellents are used on the surface so as to avoid ingress of water and other unwanted weathering agents. The use of consolidants helps in increasing the cohesion between grains of deteriorated stone. In addition to these treatments there are some more surface treatments which would reduce the decay of concrete. But, both of these conservation treatments are controversial due to their nonreversible action and their limited long-term performance. Due to incompatibility with the treated surface both water repellents and consolidants have also reported to accelerate decay (Clifton and Frohnsdorff, 1982; Delgado, 2001; Moropoulou et al., 2003; Natarajan 1995). It has also been reported that consolidants tend to produce shallow and hard crusts due to their poor penetration abilities (Clifton and Frohnsdorff, 1982; Tiano 1995; Webster and May 2006).

The feasibility of bacterial concrete is governed by the time required for production of carbonates and its efficiency depend on the speed of precipitation. (Rodriguez et al., 2003; Sutton et al., 2008; Tiano et al., 1992) reported on the importance of the type and structure of the precipitated $CaCO_3$ polymorphs (vaterite or calcite) on the efficiency of the biodeposition treatment. Organic surface treatments result in the formation of harmful incompatible surface films and due to usage of large quantities of organic solvents, pollution is increased (Camaiti et al., 1988; Rodriguez et al., 2003; Monger 2000; Peckman et al., 1999). While inorganic consolidation may be preferable consolidating materials share some physico-chemical affinity (Rodriguez et al., 2003).

It has been tried to by some researchers to reintroduce of calcite into the pores. The application of a saturated solution of calcium hydroxide has been experimented for wall painting mortars and for some deteriorated calcareous stones, so as to impart a water repellent and consolidating effect (Tiano et al., 1999; Stumm and Morgan 1981). In this method, formation small crystallites takes place which are not chemically bound to theinternal surface of the pore and which are not able to bridge the pores (Tiano et al., 2006; Whiffin, 2004). (De Muynck et al., 2010) studied the economics of biodeposition treatment on the surface of concrete. It was studied that this treatment was costlier when compared to traditional surface treatment but it had advantages of being ecological and environmental friendly.

The comparisons of control concrete with the bacterial concrete need to be studied with respect to economics and the influence on the concrete properties like permeability, water porosity, water absorption and compressive strength. Compressive strength being the most important aspect of a concrete structure should not be reduced by the introduction of bacteria. The detailed comparison of economic advantage is in Chapter 4.

2.5 EFFECT OF FLY ASH ON CONCRETE PROPERTIES

2.5.1 Compressive Strength

The compressive strength of concrete induced with fly ash depends upon some of the factors which include the ratio of addition of fly ash and the size of fly ash particles. The early age compressive strength was increased due to the addition of fly ash concrete as reported by Maslehuddin (1989). Siddique (2003a; 2003b) proposed that with the increase in percentage of fine aggregate due to replacement of cement with fly ash, the compressive strength of concrete mixtures was also increased. Electric Power Research Institute (1987) reported the percentage replacement and classification of fly ash for use in cement and concrete.

Siddique (2004) reported that there was a drastic improvement in strength properties beyond 28 days. Saraswathy et al., (2003) concluded that the compressive strength of fly ash concrete was less than that of ordinary portland cement (OPC) even after 90 days of curing but chemically activated coal fly ash (CFA) improved the compressive strength only with 10% and 20% replacements. For investigation various activation techniques like physical, thermal and chemical were adopted. To demonstrate this effect, concrete specimens were prepared with 10, 20, 30 and 40% of activated fly ash replacement levels with cement. Compressive strength was tested at the age of 7, 14, 28 and 90 days.

Lane and Best (1982) observed that when fly ash replaces cement on a one-to-one basis, the rates of hardening and strength gain at early ages were reduced whereas whenreplacement level was two or three-to-one basis, the strength reduced at 3 days ascompared to the control but the later-age strength was higher. Hence, it was ecommended that proportioning fly ash concrete on strength basis requires a replacement ratio greater than one-to-one by mass.

Cook (1982) reported that fly ash with CaO content of 30.3% at 25% replacement was able to develop the compressive strength between 55 to 75 MPa at the age of 28 days. Perry et al., (1987) also reported the benefits of using fly ash in concrete, for which a study of twelve fly ashes was carried. Further it was concluded that fly ash incorporation in concrete increased the compressive strength of concrete.

Carette and Malhotra (1984) suggested that when cement was replaced with 20% fly ash in all the mixes of concrete the compressive strength continued to increase with age upto 365 days due to the pozzolanic action of fly ashes. Nagataki et al., (1986) reported thaboth compressive strength and carbonation rate are directly inter-related. Further it was also concluded that carbonation rate decreased with an increase in compressive strength. Other authors also reported the ill-effects of improper moist curing conditions due to which more negative effects were produced (Liu 1981; Nanni 1989; Yazici et al., 2005; Bilodeau and Malhotra 1992). Swamy and Mahmud (1986) concluded that in concrete containing 50% low-calcium fly ash, the compressive strength development was between 20-30 MPa at the age of 3 days which further showed an increment to 60 MPa at the age of 28 days.

Joshi et al. (1993) reported that with replacement level variation of 40 and 60% by weight of cement with three different alberta fly ash, the compressive strength increased was found to be equal to control concrete at 120 days. Haque et al. (1988) studied when cement is replaced with 40 to 75% bituminous fly ash in concrete, there is an increase in flexural strength which is less than the increase in compressive strength between 28 days and 91 days of curing.

Klieger and Perenchio (1972) found that concrete made with Type-I fly ash cement had lower strength than the control at all ages through 3 years. Korac and Ukraincik (1983) reported concrete strengths were found to be comparable at the age of 90 days but that the early-age compressive strength of 50% fly ash concrete were lower than that of the control concrete. Saraswathy et al. (2003) concluded that when 10% and 20% coal fly ash (CFA) was replaced with cement, the compressive strength was improved and as strengthincreased so does the durability of concrete. Erdogdu and Turker (1998) reported that finer the size of fraction of fly ash more increase in the compressive strength was observed.

Siddique (2003a) concluded that Class-F fly ash is very efficient in structural concrete upto the age of 365 days as their was a significant increase in compressive strength of concrete.

Goel et al., (2012) studied self compacting concrete and reported the that flexural strength of concrete beams improved. Demirboga et al., (2007) reported that with incorporation of fly ash in concrete, the early age compressive strength was reduced which was due to slower rate of hardening. Chindaprasirt et al., (2007) studied the use of finer fly ash and it was observed that the water content was reduced but compressive strength of concrete increased due to fineness of fly ash. Termkhajornkit et al., (2006) concluded that with

50% fly ash, an increase in compressive strength was observed until 28 days beyond which it became nearly constant. Yazici and Inan, (2006) studied the effects of curing on of high-volume fly ash concrete mixtures compressive strength which diminished at later age. Swamy and Mahmud (1986) studied that there was increase in strength of fly ash concrete after one year, which between 6 to 22% of the 28-day strength of the reference concrete. Lohtia et al., (1977), Ghosh and Timusk (1981), Nasser and Marzouk (1983) have also studied the effect of fly ash replacement of cement on compressive strength. Nagataki et al. (1986) reported a direct relationship between 28-day compressive strength and extent of carbonation irrespective of fly ash replacement in concrete and also mentioned that the extent of carbonation decreased with an increase in compressive strength. Lane and Best (1982); Majko and Pistilli (1984) observed that instead of replacing cement by mass, proportional replacement of the fine aggregate, which reduces the rates of hardening and strength gain at early ages, should be done to increase strength. Cook (1982) investigated using a high-calcium fly ash with CaO content of 30.3% at 25% replacement level to develop concrete mixes. The 28-day strength was in the range of 55 to 75 MPa. It was reported that when cement factor is increased, sand content and the water-to-cementitious material ratio was reduced.

Mehta, (1994) reported that at 7 days at no significant contribution to strength development was observed but beyond 28 days, fly ash at the replacement levels of up to 30% by cement strength gain in concrete was equivalent to control concrete. Gupta et al.,(2003, 2006) reported that landfill siting effect the environment to a very large extent and developed an important relation between both landfill siting and environment. This was further improved by developing a static sunshade design for energy efficient buildings.

When concrete made with portland cement is cured at temperatures in excess of 30°C, an increase is seen in strength at early ages but it was observed that there was decrease in strength at later stage (Neville, 1973). Ravina, (1981) observed that there was an increase rate of strength due to pozzolanic action of fly ash and it was concluded that fly ash concrete at elevated temperatures produced significant improvement in strength upto 28 days. William and Owens, (1982) reported that fly ash concrete gained when compared with ordinary Portland cement concrete. Ozer and Ozukul, (2004) concluded that poor curing conditions have major affect on strength of concrete due to pozzolanic cement as compared to that of ordinary Portland cement. This effect may be due to the initial water-curing on the strength development of ordinary Portland cement and pozzolanic cement concrete. Atis, (2005) reported that fly ash cement concrete was greatly influenced by the dry curing condition when compared to the conventional concrete. These results were reported when worked on the strength properties of high-volume fly ash concrete. Haque et al., (1988) studied the role of Alberta fly ashes on the strength properties. It was concluded that when mixes with Alberta fly ashes were replaced by upto 50% cement there was less reduction. To determine the influence on strength, curing was done at 50% relative humidity at room temperature of about 23ºC.

2.5.2 Setting Time

The addition of water to concrete results in starting of hydration reaction and the cement paste begins to stiffen accompanied by heat release. The rate of stiffening of cement paste is expressed in terms of setting time. The characteristics and the amount of fly ash used play important role in setting time. The other chemical and mineral admixtures also influence the setting time. It has been found that the addition of low-calcium Class F fly ashes increase the cement setting while high-calcium fly ashes which have low carbon content and are highly reactive sometimes show an opposite effect of decrease in setting time.

The use of high-calcium fly ash in concrete resulted in increase in setting time as studied by Ramakrishan et al., (1981). But it has been studied by Lane and Best, (1982) that the influence on setting time due to cement fineness, water content, and ambient temperature is more than the influence of fly ash. Sivasundaram et al., (1990) concluded that in high-volume fly ash (HVFA) concrete mixes, the initial setting time of 7.50 hours was same as that of the control concrete but the final setting time was increased by 3 hours. Rodway and Fedirko, (1989) studied that concrete containing high calcium fly ash increased initial setting time when compared to concrete without fly ash.

2.5.3 Other Effects of Fly Ash on Properties of Concrete/mortar

The fly ash concrete shows less carbonation than the control concrete. Nagataki and Ohga, (1992) concluded that generally fly ash replacement content increases the rate ofcarbonation. Ho and Lewis, (1983) suggested that curing the concrete fly ash containing for upto 90 days, slowers the rate of carbonation. But Joshi et al., (1994) reported that after 90 days curing, the opposite trend was observed in fly ash concrete.

Schubert, (1987) showed that due to the consumption of $Ca(OH)_2$ in the pozzolanic reaction, the rate of carbonation increases but blockage of capillary pores decreases it. Kokubu and Nagataki, (1989) suggested that with the reduction in content of cementitious materials, the carbonation depth also increases. Gebauer, (1982) reported that when water-cement ratio of concrete mix is increased, it also increases the rate of carbonation. Kasai et al., (1983) reported that concrete containing fly ash showed more carbonation effect than ordinary portland cement concrete.

The pore size in cement paste containing fly ash gets reduced due to its particle size. Diamond, (1981) reported in cement paste containing fly ash, the pH of pore solution was reduced. For this two types of fly ash were studied and it was concluded that pH of pore solution was reduced from 13.75 in a control cement paste without fly ash to about 13.55 in the presence of fly ash. Saraswathy et al., (2003) concluded that concrete containing activated fly ash content of upto 50%, improved the corrosion-resistance properties. Chalee et al., (2007); Schiepl and Hardtle, (1994) reported that change in pore size distributions was observed due to the pozzolanic reactions of fly ash. (Malhotra et al., 1982; Perencho and Klieger 1976) also reported that the change in the pore structure of the cement paste was observed due to the pozzolanic reactions of fly ash, as it densifies the zone between the paste and aggregates. (Joshi 1987; Malhotra et al., 1990) conducted that all the fly ash concrete had about the same durability factor as control concrete.

The sulfate resistance is reduced when fly ash is added in normal concentrations as studied by Larsen, (1985) and Mehta (1993). (Langan et al. 1990; Dhir et al. 1991; Naik et al., 1995, 1998; Yen et al., 2007) reported that mixing of Class C fly ash with Class F fly ash showed better resistance to alkali and sulfate attack.

The reduction in temperature rise was reported by Atis, (2002, 2003, 2004) when fly ash was used as replacement of cement. Bamforth, (1980) suggested that increase in the quantity of cement replacement by fly ash and slag, the rate of heat liberated was slowed down. (Sivasundram et al., 1989; Langley et al., 1989) concluded that concrete containing low-calcium Class F fly ash reduces the rate of temperature rise more when comparedtohigh-calcium Class C fly ash (Crow and Dunstan, 1981). ACI Committee 211.1.81 (1984) reported that when cement was replaced with fly ash in the range of 15 to 30%, early age heat liberation was affected. Further it was also observed that incorporation of fly ash as replacement showed exhibits less temperature rise than concrete without fly ash.

The water demand decreased when the quantity of fly ash was between 15 and 20% but when fly ash content was more than 20%, the water demand was increased as reported by Yuan et al., (1982). Joshi and Lohtia, (1993) used alberta fly ash in making high-volume fly ash concrete mixes and further concluded that fly ash concrete was cohesive than control concrete.

The initial curing had pronounced effects on the porosity and pore structure of different types of concrete as a result of which the pore size is decreased as studied by Shafiq and Cabrera, (2004). Jiang et al., (2004) concluded that lower carbonation depth may be due to prolonged initial curing period. The effect is more marked with moist curing. Velosa and Cachim, (2009) reported the pozzolanics additions and curing conditions resulted in the strength development of concrete by improving the mechanical strength different ages. Giaccio and Malhotra, (1988) also observed that concrete containing high volume of Class F fly ash attains excellent mechanical properties.

2.6 EFFECT OF SILICA FUME ON CONCRETE PROPERTIES

2.6.1 Compressive Strength

Huang and Feldman, (1985) concluded that increase in compressive strength was due to the addition of silica fume to mortar improved the bond between the hydrated cement matrix and sand. Cong et al., (1992) reported that improvement in cement paste matrix when silica fume (18%) was replaced by cement helps in attaining increased compressive strength. The silica fume replacement along with the addition of superplasticizer increased the strength. Gleize et al., (2003) concluded that silica fume acts mainly at the interface paste-aggregate in portland cement mortars. The action grows stronger when there is a higher concentration of calcium hydroxide and greater porosity than in paste. Wolseifer, (1984) reported that addition of silica fume increases the concrete strength and hence it was concluded that silica fume may be used to produce concrete with very high-strength and low-permeability.

(Luther and Hansen 1989; Pigeon and Plante 1989; Schmidt 1992) studied the test results of air-void stability when concrete was made by replacement of silica fume with cement and further it was concluded that no influence was observed on air-void system. (Sharma et al., 2007; Yogendran et al., 1991) reported that concrete made with silica fume ability factor by 99%. Sakr,(2006) concluded that replacement of upto 15% silica fume was best and further concluded that concrete made with 15% of silica fume had better and increased compressive strength and better resistance to sulfate attack. Gutierrez et al., (2005) studied the role of silica fume on the compressive strength of fibre reinforced mortar and concluded that compressive strength of the matrix reinforced with glass fibres gained an increase of 68% when replaced with silica fume.

2.6.2 Heat of Hydration

Silica fume have higher surface area and due to its amorphous nature it is highly reactive and helps in accelerating the hydration of C_3S, C_2S, and C_4AF (Uchikawa and Uchida, 1980; Kurdowski and Nocun-wczelik, 1983). Grutzeck et al., (1983) concluded that silica fume dissolves in the presence of $Ca(OH)_2$ which than acts as a substitute on which conventional C-S-H is formed.It has been observed by Meland, (1983) observed that cumulative heat evolved is lower in paste containing silica fume and lignosulfonate. Also, it was studied that higher the amount of silica fume, the smaller is the amount of heat evolved. Lohtia and Joshi, (1996) concluded that partial replacement of cement by silica fume results in reduction of heat of hydration without any reduction in strength. ACI Committee 234 (2006) reported that silica fume accelerates the hydration of cement during early stages as it provides nucleation sites for cement hydration products. Uses of high performance silica fume concrete was studied by Scott and Singh, (2011).

2.6.3 Consistency

The consistency of cement increased with the increase in silica fume content as observed by Rao, (2003). In order to determine the influence of silica fume on the consistency of cement pastes and mortars, silica fume was varied from 0 to 30% at a constant increment of 2.5/5% by weight of cement. Further it was concluded that for cement pastes containing 20–30% silica fume an additional water requirement of 40% was observed.

Qing et al., (2007) verified the influence of nano-SiO_2 (NS) addition on consistency of cement paste incorporating silica fume and concluded that silica fume makes cement paste thinner as compared with NS. It was also observed that penetration depths (consistency value) decreased gently while increasing NS content, fresh pastes grew thicker gradually when compared with control sample but with the increase in silica fume content the pastes grew thinner and their depths increased.

2.6.4 Setting Time

The silica fume concrete was compared with non-silica fume concrete and it was observed that in the absence of water-reducer delay in setting time occurs in silica fume concrete unlike in non-silica fume concrete of equal strength as studied by Lohtia and Joshi, (1996). Further it was suggested that when 15% silica fume was added with superplasticizer, both the initial and final setting time were delayed by approximately 1 and 2 hours, respectively. Alshamsi et al., (1993) concluded that setting time of pastes can be lengthened with the addition of micro-silica. With (10%) addition of micro-silica a very little effect on setting times was observed whereas the higher percentages produced significant influence. When OPC was replaced with 20% micro-silica, a setting time with6 to 20% increase was observed. Uchikawa, (1986) mentioned that use of excessive superplasticizer may cause substantial delays in setting times of cement paste containing silica fume.

Rao, (2003) suggested that silica fume greatly influences the setting time of cement paste and concluded that initial setting time decreased with the increase in silica fume content. This may be due to the pozzolanic action of silica fume which is very active at early hours of hydration.

2.6.5 Workability

Workability (slump) of cement pastes and concrete is defined as the ease with which it can be placed in place and is a measure of the behaviour of a compacted inverted cone of concrete under the action of gravity. The consistency is measured as the time required for a given mass of concrete to be consolidated by external vibration a mould. Workability can be reduced mainly due to small particle size that leads to higher water demand. Alshamsi et al., (1993) concluded that addition of micro-silica can also influence workability in cement pastes or concrete. Sellevold and Redjy, (1983) reported that water requirement is decreased in concrete with high concentration of silica fume as concentration of contact points between the different grains is reduced which can further result in less water requirement and hence the desired consistency can be achieved. Luther, (1989) proposed that concrete incorporating silica fume reduces bleeding because of its effect on rheologic properties. Wong and Razak, (2005) suggested that workability characteristics of silica fume concrete can largely vary in mixtures due to the constant superplasticizer dosage used in mixtures with the same w/c ratio. Rao, (2003) studied the influence of silica fume on the workability of mortars and concluded that the addition of small amounts of silica fume does not require the use of extra water or super plasticizers whereas the presence of too much silica fume in mortar (>10% by weight of cement) makes the mixture stiff and lowers its workability.

Chapter 3

EXPERIMENTAL PROGRAM

This chapter deals with the

(i) Experimental program related to bacteria followed by its isolation and identification, calcite formation.

(ii) Experimental program related to bacterial concrete, the materials used with their properties, mix proportions, casting of specimens for studying various properties of concrete and methodology adopted for testing of different properties.

3.1 EXPERIMENTAL PROGRAM RELATED TO BACTERIA

3.1.1 Isolation and Identification of Bacteria

3.1.1.1 Sample collection

Samples were collected from soil. Soil samples were collected from Wakanaghat pubic toilet.

3.1.1.2 Isolation of bacterial strains

The samples were suspended in a sterile saline solution (0.85% NaCl), diluted properly and plated on agar containing urea (20 g/l), $NaHCO_3$ (2.12 g/l), NH_4Cl (10 g/l), Nutrient broth (3 g/l), $CaCl_2.2H_2O$ (25 g/l). Incubation was done at 28˚C. Colonies were assessed every 5 days with a stereo microscope and selected as positive based on visual crystal formation within 10 days.

3.1.2 Morphological Studies

3.1.2.1 Gram staining

Gram staining method was used to determine the morphology of the bacterial strains. Slide with a bacterial smear was placed on a staining rack. The slide was stained with crystal violet for 1-2 min and then the slide was flooded with Gram's iodine for 1-2 min. Decolourization was done by washing the slide slowly with acetone (2-3 seconds). Slide was then thoroughly rinsed with water to remove the acetone. The slide was flooded with safranin counter stain for 2 min and then again washed with water. The excess water was removed and slide was air dried. Finally samples were visualized under microscope.

In Gram-positive bacteria, the dark purple crystal violet stain was retained by the thick layer of peptidoglycan and the Gram-negative bacteria, the thin peptidoglycan layer in the periplasm does not retain the dark stain, and the pink safranin counterstain stains the peptidoglycan layer.

3.2 EXPERIMENTAL PROGRAM RELATED TO CONCRETE

3.2.1 Materials Used in Concrete

3.2.1.1 Ordinary portland cement

Ordinary portland cement was used in concrete. It was tested as per Indian Specifications IS: 8112-1989. Its chemical properties are shown in Table 3.1, respectively.

Table 3.1: Chemical Properties of Ordinary Portland Cement (OPC)

Chemical	Constituent %
SiO_2	21.04
Al_2O_3	5.02
Fe_2O_3	3.12
CaO	62.11
MgO	2.44
$K_2O + Na_2O$	1.04
SO_3	3.12

3.2.1.2 Fine aggregates

Natural sand with 4.75mm maximum size was used as fine aggregate. It was tested as per Indian Standard Specifications IS: 383-1970.

Table 3.2: Sieve Analysis of Fine Aggregates

Weight of the sample taken = 1.0 kg

I.S. Sieve	Weight (gm)	% weight (gm)	Cumulative % weight	% passing
10.0mm	00	00	00	100
4.75mm	10	1.0	1.0	99
2.36mm	60	6.0	7.0	93
1.18mm	200	20.0	27.0	73
600µm	190	19.0	46.0	54
300µm	350	35.0	81.0	19
150µm	150	15.0	96.0	4.0
Pan	40	4.0	100	0

3.2.1.3 Coarse aggregates

Crushed stone with maximum 12.5mm graded aggregates (nominal size) was used. Sieve analysis results are given in Tables 3, respectively.

Table 3.3: Sieve Analysis of Coarse Aggregates

Weight of the sample taken = 2.0 kg.

I.S. Sieve	Weight (gm)	% weight (gm)	Cumulative % weight	% passing
80mm	00	00	00	100
40mm	00	00	00	100
20mm	00	00	00	100
12.5mm	.97	4.8	4.8	95.2
10mm	642	32.1	36.9	63.1
4.75mm	1184	59.2	96.1	3.9
Pan	77	3.85	100	00

3.2.1.4 Properties of fly ash

Physical and chemical properties of fly ash from Bathinda thermal power plant (Punjab, India) was analyzed as per ASTMC 618. Fly ash has a very high content of amorphous silicon dioxide and consists of fine spherical particles along with small amounts of iron, magnesium, and alkali oxides were found. Chemical results are given in Tables 3.7 and Table 3.8 respectively

Table 3.4: Chemical Properties of Fly Ash (ASTM C618)

Compound	% By mass
SiO_2	58.11
Al_2O_3	27.21
Fe_2O_3	5.23
CaO	2.14
MgO	0.72
K_2O+ Na_2O	1.0
Loss on Ignition	1.52

3.2.1.5 Properties of silica fume

Physical and chemical properties of silica fume were analyzed as per ASTM C1240. Silica fume is composed primarily of pure silica in non-crystalline form. Chemical properties of silica fume includes very high content of amorphous silicon dioxide. Small amounts of iron, magnesium, and alkali oxides were also found. Chemical properties results are given in Tables 3.5, respectively

Table 3.5: Chemical Properties of Silica Fume (ASTM 1240)

Compound	% By mass
SiO_2	92.65
Al_2O_3	0.36
Fe_2O_3	0.53
CaO	0.48
MgO	2.5
$K_2O + Na_2O$	2.50
Loss on Ignition	1.77

3.2.2 Design of Concrete Mix

The compressive strength of concrete is considered as the strength and index of its quality. Therefore the mix design is generally carried out for a particular compressive strength of concrete with adequate workability so that the fresh concrete can be properly mixed, placed and compacted. The proportions for the mix were calculated adopting the requirements of water as specified in BIS: 10262-1982.

The proportioning of concrete mixes consists of three interrelated steps.

(i) Selection of suitable materials and ingredients-cement, supplementary cementing materials, water, coarse and fine aggregates.

(ii) Determination of the relative quantities of these materials in order to produce a concrete that has desired strength and durability.

(iii) Careful quality control of every phase of the concrete making process.

In the present study Mix Design for M20 (Design value at the age of 28 days) grade concrete is done according to BIS: 10262-1982.

M20 design mix

Data

Characteristic strength at 28 days = 20 N/mm^2

Maximum size of aggregate = 12.5mm

Type of exposure = Mild, no sulfate attack

Concrete use = Concrete structure

Ingredients of M20 concrete mix are given in Table 3.6

Table 3.6: Mix Proportion M20

Unit of Batch	Water(Liters)	Cement(Kg)	F.A.(Kg)	C.A.(Kg)
Cubic meter content	195	390	569	1165
Ratio of ingredients	0.5	1	1.45	2.98

F.A: denotes fine aggregates; C.A: denotes coarse aggregates

Mix composition

The concrete mixes were designed with constant cement, fine aggregate, coarse aggregate. Control concrete mixture was designed as per IS 10262-1982 to have 28-day compressive strength of 28 MPa. Then cement was partially replaced with 0, 10, 20, and 30% fly ash in addition to 5 and 10% silica fume by weight of cement with varying concentration of bacterial culture, (*Bacillus sphaericus*) 10^3cells/ml; 10^5cells/ml. The detailed description of all mixes is given in Table 3.7.

Table 3.7: Concrete Mix Proportions with and without Fly Ash (FA) and Silica fume (SF)

Mixture No.	M-1	M-2	M-3	M-4
Cement (kg/m^3)	390	351	312	273
Natural sand (kg/m^3)	568.7	568.7	568.7	568.7
Fly ash (%)	0	10	20	30
Coarse aggregate (kg/m^3)	1164.12	1164.12	1164.12	1164.12
W/C ratio	0.5	0.5	0.5	0.5
Water (kg/m^3)	185	185	185	185
Slump (mm)	90	85	80	80

M : denotes Mix

* In each of the above mixes 5 and 10% Silica fume was added

For these mix proportions, required quantities of materials were weighed. The mixing procedure adopted was as follows:

1. The cement, fly ash and silica fume were dry mixed in a tray for about 15 minutes to obtain a uniform color.
2. Weighed quantities of coarse aggregates and sand were then mixed in dry state.

3. The mix of cement, fly ash and silica fume was added to the mix of coarse aggregates and sand and these were mixed thoroughly for a homogeneous mix.
4. Water and bacterial culture was then added.

All the moulds were properly oiled before casting the specimens. The casting immediately followed mixing, after carrying out the tests for fresh properties. The top surface of the specimens was scraped to remove excess material and achieve smooth finish. The specimens were removed from moulds after 24 hours and cured in water till testing or as per requirement of the test.

After required period of curing i.e 28 and 91 days, the specimens were taken out of the curing tank and their surfaces were wiped off.

3.2.3 Preparation of Test Specimens

Concrete cubes were prepared with different concentrations of bacterial cells (*Bacillus sphaericus).* The cell concentration was determined from the bacterial growth curve made by observing optical density at 600 nm. Control concrete cubes were cast without the addition of microbes. All the experiments were performed in triplicates..

3.2.4 Testing Procedure for Concrete

Following tests were performed on hardened concrete

- Compressive strength (IS: 516 – 1959)

The specimen properties were determined at the age of 28 and 91days

3.2.4.1 Compressive strength

Compressive strength is the capacity of a material or structure to withstand axially directed pushing forces. When the limit of compressive strength is reached, materials are crushed. Concrete can be made to have high compressive strength. Fly ash and silica fume was added by replacing the amount of cement at the concentrations of 0%, 10%, 20% and 30% (for fly ash) and 5% and 10% (for silica fume).To study the compressive strength test of cement mortar, *Bacillus sphaericus* was grown in medium (described above). Concrete as per specifications of compressive strength cubes were cast. Sand and cement were thoroughly mixed, adding along with grown culture of *Bacillus sphaericus*. Cubes were cast and compacted in a vibration machine. After de-molding, allspecimens were cured compression testing at 28 and 91 days. Control specimens were also prepared in similar way where water and medium (described above) replaced bacterial culture. Compressive strength casting in moulds is shown in Figure 3.1.

Figure 3.1 Casted Samples for Compressive Strength

Compressive strength is often measured on a universal testing machine. Measurements of compressive strength are affected by the specific test method and conditions of measurement. Compressive strengths are usually reported in relationship to a specific technical standard. When a specimen of material is loaded in such a way that it extends it is said to be in tension. On the other hand if the material compresses and shortens it is said to be in compression. Cube specimens of size 150mm were cast for compressive strength as per Indian standard specifications IS: 516-1959. After casting, all tests specimens were finished with steel trowel. Immediately after finishing, the specimens were covered with sheets to minimize the moisture loss from them. Specimens were demoulded after 24-hours and then cured in water at approximately room temperature till testing. Compressive strength tests for cubes were carried out at 28 and 91 days. The compressive strength was then calculated according to the formula:

$$\sigma = P / A \qquad (3.1)$$

Where σ = Compressive Strength (N/mm^2)
P = Maximum load (N)
A = Cross section area of cube (mm^2)

Chapter 4

RESULTS AND DISCUSSION

This chapter presents the results and discussion related to :-

(i) Bacterial isolation, identification, estimation of calcite and urease activity followed by DNA isolation and sequencing analysis of bacteria, optimization of bacteria inoculums based on calcite fornation

(ii) (ii) Effect of bacteria on concrete properties such as compressive strength of cube.

4.1 RESULTS AND DISCUSSION RELATED TO BACTERIA

4.1.1 Isolation of Calcium Carbonate Producing Bacteria.

The pH of cement is alkaline, so the bacteria have to be isolated from a similar medium in order to survive in the pH of cement. Taking this into account the bacteria were isolated from alkaline soil.

4.1.2 Crystal Nucleation Site Development

The Bacillus strains was based on visual differences in the precipitate morphology.

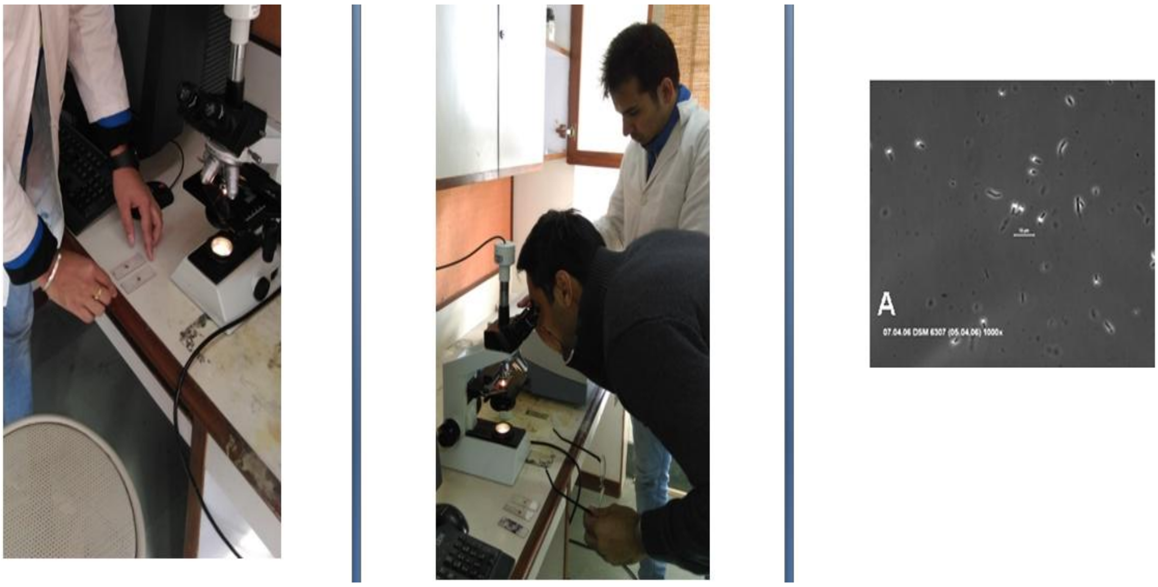

Figure 4.1 : Physical identification of Bacterial strains using Stereomicroscope

(a) Strain 1 **(b) Strain 2**

Figure 4.2 : Stereomicroscopic Images of Calcite Crystals Precipitated Within Different Bacterial Strains.

Crystal precipitating colonies were studied after 5 days of cultivation with stereomicroscopy. Digital images were captured with a C.C.D. camera. Large crystal aggregates that precipitated within a single colony of these isolates showed crystal nucleation site development. The Bacillus strains were selected for further study. Based on morphological differences it was observed that large crystal aggregates of bacteria which precipitated within single colonies on the precipitation agar were termed as calcium precipitating bacteria. Five basic morphologically distinct groups of crystal aggregates were distinguished

4.2 RESULTS RELATED TO INFLUENCE OF BACTERIA ON PROPERTIES OF CONCRETE

4.2.1 Compressive Strength

The objective of this research work is to highlight the effect of bacteria on the compressive strength of concrete. Results of the influence of bacteria (*Bacillus sphaericus*) on the compressive strength of concrete containing fly ash and silica fumeisgiven in Table 4.1 and shown.

Table 4.1: Compressive Strength of Concrete. Values are ± S.D (n=3)

Mix	Without Bacteria		Bacteria (10^3 cells/ml)		Bacteria (10^5 cells/ml)	
	28 Days	91 Days	28 Days	91 Days	28 Days	91 Days
10% FA	26.4±0.10	27±0.13	27.1±0.15	28±0.17	27.6±0.21	28.6±0.16
20% FA	25±0.12	26±0.22	26.1±0.23	27±0.22	26.5±0.24	27.5±0.21
10% FA + 10% SF	30±0.54	31±0.53	31±0.42	32±0.21	32.7±0.5	36±0.1
20% FA + 10% SF	29±0.32	29.7±0.34	29.4±0.28	30±0.12	30±0.11	31±0.1

FA: Fly ash; SF: Silica fume

It can be seen from Table 4.1 that compressive strength of fly ash concrete without bacteria was 26.4 MPa, 25 MPa and 24 MPa, respectively with 10, 20, and 30% fly ash content at the age of 28 days, and strength increased marginally at the age of 91 days (Table 4.1).

Further, with the inclusion of bacteria, compressive strength of fly ash concrete increased with increase in bacteria cell concentration up to 10^5 cells/ml Maximum increase in compressive strengths was achieved at 10^5 cells/ml for all fly ash concretes; 10^3 cells/ml shows least compressive strength. Compressive strength of concrete containing both fly ash and silica fume is given,it can be seen the compressive strength of concrete (containing varying percentages of fly ash & silica fume) without bacteria was between 28 and 30 MPa at 28 days and between 29 and 31 MPa at 91 days. Concrete made with 10% fly ash and 10% silica fume gave the best results; 30 MPa at 28 days and 31 MPa at 91 days.

Inclusion of bacteria enhanced the compressive strength of concretes made with fly ash and silica fume. With 10% fly ash+10% silica fume, compressive strength of concrete with 10^3 cells/ml bacterial concentration was 31 and 32 MPa at 28 and 91 daysrespectively. Further with 30% fly ash + 10% silica fume, the strength achieved was 28.3 MPa and 29 MPa respectively at 28 and 91 days. Even in the combination of 30% fly ash with 5% silica fume minimum strength (26 MPa)

was observed, compared to 10% and 20% fly ash. In case of bacterial concrete with concentration of 10^7 cells/ml, 10% fly ash
+ 10% silica fume gave best strength when compared to other combinations; 20% fly ash
+ 10% silica fume and 30% fly ash + 10% silica fume.

In fly ash and silica fume concrete, there was 23% improvement in compressive strength of concrete (10% fly ash and 10% silica fume) with the inclusion of 10^5 cells/ml bacterial cells. Similarly, there was 19% and 12% improvement in compressive strength of concretes with 20 and 30% fly ash and 10% silica fume contents (each) with the addition of 10^5 cells/ml bacterial cells shown, respectively.The improvement of compressive strength of Portland cement mortar was due to microbiologically induced calcite precipitation, as was reported by Ramachandran et al., (2001). It was found that the 28-day compressive strength of the control cubes was about 55±1MPa while the specimens made with 10^3 cellscm^{-3} had a compressive strength of about 65±1MPa. The compressive strength and stiffness of cracked concrete specimens can be improved by use of industrial by-products as a good nutrient source to produce microbial concrete as studied by Zhong and Yao (2008); Achal et al., (2009, 2010). The cement was replaced with three percentages of fly ash which reduced the 28-day compressive strength but there was continuous improvement of compressive strength after 28 days. This indicated the pozzolanic action of fly ash. The study was carried out by (Carette and Malhotra, 1984; Saraswathy et al., 2003; Siddique, 2004). Study on compressive strength was carried out by (Wolseifer 1984; Huang and Feldman 1985; Luther and Hansen 1989; Gleize et al., 2003; Sakr 2006; Zhang 2008) and concluded that concrete mixed with silica fume had the high compressive strength.

In our research work, the improvement in compressive strength by *Bacillus sphaericus* was probably due to deposition of $CaCO_3$ on the microorganism cell surfaces and within the pores of, which plug the pores within the mortar. These results have demonstrated that concrete with enhanced strength and low-permeability concrete could be produced. The increase in the matrix strength (for concrete made with bacterial cells) would have resulted in lesser mean expansion and would have eventually increased the overall durability performance of the concrete. Thus, increase in compressive strengths is mainly due to consolidation of the pores inside the cement mortar cubes with microbiologically induced calcium carbonate precipitation.

4.3 ECONOMICS OF BACTERIAL CONCRETE

Two of the most important properties; compressive strength and permeability were considered for initial comparison of bacterial concrete with that of control concrete. Another important aspect considered for the comparison was of the overall costs involved in making one cubic meter of concrete. In concrete, quantity of cement, fine aggregates and coarse aggregates per cubic meter were 390, 569 and 1165 kg, respectively.

In this study, bacteria concentration of 10^5cells/ml has been found to be optimum. Because of this reason, for economics of bacterial concrete, bacteria concentration of 10^5cells/ml has been taken into account.

The amount of cement varied with percentage replacement with fly ash and silica fume. In each sample, the amount of bacteria added was 6 gm. The rate (price) of the different components of concrete are taken as per the prevailing rates in typical Indian market and are as follows:

Rate of cement = Rs.7 per kg
Price of aggregates per m^3 =Rs. 957/-
Rate of Fly Ash = Rs. 0.00/-(being a byproduct of thermal power plant)
Rate of Bacteria = Rs. 88 per gm
Rate of Silica Fume = Rs. 44 per kg

The comparison of cost, permeability and compressive strength of concrete using different ratio of fly ash, silica fume and bacteria was done for 1.0 cubic meter of concrete and is shown in Table 4.2.

In Table 4.2, first three columns in the table refer to the cost, permeability and compressive strength of the specific mixture. The next three columns refer to the percentage change of cost, permeability and compressive strength with respect to that of control concrete. The negative sign represent percentage decrease and positive values reflect the increase in percentage. However, in case of permeability, decrease in its value(negative sign) indicates that concrete quality(durability) has improved and further for compressive strength positive sign indicate good quality concrete. Negative in case of cost represents an economic advantage.

Table 4.2: Comparison of cost, permeability and compressive strength of bacterial concrete with control concrete

Sr. No.	Mix	Cost (INR)	Compressive Strength (MPa)	Change in cost (%)	Change in Compressive Strength(%)
1	**Cem.+Agg.**	3687	28		
2	**Cem.+Agg.+Fly Ash 10%**	3414	27	-7.40	-3.57
3	**Cem.+Agg.+Fly Ash 10%+Bac.**	3942	28.6	6.92	2.14
4	**Cem.+Agg.+Fly Ash 10%+Silica Fume 10%**	4135.5 4663.5 4857	29 33 31	12.16 26.48 31.73	3.57 17.86 10.71
5	**Cem.+Agg.+Fly Ash 10%+Silica Fume 10%+Bac.**	5385	36	46.05	28.57
6	**Cem.+Agg.+Fly Ash 20%**	3141	26	-14.81	-7.14
7	**Cem.+Agg.+Fly Ash 20%+Bac.**	3669	27.5	-0.49	-1.79

Agg: Aggregates; Cem:Cement;

From practical point of view, economics plays a very important role. The perfect concrete mixtures with bacteria would be one(s) that do not enhance cost significantly with the inclusion of bacteria, but significantly improve the compressive strength and reduce the permeability. Keeping this parameter in mind, the concrete mixtures at Sr.No. 3,9,11,15 and 17 were found to be optimum. The reason for selection of these mixtures is due to the following reasons:

a. Decrease in percentage of permeability.It indicates improvement in concrete quality (microstructure), thereby increasing the life of structure

b. Economically viable with respect to cost. The mixture at Sr. No. 3 has cost increase of 6.92%, Sr. No. 9 has cost decrease of -0.49%, Sr. No. 11 has cost increase of 19.08% Sr. No. 15 has cost decrease of -7.89% and Sr. No. 17 has cost increase of 11.68%.

c. Concrete have high compressive strength, therefore, marginally negative compressive strength mixtures have been considered.

It is known that there exists an inverse relationship between permeability and durability of concrete. The decrease in permeability of concrete will increase the durability of the same and vice versa. In our case, the decrease in permeability by using bacteria, fly ash and silica fumes in the specific quantities has been around 50% and more in all the selected samples. While the exact increase in life of concrete in number of years with decrease in permeability has not been established, still research in this field is in progress.

But it has been studied for high performance concrete by Cusson et al., (2012) that the service life of concrete bridge decks can increase over 100 years with low permeability high performance concrete as compared to only 20 years for normal concrete decks. The permeability for such concrete reduced nearly 30% from the control concrete thus giving favourableresults.In respect to the cost, it was concluded by Jonkers et al., (2012) that if the bacteria adds 50% to the concrete cost, it would increase the total cost of construction by around 1 to 2% which will be much less than the maintenance costs incurred. It was also concludedthat the bacteria could remain as dormant spore for a period upto 50 years without mediaor water, which can again become active upon receiving optimum conditions for its revival and growth which would be beneficial for enhancing the durability by reducing the permeability through calcite production.

The benefit/cost ratio for the samples selected above (Sr.No. 3, 9, 11, 15 and 17) is given in Table 4.3 wherein,

A: Value of specific property for mixture (from previous Tables 4.4, 4.5, 4.6 and 4.7)

B: Improvement of value with respect to control concreteFor**compressive strength**:

B = Value of property of mixture / Value of property of Control concrete

(Increase in compressive strength is improvement)

For **permeability, water porosity and water absorption**

B= 1- (Value of property of mixture / Value of property of Control concrete)

(Since, Decrease in permeability, water porosity and water absorption is considered improvement)

C: Benefit for specific property. Calculated as product of (B) and weightage factor.

Weightage factor is a measure of importance of specific property of concrete to bringthem to same scale for calculations. In our case, all four properties compressive strength, permeability, water porosity and water absorption have been considered equally important; therefore, highest weightage factor of 10 is given to each.

Note:

Benefit = Sum of (C) for compressive strength, permeability, water porosity and water absorption.

Table 4.3 Benefit/Cost Ratio for selected samples

Property	**Weightage Factor**	**Cem. +Agg. +Fly Ash 10%+Bac.**			**Cem. +Agg.+Fly Ash 20%+Bac.**		
		A	B	C	A	B	C
Compressive Strength(MPa)	**10**	28.6	1.02	10.21	27.5	0.98	9.82
Water Absorption (%)	**10**	2	0.89	8.9	3	0.83	8.3
Benefit		**24.41**			**26.32**		
Cost (Rs.)		3942			3669		
Benefit/Cost		**0.0062**			**0.0072**		

Agg: Aggregates; Cem:Cement; Bac:Bacteria.

The total benefits, sum of (C) for compressive strength, permeability, water porosity and water absorption divided by the cost of give the Benefit/Cost Ratio. Out of the ratios, highest **0.0076** is of mixture Cement+Aggregate+Fly Ash 30%+Silica Fume 5%+Bacteria which is the optimum mixture while ratio for control concrete taking into account the improvement of property isone third of this mixture.

CHAPTER 5

CONCLUSIONS

5.1 GENERAL

The present work investigated influence of bacteria on the permeation properties of concrete containing supplementary cementing materials. The supplementary cementing materials used were fly ash and silica fume which replaced cement partially by percentage of its weight. The presence of bacteria played significant role on permeation properties of concrete. The properties studied were on M20 grade of concrete. On the basis of the results from the present study, following conclusions are drawn.

5.2 IDENTIFICATION AND SELECTION OF BACTERIA

5.2.1 Bacterial Isolation

i. The bacteria in order to survive in the pH of cement required to be isolated from a similar type of medium. Since, the pH of cement is on alkaline side it was concluded to isolate the bacteria from alkaline medium.

ii. Taking into account the pH, alkaline media commonly available for isolation of bacteria were taken into consideration. It was concluded to isolate bacteria from rhizopheric soil (tulsi plant), alkaline soil and sewage sludge.

5.2.2 Sequencing and Identification of Bacteria

The comparison of a nucleotide query sequence with the nucleotide sequence database revealed that query sequence obtained showed similarity with *Bacillussubtilis*(Strain 3), *Bacillus lichniformis*(Strain 4),*Bacillussphaericus*(Strain5). It was concluded that the bacterial strain 5, namely *Bacillus sphaericus*, would be used for further research and experimentation of this work.

5.2.3 Optimization of Bacteria

i. Bacterium was suspended in a sterile saline solution (0.85% NaCl), dilutedproperly and plated on agar containing urea (20 g/l), $NaHCO_3$ (2.12 g/l), NH_4Cl (10 g/l), Nutrient broth (3 g/l), $CaCl_2.2H_2O$ (25 g/l). Incubation was done at 28˚C. Further studies were performed for optimization of bacteria. To check the effect of bacteria on concrete properties, the bacterial culture of 10^3, 10^5cells/ml were prepared

(*Bacillus sphaericus*). Out of these doses 10^5 cells/ml was considered to be optimum.

5.3 SUPPLEMENTARY CEMENTING MATERIALS

i. The concrete mixes were designed with constant cement, fine aggregate, coarse aggregate. Control concrete mixture was designed as per IS 10262-1982 to have 28-day compressive strength of 30 MPa. The cement was partially replaced with 0, 10 and 20%fly ash in addition to 0, and 10% silica fume by weight of cement. All the different combinations of fly ash with silica fume and bacterial concentrations were studied for various properties. Varying amount of bacterial culture (*Bacillus sphaericus*) used were of 10^3, 10^5 cells/ml.

5.4 PROPERTIES OF CONCRETE

5.4.1 Compressive Strength

i. Compressive strengths for all concrete mixtures were designed for M20 grade. The compressive strengths observed for 28 days and 91 days with bacterial addition of concrete mixes were found to be more than 24 MPa.

ii. The maximum compressive strength after 91 days was observed for a mixture with 10% fly ash and 10% silica fume which had 10^5 cells/ml bacterial concentrations. The compressive strength for 10^5 cells/ml was 11% more than that in 10^3 cells/ml. It may be concluded that increase in compressive strength was result of optimum mixture of SCMs and optimum doze of bacteria.

iii. The minimum compressive strength observed after 28 days and 91 days was in the mixture which had 30% fly ash only. The compressive strength increased but marginally, with the introduction of bacteria in mixtures at different concentrations. It may be concluded that the addition of bacteria has a limitation to increase the compressive strength when high percentage of cement is replaced by fly ash.

iv. Compressive strength in all cases increased with increase in age for all mixtures with or without bacteria. However, the rate of compressive strength increase was higher in case of mixtures with bacteria than mixtures without bacteria.

v. From the above it has been concluded that the improvement in compressive strength was due to deposition of calcite on the bacteria cell surfaces within the pores which and confirmed which revealed calcium carbonate precipitation.

5.5 ECONOMIC STUDY OF BACTERIAL CONCRETE

i. Different mixtures were compared based on cost incurred in making one cubic metre of mixture. Mixtures having low increase in cost, increase in compressive strength and decrease in permeability were selected for further comparison.

ii. Mixtures were compared for benefit to cost ratios considering equal weightage factors for increase in compressive strength and decrease in permeability, water porosity and water absorption.

iii. Mixture made with Cement + Aggregates + Fly Ash 30%+Silica Fume 10%+Bacteria showed the highest benefit/cost ratio and was considered as optimum mixture.

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