Influence of Bacteria on the Permeation Characteristics of Concrete made with Supplementary Cementing Materials

A Thesis

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By

Navneet Chahal

(Roll No. 900800008)

Department of Biotechnology and Environmental Sciences

Thapar University, Patiala-147004

Punjab (India)

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Dedication

This thesis is dedicated to my lovable son (Naaz), my husband Jastej Singh Sra and My parents whose endless efforts helped me to attain the Doctor of Philosophy.

CERTIFICATE

Certified that the thesis **"Influence of Bacteria on the Permeation Characteristics of Concrete made with Supplementary Cementing Materials"** which is submitted by Ms. Navneet Chahal, in fulfillment of the requirement for the award of degree of **Doctor of Philosophy** in the Department of Biotechnology and Environmental Sciences (DBTES), Thapar University, Patiala, is a record of candidates's own original and independent research work carried under our supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any university or institute for award of any degree.

(Dr. Rafat Siddique)
Senior Professor & Dean of Faculty Affairs
Department of Civil Engineering
Thapar University, Patiala
Punjab (India)

(Dr.Anita Rajor)
Assistant Professor
DBTES, Thapar University
Patiala, Punjab (India)

DECLARATION

I hereby declare that the research work presented in this thesis entitled "*Influence of Bacteria on the Permeation Characteristics of Concrete made with Supplementary Cementing Materials*" submitted for the award of the degree of Doctor of Philosophy in the Department of Biotechnology and Environmental Sciences, Thapar University, Patiala 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.

Navneet ...

(Navneet Chahal)

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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. The initial objective of the research work involved the isolation of urease producing bacteria from alkaline, rhizospheric soil and sewerage sludge. The bacteria were identified by the ability to sustain itself in alkaline environment of cement/concrete. All the bacterial isolates were analysed through DNA sequencing and the bacteria identified as *Sporosarcina pasteurii*, showed maximum urease production when it was grown on urease agar and broth. The sufficient urease activity allowed application of *Sporosarcina pasteurii* for biocementation.

The significant objective of the research work further involved the use of ureolytic bacteria (*Sporosarcina pasteurii)* 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 10^3,10^5 and 10^7 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, 20 & 30%, and that of silica fume was 0, 5 & 10%. The experiments were carried out to evaluate the effect of *Sporosarcina pasteurii* on the compressive strength, water absorption, water porosity and rapid chloride permeability of concrete made with fly ash and silica fume up to the age of 91 days. The test results indicated that inclusion of *Sporosarcina pasteurii* 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 within the pores which was scanned by electron microscopy and confirmed by XRD which revealed calcium carbonate precipitation. This precipitation reduced the chloride permeability in concrete with fly ash and silica fume.

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 BACTERIA

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 influence the permeation properties of concrete.

1.1.1 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.

1.1.2 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 of non-essential cellular proteins starts.

1.1.3 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).

Bacterial growth needs nutrients for its sustenance which is further affected by the type and amount of nutrient in system. Some of the nutrients helpful in the growth of bacteria are CO_2, N, P, K, Mg (Mitchell and Ferris, 2005). The activity of bacteria also depends upon amount and type of water which includes soluble materials, 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).

1.2 UREOLYTIC AND CARBONATE BIOMINERALIZATION

Biomineralization is a biologically induced precipitation in which an organism creates a local micro-environment by providing optimal extracellular chemical precipitation of mineral phases (Hamilton, 2003). Various natural environments have 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 in attaining 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 give rise 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.

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

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

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

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

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

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

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

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 while 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 which increases the mineral saturation and induces nucleation (Schultze et al., 1996). In the ureolysis, 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

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 or protection (Ghannoum and O'Toole, 2004). The EPS matrix is composed primarily of polysaccharides. 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 and bioremediation (Mitchell and Ferris, 2005). 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*, also reclassified as *Sporosarcina pasteurii* by 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, endospore forming bacteria commonly found in soil (Todar and Kenneth, 2005). *Sporosarcina pasteurii*, a member of this genus, converts urea to ammonium carbonate more actively than any other known bacterium. Therefore, *Sporosarcina 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 *Sporosarcina 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 *Sporosarcina pasteurii*, *Bacillus psychrophilus*, *Bacillus globisporus*, *Planococcus okeanokoites*, and *Filibacter limicola* .

Microbiologically induced (also called "bacteriogenic") calcium carbonate precipitation is comprised of a series of complex biochemical reactions, including concomitant participations of a bacterium like *Sporosarcina pasteurii*, urease (urea amidohydrolase; EC 3.5.1.5), and high pH. In this process, an alkalophilic soil microorganism, like *Sporosarcina pasteurii*, plays a key role by producing urease that hydrolyzes urea to ammonia and carbon 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. 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):

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

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

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

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 by 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 bioremediation processes can be more environmentally friendly, efficient, and cost effective as the use of costly reagents would be eliminated.

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 possible by bacteria as it helps in 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 (Adolphe et al., 1990).

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. Concrete is designed to crack in order to activate the steel reinforcement bars inside the structure. The steel reinforcement bars will prevent cracks to open too much. But if the entry of water chloride ions, CO_2, sulfide ions, nitrate ions, etc is not avoided it results in rusting of steel reinforcement causing extensive damage to the structure. 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 as well as environmentally safe method to repair cracks 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 *Sporosarcina pasteurii* 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). Rodriguez et al. (2003) studied that *Myxococcus xanthus* is capable of precipitating calcium carbonate in limestone statues and carvings by depositing calcite grains on the pore of walls without completely plugging the pore. This is possible as the bacteria helps in improving the impermeability through deposition of calcite. The resulting crystals are strongly attached and more resistant to stress.

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 1.1.

Table 1.1: Bacteria used in concrete

Applications	Types of Bacteria
Bacterial concrete as crack healer	*Sporosarcina pasteurii* *Deleya halophila* *Halomonas eurihalina* *Myxococcus xanthus* *Bacillus megaterium*
Bacterial concrete as surface treatment	*Bacillus sphaericus*
Bacterial concrete as water purifier	*Bacillus subitilis* *Bacillus sphaericus* *Thiobacillus*

1.4 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.

1.4.1 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.

1.4.1.1 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.

1.4.1.2 Different methods to improve concrete durability

- **Chemical methods:** By applying epoxy coating which thereby reduces steel contact 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 the concrete 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 the remediation 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.

1.5 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.

1.5.1 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 collection

systems 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.

1.5.1.1 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 (Figure 1.1). 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.

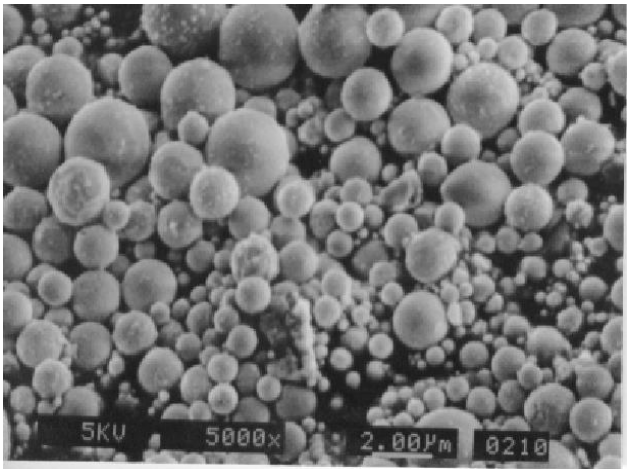


Figure 1.1: SEM Image of Fly Ash Particles at 5,000x Magnification.

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 pozzolan and

calcium hydroxide in presence of water. 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).

Particle morphology

Fly ash particles consist of different shapes and surface characteristics which has been investigated using scanning electron microscope (SEM) and energy dispersive x-ray analysis (EDX) (Joshi, 1970; Diamond, 1985; Joshi and Lam, 1987; and Mehta, 1988). It has been established that some particles are solid while others are hollow. The surface of the solid and hollow spherical particles of low-calcium oxide fly ashes are generally smooth than those of high-calcium oxide fly ashes.

1.5.1.2 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 opaline chert, 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 1.2.

Table 1.2: 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

1.5.1.3 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 Siddique et al., (2003a).

1.5.1.4 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 as suggested by Siddique et al. (2003b).

1.5.1.4.1 High volume uses

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

1.5.1.4.2 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

1.5.1.4.3 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.

1.5.1.4.4 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.

1.5.1.5 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 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.

1.5.2 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.

1.5.2.1 Properties of silica fume

1.4.2.1.1 Physical properties

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

1.5.2.1.2 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.

1.5.2.2 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.

1.5.2.3 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 concrete 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 or secondary applications (remedial operations including leak repairs, 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 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.

1.5.2.4 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.

1.6 OBJECTIVES OF PRESENT WORK

- Isolation of bacteria from soil and Sewage sludge. Screening and its identification on the basis of calcite formation, Gram character and 16S r DNA analysis.
- Addition and Optimization of the bacterial inoculum on the basis of calcite formation.
- Preparation of concrete mixtures by partial replacement of cement with 0, 10, 20, and 30% fly ash, and also the addition of silica fume (5 and 10%) by weight of cement in M20 or 25 grade of concrete with varying bacterial inoculum.
- Determination of influence of bacteria on the porosity, water absorption, compressive strength and permeability 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 and

recovery 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 was

observed 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 as suggested by 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 carbonate

precipitation 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 ureolytic biodeposition 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 of

biological 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. In this study living and dead cells of *Sporosarcina pasteurii* and *Pseudomonas aeruginosa* were investigated by storing the mortar specimens in a solution containing urea and calcium chloride for 7 days. The presence of *Sporosarcina pasteurii* was shown to increase the compressive strength of mortar cubes while the contribution of *Pseudomonas aeruginosa* to the strength was found to be insignificant.

Intracellular urease constitutes close to 1 % of the cell dry weight as produced by *Sporosarcina pasteurii* (Bachmeier et al., 2002). An organism creates a local environment with conditions 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 by SEM and XRD analysis.

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).

2.3.2 Water Absorption and Permeability

Water absorption is defined as the amount of water absorbed by a material when immersed in water for a stipulated period of time. It is calculated as the ratio of the weight of water absorbed by a material, to the weight of the dry materials. Permeability may be defined as the measure of the ability of a material to allow fluids to pass through it.

Bacterial deposition of a layer of calcite on the surface of the specimens resulted in a decrease of capillary water uptake and gas permeability (De Muynck et al., 2008a). The effects of bacterial carbonate precipitation (biodeposition) on the durability of mortar specimens with different porosity. The surface deposition of calcium carbonate crystals decreased the water absorption with 65 to 90% depending on the porosity of the specimens. As a consequence, the carbonation rate and chloride migration decreased by about 25–30% and 10–40% respectively.

Nemati et al. (2005) reported that due to calcium carbonate forming reactants, the decrease in permeability was observed. Nolan et al. (1995) concluded that application of bacteria on top of concrete helps in lowering the permeation properties making the concrete more durable. The calcite crystals when precipitated helps in reducing the water permeability moreover reduction in other harmful substances was observed. The calcite deposition on the surface of specimens by bacteria helps in decreasing the capillary water uptake (Van Tittelboom et al., 2010). Other author named Ferris et al. (1987) reported that bacteria served as nucleation sites in a metal. Achal et al. (2011) concluded that bacteria played an important role in reducing the chloride permeability of concrete. The effects of *Sporosarcina pasteurii* (Bp M-3) on the permeability of concrete was determined by the average charge passed through the specimens. For the concrete samples treated with bacteria, the permeability class type was ‘‘low’’ whereas for control concrete specimens the class changed to ‘‘moderate’’. The average charge passed was 3,177 C for the control samples treated with bacteria, whereas for samples prepared with bacterial cells in Nutrient Broth and Corn Steep Liquor media it was 1,019 and 1,185 C, respectively.

Chandramouli et al. (2010) studied the permeability profile for different grades of concrete with varying percentages of addition of glass fibres. It was observed that plain concrete increased permeability whereas with increased percent replacement of fibre 0.10% there was a consistent reduction in permeability. It was observed that chloride permeability at the age of 90 days for M30, M40 and M50 grade of concrete was 2345 C, 1840 C and 1650 C respectively; which fall under the ‘moderate’ for M30 and ‘low’ for M40 and M50 grade of concrete.

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) studied the importance of 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 the

internal 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 when

replacement level was two or three-to-one basis, the strength reduced at 3 days as compared to the control but the later-age strength was higher. Hence, it was recommended 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 tha both 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 strength increased 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 there 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 Permeability

There have been studies about the effects of fly ash on permeability of concrete. The size of particles of fly ash have effect on permeability of concrete. The finer the size of fly ash the lower will be the permeability. Achal et al. (2009, 2010) suggested a technique incorporating usage of Industrial by-products to improve the compressive strength and stiffness of cracked concrete specimens. It was observed that Lactose Mother Liquor (LML) and Corn Steep Liquor (CSL) can be used as a successful nutrient source to produce microbial concrete. These by-products supported fast growth of *Bacillus* species and further helped in attaining maximum urease and calcite production.

Achal et al. (2011) studied the influence of the microbially induced calcite precipitation on the water absorption and permeability of mortar cubes. It was observed that over a period of 168 h (7 days), the cubes containing fly ash (0%, 10% and 20%) with bacterial cells absorbed nearly 3.5 times less water than the control cubes. But in case of cubes containing 40% fly ash, mortars absorbed two times less water compared to control. The presence of bacteria resulted in a significant decrease of the water uptake compared to control specimens. The deposition of a layer of calcium carbonate on the surface and inside pores of the mortar specimens resulted in a decrease of water absorption and permeability. Once the pores are filled with inert material like calcium carbonate, continuous channels for passage of water, air and pollutants are sealed and reduction in ingress of water and chlorides is observed. This deposition by microbial action can seal the voids, pores, and cracks of microscopic size where other sealants cannot go. This resulted in limited ingress of harmful substances.

Admixtures for Concrete, (1963) revealed that RCPT values for Latex-modified concrete and Silica-fume concrete fall under the Class type of "Very Low". Fly ash minimizes water demand and by reducing the bleed channels; all of which increase concrete density. These factors yielded concrete of low permeability with low internal voids.

The water flow is restricted when there is calcite precipitation which reduces the pore throats. Nemati et al. (2005) observed that there was reduction in permeability by 98% within a Berea sandstone core, and Whiffin et al. (2007) also found reduction in permeability to an extent of 22% to 75% of the initial permeability.

Naik et al. (1994) studied the influence of adding large amounts (50 and 70% cement replacement) of Class C fly ash on the chloride permeability of concrete and found that it decreased with age. At the age of 2 months, all concrete mixtures except the 70% fly ash mixture exhibited moderate (2000-4000 coulombs) permeability in accordance with ASTM C1202 specifications. The 50% fly ash concrete mixture showed lower permeability compared to control concrete at all ages. The 70% fly ash mixture also showed low permeability than control concrete after 3 months.

The durability with regard to frost action is improved substantially by addition of high-volumes of low-calcium fly ash in concrete. This increased durability was the results of

low permeability of the concrete mixture Malhotra (1990). Good durability and excellent mechanical properties in regard to repeated freezing and thawing were shown by the concrete mixture when Class F fly ash was used Langley et al. (1992); Mehta (1988). The mixture had shown low permeability to chloride-ions. It was also observed by Ellis et al. (1991) that the permeability of concrete decrease with increase in usage of Class C fly ash.

The permeability was measured by cylindrical concrete samples of 50 mm diameter and 40 mm thick containing fly ash and it was reported it showed decreased permeability Cabrera and Lynsdale (1988). It was reported by Hui-sheng et al. (2009) that with increase in fly ash, the permeability coefficients increased significantly. Similar results of reduced permeability in fly ash induced concrete as compared to normal concrete were also reported by Bamforth (1991); McCarthy et al. (1988); McCarthy and Dhir (2005). But in case of high performance concrete the incorporation of fly ash resulted in marginal decrease in permeability according to Khan (2002). Concrete mixtures made with 15, 30 and 50% fly ash as cement replacements, exhibited reduction in the permeability values by 50, 60 and 86%, respectively, compared to concrete without fly ash as studied by Thomas and Matthews (1992).

2.5.3 Water Absorption

The increase in fly ash content would reduce the water absorption. The replacement of 35 to 50% of cement with fly ash, resulted in 5 to 7% reduction in the water requirement for obtaining the designated slump as studied by Ravina and Mehta (1986). It was also studied that the rate and volume of the bleeding water compared to control mixture was either higher or the same compared. The concrete containing fly ash usually reduces the water content for a given consistency as concluded by Lane and Best (1982). Studied have also been carried out for the effect of shape of fly ash effecting water absorption and water reduction. It has been reported by Helmuth (1987) that in case the water reduction was due to the spherical shape of the fly ash particles, it would increase with increase in fly ash content. The percentage replacement has effect on the water demand as per Yuan et al. (1982). As per the study, the water demand reduced when the quantity of fly ash was between 15 and 20%, and increased when fly ash content was more than 20%. The increase in water demand was due to additional specific surface and porous grains, and decrease due to deflocculation by adsorption of fine grains of fly ash on cement clusters.

The use of fly ash having particles coarser than 45μm and fly ash with high amount of unburned carbon showed higher water demand as per study of Owens (1979).

2.5.4 Sulfate Resistance

The sulphate resistance of concrete can be reduced by sulphate attack. Larsen (1985) reported that there are many reasons for the alteration of pore structure of cement paste. This may be due to addition of fly ash which include chemical attack, sulfate or with the additional chemical-mineral interaction. Joshi (1987) reported that the sulfate resistance of cement-sand mortar improved significantly at percentage replacement below 10%. Prusinski and Carrasquillo (1995) concluded that sulfate resistance of concrete made with class C fly ash may vary due to the gypsum content of the mix. Mehta (1993) concluded that fly ash increases the durability of concrete exposed to sulfate attack. Further it was observed that value or degree of improvement may vary with either the cement used or the fly ash.

2.5.5 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.

2.5.6 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 of carbonation. 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) and 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) and Langley et al. (1989) concluded that concrete containing low-calcium Class F fly ash reduces the rate of temperature rise more when ompared to high-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) and 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 Water Absorption and Permeability

Water absorption is defined as the amount of water absorbed by a material when immersed in water for a stipulated period of time. It is calculated as the ratio of the weight of water absorbed by a material, to the weight of the dry materials. Water absorption tests were carried out at the ages of 28 & 91 days as per ASTM C 642.

Silica fume has been used in concrete with an additional replacement of upto 15 percent. But it was also observed that with an addition of 15 percent, the concrete becomes more strong and brittle in nature. Ozyildirim (1986) and Plante and Bilodeau (1989) showed that addition of 8% of silica fume reduces the chloride permeability. The density of the matrix was increased due to the presence of silica fume. Sellevold and Redjy (1983) concluded that concrete containing silica fume demands less water requirements and this decrease may be due to reduction of concentration of contact points between the matrix.

2.6.3 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 were studied by Scott and Singh (2011).

2.6.4 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.

2.6.5 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 with 6 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.

2.6.6 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. Tenoutasse and Marion (1987) also concluded that silica fume being pozzolan can reduce alkali-sulfate resistance. 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, estimation of urease activity and optimization of the bacterial inoculums on the basis of 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.

3.1EXPERIMENTAL PROGRAM RELATED TO BACTERIA

3.1.1 Isolation and Identification of Bacteria

3.1.1.1 Sample collection

Samples were collected from rhizopheric soil, alkaline soil and sewage sludge. Rhizopheric soil samples were collected from Tulsi plant from Thapar University, Patiala (Punjab). Alkaline soil and sewage sludge was collected from Nirol factory, Tapa (Punjab). Samples were collected by sterilized tools and then further stored in sterile containers and kept at 4˚C until their use.

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 (Zeiss) and selected as positive based on visual crystal formation within 10 days. Positive isolates were purified through repetitive dilution and plating (as described above).

3.1.1.3 Microscopy and crystal nucleation site development

Crystal precipitating colonies were studied after 5 days and 10 days cultivation with stereomicroscopy. Digital images were captured with a ccd (Charged Coupled Device) camera. Large crystal aggregates that precipitated within a single colony of these isolates

were subsequently harvested from the agar surface, washed in sterile water and dried (28˚C, 3 days).The dried aggregates were ground to be appropriate particle size for XRD analysis, using a McCrone micronising mill. The grounded samples were then mounted in a sample holder and analysed (Xpert software).

3.1.1.4 Urease activity

All the isolates were tested for urease activity. This was done by streaking the purified cultures on urease test agar (Himedia) and inoculating test broth with viable liquid cultures as well as filtrates of the liquid culture.

3.1.1.5 Phenol hypochlorite assay method

The urease positive isolates were further tested for the urease activity. This was determined in the media according to the Phenol hypochlorite assay method. Ammonium chloride (50-100µM) was used as standard. The culture filtrates (250µl) were added to the mixture containing 1ml of 0.1M potassium phosphate buffer (pH 8.0) and 2.5ml of urea (0.1M). The mixture was incubated at 37˚C for 5 min followed by addition of phenol nitroprusside and alkaline hypochlorite, 1ml each and incubated at 37˚C for 25 min. Optical density was measured at 626 nm and one unit of urease is defined as the amount of urease is defined as the amount of enzyme hydrolyzing 1µmole urea/min (Natarajan, 1995).

3.1.2 Physiological and Biochemical Characterization

3.1.2.1 Growth profile of bacterial strains

The growth profile of bacterial strains was tested by taking the absorbance at 425nm at regular time intervals for 120 hours and corresponding cfu/ml were calculated.

3.1.2.2 Biochemical tests

3.1.2.2.1 Nitrate reduction test: To carry out the nitrate reduction test, nitrate broth was used. After incubating the nitrate broth, 2-3 drops of sulfanilic acid and alpha napthylamine were added. If the organisms have the ability to reduce the nitrate to nitrite, the nitrites in the medium will form nitrous acid. If the medium turns red, the organism is termed to be positive in reducing nitrate or otherwise termed as negative.

3.1.2.2.2 Oxidase test : Bacterial culture was grown on agar plates. Then one drop of reagent (N,N,N',N'-tetra- methyl-p-phenyenediamine dihydrochloride) was added. If the medium turns violet to purple immediately or in less than 30 seconds, the organism is termed as positive in oxidase test and if the reaction is delayed it was considered to be negative.

3.1.3 Morphological Studies

3.1.3.1 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.

3.1.3.2 SEM and XRD

The morphology and chemical constituents of the bacteria were analyzed with SEM and XRD. Samples were completely dried at room temperature, then examined at accelerating voltages ranging from 30 to 35 kV by a SEM (Zeiss EVO50). Samples were gold coated with a sputter coating Emitech K575 prior to examination. XRD spectra were obtained using an X'Pert PRO diffractometer with a Cu anode (40 kV and 30 mA) and scanning from 3° to 60°. Each bacterial sample was crushed and ground before mounting onto a glass fibre filter using a tubular aerosol suspension chamber (TASC). The components of the sample were identified by comparing them with standards established by the International Center for Diffraction Data. All experiments were performed in triplicate.

3.1.3.3 EDX analysis of ureolytic bacteria

Mineral constituents of the isolates were further characterized by EDX analysis. Presence of high amounts of calcium in the bacterial isolates confirmed the presence of calcite in the form of calcium carbonate. The isolates were grown at a higher rate in the presence of oxygen and consequently induced active precipitation of calcium carbonate around the surface area.

3.1.4 Extraction of DNA

3.1.4.1 Isolation of Genomic DNA

Bacterial cultures were inoculated into 25 ml of nutrient broth in a 250 ml Erlenmeyer flask and incubated for 14-19 hrs at 37˚ C under shaking shaking condition (120 rpm). Then by centrifugation the liquid culture (2.0 ml) of bacteria were harvested at 8,000 rpm for 1 min. The pellets were resuspended with 800µl saline-EDTA, 10µg lysozyme was added. This was incubated at 37˚ C (30 min), suspension was mixed thoroughly, followed by addition of 200µl SDS (10%), then the cell suspension was incubated at 65˚C (15 min).

To remove the cell debris and proteins, the suspension was extracted with organic solvents [Phenol: Chloroform: Isoamyl alcohol (25: 24:1)].Then again the suspension was centrifuged for 10 min at 12,000 rpm. The upper aqueous phase was then extracted with an equal volume of phenol:chloroform:isoamyl alcohol (25:24:1). The nucleic acids were extracted in order to precipitate, Isopropanol (0.7) was added to the aqueous phase, followed by centrifugation for 10 min (12,000 rpm). The DNA pellets were washed with 750µl EtOH (70%) and microfuged for 5 min. Then the pellets were resuspended in 40µl TE buffer/milliQ water and stored at 4˚C.

3.1.4.2 DNA purification

The DNA was purified by elution through DNA clean up system (Promega,USA), according to manufacturer's instructions to remove contaminants.

3.1.4.3 Electrophoresis of DNA on agarose gels

DNA was loaded on agarose gels prepared in 0.5X TBE, pH 8.0 using a 6X loading dye. Ethidium bromide (0.5µg/ml) was added in order to stain the gel prior to pouring. The

nucleic acids were then electrophoresed at 3 volts/cm for 45-60 mins and visualized on a U.V transilluminator.

3.1.4.4 Amplificaion of 16S r DNA and purification of PCR products

A DNA template for PCR amplification from pure cultures was obtained by extracting total genomic DNA. The PCR master mixture contained each deoxynucleoside triphosphate at a concentration of 200 µM, 1.5 mM $MgCl_2$, 10 µl of thermophilic DNA polymerase 10x reaction buffer ($MgCl_2$ free), 2.5 U of *Taq* DNA polymerase 400 ng of bovine serum albumin per µl, and enough DNase- and RNase-free filter-sterilized water (Sigma-Aldrich) so that the final volume was 100 µl. One microliter of DNA template was added to 24 µl of the master mixture.

PCR was performed in a 9600 thermal cycler (Perkin-Elmer, Norwalk, Conn.) with a program consisting of 10 min at 95°C, followed by 30 cycles of 1 min at 94°C, 1 min at 53°C, and 2 min at 72°C and a final elongation step for 10 min at 72°C.

Its quality was evaluated on 1.2% Agarose Gel, a single band of high-molecular weight DNA has been observed. Fragment of 16S rDNA gene was amplified by PCR from the above isolated DNA. A single discrete PCR amplicon band of 1500 bp was observed when resolved on Agarose Gel (Gel Image-1).The PCR amplicon was purified to remove contaminants. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out with 8F and 1492R primers using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of 1341 bp 16S rDNA gene was generated from forward and reverse sequence data using aligner software.

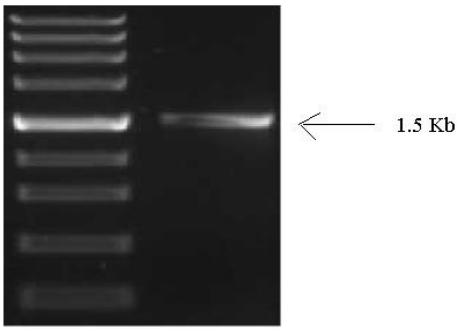


Figure 3.1 16S r DNA Amplification of *Sporosarcina pasteurii*

3.1.4.5 Analysis of sequence data

The 16S rDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 4

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 physical and chemical properties are shown in Table 3.1 and Table 3.2, respectively.

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

Physical Property	Value
Consistency of standard cement paste (%)	36
Initial setting time (minutes)	123
Final setting time (minutes)	174
Compressive strength (MPa) 3 day 7 day 28 day	 16 35 46
Specific gravity	2.9
Standard consistency (%)	34

Table 3.2: 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. Its physical properties and sieve analysis are given in Tables 3.3 and Table 3.4, respectively.

Table 3.3: Physical Properties of Fine Aggregate

Properties	Observed values
Bulk density , kg/m^3	1672
Specific gravity	2.15
Water absorption (%)	1.02
Moisture content (%)	0.16
Material finer than 75µ (%)	0.5
Fineness modulus	2.58

Table 3.4: 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. Physical properties and sieve analysis results are given in Tables 3.4 and Table 3.5, respectively.

Table 3.5: Physical Properties of Coarse Aggregates

Properties	Observed values
Maximum size (mm)	12.5
Bulk density (kg/m^3)	1650
Specific gravity	2.7
Total water absorption (%)	1.14
Moisture content (%)	Nil

Table 3.6: 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 and physical properties results are given in Tables 3.7 and Table 3.8 respectively

Table 3.7: 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

Table 3.8: Physical Properties of Fly Ash (ASTM C 618)

Colour	**Dark Grey**
Specific gravity	2.4
Bulk density (kg/m^3)	700
Surface area (kg/m^2)	19,000

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 include very high content of amorphous silicon dioxide. Small amounts of iron, magnesium, and alkali oxides were also found. Physical and chemical properties results are given in Tables 3.9 and Table 3.10, respectively

Table 3.9: Physical Properties of Silica Fume (ASTM 1240)

Colour	light grey
Specific gravity	2.5
Bulk density (kg/m^3)	700
Surface area (kg/m^2)	22,000

Table 3.10: 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

3.2.1.6 Water

Water used for casting specimens conformed to the requirements of BIS: 456-2000. Test results are given in Table 3.11

Table 3.11: Properties of Water

Properties	Observed value
PH	8.1
Dissolved solids (mg/l)	295
Suspended solids	Nil
Chlorides (mg/l)	22
Sulphates (mg/l)	71
MPN value/100 ml.	Nil

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 3.12

Table 3.12: 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, (*Sporosarcina pasteurii*) 10^3 cells/ml; 10^5 cells/ml and 10^7 cells/ml of inoculums. The detailed description of all mixes is given in Table 3.13.

Table 3.13: 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 (*Sporosarcina pasteurii).* 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)
- Water Absorption and Porosity (ASTM 642)
- Rapid chloride permeability test (ASTM C 1202)

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, *Sporosarcina pasteurii* 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 *Sporosarcina pasteurii*. Cubes were cast and compacted in a vibration machine. After de-molding, all

specimens 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 3.2.

Figure 3.2 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)

3.2.4.2 Water absorption and porosity

The water absorption test was conducted as per ASTM C 642 in order to determine the increase in resistance towards water penetration in concrete. The cube moulds of 70 mm

were prepared both with and without bacteria and fly ash+ silica fume. The concrete specimens were cured for 28 days and 91 days. After curing, the specimens were oven dried at 110˚C in oven, establishing a mass equilibrium of less than 0.5% between two measurements at 24 hours intervals. Then the specimens were immersed in water at approximately 21˚C for 48 hours and saturated mass after immersion was calculated. Then the specimens were placed in suitable receptacle, covered with tap water and were boiled for 5 hours, further the saturated mass after boiling was calculated. The specimens were suspended by a wire and the apparent mass in water was calculated as per the formula:

Volume of permeable voids % = (C-A) / (C-D) × 100

Where in; A = mass of the oven dried sample in air, grams

C = mass of sample after immersion and boiling, grams

D = apparent mass of sample in water after immersion and boiling, grams.

The total porosity (P) measurements are based on Archimedes Principle. The total porosity can be calculated from water saturated surface dry mass (m_{sat}), mass susupended in water (m_{water}) and oven dry mass (m_{dry}) :

$$P\ (\%) = (m_{sat} - m_{dry} / m_{sat} - m_{water}) \times 100$$

These moisture states are shown in Figure 3.3. Impact of water absorption is clearly illustrated in Figure 3.4.

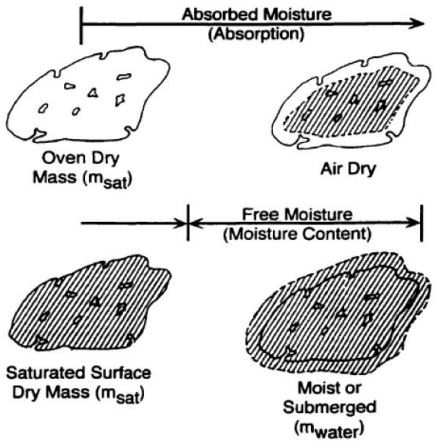


Figure 3.3 Schematic Diagram of Moisture States of Cement Based Materials (Nevielle 1973)

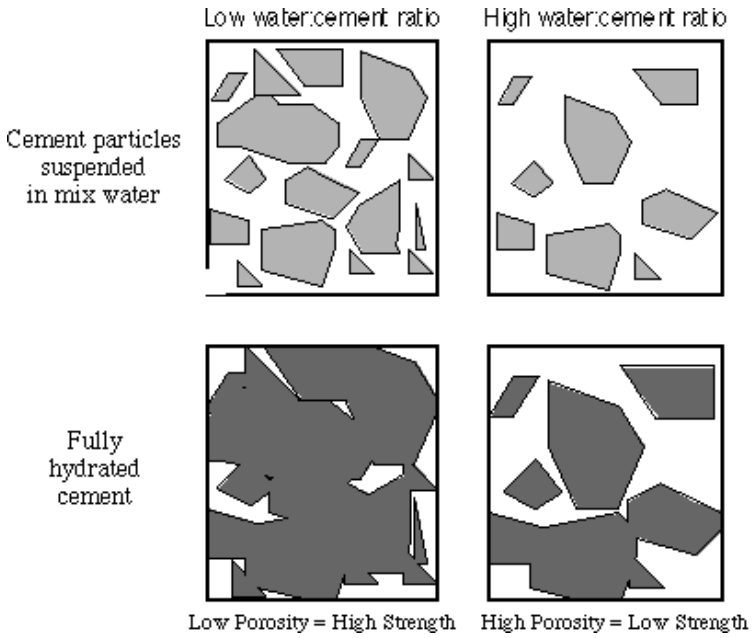


Figure 3.4 Impact of Water Absorption

3.2.4.3 Rapid chloride permeability

Corrosion is mainly caused by the ingress of chloride ions into concrete annulling the original passivity present. Rapid chloride permeability test (RCPT) has been developed as a quick test able to measure the rate of transport of chloride ions in concrete. One of the main characteristics influencing the durability of concrete is its permeability to the ingress of chloride. The chloride ion present in the concrete can have harmful affect on concrete as well as on the reinforcement. Swelling of concrete due to chloride ion penetration is 2 to 2.5 times larger than that observed with water penetration. So this test covers the experimental evaluation of electrical conductance of concrete to provide rapid indication of concrete resistance against chloride ion penetration.

Preparation of Samples

Cylindrical concrete disc of thickness (100mm×200mm) with and without bacterial culture were casted and cured in water for 28 and 91 days. Concrete mixture was designed using M-20 grade. Control mixture was 1:1.5:3, with w/c of 0.5. Then cement was partially replaced with 0, 10, 20, and 30% fly ash in addition to 0, 5 and 10% of silica fume to obtain other 3 with varying amount of bacterial culture of 10^3, 10^5 10^7 cells/ml (*Sporosarcina pasteurii*). Casting and Cross sectional view of samples is shown in Figure 3.5 and Figure 3.6 respectively.

Figure 3.5 Casted Samples for RCPT [ASTM C 1202]

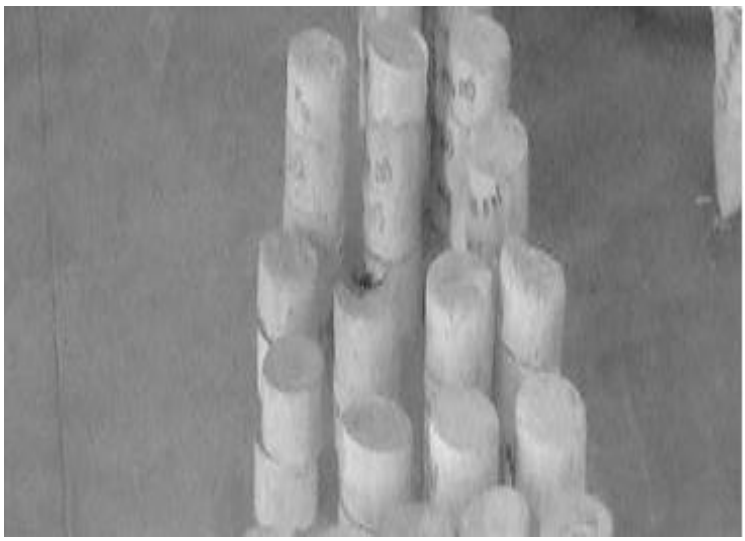


Figure 3.6 Cross-sectional Views of RCPT Samples

Conditioning and Testing of Concrete Specimen

The cylinders (100mm×200mm) thickness with and without bacterial culture were cast and allowed to cure. Specimens were placed in the vacuum desiccator's bowl as shown in Figure 3.6 which illustrates the setup of the vacuum pump, desiccator with stopcock, vacuum gauge and valve and the deaerated water container after the water has filled the desiccators. The vacuum was maintained in the desiccators bowl for 3 hours. The de-aerated water was allowed to flow into the desiccator, so that it completely covers the

specimens and no air was allowed to enter. Again the vacuum was maintained for another one hour. Then the specimens were left to soak in the container water for another 18 hours. The specimens were removed from the dessicator, dried and placed in gasket. The liquids (3.0% NaCl and 0.3 N NaOH solutions) were filled in the two cells. Power supply was set to 60V, and initial current reading was recorded. Temperatures of the specimen, applied voltage cell and solutions were maintained at 68 to 77°F (20 to 25°C) at the time the test was initiated (when the power supply was turned on). During the test, the air temperature around the specimens was maintained in the range of 68 to 77°F (20 to 25°C). The values for the current were recorded. Schematic diagram and RCPT test set up is shown in Figure 3.7 and Figure 3.8 respectively.

The interpretation is that the larger the Coulomb number or the charge transferred during the test, the greater the permeability of the sample. The more permeable is the concrete, the higher the coulombs; the less permeable the concrete, the lower the coulombs. The method has shown good correlation with chloride tests. Chloride penetrability as per ASTM 1202 is given in Table 3.14. The following formula, based on the trapezoidal rulecan be used to calculate the average current flowing through one cell.

$$Q = 900(I_0+2I_{30}+2I_{60}+2I_{90}+2I_{120}+\ldots+2I_{300}+2I_{330}+I_{360})$$

Where, Q = current flowing through one cell (coulombs)

I_0 = Current reading in amperes immediately after voltage is applied, and

I_t = Current reading in amperes at t minutes after voltage is applied

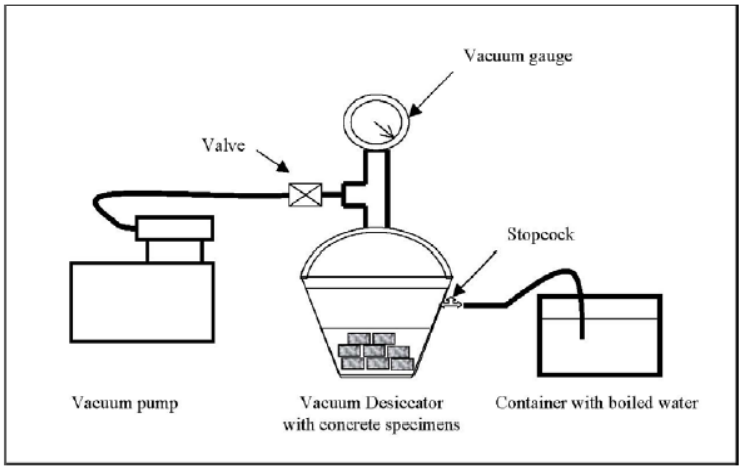


Figure 3.7: Conditioning of RCPT Samples

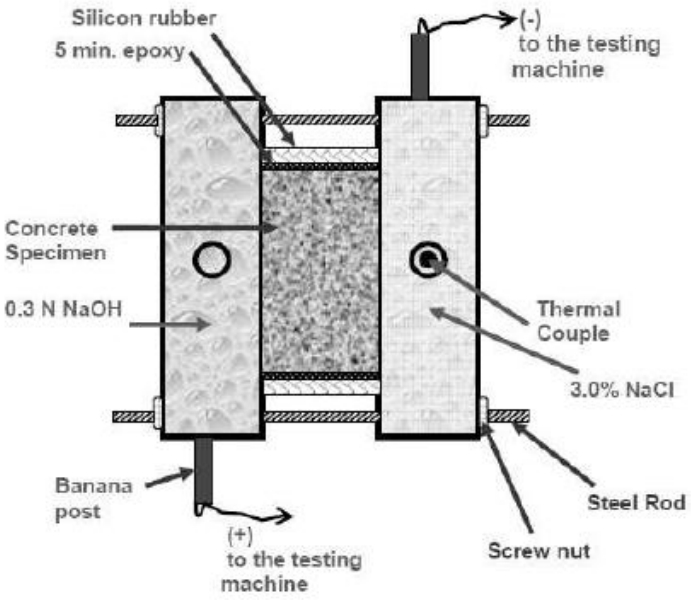


Figure 3.8 Schematic diagram of RCPT [ASTM C 1202]

Figure 3.9 Rapid Chloride Permeability Test Set Up

Table 3.14: Chloride Ion Penetrability Based on Charge Passed (ASTM 1202)

Charge passed (Coulomb)	Chloride Ion Penetrability
> 4000	High
2000 – 4000	Moderate
1000- 2000	Low
100 – 1000	Very Low
< 100	Negligble

3.2.4.4 SEM / EDX of concrete samples

An SEM is essentially a high magnification microscope, which uses a focused scanned electron beam to produce images of the sample, both top-down and, with the necessary sample preparation, cross-sections. The morphology and chemical constituents of the concrete samples were analyzed with SEM and EDX respectively. The Scanning Electron Microscope (SEM) is a powerful instrument which permits the characterization of heterogeneous materials and surfaces. Samples were completely dried at room temperature, then examined at accelerating voltages ranging from 30 to 35 kV by a SEM (Zeiss EVO50).

In the present investigation, the SEM was used in its most common mode, the emissive mode. Samples were gold coated with a sputter coating Emitech K575 prior to examination which is shown in Figure 3.10. Primary electrons generate low energy secondary electrons, which tend to emphasize the topographic nature of the specimen. Primary electrons can be backscattered which produces images with a high degree of atomic number (Z) contrast. Ionized atoms can relax by electron shell-to-shell transitions, which lead to either X-ray emission or Auger electron ejection. The X-rays emitted are characteristic of the elements in the top few μm of the sample and are measured by EDX detector. Mineral constituents of the isolates were further characterized by EDX analysis. Presence of high amounts of calcium in the samples confirmed the presence of calcite in the form of calcium carbonate. Test set of SEM/EDX is shown in Figure 3.11

Figure 3.10 Gold Coating of SEM Sample

Figure 3.11 SEM / EDX Test Set Up (Zeiss EVO50).

3.2.4.5 XRD

X-ray diffraction is a non-destructive technique used to determine the elements present in any particular substance. X-ray powder diffraction technique is the most prominent technique used for unraveling the structure of the materials in bulk and thin film forms. XRD spectra were obtained using an X'Pert PRO diffractometer with a Cu anode (40 kV and 30 mA) and scanning from 3° to 60°. Each sample was crushed and ground before mounting onto a glass fibre filter using a tubular aerosol suspension chamber (TASC). The components of the sample were identified by comparing them with standards established by the International Center for Diffraction Data. All experiments were performed in triplicate. X-ray diffraction is based on the fact that, in a mixture, the measured intensity of a diffraction peak is directly proportional to the content of the substance producing it. The samples for X-Ray diffraction analysis were prepared in powdered form. The concrete sample was taken from the inner core of the matrix. X-ray diffractometer is shown in Figure 3.12.

Procedure for Deduction of Minerals

(i) For a given sample, XRD graphs are obtained with intensities on Y-axis and 2θ on X-axis.

(ii) Determine sharp peaks in the graph and represent them on an arbitrary scale as very strong (VS), strong (S), moderate (M), weak (W) and very weak (VW). The intensities corresponding to above are:

VS	75-100
S	50-75
M	25-50
W	10-25
VW	0-10

(iii) Match the d values and intensities of peaks of respective minerals with the fundamental peaks of X-ray powder diffraction files given in the software.

(iv) For any mineral to be present, all the strong peaks should be present in the XRD graph, else the mineral is not present.

Figure 3.12 X-ray Diffractometer.

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 formation (ii) Effect of bacteria on concrete properties such as compressive strength of cubes, micro structure analysis using XRD, SEM and EDX. The durability properties like water absorption, water porosity and rapid chloride permeability of the mixes are studied and analyzed.

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 rhizopheric soil (tulsi plant), alkaline soil and sewage sludge.

In our case, a total of 10 Bacillus strains which were capable of producing calcium carbonate were isolated. All the Bacillus strains utilized urease enzyme obtained from jack bean medium. Jack Bean is a source or media from which synthetic urea is prepared. In our case, Bacillus strains 3, 4 and 5 found to be best on the basis of calcite formation.

4.1.2 Growth Profile of Ureolytic Bacteria

In our case, the growth profile was studied upto 120 hour. It was observed from graph that in Bacillus 1 and 2 the optical density increased up till 48 hr to value of 0.65 and 0.601 respectively which keep on decreasing uptill 120 hr linearly, whereas in Bacillus strains 3,4 and 5 the optical density kept on increasing till 120 hr. The maximum growth was observed 1.084 in Bacillus strain 4 followed by Bacillus strain 5 and Bacillus strain 3 with values 1.013 and 0.867 respectively Figure 4.1.

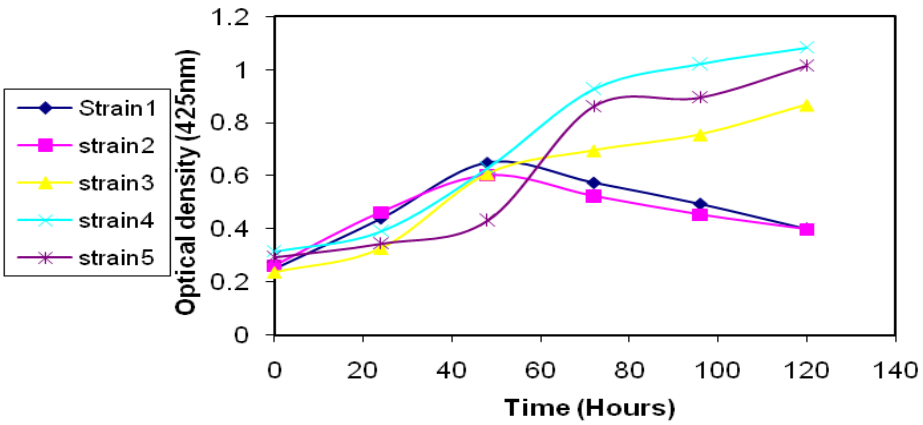


Figure 4.1: Growth Profile of Bacillus Strains

The pH of the medium was significantly increased with the increase in growth of these isolates. The ability to grow at high pH by Bacillus strain 3, 4 and 5 suggests that it can be used in building materials such as cement to enhance the calcite precipitation where the pH of the proximal environment is highly alkaline (pH 11–12). Similarly, Castainer et al., (2000) also observed that how the bacteria are encased within the calcium carbonates systematically Figure 4.2.

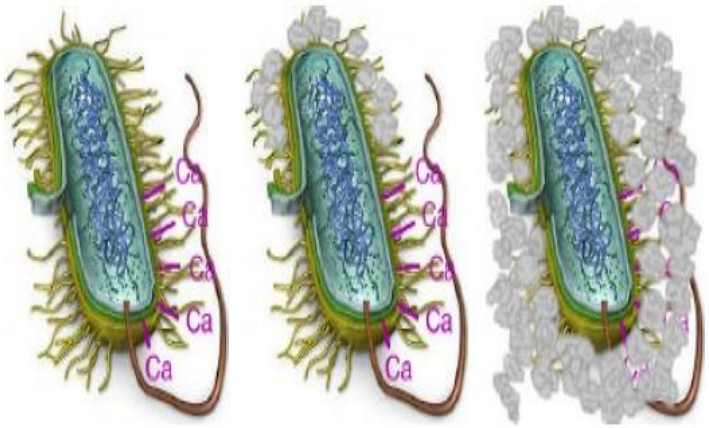
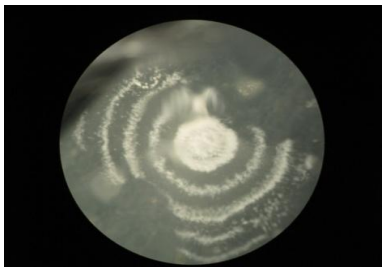


Figure 4.2: Calcium Carbonate Formation by Bacteria (Castainer et al., 2000)

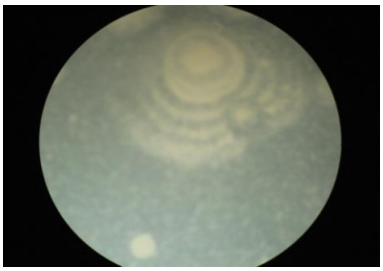
The pH of the medium was significantly increased with the increase in growth of the isolates i.e Bacillus strains 3, 4 and 5. The ability to grow at high pH by Bacillus strain 3, strain 4 and strain 5 suggests that it can be used in building materials such as cement to enhance the calcite precipitation where the pH of the surrounding environment is highly alkaline (pH 11–12).

4.1.3 Crystal Nucleation Site Development

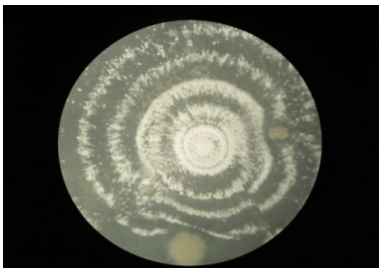
The selection of 10 Bacillus strains was based on visual differences in the precipitate morphology.



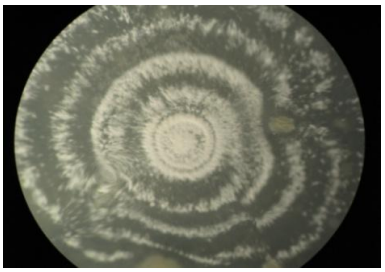
(a) Strain 1



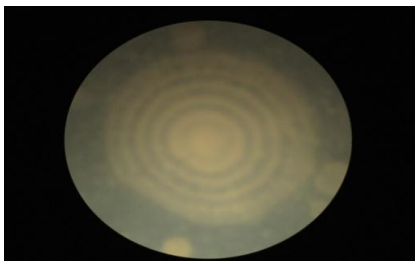
(b) Strain 2



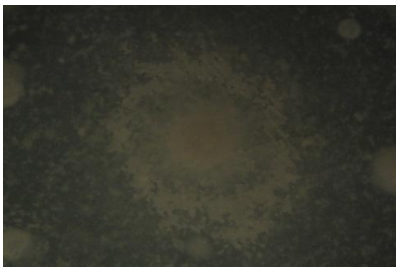
(c) Strain 3



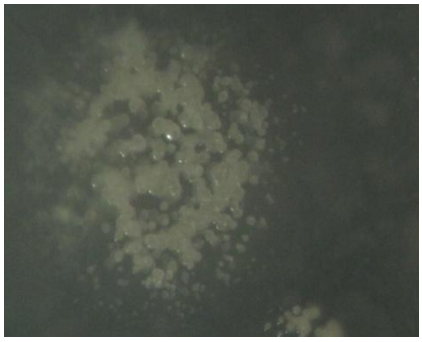
(d) Strain 4



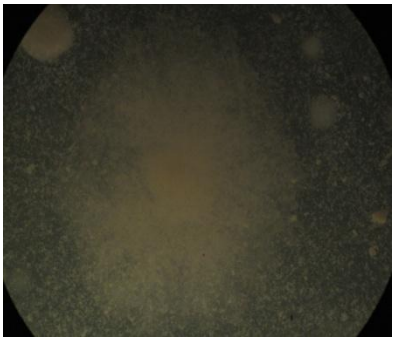
(e) Strain 5



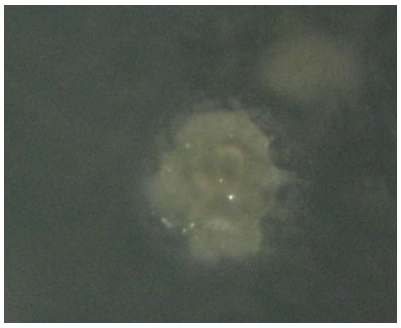
(f) Strain 6



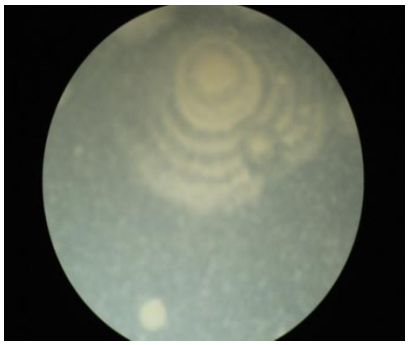
(g) Strain 7



(h) Strain 8



(i) Strain 9



(j) Strain 10

Figure 4.3 (a-j): Stereomicroscopic Images of Calcite Crystals Precipitated Within Different Bacterial Strains

Crystal precipitating colonies were studied after 5 days and 10 days of cultivation with stereomicroscopy. Digital images were captured with a ccd 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. Bacillus strains 1-5 all produced large, white structures which formed rapidly (20-48 h for crystallization) with aggregates taking up as much as

98% of the total colony surfaces Figure 4.3(a-j). Bacillus strains 6-7 precipitated at similar rates, and produced whitish and transparent crystal aggregates. Bacillus strains 8-10 precipitated at noticeably slower rates (about 3-5 days for crystallization). Similar results were reported by Dick et al., (2006) who found calcium carbonate production by different Bacillus strains in different amount. Even Boquet et al., (1973) demonstrated the ability of soil bacteria to precipitate calcium carbonate under laboratory conditions.

Large crystal aggregates that precipitated showed crystal nucleation site development. Thus it was concluded that crystal formation is a function of the medium, and that under suitable conditions most bacteria can form crystals. It was suggested that there is considerable geological evidence that microorganism function as crystal nucleating agents during mineral precipitation.

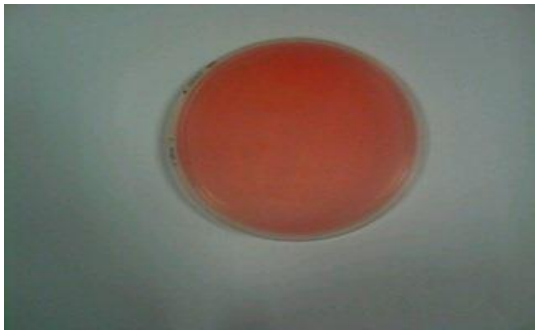
4.1.4 Urease Activity

The urease activity was measured when urea hydrolyzed to form carbon dioxide and ammonia. The change in coloration on urease agar and urea broth when incubated for 5 days at 28˚C was recorded as a urease positive reaction. Bacteria utilize urea, ammonia is formed which in turns makes media alkaline by producing red pink color Figure 4.4(a-j). The urease activity is Bacillus strain specific, some bacterial strains have high urea affinities where as some have lower affinities. By using phenol hypochlorite assay method it was estimated that Bacillus strain 3, Bacillus strain 4 and Bacillus strain 5 showed maximum urease activity as it did not decrease from 72 hr to 120 hr. The highest productivities in all media were obtained in 120 h. After 120 h urease production was decreased in the biomineralisation media Figure 4.5. The bacillus strains (strain 1-5) are able to precipitate calcite on their cell constituents and in their micro environment by conversion of urea into ammonia and carbon dioxide. It was observed from Figure 4.5 that maximum urease activity found in Bacillus strain1 and Bacillus strain 2 was 578U/ml and 512U/ml respectively in 72 hr which kept on decreasing uptill 120 hr in biomineralization media.

The same trend was found in growth curve of these two Bacillus strains 1 and 2 which indicates as the multiplication of bacteria decreases after 72 hr even the urease activity also decreased. The maximum urease activity found in Bacillus strains 3, 4 and 5 was

estimated to be 589U/ml, 598U/ml and 593U/ml respectively in 120 hr after which there was marginal increase due to which the experiment was terminated Figure 4.5. The percent increase was found in 54.68%, 58.5% and 58.9% in Bacillus strain 3 in 72 hr to 120 hr. In Bacillus strain 4, 59%, 59.7% and 60.5% and in Bacillus strain 5 it was 56.72%, 56.4% and 57.2 % in which allows the termination of the experiment.

(a) Strain 1



(b) Strain 2

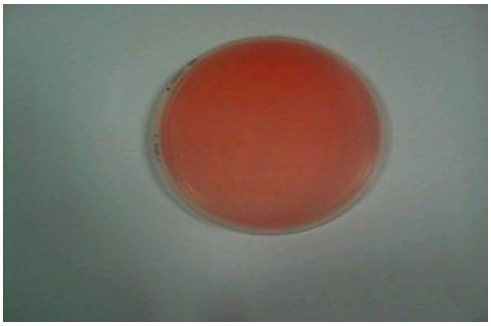
(c)Strain 3

(d) Strain 4

(e) Strain 5

(f) Strain 6

(g) Strain 7



(h) Strain 8

(i) Strain 9

(j) Strain 10

Figure 4.4 (a-j): A Change in Coloration By Bacterial Strains on Urease Agar

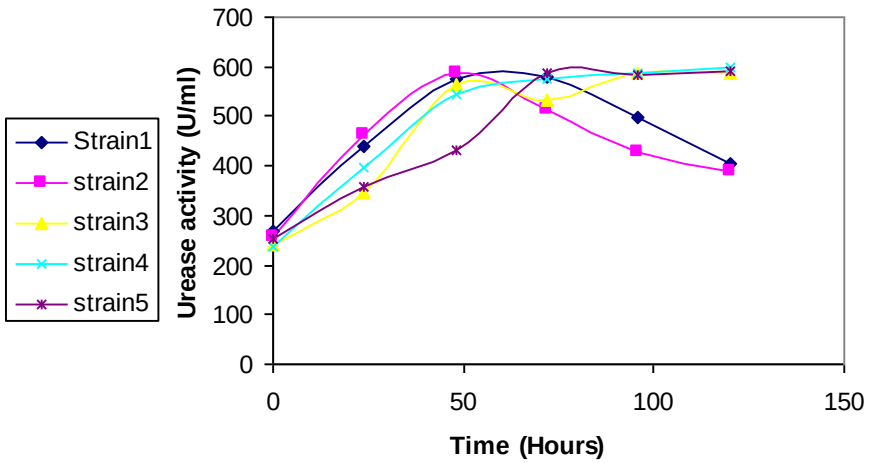


Figure 4.5: Urease Activity of Different Bacterial Strains

Bacillus strains isolated from different sources like alkaline soil, rhizopheric soil and sewage sludge were screened for their ability to precipitate calcite on agar plates

containing urea and calcium chloride. Hammes et al. (2003) also observed that microorganisms closely related to the *Bacillus sphaericus* group express the urease gene under the given cultivation conditions. The hydrolysis of urea is catalyzed by means of urease. As a consequence, urea is degraded to carbonate and ammonium, resulting in an increase of the pH and carbonate concentration in the bacterial environment (Stocks et al., 1999).

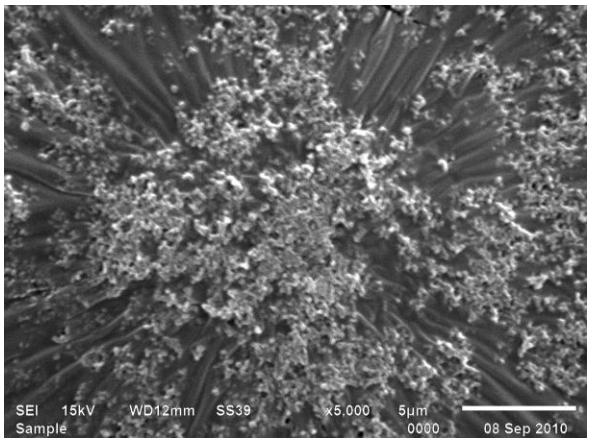
In our research the isolated bacteria from alkaline soil and sewage sludge belonged to *Bacillus* genera. There was significant difference among the strains in urease production. The hydrolysis of urea was selected as a very suitable pathway for the production of carbonate ions due to its ability to alkalinize the environment.

4.1.5 SEM and XRD Analysis of Bacillus Strains

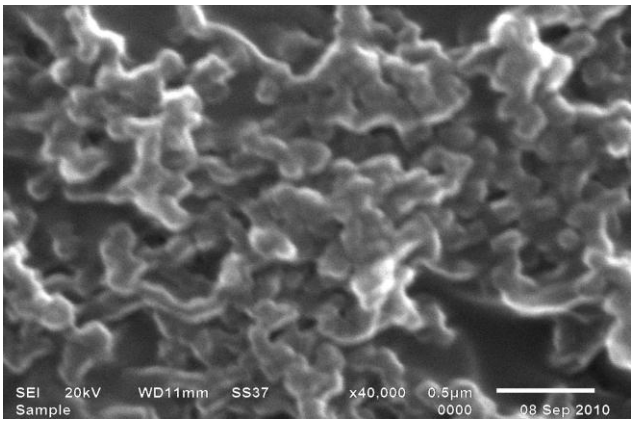
To confirm the presence of bacterial calcite precipitation the Bacillus strains 3, 4 and 5 were examined under SEM and XRD. The SEM analysis revealed in Figure 4.6 (a-e) shows distinct calcite crystals embedded with bacteria which indicated that the bacteria served as the nucleation sites for the mineralization process. High calcium amounts in all the bacterial samples confirmed that calcite was present in the form of calcium carbonate. The presence of crystalline calcite associated with bacteria which indicated that bacteria served as nucleation sites during mineralization process. For further confirmation of the carbonate deposits as calcite crystals XRD analysis was performed Figure 4.7 (a-c).

The maximum number of calcite peaks were observed in Bacillus strain 3, strain 4 and strain 5. So; from the above results it was concluded that Bacillus strain 3, strain 4 and strain 5 were more efficient than other isolated strains that were strain 1 and strain 2 with respect to calcite precipitation which was present in the Figure 4.6 (a-e).The influence of the calcium source was limited to the morphology of the crystals.

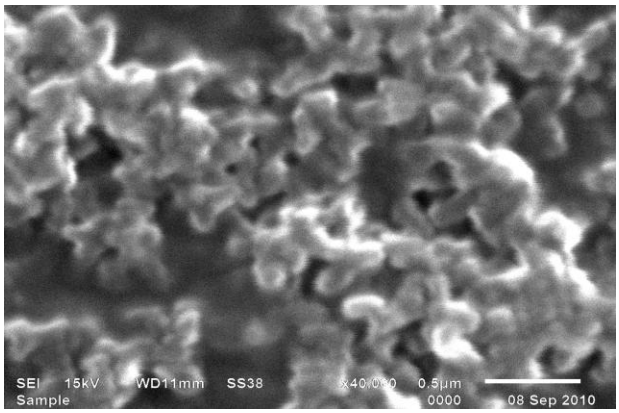
As significant increase in the urease activity was observed in Bacillus 3, 4 and 5. These Bacteria can be used commercially for the crack remediation process in buildings. Bio-mineralization process by *Sporosarcina pasteurii* by SEM and XRD indicated that calcite is the dominated mineral phase when bacteria are present.



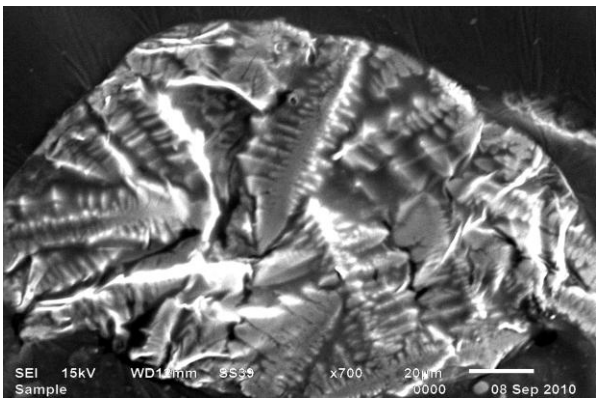
(a)



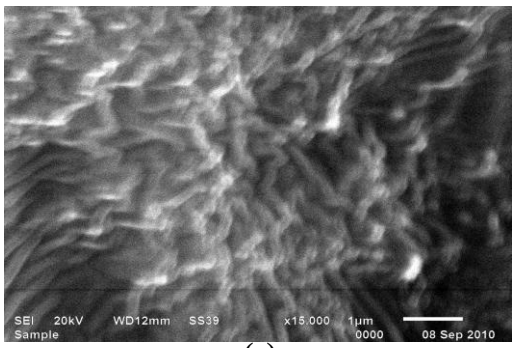
(b)



(c)

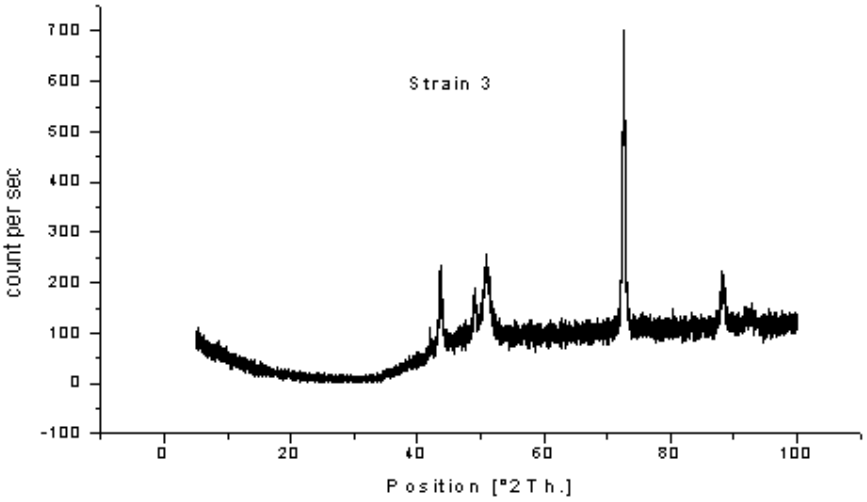


(d)

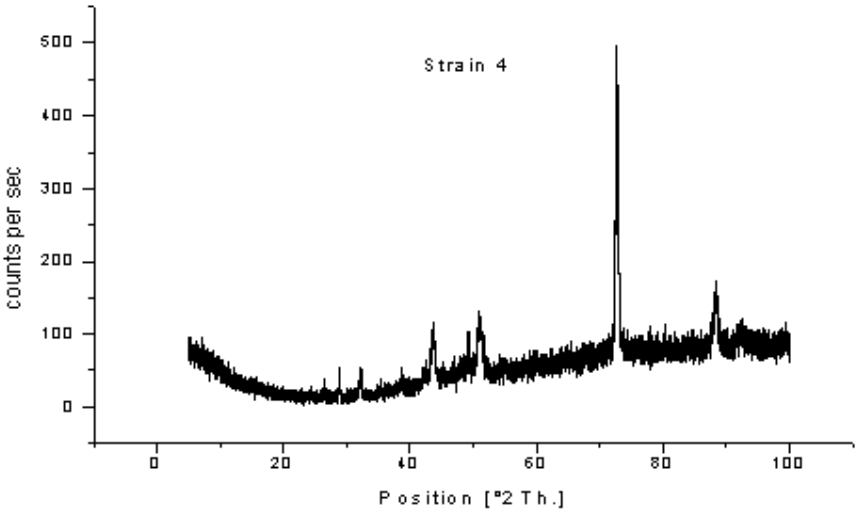


(e)

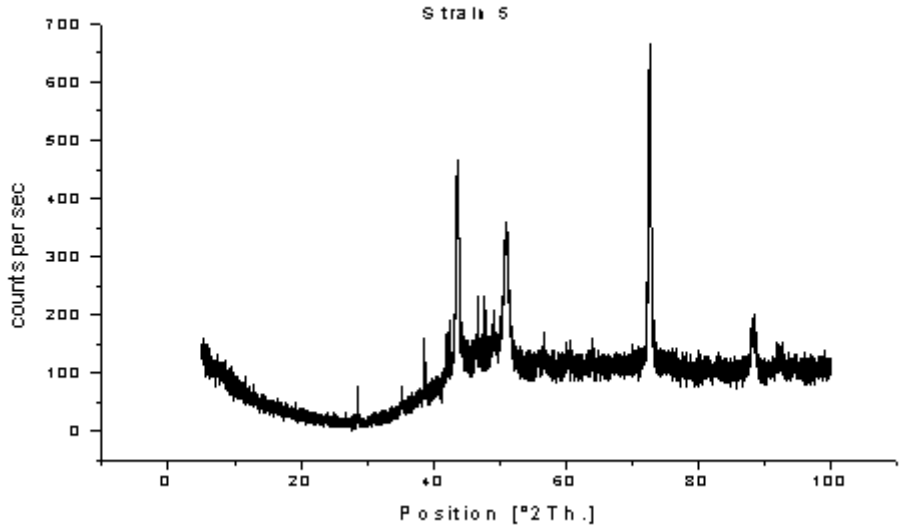
Fig 4.6 (a-e) shows SEM Images of Bacillus 1, 2,3,4,5


Strain 3
count per sec
700
600
500
400
300
200
100
0
-100
0
20
40
60
80
100
Position [°2 Th.]

(a)


Strain 4
counts per sec
500
400
300
200
100
0
0
20
40
60
80
100
Position [°2 Th.]

(b)



(c)

Figure 4.7 (a-c) XRD Analysis for Bacillus (Strain 3, 4 and 5)

The bacterium used in our research study was capable of surviving within the concrete when mixed with it. In the growth medium, the bacterium grows well within 3–4 days at 28˚C temperatures and at pH 8.0. The pH tolerance of the bacterium was found to be 12.0. During its growth, the bacterium secretes some proteins in the medium. One of the proteins possesses silicate in like catalytic activity. With increasing concentrations of precipitated carbonate, increasing bond formation and hence consolidation can be obtained. Therefore, increasing amounts of carbonate precipitates could result in an increased protective effect of the biodeposition treatment.

4.1.6 EDX Analysis of Bacterial Isolates

As these three Bacterial strains 3, 4 and 5 were found to produce the maximum urease activity they were further analysed by EDX spectra Figure 4.8(a-c). The presence of crystalline calcite associated with bacteria indicated that bacteria served as nucleation sites during the mineralization process. The maximum amount of calcium was found to be (in weight %) 52.54% Bacillus strain 5, 46.42% in Bacillus strain 4 and 42.25% Bacillus strain 3 indicated by EDX spectra. The majority of the carbonate deposits were present as

calcite crystals as confirmed by XRD analysis. From these results it can be concluded that theses strains are more efficient with respect to calcite precipitation.

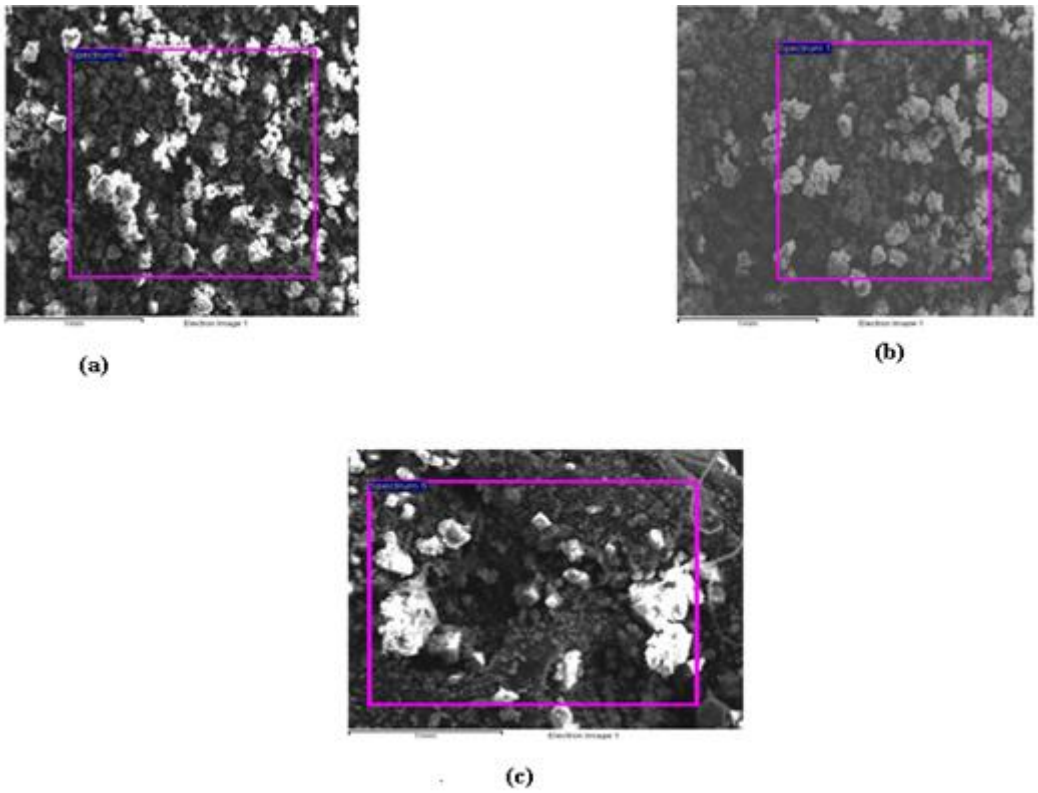


Figure 4.8 (a-c): EDX Spectra of Bacillus (Strain 3, 4 and 5 respectively)

4.1.7 DNA Sequencing and Sequence Analysis

Analysis of DNA sequences and homology searches was completed using standard BLAST server of the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov) with the BLAST algorithm and specially the blastn program for comparison of a nucleotide query sequence with the nucleotide sequence database. The query sequence obtained showed similarity with *Bacillus subtilis (Strain 3), Bacillus lichniformis (Strain 4), Sporosarcina pasteurii (Strain 5).* The query sequence obtained showed similarity with *Sporosarcina pasteurii* (B-1). Figure 4.9 shows Phylogenetic tree for *Sporosarcina pasteurii* as it was the only perfect bacteria which is used in our research work.

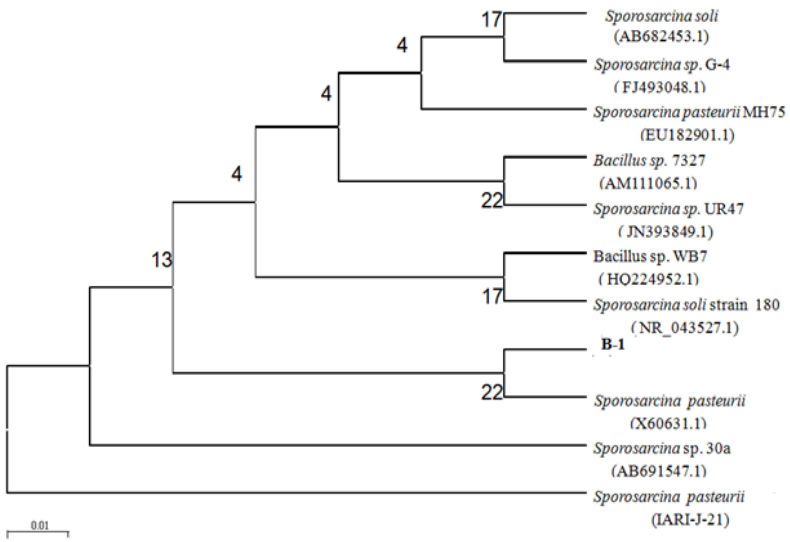


Figure 4.9 Phylogenetic Tree for *Sporosarcina pasteurii*

The Phylogenetic tree of 1341 bp sequence was analysed using the Neighbor-Joining method in MEGA4. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. Sequence of Bacteria Producing Significant Alignments is given in Table 4.1 and its Distance Matrix is shown in Table 4.2.

Table 4.1: Sequence of Bacteria Producing Significant Alignments

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
AB682453.1	***Sporosarcina soli***	2477	2477	100%	0.0	100%
FJ493048.1	***Sporosarcina sp* G-4**	2477	2477	100%	0.0	100%
EU182901.1	***Sporosarcina pasteurii* MH75**	2477	2477	100%	0.0	100%
AM111065.1	***Bacillus sp* 7327**	2477	2477	100%	0.0	100%
JN393849.1	***Sporosarcina sp* UR4**	2477	2477	100%	0.0	100%

Accession	Description	Max score	Total score	Query coverage	E value	Max ident
HQ224952.1	***Bacillus sp* WB7**	2477	2477	100%	0.0	100%
NR043527.1	***Sporosarcina soli* strain 180**	2477	2477	100%	0.0	100%
X60631.1	***Sporosarcina pasteurii***	2477	2477	100%	0.0	100%
AB691547.1	***Sporosarcina sp* 30a**	2477	2477	100%	0.0	100%
IARI-J-21	***Sporosarcina pasteurii***	2477	2477	100%	0.0	100%

Table 4.2: Distance Matrix

B-1	1		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
AB682453.1	2	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
FJ493048.1	3	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
EU182901.1	4	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000	0.000
AM111065.1	5	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000	0.000
JN393849.1	6	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000	0.000
HQ224952.1	7	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000	0.000
NR043527.1	8	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000	0.000
X60631.1	9	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000	0.000
AB691547.1	10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000		0.000
IARI-J-21	11	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	

The evolutionary history was inferred using the Neighbor-Joining method. The bootstrap consensus tree inferred from 500 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches. The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site. Codon positions

included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated from the dataset (Complete deletion option). There were a total of 1341 positions in the final dataset. Phylogenetic analyses were conducted in MEGA4. Biochemical characterization of all the three isolates were performed and found that all the three strains had similar results. The sequences were submitted in NCBI with accession No. JX 081255, JX 08125, JX 08126. Results are shown in Table 4.3.

Table 4.3: Biochemical Characterization of the Bacterial Isolates

Bacterial Isolates	Oxidase	Nitrate Reduction	Fermentation of Glucose	Fermentation of Mannitol	Fermentation of Sucrose
Bacillus (Strain 3)	+	-	+	+	+
Bacillus (Strain 4)	+	-	+	+	+
Bacillus (Strain 5)	+	-	+	+	+

4.2 RESULTS RELATED TO INFLUENCE OF BACTERIA ON PROPERTIES OF CONCRETE

4.2.1 Compressive Strength

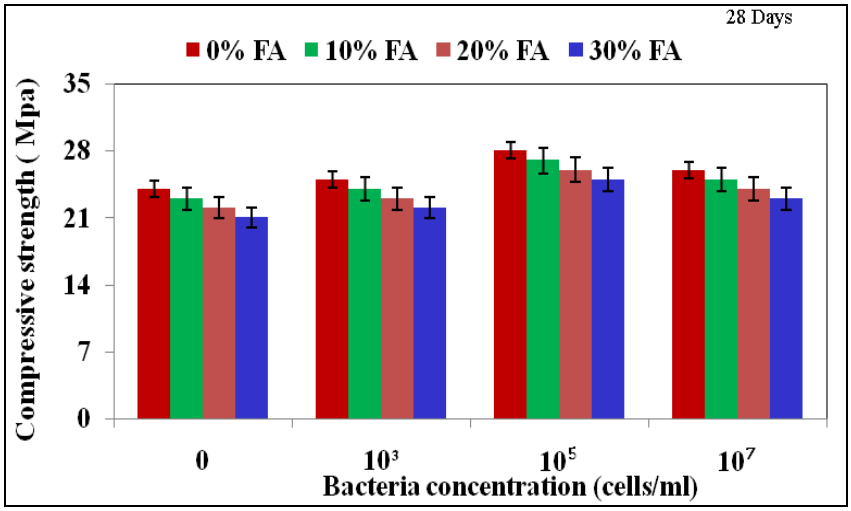
The objective of this research work is to highlight the effect of bacteria on the compressive strength of concrete. Results of the influence of bacteria (*Sporosarcina pasteurii*) on the compressive strength of concrete containing fly ash and silica fume is given in Table 4.2 and shown in Figures 4.10-4.13.

Table 4.4: Compressive Strength of Concrete. Values are ± S.D (n=3)

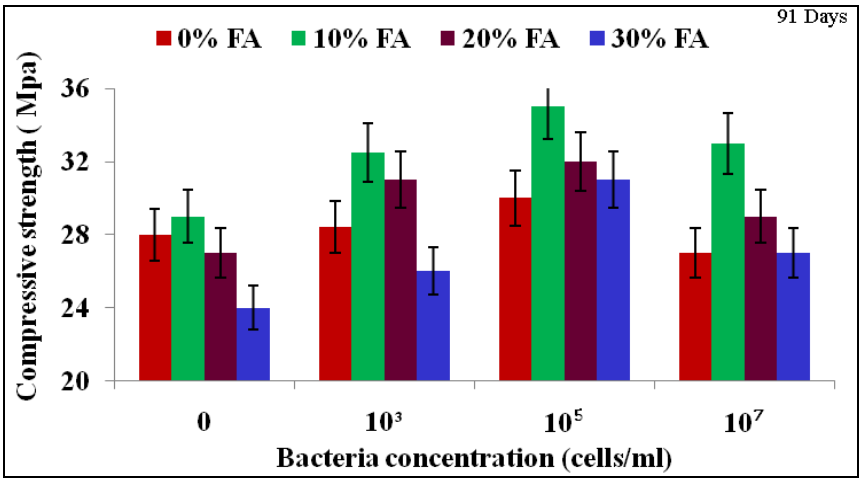
Mix	Without Bacteria		Bacteria (10^3 cells/ml)		Bacteria (10^5 cells/ml)		Bacteria (10^7 cells/ml)	
	28 Days	91 Days	28 Days	91 Days	28 Days	91 Days	28 Days	91 Days
10% FA	26.4±0.10	27±0.13	27.1±0.15	28±0.17	27.6±0.21	28.6±0.16	27±0.18	27.2±0.19
20% FA	25±0.12	26±0.22	26.1±0.23	27±0.22	26.5±0.24	27.5±0.21	26±0.23	26.7±0.23
30% FA	24±0.15	25±0.5	24.4±0.15	25.5±0.16	24.7±0.15	26.4±0.5	24.2±0.11	25.1±0.12
10% FA + 5% SF	28.2±0.11	29±0.05	28.4±0.10	30±0.10	29.8±0.11	33±0.15	28.2±0.14	30.1±0.15
20% FA + 5% SF	28.2±0.12	30±0.21	28.1±0.23	30.2±0.22	28.8±0.5	32±0.5	28±0.12	30±0.13
30% FA + 5% SF	26±0.43	28.8±0.34	27±0.25	28±0.26	26.6±0.17	30±0.15	27±0.22	28.2±0.24
10% FA + 10% SF	30±0.54	31±0.53	31±0.42	32±0.21	32.7±0.5	36±0.1	29.1±0.34	31±0.45
20% FA + 10% SF	29±0.32	29.7±0.34	29.4±0.28	30±0.12	30±0.11	31±0.1	26.2±0.21	28.5±0.19
30% FA + 10% SF	28±0.21	29±0.53	28.3±0.19	29±0.17	28.7±0.3	29.5±0.3	28.1±0.38	29±0.31

FA: Fly ash; SF: Silica fume

It can be seen from Table 4.4 and Figure 4.10 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 4.4 & Figure 4.10).



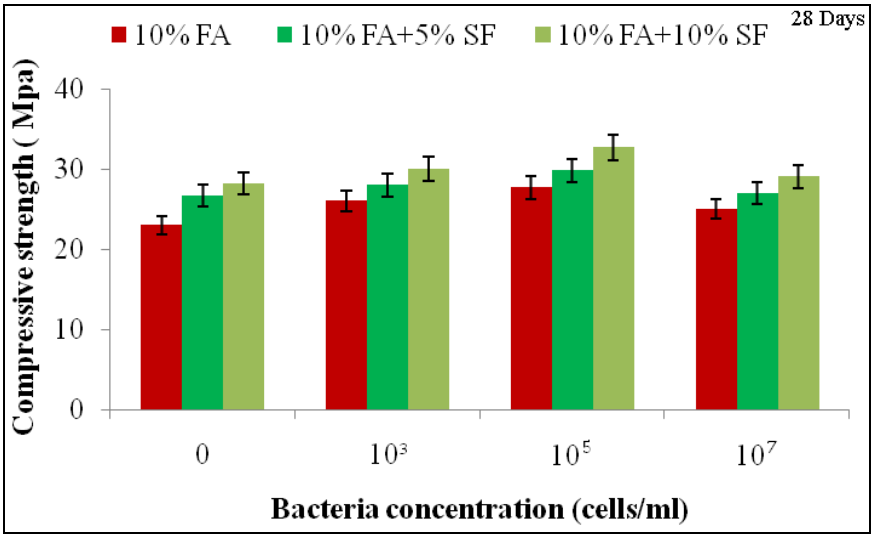
(a)



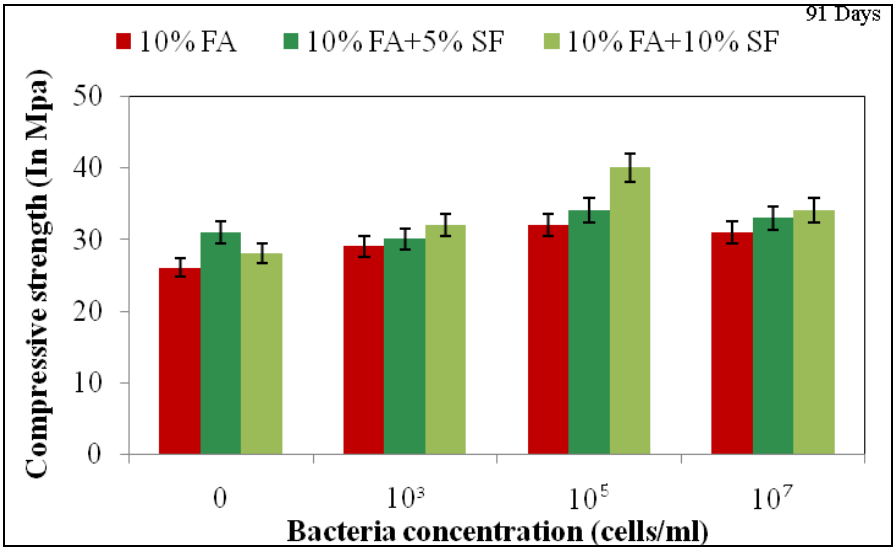
(b)

Figure 4.10: Effect of Bacteria (*Sporosarcina pasteurii)* on Compressive Strength of Concrete

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, and then, there was reduction in the strength with 10^7 cells/ml of bacteria. Maximum increase in compressive strengths was achieved at 10^5 cells/ml for all fly ash concrete; 10^3 cells/ml shows least compressive strength. Compressive strength of concrete containing both fly ash and silica fume is given in Table 4.2 and shown in Figures 4.11-4.13.



(a)



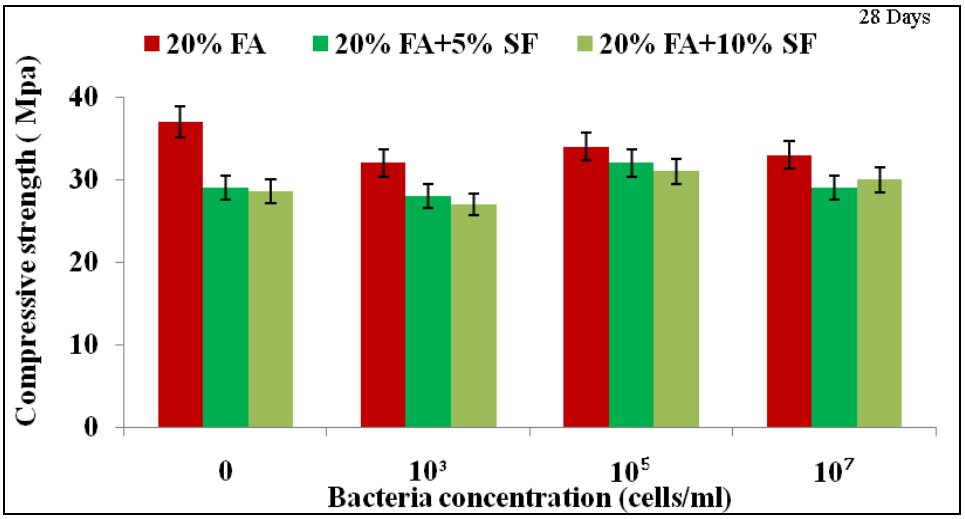
(b)

Figure 4.11: Effect of Bacteria (*Sporosarcina pasteurii)* on Compressive Strength of (10%) Fly Ash and (5%, 10%) Silica Fume Concrete.

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 days

respectively. 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.



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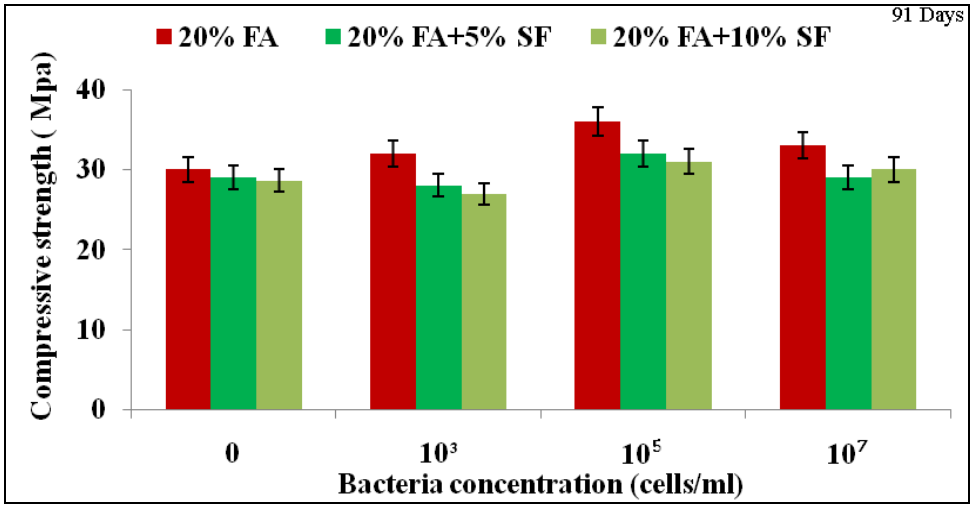
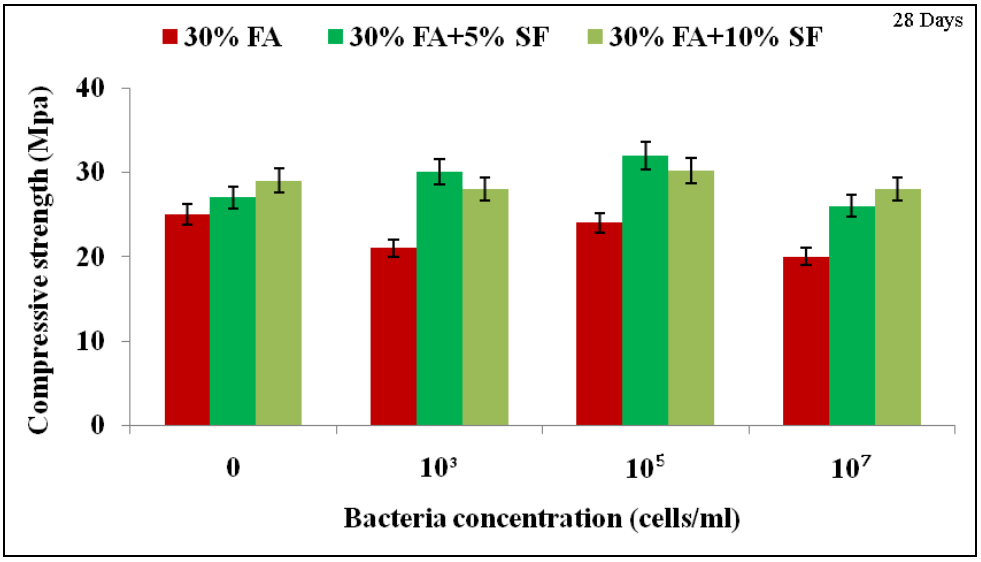


(b)

Figure 4.12: Effect of Bacteria (*Sporosarcina pasteurii)* on Compressive Strength of (20%) Fly Ash and (5%, 10%) Silica Fume Concrete.



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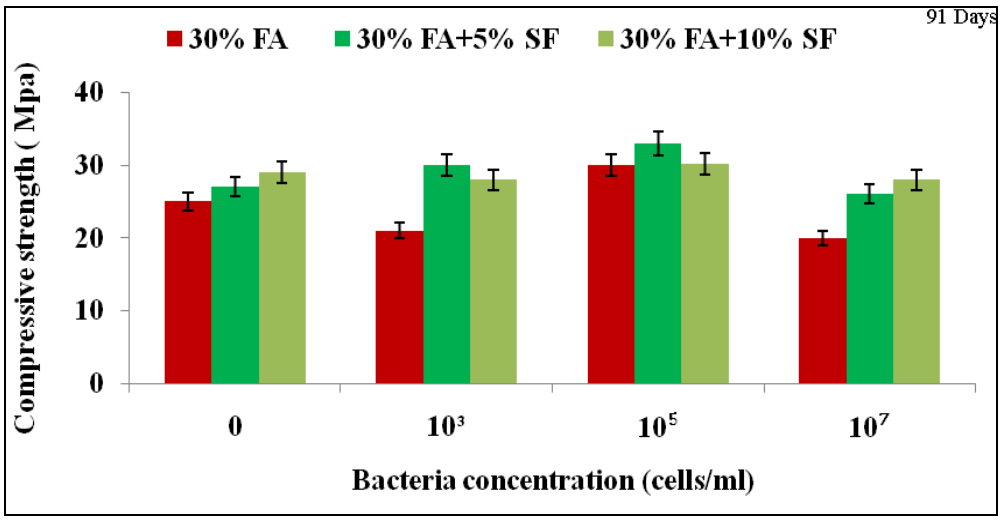


(b)

Figure 4.13: Effect of Bacteria (*Sporosarcina pasteurii)* on Compressive Strength of (30%) Fly Ash and (5%, 10%) Silica Fume Concrete.

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 in Figures 4.12 and 4.13, 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 it was concluded that concrete mixed with silica fume had the high compressive strength.

In our research work, the improvement in compressive strength by *Sporosarcina pasteurii* 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.

4.2.2 Water Porosity

In this research work, the effect of bacteria (*Sporosarcina pasteurii*) on water porosity of concrete was studied. Metabolic activities by bacteria led to the precipitation of calcium carbonate. Concrete specimens were tested for water porosity at the age of 28 and 91 days. It was observed that bacteria produced calcite which filled the voids or pores of concrete resulting in reduction of water retaining capacity of concrete. The decrease in porosity of concrete treated with and without bacteria is given in Table 4.5.

Table 4.5: Water Porosity of Concrete with and without Bacteria.

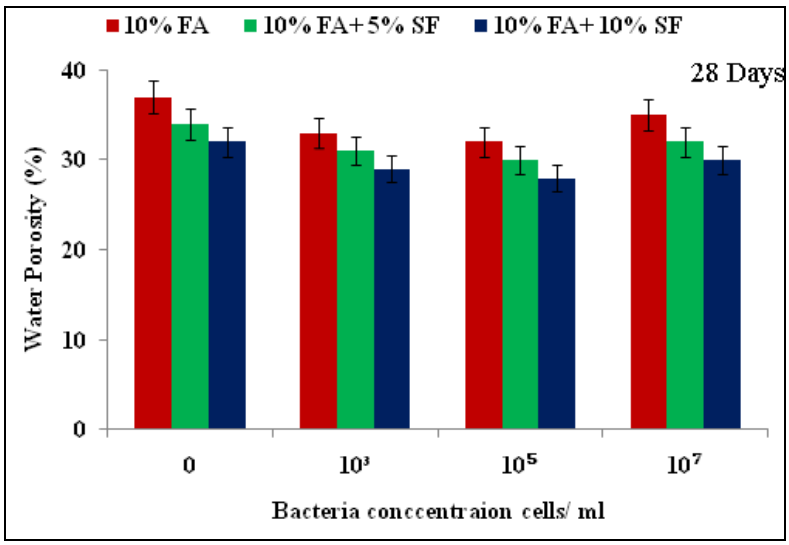
Mix	Without Bacteria		With Bacteria (10^3 cells/ml)		With Bacteria (10^5 cells/ml)		With Bacteria (10^7 cells/ml)	
	28 Days	91 Days	28 Days	91 Days	28 Days	91 Days	28 Days	91 Days
10% FA	37±0.21	34±0.22	33±0.23	27±0.23	32±0.30	26±0.22	35±0.31	28±0.31
20% FA	34±0.20	32±0.21	27±22	22±0.23	26±0.28	19±0.21	28±0.22	24±0.23
30% FA	32±0.31	30±0.30	22±0.31	20±0.33	19±0.31	17±0.38	24±0.37	23±0.37
10% FA + 5% SF	35±0.41	34±0.42	31±0.5	25±0.51	30±0.81	24±0.71	32±0.82	27±0.82
20% FA + 5% SF	32±0.51	31±0.52	25±0.53	20±0.53	24±0.53	17±0.51	27±0.54	23±0.54
30% FA + 5% SF	28±0.58	26±0.57	20±0.58	18±0.58	17±0.61	15±0.60	23±0.60	21±0.61
10% FA + 10% SF	32±0.28	30±0.27	29±0.28	23±0.28	28±0.29	22±0.27	30±0.29	25±0.30
20% FA + 10% SF	30±0.30	28±0.30	23±0.32	19±0.31	22±0.33	14±0.35	25±0.34	21±0.34
30% FA + 10% SF	28±0.12	26±0.13	19±0.15	17±0.16	14±0.11	13±0.13	21±0.17	19±0.17

FA: Fly ash; SF: Silica fume

The presence of bacteria resulted in reduction of the water uptake when compared to control specimens. The deposition of a layer of calcium carbonate on the surface and inside pores of the concrete specimens resulted in a decrease of water porosity. The bacterial action deposition seals the pores, voids and cracks of very minute size. Water porosity of control concrete (without bacteria) was high. Inclusion of bacteria resulted in lower porosity of concrete when compared to the untreated specimens of concrete. It can be seen from Table 4.3 that at the age of 28-day, water porosity of fly ash (10, 20, and 30% fly ash content) concrete without bacteria was 37%, 34% and 32%., and porosity decreased marginally at the age of 91 days.

Further, with the action of bacteria, water porosity of fly ash concrete decreased with increase in bacteria cell concentration up to 10^5 cells/ml, and then, there was reduction in the porosity with 10^7 cells/ml of bacteria. Maximum decrease in water porosity was achieved at 10^5 cells/ml for all fly ash concrete; 10^3 cells/ml showed maximum water porosity. The porosity values were 35% in 28 day which reduced to 26% in 91 days

which decreased with increasing percentage of fly ash (10%, 20% and 30%) with different concentration of silica fume and bacteria as shown in Figures 4.14- 4.16.



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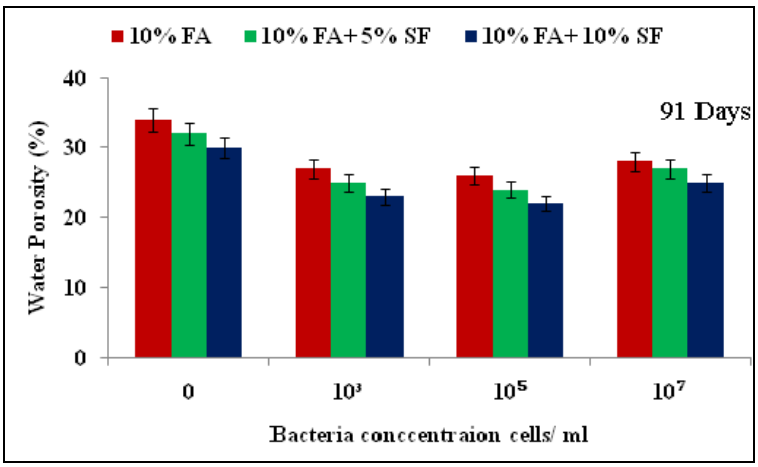
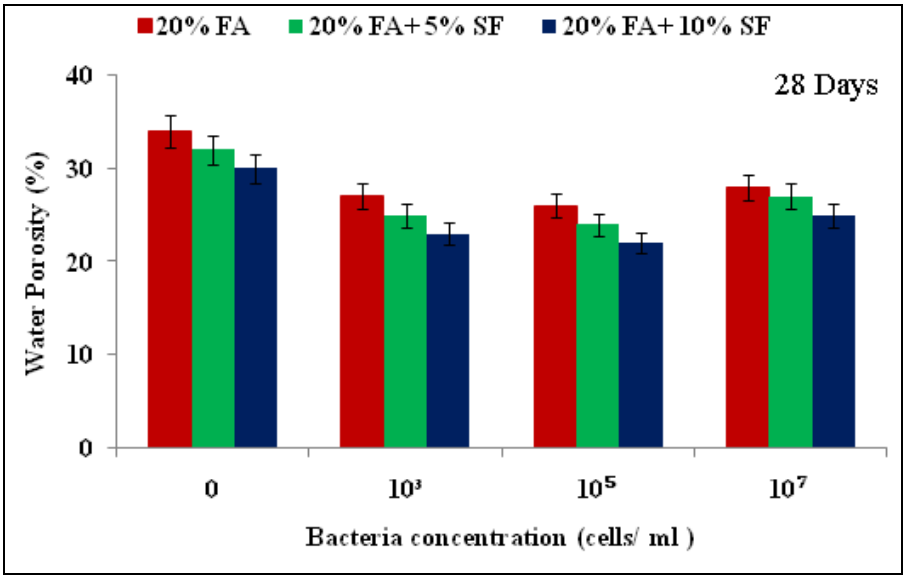


(b)

Figure 4.14: Effect of Bacteria (*Sporosarcina pasteurii)* on Water Porosity of (10%) Fly Ash and (5%, 10%) Silica Fume Concrete.

The presence of bacteria resulted in significant reduction of water uptake when compared to control concrete without bacteria. The decrease in porosity of concrete treated with bacteria was due to deposition of calcium crystals on the surface which further resulted in decrease of the permeation properties.



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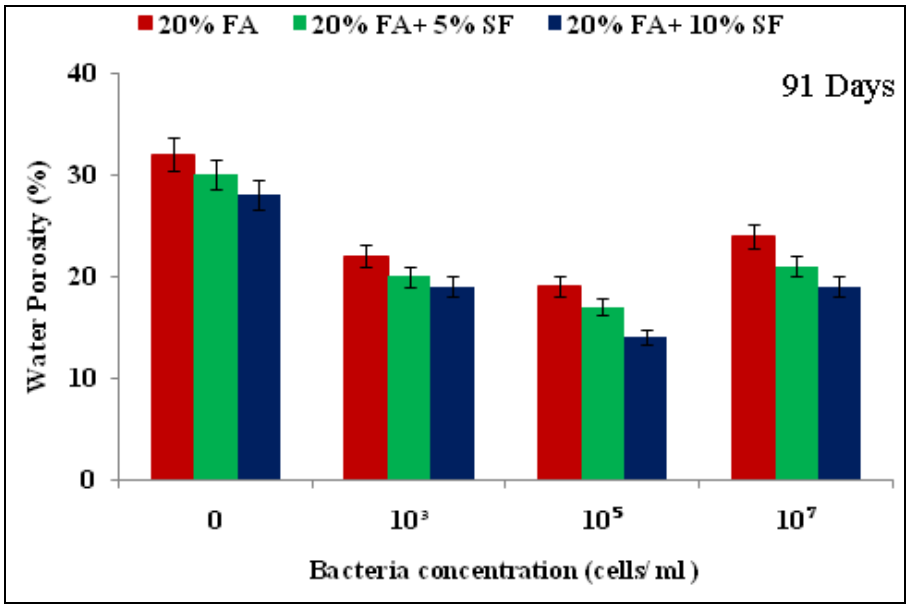

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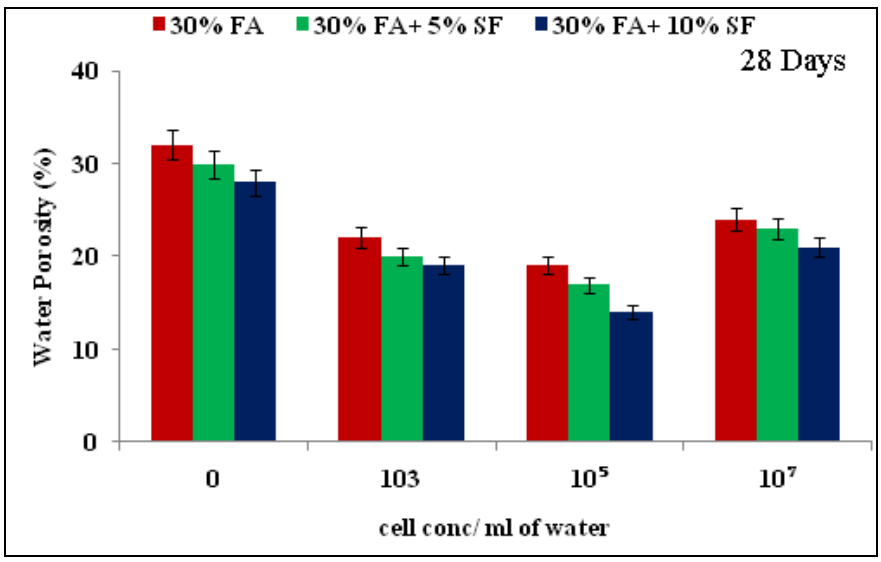
Figure 4.15: Effect of Bacteria (*Sporosarcina pasteurii)* on Water Porosity of (20%) Fly Ash and (5%, 10%) Silica Fume Concrete.

It can be seen from Figures 4.14- 4.16 that water absorption of concrete (containing varying percentages of fly ash & silica fume) without bacteria was between 28% and 35% at 28 days and between 26% and 34% at the age of 91 days. Concrete made with 10% fly ash and 10% silica fume was observed to reduce water uptake capacity from 32% at 28 days to 30% at the age 91 days. Inclusion of bacteria, thereby, decreased the water

porosity of concrete made with fly ash and silica fume. With 10% fly ash & 10% silica fume, water absorption of concrete with 10^3 cells/ml bacterial concentration was 29% and 23% at 28 and 91 days respectively. Further with 30% fly ash & 10% silica fume, the porosity percentage reduction achieved was 19% and 17%, respectively at 28 and 91 days. Even in the combination of 30% fly ash with 5% silica fume porosity (18%) was observed, compared to 10% and 20% fly ash. In case of bacterial concrete with concentration of 10^7 cells/ml, 30% fly ash & 10% silica fume gave best reduction in porosity when compared to other combinations; 10% fly ash & 10% silica fume and 20% fly ash & 10% silica fume.

Reduction in water porosity was observed due to bacterial deposition in concrete although the extent of reduction depends on concentration of bacterial cells used. The optimum concentration of bacteria which resulted in best decrease in water uptake was 10^5 cells/ml. Ureolytic activity by bacteria produce calcite which clogges the pores at the surface of concrete to decrease the water absorption. Improvement of the aggregate cement paste interface by the bacterial induced calcium carbonate precipitation plays a very important role in decreasing the water porosity of concrete incorporating supplementary cementing materials (i.e. fly ash and silica fume). The decrease in water uptake of concrete containing bacteria (10^5 cells/ml) results in a denser microstructure due to calcium carbonate precipitation by bacteria.



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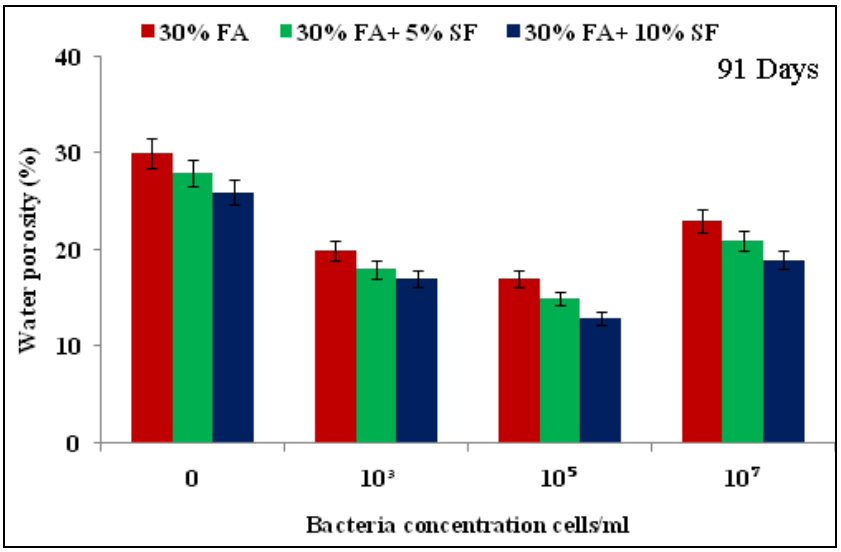


(b)

Figure 4.16: Effect of Bacteria (*Sporosarcina pasteurii)* on Water Porosity of (30%) Fly Ash and (5%, 10%) Silica Fume Concrete.

Same trend of water porosity values was found with bacterial cell concentration of 10^3 cells/ml and 10^7 cells/ml, but minimum water porosity was found in 10^5 cells/ml where maximum water porosity was found 17% in 30% FA + 5% SF in 28 days and 15% in 91 days. The percentage decrease was found maximum compared to control in the mixes e where 10% Silica fume was added rather than the 5% Silica fume. From these results, it can be well predicted that water porosity reduces substantially in bacterial concrete as compared to control concrete. Calcite deposition naturally sealed the pores in concrete. Permeation properties of concrete were improved due to bacterial calcite precipitation.

Similar results were quoted by Tiano et al. (1999) who reported that water porosity was reduced by the process of biomineralization by bacteria, which would increase the durability of the concrete structure. It was studied by Warren et al. (2001) that the degradation of urea gives calcium carbonate precipitates. Due to calcite precipitation by bacteria, there was significant decrease in water porosity of concrete as supported by previous studies of Bang et al. (2001); De Muynck et al. (2008a); Ramachandran et al., (2001); Ramakrishnan et al. (2001). Thus, it was concluded that due to pore blockage inside concrete cubes water porosity decreased drastically. Results were reported by

Nemati et al. (2005); Nolan et al. (1995) that a concrete which shows low permeation properties has more durability and show less signs of deterioration. De Muynck et al. (2008a) suggested that depending upon the water porosity of the specimens, the water absorption reduced to the extent of 65 to 90% due to the surface deposition of calcium carbonate crystals by bacteria. The porosity of harmful substances is reduced with the precipitated crystals of calcite which increases the durability of concrete as concluded by Van Tittelboom et al. (2010). The results from this research objective tend to confirm the transport mechanisms occurring in concrete influenced by calcium carbonate precipitation by bacteria and calcium salt. The bacterial mediation of calcite deposit*i*on resulted in resistance towards the water porosity. Bacterial (*Sporosarcina pasteurii*) calcite precipitation helped in clogging of pores providing a promising solution for durability of concrete.

4.2.3 Rapid Chloride Permeability

The ability of concrete to resist the penetration of chloride ions is a critical parameter in determining the durability of concrete structures. The concrete with supplementary cementing materials was used along with different concentrations of *Sporosarcina pasteurii*. The results of the mixes for rapid chloride penetration was tested at two different ages (28 & 91 days) of concrete. The results are given in Table 4.6.

Table 4.6: RCPT of Concrete. Values are ±S.D (n=3)

Mix	Without Bacteria		With Bacteria (10^3 cells/ml)		With Bacteria (10^5 cells/ml)		With Bacteria (10^7 cells/ml)	
	28 Days	91 Days	28 Days	91 Days	28 Days	91 Days	28 Days	91 Days
10% FA	1109±1.2	1041±1.1	991±1.1	830±1.0	680±1.53	590±1.20	1030±1.7	900±1.8
20% FA	980±1.0	820±1.2	730±1.3	680±1.4	600±1.21	550±1.21	770±1.20	750±1.1
30% FA	710±1.4	640±1.5	680±1.1	610±1.2	570±1.0	490±1.2	700±1.2	670±1.1
10% FA + 5% SF	890±1.6	700±1.6	680±2.56	590±2.53	651±3.1	467±3.2	690±3.12	670±3.0
20% FA + 5% SF	820±3.0	570±3.0	630±3.41	600±3.21	490±3.5	431±3.4	670±3.1	650±3.4
30% FA + 5% SF	810±3.8	656±3.7	705±3.25	501±3.56	630±4.1	391±4.1	660±4.23	580±4.23
10% FA + 10% SF	640±3.0	470±3.0	580± 3.2	600± 4.0	490±3.1	330±3.2	620±3.21	570±3.23
20% FA + 10% SF	720±2.0	540±2.1	600±2.1	490±3.2	580±4.0	300±4.0	610±4.12	560±4.23
30% FA + 10% SF	780±2.0	567±2.1	605±2.5	415±2.5	600±2.3	220±4.5	600±4.1	540±4.2

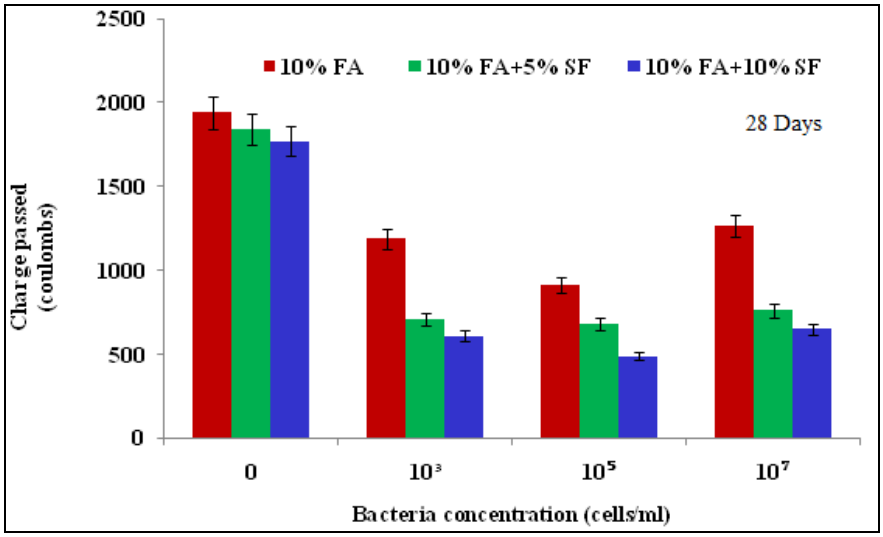
FA: Fly ash; SF: Silica fume

The concentrations of bacteria used were (10^3 cells/ml; 10^5 cells/ml; 10^7 cells/ml) along with different percentage of silica fume and fly ash. The different mixes showed resistance to the chloride penetration. The inclusion of bacteria with different concentrations affected the chloride penetration resistance of concrete. The same reduced for the concentration from 0 to 10^5 cells/ml, but there was an increase in case of bacterial concentration of 10^7 cells/ml. The Ca/Si ratio within the Calcium silicate hydrate (CSH) gel of concrete matrix is increased by the treatment of bacteria and is optimum at bacterial concentration of 10^5 cells/ml. At higher cell concentration of bacteria that is 10^7 cells/ml, the matrix integrity may disrupt due to excessive bacterial activity and thus the decrease in chloride penetration of concrete at higher cell concentration was observed.

The concrete with supplementary cementing materials along with optimized dose of *Sporosarcina pasteurii* mixes showed good resistance to rapid chloride penetration and were observed under the category of "very low" as per ASTM standard. Summary of average results of the effect of bacteria on the rapid chloride permeability of concrete at the age of 28 and 91 days is given in Table 4.6.

Maximum reduction in chloride ions was observed with 10^5 cells/ml for all fly ash and silica fume concretes; however, concrete with 10% fly ash and 10% silica fume concrete gave 490 coulombs penetration which is considered to be "very low". In control concrete, where no bacteria was added, the maximum chloride permeability was found to be 1041 coulombs at 91 days which kept on decreasing when 30% Fly ash was added (640 coulombs). Similar trends were observed in Fly ash mixture 10%, 20% and 30% with 5% and 10% Silica fume as shown in Figures 4.17- 4.19.

The maximum 890 coulombs was observed in 10% FA + 5% SF which kept on decreasing uptil 790 coulombs in 91 days in different combination of Fly ash and Silica fume except 10% Fly ash and 10% Silica fume which was 640 in 28 days and 470 coulombs in 91 days which is lowest in control. The similar trends were observed in 10^3 cells/ml where minimum 991 chloride permeability was observed in 10% Fly ash in 28 days and 830 in 91 days which kept on decreasing to 415 coulombs in 91 days with 30% FA+ 10% SF.



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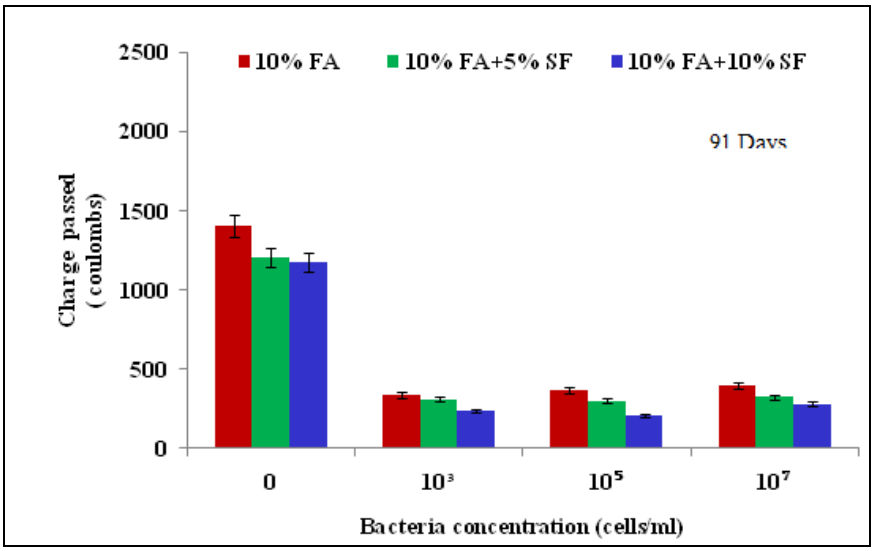
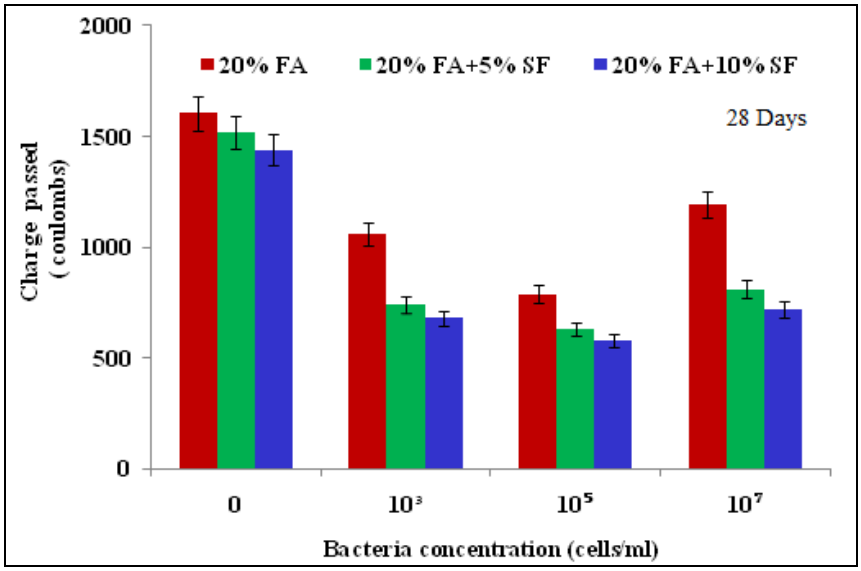


(b)

Figure 4.17: Effect of Bacteria (*Sporosarcina pasteurii)* on RCPT of (10%) Fly Ash and (5%, 10%) Silica Fume Concrete.



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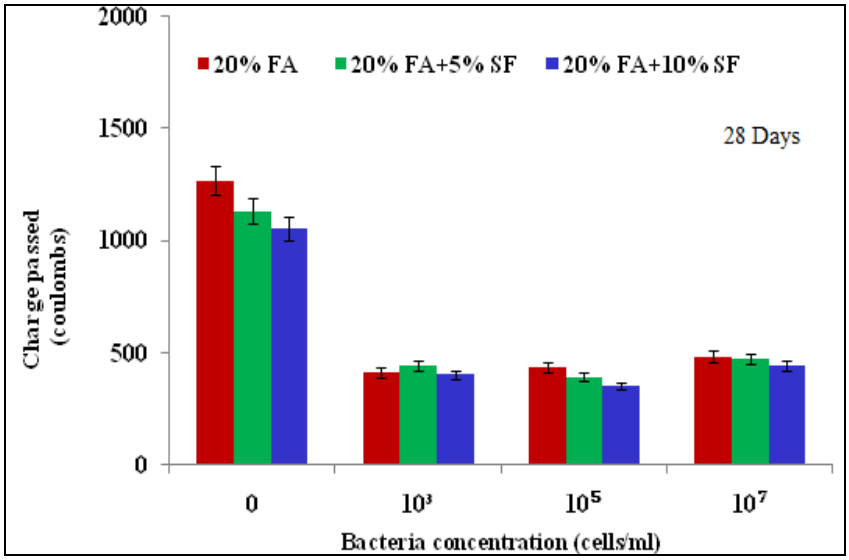

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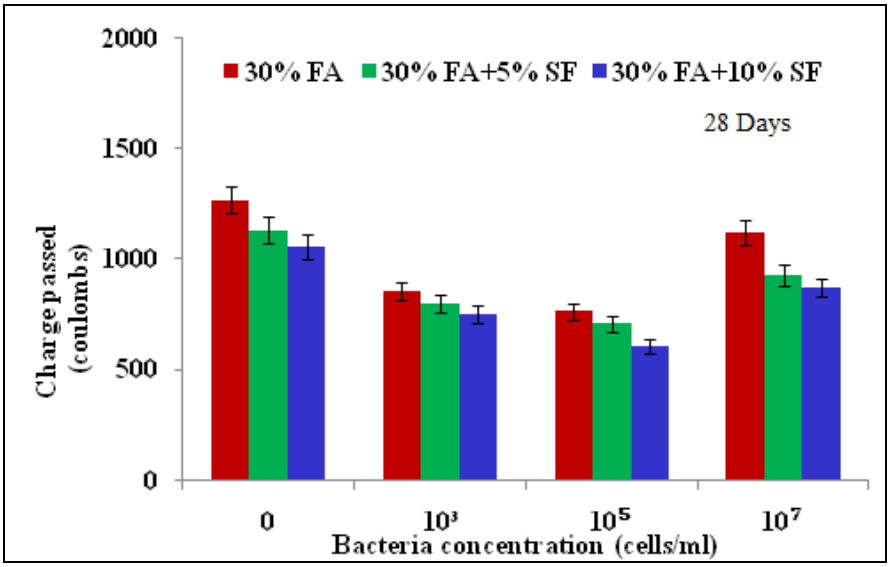
Figure 4.18: Effect of Bacteria (*Sporosarcina pasteurii)* on RCPT of (20%) Fly Ash and (5%, 10%) Silica Fume Concrete.

The minimum 540 coulombs of chloride permeability was gained in 91 days in 10^7 cells/ml which is higher than 200 when bacterial concentration 10^5 cell/ml.



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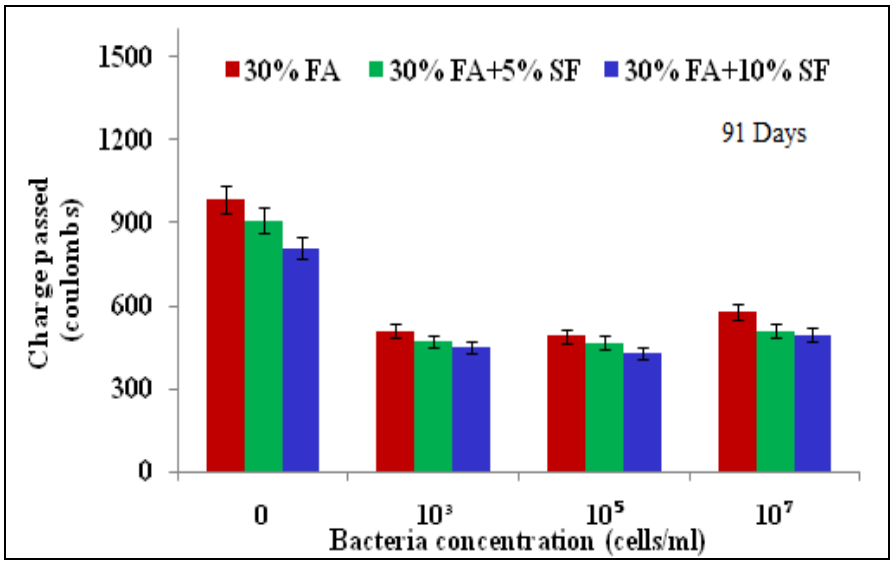

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Figure 4.19: Effect of Bacteria (*Sporosarcina pasteurii)* on RCPT of (30%) Fly Ash and (5%, 10%) Silica Fume Concrete.

Maximum reduction in chloride ions was observed with 10^5 cells/ml for all fly ash and silica fume concrete; however, concrete with 10% fly ash and 10% silica fume concrete gave 490 coulombs penetration which is considered to be "very low". De Muynck et al. (2008b) concluded that there was decrease in permeation properties of mortar and concrete due to calcite precipitation by bacteria. Nolan et al. (1995) suggested that concrete with less permeation properties has more durability and last longer showing less signs of distress. DeJong et al. (2006) reported that carbonate precipitates provide a protective effect which could thereby increase the life of concrete structures due to

increase amount of carbonate precipitation. De Muynck et al. (2009) had observed that the waterproofing be increased by the number of surface treatments or by increasing the concentration of crystals in one treatment. Similar results were summarized by other researchers Abou-Zeid et al. (2003); Zhang et al. (2008); Bickley et al. (2006); Whiffin et al. (2007) who concluded that silica fume content of up to 7% significantly reduced the chloride ion penetrability.

From the discussions presented by above results it can be concluded that more chloride ion penetrability can thereby reduce the durability of concrete structures. The bacterial pore blockage by calcite deposition resulted in resistance towards the chloride permeation.

4.2.4 Water Absorption

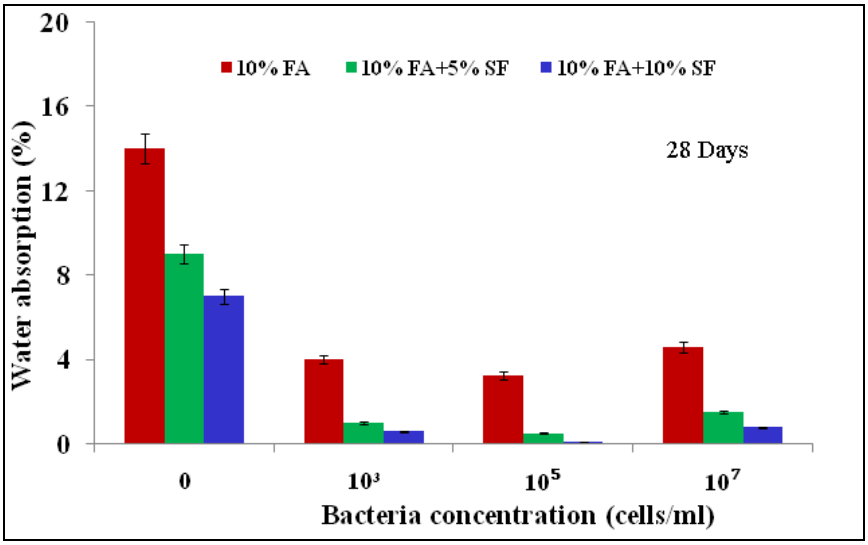
The influence of bacteria on the water absorption of fly ash and silica fume concrete is given in Table 4.7, and shown in Figures 4.20-4.22. It can be observed from the table that with the inclusion of bacteria, water absorption capacity of fly ash and silica fume concrete decreased.

Table 4.7: Water Absorption of Concrete. Values are ± S.D (n=3)

Mix	Without Bacteria		With Bacteria (10^3 cells/ml)		With Bacteria (10^5 cells/ml)		With Bacteria (10^7 cells/ml)	
	28 Days	91 Days	28 Days	91 Days	28 Days	91 Days	28 Days	91 Days
10% FA	14±0.15	11.8±0.17	4±0.16	3±0.18	3.25±0.43	2±0.25	4.6±0.44	3.4±0.45
20% FA	15.8±0.15	9±0.15	6.9±0.15	5±0.17	5.2±0.47	3±0.45	7±0.46	5±0.44
30% FA	17.4 ±0.15	14.5±0.15	7.8±0.16	7±0.17	6.9±0.36	6±0.28	8±0.37	7.9 ±0.38
10% FA + 5% SF	9±0.15	6±0.10	1±0.12	0.4±0.12	0.5±0.30	0.4±0.1	1.5±0.32	1±0.33
20% FA + 5% SF	11±0.05	7.5±0.17	1.5±0.9	0.09±0.0.1	0.9±0.55	0.3±0.07	2±0.48	0.9±0.49
30% FA + 5% SF	12±0.15	9±0.17	1.6±0.14	1±0.13	1.2±0.47	0.7±0.45	1.9±0.49	1±0.49
10% FA + 10% SF	7±0.10	4±0.05	0.6±0.11	0.2±0.13	0.1±0.50	0.09±0.50	0.8±0.10	0.5±0.11
20% FA + 10% SF	9±0.36	7±0.11	0.8±0.23	0.5±0.21	0.4±0.35	0.2±0.34	1.4±0.36	0.7±0.37
30% FA + 10% SF	10±0.25	6.9±0.15	2.3±0.22	0.9±0.20	0.6 ±0.23	0.4±0.50	2.8±0.51	2±0.52

FA: Fly ash; SF: Silica fume

Figure 4.20 shows the effect of Bacteria (*Sporosarcina pasteurii)* on the water absorption of concrete made with fly ash. The presence of bacteria resulted in a significant decrease in the water uptake compared to control specimens. The deposition of a layer of calcium carbonate on the surface and inside pores of the concrete specimens resulted in a decrease of water absorption and permeability. Once the pores are sealed, reduction in water ingress was observed.



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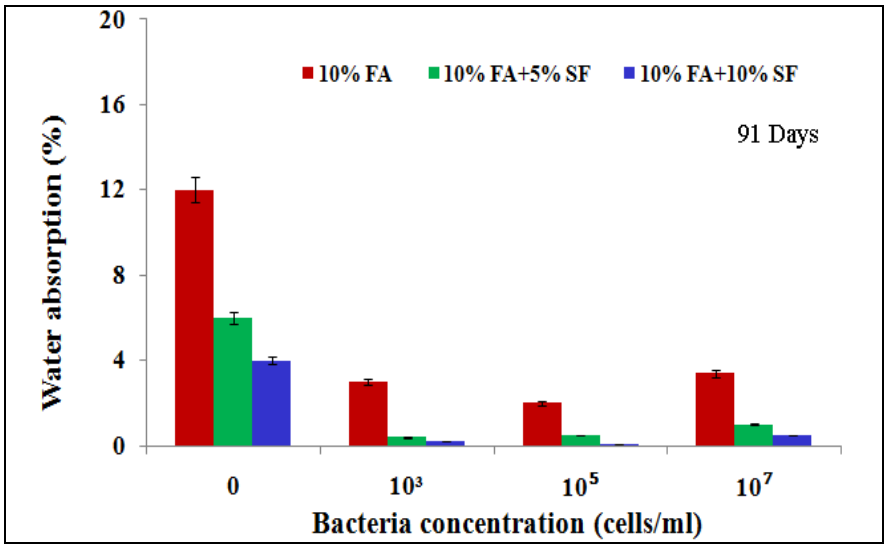
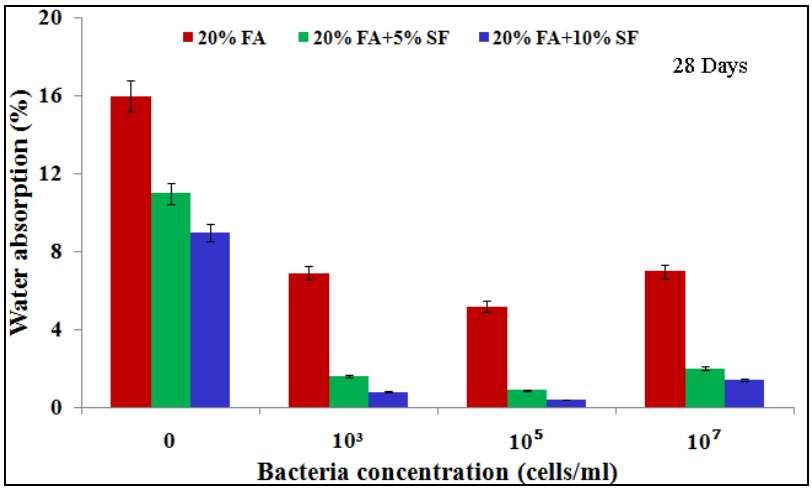


(b)

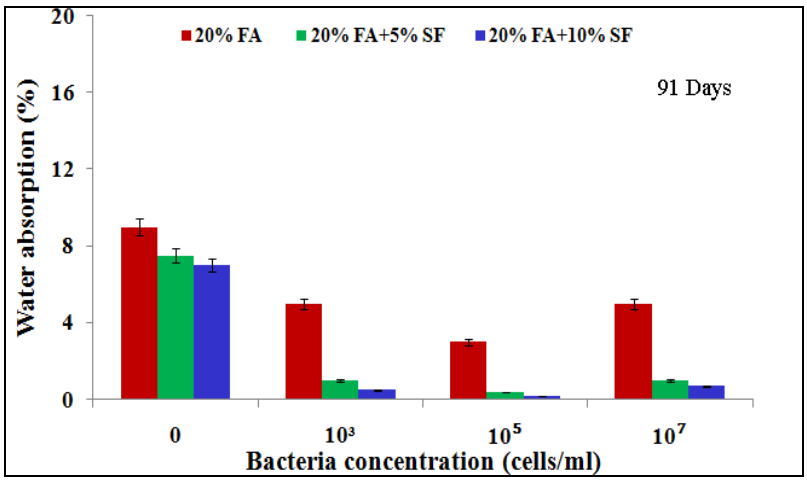
Figure 4.20: Effect of Bacteria (*Sporosarcina pasteurii)* on Water Absorption of (10%) Fly Ash and (5%, 10%) Silica Fume Concrete.

Water absorption capacity of concrete was high in case of control where bacteria were not added. Bacteria resulted in lowered water absorption capacity of concrete when compared to the control (untreated with bacteria) specimens of concrete. It can be seen from Table 4.7 and Figure 4.20 that water absorption of fly ash concrete without bacteria was 14%, 15.8% and 17.4%, respectively with 10, 20, and 30% fly ash content at the age of 28 days, and further water absorption decreased marginally at the age of 91 days. Bacteria played a significant role in decreasing the water absorption of fly ash concrete which decreased with increase in bacteria cell concentration up to 10^5 cells/ml, and then, there

was reduction in the absorption capacity with 10^7 cells/ml of bacteria whereas 10^3 cells/ml was considered as optimum concentration of bacterial dose in decreasing the water absorption. Figures 4.21-4.22 show the Influence of bacteria (*Sporosarcina pasteurii*) on water absorption of concrete containing both fly ash and silica fume. The maximum value of water absorption (12%) was observed for concrete mix with 30% fly ash and 5% silica fume content at the age of 28 days, and 4% at 91 days for mix with 10% fly ash and 10% silica fume. With inclusion of various dosages of bacteria, the water absorption capacity of the concrete mixes decreased significantly, as could be seen from Table 4.7 and Figure 4.21-4.22.

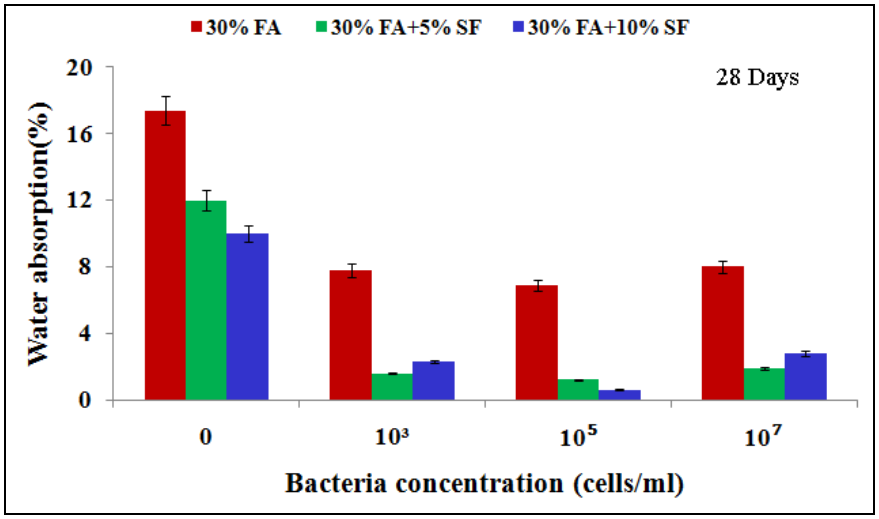


(a)

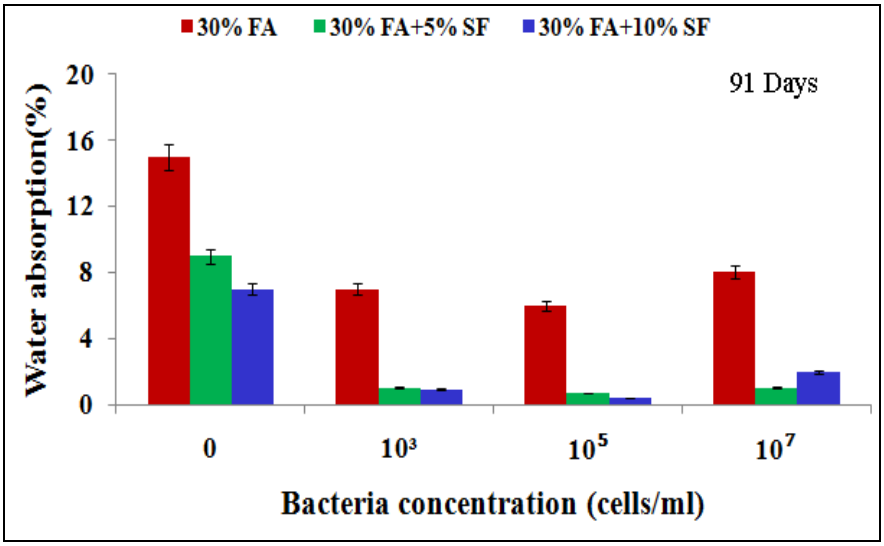


(b)

Figure 4.21: Effect of Bacteria (*Sporosarcina pasteurii)* on Water Absorption of (20%) Fly Ash and (5%, 10%) Silica Fume Concrete.



(a)



(b)

Figure 4.22: Effect of Bacteria (*Sporosarcina pasteurii)* on Water Absorption of (30%) Fly Ash and (5%, 10%) Silica Fume Concrete.

It can be seen from Figures 4.20- 4.22 that the water absorption of concrete (containing varying percentages of fly ash & silica fume) without bacteria was between 9% and 12% at 28 days and between 6% and 9% at the age of 91 days. Concrete made with 10% fly ash and 10% silica fume was observed to reduce water absorption from 7% at 28 days to 4% at the age 91 days. Bacterial inclusion thereby decreased the water absorption of concrete made with fly ash and silica fume. With 10% fly ash & 10% silica fume, water

absorption of concrete with 10^3 cells/ml bacterial concentration was 0.6% and 0.2% at 28 and 91 days, respectively. Further, with 30% fly ash & 10% silica fume, the water absorption percentage reduction achieved was 2.3% and 0.9% respectively, at 28 and 91 days. Even in the combination of 30% fly ash & 5% silica fume porosity (1%) 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 reduction in porosity when compared to other combinations; 20% fly ash & 10% silica fume and 30% fly ash & 10% silica fume. The decrease in water absorption by concrete containing 10^5 cells/ml of bacteria results in a denser microstructure due to calcium carbonate precipitation by bacteria. Same trend of water absorption values was found with bacterial cell concentration of 10^3 cells/ml and 10^7 cells/ml, but minimum water absorption was found in 10^5 cells/ml where maximum water porosity was found 1.2% in 30% FA & 5% SF in 28 days and 0.7% in 91 days. The percentage decrease was found maximum compared to control where 10% silica fume was added rather than the 5% silica fume. From these results, it can be concluded that water absorption reduces substantially in bacterial concrete as compared to control concrete. The presence of the calcite decreased the water absorption rate to a significant extent while retaining the permeability. Maximum reduction in water absorption was observed with 10^5 cells/ml for all fly ash and silica fume concrete.

Similar results were reported by Le Metayer-Levrel et al. (1999); Orial (2000) which concluded that bacteria deposits calcite which serves as an important factor to enhance the durability of concrete structures. Nemati et al. (2005) observed bacteria have the ability to decrease the water absorption capacity of sandstone cores thereby decreasing the permeability. From the experiment, it was studied that the resistance of cementitious materials towards degradation was improved by the presence of a layer of carbonate crystals on the surface by bacterial cells. De Muynck et al. (2008a); Achal et al. (2010) had confirmed that there was significant decrease of water uptake in the presence of bacteria as compared to control specimens. The results from this research objective conclude that deposition of a layer of calcium carbonate in the pores of the concrete specimens resulted in a decrease of water absorption. When the pores are blocked by inert material like calcium carbonate, the passage for water, air and pollutants is sealed which reduces the permeation of water and chloride in concrete. Bacterial induced calcite

precipitation can be regarded as a new potential method to increase and enhance the durability of concrete incorporating supplementary cementing materials. Bacterial usage in concrete may be highly desirable because the calcite precipitation induced by the metabolic activities is natural and pollution free.

4.2.5 EDX Analysis of Bacterial Concrete

In our case, formation of calcite in concrete is detected using EDX. The sample with best compressive strength, porosity low, low water absorption and low chloride permeability was checked through the EDX technique for the change in its compound components. The samples with bacterial concentration of 10^5cells/ml qualified the criteria of these concrete properties and showed the best results as compared to others. These samples were tested for EDX.

Table 4.8: EDX Analysis of Bacterial Treated Concrete Containing Fly Ash (10%) and of Silica Fume (5%, 10%)

Compounds	Fly Ash (10%)			
	Silica Fume (5%)		Silica Fume (10%)	
	28 Days (Weight %)	91 Days (Weight %)	28 Days (Weight %)	91 Days (Weight %)
$CaCO_3$	0.88	8.14	1	14.63
O	33.81	45.66	35.76	56.83
Na_2O	0.88	0.61	0.46	0.21
MgO	0.93	1.46	0.43	0.59
Al_2O_3	2.81	0.13	3.98	2.41
SiO_2	7.48	6.3	9.09	8.8
SO_2	0.5	0	1.59	0.72
K_2O	0.74	0	0.75	0.72
CaO	50.69	37.42	44.17	14
Fe_2O_3	1.28	0.28	2.77	1.09

The presence of crystalline calcite associated with bacteria indicated that bacteria served as nucleation sites during the mineralization process. For the calcite producing bacteria the results shall show CaO (Calcium oxide) converted to $CaCO_3$ which was revealed from the results thereafter. Samples with different mixtures for 10^5cells/ml bacteria concentration were tested for 28 and 91 days.

In reference to the Table 4.8, the EDX spectra for 10% fly ash with 5% and 10% silica fume showed that maximum CaO was found as 50.69 at 28 days, 37.42 at 91 days with 5% silica fume and 44.17 and 14% CaO in 10% Silica fume at 28 and 91 days, respectively.

Table 4.9: EDX Analysis of Bacterial Treated Concrete Containing Fly Ash (20%) and of silica fume (5%, 10%)

Compounds	**Fly Ash (20%)**			
	Silica Fume (5%)		**Silica Fume (5%)**	
	28 Days (Weight %)	**91 Days (Weight %)**	**28 Days (Weight %)**	**91 Days (Weight %)**
$CaCO_3$	0.68	8.52	1.01	10.12
O	36.13	55.73	37.62	52.4
Na_2O	0	0	0	0
MgO	0.54	0.89	0.33	0.66
Al_2O_3	2.86	1.75	2.67	1.75
SiO_2	12.16	11.6	14.46	12.4
SO_2	0.78	0.68	1.32	0.15
K_2O	0.81	0.28	1.32	0.47
CaO	41.4	18.69	37.25	21.05
Fe_2O_3	4.64	1.86	4.02	1

Considering the 10% fly ash and 10% silica fume, it is clear that $CaCO_3$ increased nearly 14 times after 91 days, which was considered as highest increase as compared to other samples with different combination of silica and fly ash. As the %age of fly ash was increased, the formation of calcite reduced. The maximum calcite was produced with 10% fly ash and with 10% silica fume after 91 days.

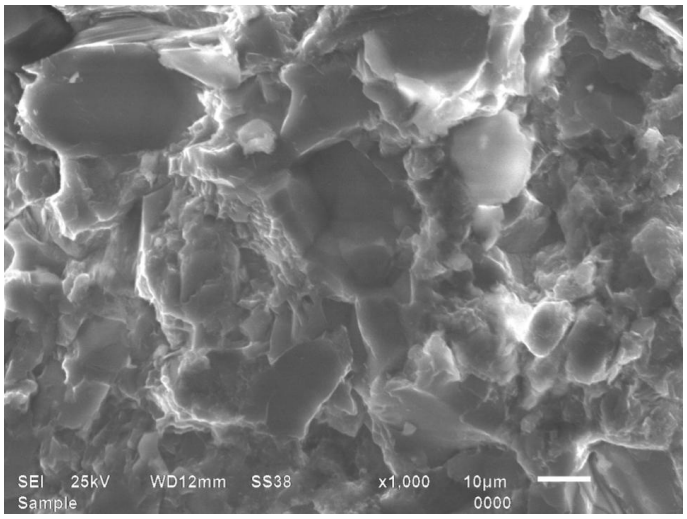
Consider the results tabulated in Table 4.8 and 4.9, 20% Fly ash EDX spectra range is 18.69 with 5% silica fume and 21.05 with 10% silica fume which further decreased with 30% fly ash where CaO ranges 10.76 to 36.8 CaO in 28 and 91 days, respectively.

Table 4.10: EDX Analysis of Bacterial Treated Concrete Containing Fly Ash (30%) and of Silica Fume (5%, 10%)

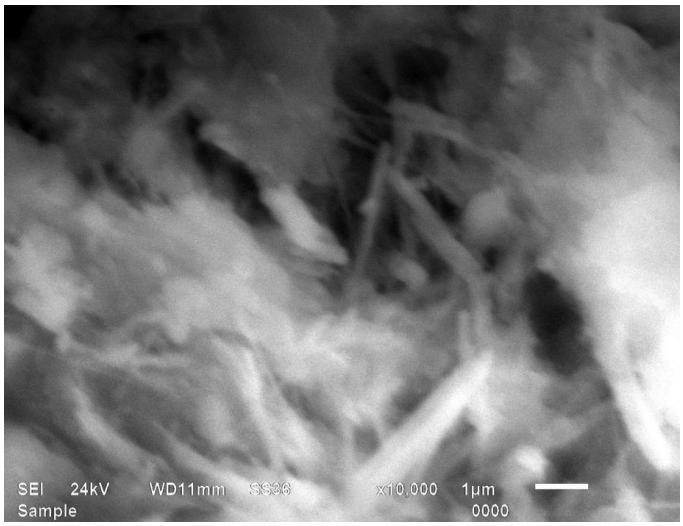
Compounds	**Fly Ash (30%)**			
	Silica Fume (5%)		**Silica Fume (10%)**	
	28 Days (Weight %)	**91 Days (Weight %)**	**28 Days (Weight %)**	**91 Days (Weight %)**
$CaCO_3$	0.61	5.01	0.92	10.03
O	35.4	53.42	40.68	56.1
Na_2O	0	0	0.88	0.42
MgO	0.55	0.43	0.37	0.25
Al_2O_3	3.72	2.1	8.71	3.72
SiO_2	11.31	10.5	16.48	15.9
SO_2	0.85	0.72	0.86	0.11
K_2O	1.1	0.26	0.72	0.62
CaO	36.8	24.36	24.62	10.76
Fe_2O_3	9.66	3.2	5.76	2.09

4.2.6 SEM Analysis of Bacterial Concrete

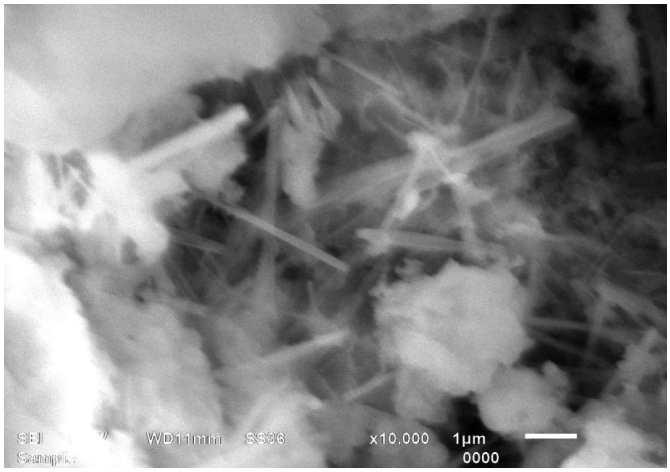
The concrete samples treated with bacteria and the untreated ones were analyzed using this technique, and SEM pictures are shown in Figure 4.23.The SEM analysis revealed the presence of distinct calcite crystals in the concrete samples. The high calcium amounts in all the bacterial samples confirmed that calcite was present in the form of calcium carbonate. The presence of crystalline calcite associated with bacteria indicated that bacteria served as nucleation sites during mineralization process.



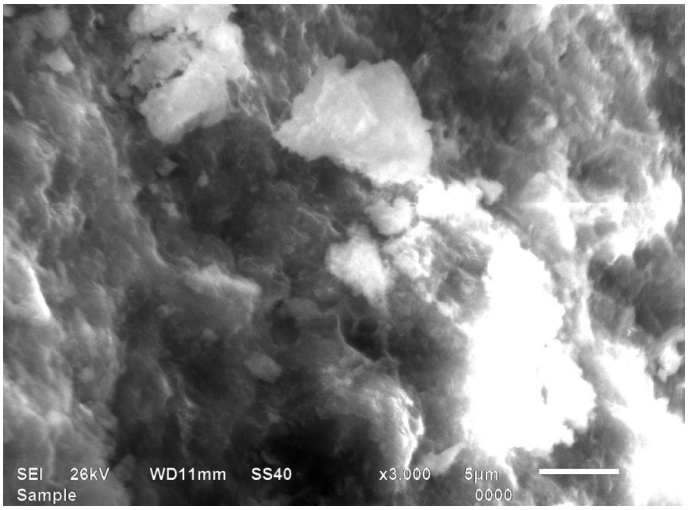
(a)



(b)



(c)



(d)

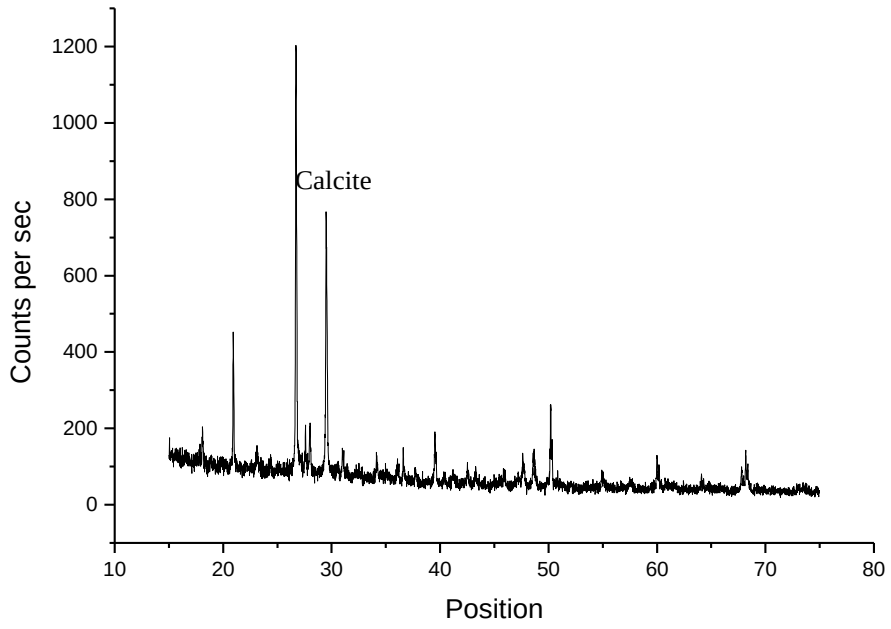
Figure 4.23: SEM Pictures of Control Concrete (a) and (b,c,d) depicts Calcite Precipitation By Bacteria

The treated and the untreated samples with bacteria were analyzed for the growth of calcite crystals. The matrix of the untreated samples (without bacteria) appears to be amorphous, showing no sign of crystal growth while the concrete samples that were treated with the microorganism shows crystalline matrix, where individual crystals could be recognized. The degree of formation of crystals in the matrix of treated samples is somewhat heterogeneous. There occur concentrations of relatively large crystals at the interfaces of sand particles and the matrix. This type of textural setting suggests that the

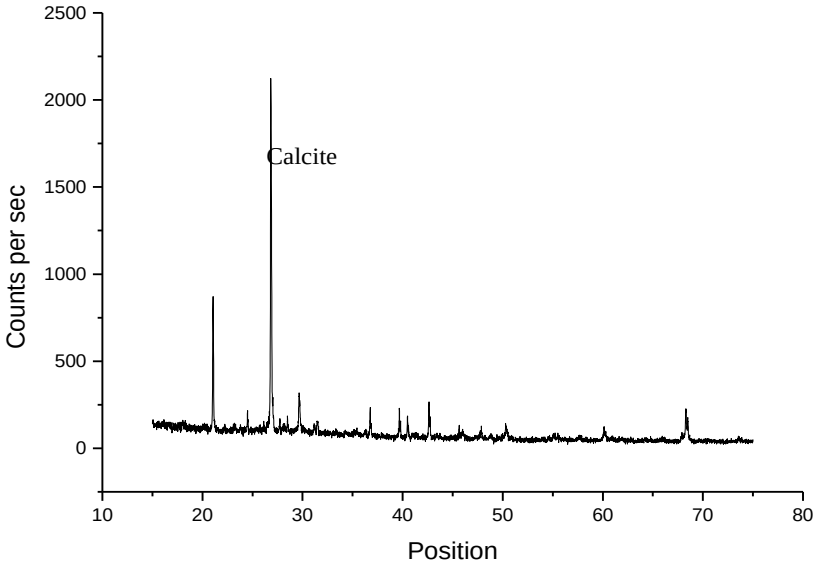
coherence between cement particles and the matrix in micro-scale is probably enhanced due to preferential crystallization at the concrete –matrix interfaces.

4.2.7 XRD Analysis of Bacterial Concrete

In our case, this technique was used to observe concrete for calcite formation and the comparison thereof, between the different samples. XRD analysis is shown in Figure 4.24. The graphs made using the technique were analyzed to get the sample which gave maximum number of calcite peaks. It was observed after comparison, that the maximum numbers of calcite peaks were observed concrete containing 10% fly ash and 10% silica fume. from the above results it was concluded that *Sporosarcina pasteurii* is more efficient with respect to calcite precipitation. The influence of the calcium source was limited to the morphology of the crystals. As significant increase in the urease activity was observed *Sporosarcina pasteurii* can be used commercially for the crack remediation process in buildings.



(a)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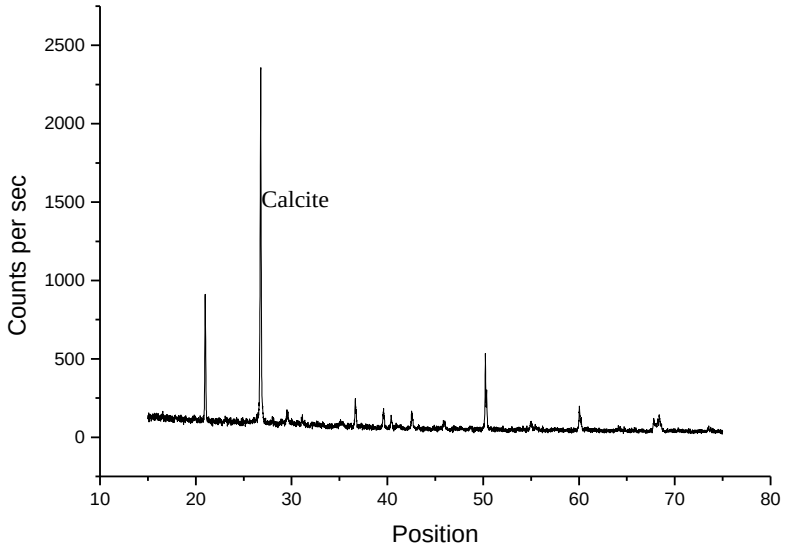
(b)


Calcite
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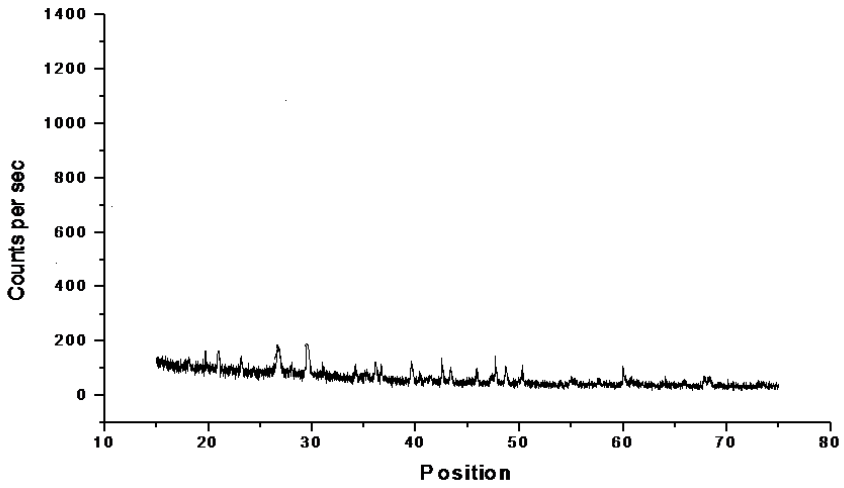
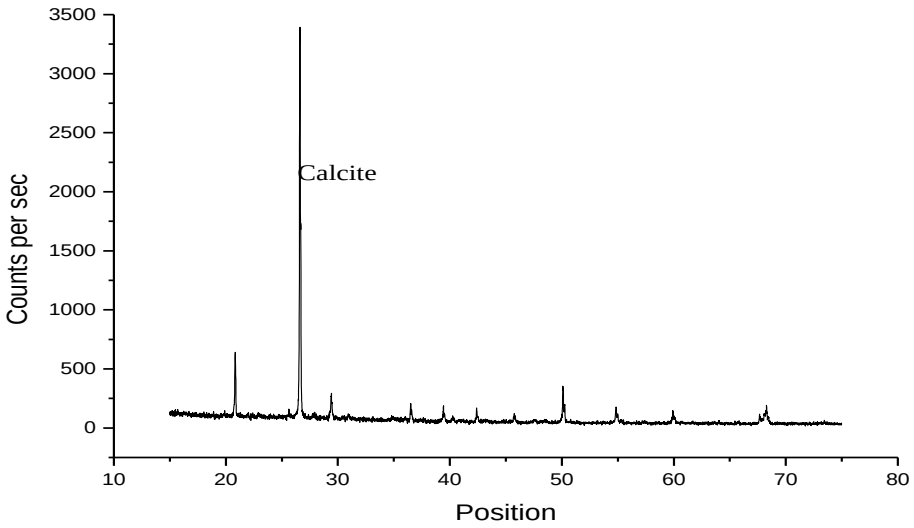
(c)

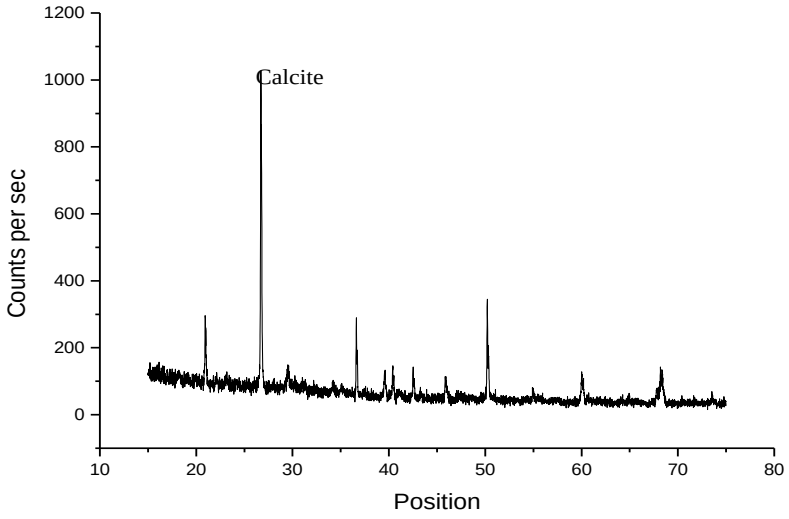


(d)

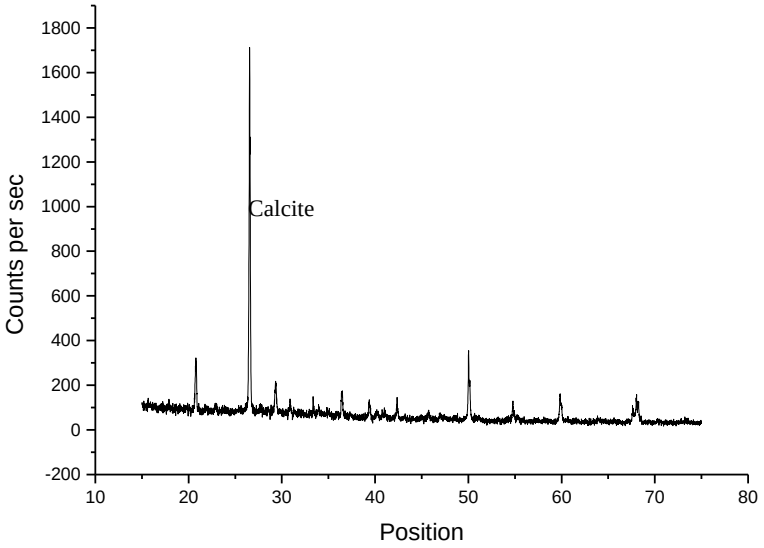
Figure 4.24: XRD Analysis of Bacterial Treated Concrete Containing (a) Fly Ash (10%) + Silica Fume (10%) (b) Fly Ash (20%)+Silica Fume(10%) (c) Fly Ash (30%) + Silica Fume (10%) (d) Control concrete.



(a)



(b)



(c)

Figure 4.25: XRD Analysis of Bacterial Treated Concrete Containing (a) Fly Ash(10%) + Silica Fume(5%) (b) Fly Ash (20%) +Silica Fume (5%) (c) Fly Ash (30%) +Silica Fume (5%).

The powder crystal X-ray analysis of the samples with and without bacteria shows that there were some extra peaks in the XRD spectra of the bacteria treated samples, which are absent in the control samples. An elaborate search of the minor peaks [comparing the values of 2h/d/(I/I0)] showed that these new peaks match with the minor peaks of pure calcium aluminum silicate phase. This result suggests that the bacteria is capable of formation new silicate phase within the concrete matrix. The microstructural in homogeneities can lead to serious effects on strength and other related mechanical properties because these properties are controlled by the microstructural extremes. The data obtained indicates that the overall distributions of the oxides of Si, Al and Fe within the concrete matrices of control sample are non-uniform. As the concentration of the bacterial cell was 10^5cells/ml, uniformity of the concentration of SiO_2 over the matrix gets increased. It is also noted that the distributions of other oxides are less uniform in the bacteria treated samples.

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. Considering Rs.50 (INR) equal to 1 US $. The rate (price) of the different components of concrete are taken as per the prevailing rates in typical Indian and US market and are as follows:

Rate of cement = Rs.7 per kg (US $=0.14)
Price of aggregates per m^3 = Rs. 957/- (US $=19.14)
Rate of Fly Ash = Rs. 0.00/-(being a byproduct of thermal power plant)

Rate of Bacteria = Rs. 88 per gm (US $=1.76)

Rate of Silica Fume = Rs. 44 per kg (US $=0.88)

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 4.11(a) (in INR) and 4.11 (b) (in US $).

In Table 4.11(a) and 4.11(b), first three columns 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 4.11 (a): Comparison of cost, permeability and compressive strength of bacterial concrete with control concrete (in INR)

Sr. No.	Mix	Cost (INR)	Permeability (Coulombs)	Compressive Strength (MPa)	Change in cost (%)	Change in Permeability (%)	Change in Compressive Strength(%)
1	**Cem.+Agg.**	3687	1150	28			
2	**Cem.+Agg.+Fly Ash 10%**	3414	1041	27	-7.40	-9.48	-3.57
3	**Cem.+Agg.+Fly Ash 10%+Bac.**	3942	590	28.6	6.92	-48.70	2.14
4	**Cem.+Agg.+Fly Ash 10%+Silica Fume 5%**	4135.5	700	29	12.16	-39.13	3.57
5	**Cem.+Agg.+Fly Ash 10%+Silica Fume 5%+Bac.**	4663.5	467	33	26.48	-59.39	17.86
6	**Cem.+Agg.+Fly Ash 10%+Silica Fume 10%**	4857	470	31	31.73	-59.13	10.71
7	**Cem.+Agg.+Fly Ash 10%+Silica Fume 10%+Bac.**	5385	330	36	46.05	-71.30	28.57
8	**Cem.+Agg.+Fly Ash 20%**	3141	820	26	-14.81	-28.70	-7.14
9	**Cem.+Agg.+Fly Ash 20%+Bac.**	3669	550	27.5	-0.49	-52.17	-1.79
10	**Cem.+Agg.+Fly Ash 20%+Silica Fume 5%**	3862.5	570	30	4.76	-50.43	7.14
11	**Cem.+Agg.+Fly Ash 20%+Silica Fume 5%+Bac.**	4390.5	431	32	19.08	-62.52	14.29
12	**Cem.+Agg.+Fly Ash 20%+Silica Fume 10%**	4584	540	29.7	24.33	-53.04	6.07
13	**Cem.+Agg.+Fly Ash 20%+Silica Fume 10%+Bac.**	5112	300	31	38.65	-73.91	10.71
14	**Cem.+Agg.+Fly Ash 30%**	2868	640	25	-22.21	-44.35	-10.71
15	**Cem.+Agg.+Fly Ash 30%+Bac.**	3396	490	26.4	-7.89	-57.39	-5.71
16	**Cem.+Agg.+Fly Ash 30%+Silica Fume 5%**	3589.5	656	28.8	-2.64	-42.96	2.86
17	**Cem.+Agg.+Fly Ash 30%+Silica Fume 5%+Bac.**	4117.5	391	30	11.68	-66.00	7.14
18	**Cem.+Agg.+Fly Ash 30%+Silica Fume 10%**	4311	567	29	16.92	-50.70	3.57
19	**Cem.+Agg.+Fly Ash 30%+Silica Fume 10%+Bac.**	4839	220	29.5	31.24	-80.87	5.36

Agg: Aggregates; Cem:Cement; Bac:Bacteria; INR-Indian Rupees

Table 4.11 (b): Comparison of cost, permeability and compressive strength of bacterial concrete with control concrete (in US $)

Sr. No.	Mix	Cost ($)	Permeability (Coulombs)	Compressive Strength (MPa)	Change in cost (%)	Change in Permeability (%)	Change in Compressive Strength(%)
1	**Cem.+Agg.**	73.7	1150	28			
2	**Cem.+Agg.+Fly Ash 10%**	68.3	1041	27	-7.40	-9.48	-3.57
3	**Cem.+Agg.+Fly Ash 10%+Bac.**	78.8	590	28.6	6.92	-48.70	2.14
4	**Cem.+Agg.+Fly Ash 10%+Silica Fume 5%**	82.7	700	29	12.16	-39.13	3.57
5	**Cem.+Agg.+Fly Ash 10%+Silica Fume 5%+Bac.**	93.3	467	33	26.48	-59.39	17.86
6	**Cem.+Agg.+Fly Ash 10%+Silica Fume 10%**	97.1	470	31	31.73	-59.13	10.71
7	**Cem.+Agg.+Fly Ash 10%+Silica Fume 10%+Bac.**	107.7	330	36	46.05	-71.30	28.57
8	**Cem.+Agg.+Fly Ash 20%**	62.8	820	26	-14.81	-28.70	-7.14
9	**Cem.+Agg.+Fly Ash 20%+Bac.**	73.4	550	27.5	-0.49	-52.17	-1.79
10	**Cem.+Agg.+Fly Ash 20%+Silica Fume 5%**	77.3	570	30	4.76	-50.43	7.14
11	**Cem.+Agg.+Fly Ash 20%+Silica Fume 5%+Bac.**	87.8	431	32	19.08	-62.52	14.29
12	**Cem.+Agg.+Fly Ash 20%+Silica Fume 10%**	91.7	540	29.7	24.33	-53.04	6.07
13	**Cem.+Agg.+Fly Ash 20%+Silica Fume 10%+Bac.**	102.2	300	31	38.65	-73.91	10.71
14	**Cem.+Agg.+Fly Ash 30%**	57.4	640	25	-22.21	-44.35	-10.71
15	**Cem.+Agg.+Fly Ash 30%+Bac.**	67.9	490	26.4	-7.89	-57.39	-5.71
16	**Cem.+Agg.+Fly Ash 30%+Silica Fume 5%**	71.8	656	28.8	-2.64	-42.96	2.86
17	**Cem.+Agg.+Fly Ash 30%+Silica Fume 5%+Bac.**	82.3	391	30	11.68	-66.00	7.14
18	**Cem.+Agg.+Fly Ash 30%+Silica Fume 10%**	86.2	567	29	16.92	-50.70	3.57
19	**Cem.+Agg.+Fly Ash 30%+Silica Fume 10%+Bac.**	96.8	220	29.5	31.24	-80.87	5.36

Agg: Aggregates; Cem:Cement; Bac:Bacteria; $-US Dollars

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 favourable results.

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 concluded that the bacteria could remain as dormant spore for a period upto 50 years without media

or 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 4.12 (a) and 4.12 (b) 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 concrete

For **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 bring them 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 4.12 (a): Benefit/Cost Ratio for selected samples(in INR)

Property	Weightage Factor	Cem. +Agg. +Fly Ash 10%+Bac.			Cem. +Agg.+Fly Ash 20%+Bac.			Cem. +Agg.+Fly Ash 20%+Silica Fume 5%+ Bac.			Cem. +Agg.+Fly Ash 30%+Bac.			Cem. +Agg.+Fly Ash 30%+Silica Fume 5%+Bac.		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Compressive Strength(MPa)	**10**	28.6	1.02	10.21	27.5	0.98	9.82	32	1.14	11.43	26.4	0.94	9.43	30	1.07	10.71
Permeability (Coulumbs)	**10**	590	0.49	4.9	550	0.52	5.2	431	0.63	6.3	490	0.57	5.7	391	0.66	6.6
Water Porosity (%)	**10**	26	0.04	0.4	19	0.30	3.0	17	0.37	3.7	17	0.37	3.7	15	0.44	4.4
Water Absorption (%)	**10**	2	0.89	8.9	3	0.83	8.3	0.3	0.98	9.8	6	0.67	6.7	0.7	0.96	9.6
Benefit				**24.41**			**26.32**			**31.23**			**25.53**			**31.31**
Cost (Rs.)		3942			3669			4390.5			3396			4117.5		
Benefit/Cost		**0.0062**			**0.0072**			**0.0071**			**0.0075**			**0.0076**		

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

Table 4.12 (b): Benefit/Cost Ratio for selected samples(in US $)

Property	Weightage Factor	Cem. +Agg. +Fly Ash 10%+Bac.			Cem. +Agg.+Fly Ash 20%+Bac.			Cem. +Agg.+Fly Ash 20%+Silica Fume 5%+ Bac.			Cem. +Agg.+Fly Ash 30%+Bac.			Cem. +Agg.+Fly Ash 30%+Silica Fume 5%+Bac.		
		A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Compressive Strength(MPa)	**10**	28.6	1.02	10.21	27.5	0.98	9.82	32	1.14	11.43	26.4	0.94	9.43	30	1.07	10.71
Permeability (Coulumbs)	**10**	590	0.49	4.9	550	0.52	5.2	431	0.63	6.3	490	0.57	5.7	391	0.66	6.6
Water Porosity (%)	**10**	26	0.04	0.4	19	0.30	3.0	17	0.37	3.7	17	0.37	3.7	15	0.44	4.4
Water Absorption (%)	**10**	2	0.89	8.9	3	0.83	8.3	0.3	0.98	9.8	6	0.67	6.7	0.7	0.96	9.6
Benefit				24.41			26.32			31.23			25.53			31.31
Cost ($)		78.8			73.4			87.8			67.9			82.4		
Benefit/Cost		0.31			0.36			0.35			0.37			0.38		

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 (0.38 when cost is in US $) 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 is one 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.

iii. A total of 10 Calcium carbonate precipitating strains were isolated from three alkaline media :- rhizopheric soil (tulsi plant), alkaline soil and sewage sludge. In a comparative study of 10 Bacterial strains, it was observed that in some strains urease activity started to decrease after 72 hours while in three bacterial strains (Bacillus strain 3, 4 and 5) this activity increased till 120 hours. . The maximum urease activity found in Bacillus strain 3, 4 and 5 and was 589U/ml, 593U/ml and 598U/ml, respectively after 120 hours. Based on XRD analysis, Biochemical characterization, and EDX analysis, it was found that the maximum amount of calcium content (by weight %) 52.54% in Strain 5, 46.42% in Strain 4 and 42.25% Strain 3 as indicated by EDX spectra. It was concluded that Strain 5 *(Sporosarcina pasteurii)* was more efficient with respect to calcite precipitation.

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 *Bacillus subtilis* (Strain 3), *Bacillus lichniformis* (Strain 4), *Sporosarcina pasteurii* (Strain 5). It was concluded that the bacterial strain 5, namely *Sporosarcina pasteurii,* 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), 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. Further studies were performed for optimization of bacteria. To check the effect of bacteria on concrete properties, the bacterial culture of 10^3, 10^5 10^7 cells/ml were prepared (*Sporosarcina pasteurii*). 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, 20, and 30% fly ash in addition to 0, 5 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 (*Sporosarcina pasteurii*) used were of 10^3, 10^5 and 10^7 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 and 13.5% more than that in 10^7 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 was scanned by electron microscopy and confirmed by XRD which revealed calcium carbonate precipitation.

5.4.2 Water Absorption

i. Water absorption was observed for all concrete mixtures. It was found that water absorption was less in mixtures with bacterial addition as compared to the mixtures without bacteria. This indicates that the voids of concrete may have been blocked due to calcite precipitation by bacteria.

ii. The minimum water absorption after 91 days was observed for mixtures with optimum bacterial concentration of 10^5 cells/ml. The absorption of water by *Sporosarcina pasteurii* causes nearly four to five times reduction in water absorption at 28 and 91 days respectively with 10^5 cells/ml of bacteria which could inturn increase durability of concrete structures. It may be concluded that reduction in water absorption was result of presence of SCMs in high percentage and optimum doze of bacteria.

iii. The maximum water absorption after 28 days and 91 days for mixtures with bacteria was in the mixture which had 10% fly ash only followed by 20% and

30% fly ash content. The water absorption was minimum for 10^5 cells/ml concentration mixtures. It showed decrease for all mixtures from concentration 10^3 cells/ml to 10^5 cells/ml and while it showed increase from concentration 10^5 cells/ml to 10^7 cells/ml. It may be concluded that the addition of bacteria has a limitation to reduce the water porosity with increase in bacterial concentration after 10^7cells/ml.

iv. Water absorption in all cases decreased with increase in age for all mixtures with or without bacteria. However, the rate of absorption decrease was higher in case of mixtures with bacteria than mixtures without bacteria.

5.4.3 Water Porosity

i. Water porosity was observed for all concrete mixtures. It was found that water porosity was less in mixtures where bacteria was added as compared to the mixtures without bacteria. This indicates that the pores of the concrete may been reduced in number or may have been blocked, which was the result of calcite precipitation by bacteria.

ii. The minimum water porosity after 91 days was observed for a mixture with 30% fly ash and 10% silica fume which had 10^5 cells/ml bacterial concentration. The porosity for 10^5 cells/ml concentration bacteria reduced by 50% than that in same mixture without bacteria. It may be concluded that reduction in water porosity was result of presence of SCMs in high percentage and optimum doze of bacteria.

iii. The maximum porosity after 28 days and 91 days for mixtures with bacteria was in the mixture which had 10% fly ash only. The water porosity was minimum for 10^5 cells/ml concentration mixtures. It showed decrease for all mixtures from concentration 10^3cells/ml to 10^5 cells/ml and while it showed increase from concentration 10^5 cells/ml to 10^7 cells/ml. The reason for increase for 10^7cells/ml that the Ca/Si ratio within the Calcium silicate hydrate (CSH) gel of concrete matrix gets increased when treated by bacteria and is optimum at bacterial concentration of 10^5cells/ml. It may be concluded that the addition of bacteria has a limitation to reduce the water porosity with increase in bacterial concentration after 10^7cells/ml.

iv. Water porosity in all cases decreased with increase in age for all mixtures with or without bacteria. However, the rate of water porosity decrease was higher in case of mixtures with bacteria than mixtures without bacteria.

5.4.4 Rapid Chloride Permeability Resistance

i. Chloride permeability resistance was observed for all concrete mixtures. It was found that chloride permeability resistance was less in mixtures where bacteria was added as compared to the mixtures without bacteria which indicates resistance to the flow through the voids of concrete as a result of reduction in their umber or blockage.

ii. The inclusion of bacteria with different concentrations affected the chloride penetration resistance of concrete. The same reduced for the concentration from 0 to 10^5 cells/ml.

iii. The minimum RCPT value (Coulombs) was for the mixture of 30% fly ash and 10% silica fume for bacterial concentration of 10^5cells/ml. The value reduced nearly 2.5 times from the value of same SCMs mixture without bacteria.

iv. RCPT value was more in case of bacterial concentration of 10^7cells/ml than 10^5cells/ml. Reason being, the Ca/Si ratio within the Calcium silicate hydrate (CSH) gel of concrete matrix is increased by the treatment of bacteria and is optimum at bacterial concentration of 10^5cells/ml. At higher cell concentration of bacteria that is 10^7 cells/ml, the matrix integrity gets disrupted due to excessive bacterial activity and thus the decrease in chloride penetration of concrete at this higher cell concentration was observed.

v. RCPT values in all cases decreased with increase in age for all mixtures with or without bacteria. However, the rate of chloride penetration decrease was much higher in case of mixtures with bacteria than mixtures without bacteria.

5.5 STATISTICAL ANALYSIS

i. All the experiments were performed in triplicate. The data was analyzed by SPSS (software). It concluded that inclusion of bacteria in concrete led to decrease water absorption, porosity and chloride permeability resistance.

ii. The standard deviation showed minimum error and the values are significant enough to increase the compressive strength of concrete containing bacteria.

5.6 X-RAY DIFFRACTION STUDIES

ii. Microstructure analysis was done using XRD for confirmation of calcite which was present in the form of calcium carbonate at age of 28 and 91 days. Calcite precipitation was more at 91 days and in all concrete mixes for M20 grade of concrete, calcite was present.

iii. The maximum numbers of calcite peaks were observed concrete containing 10% fly ash and 10% silica fume. Therefore, from the above results it can be concluded that *Sporosarcina pasteurii* is more efficient with respect to calcite precipitation after 91 days.

iv. Calcium hydroxide if leached out causes durability problems. But in our research work the presence of calcium hydroxide ($Ca(OH)_2$) is negligible in all concrete mix which confirm the maximum consumption in the hydration reaction and formation of calcium carbonate with additional development of C-S-H gel which led to improve the strength and durability properties.

5.7 SEM/EDX STUDIES

i. The SEM analysis of bacterial concrete revealed distinct calcite crystals embedded with bacteria which indicated that the bacteria served as the nucleation sites for the mineralization process.

ii. High calcium amounts in all the bacterial concrete samples confirmed that calcite was present in the form of calcium carbonate.

iii. In the EDX analysis, the spectra for CaO and $CaCO_3$ for 28 days was noted for all the mixtures. The study after 91 days revealed that the spectra for CaO decreased while that of $CaCO_3$ increased. It was concluded that the calcium carbonate spectra was increased due to presence of the bacteria.

iv. The concrete without bacteria was also analyzed with SEM/EDX. Its matrix appeared to be amorphous and showed minor no sign of calcium carbonate crystals after 91 days.

v. From the analysis it was clear and concluded that the concrete samples treated with the bacteria show crystalline matrix, where individual crystals could be recognized. The degree of formation of crystals in the matrix of treated samples is heterogeneous. This type of textural setting conclude that the coherence between cement particles and the matrix in micro-scale is enhanced due to preferential crystallization at the concrete–matrix interfaces.

5.8 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 5%+Bacteria showed the highest benefit/cost ratio and was considered as optimum mixture.

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APPENDIX –I

16S rRNA sequences of bacterial strains

Uncultured Bacillus sp. clone NKC5 16S ribosomal RNA gene, partial sequence

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LOCUS       JX081257       909 bp    DNA     linear   ENV 01-OCT-2012
DEFINITION  Uncultured Bacillus sp. clone NKC5 16S ribosomal RNA gene,
            partial  sequence.
ACCESSION   JX081257
VERSION     JX081257.1  GI:407020966
KEYWORDS    ENV.
SOURCE      uncultured Bacillus sp.
  ORGANISM  uncultured Bacillus sp.
            Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae;
            Bacillus;environmental samples.
REFERENCE   1 (bases 1 to 909)
  AUTHORS   Chahal,N.K., Rajor,A. and Siddique,R.
  TITLE     Isolation and identification of calcite producing bacteria
  JOURNAL   Unpublished
REFERENCE   2 (bases 1 to 909)
  AUTHORS   Chahal,N.K., Rajor,A. and Siddique,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-MAY-2012) Department of Biotechnology and
            Environmental Sciences, Thapar University, Patiala, Punjab
            147004,India
COMMENT     Sequences were screened for chimeras by the submitter using
            Bellerophon version 3.
FEATURES             Location/Qualifiers
     source          1..909
                     /organism="uncultured Bacillus sp."
                     /mol_type="genomic DNA"
                     /isolation_source="alkaline soil"
                     /db_xref="taxon:83428"
                     /clone="NKC5"
                     /environmental_sample
     rRNA            <1..>909
                     /product="16S ribosomal RNA"
ORIGIN
        1 ttcgaatagg ttcggtatca tagaccggta gcttaccaga atagccaggg cccagtacat
       61 tccagcagtt gggttatttc gtagggtggc tagcgttgtc gggtattatt gggcgtaaag
      121 cgcgcgctgg cgggtttgtt aactctgatg tgatagccct cggctcttac cgggggaggg
      181 tcattggaga ctggggaact tgagtgcaga agaggagagt ggacttacac gtgtagcggt
      241 gaaatggttt agatgtggcg gaacaccagt ggcgaaggcg actctctggt ctgtaaccga
      301 cggtgagtcc cgatggtttg tgggggggaca ggtttacata tctcggtggt ccacccccgta
      361 aacgccgagt gctaagtgtt agagggtttc gccctttagt gctgcagcta acgccttaaa
      421 cactcggcct ggggagtacg gtcgcaagac tgaaagtcaa aggaatggac gggggcccgc
      481 acaagcggtg gagcatgtgg tttgtttcgc accaactcga aggaccttac caggtcttga
      541 catcctatga caaccattca gatacgtttt ccccttcggg ggcagagtga caggtggtgc
      601 attcccttgt caagatttcc ttgagatgtt gggttaagtc ccgcaacgag ctcaaccttc
      661 ctgttagccc cagcattcag ttgggcactc taaggtgact gccggtgaca aaccggagga
      721 aggtggggat gaagtcaaat catcatcccc ttataacggg gctacacacc tccttcaatg
      781 ggcagaccaa agggcagcga agcggtgagg gtaaaccaat cccacacatg attatcagtt
      841 tggatcgcag tggcaactcg agtgtggagc ggattgctag actcgagtta gccttcgccg
      901 ccccccgcct
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Uncultured Bacillus sp. clone NK2 16S ribosomal RNA gene, partial sequence

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LOCUS       JX081255    1341 bp    DNA     linear   ENV 01-OCT-2012
DEFINITION  Uncultured Bacillus sp. clone NK2 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   JX081255
VERSION     JX081255.1  GI:407020964
KEYWORDS    ENV.
SOURCE      uncultured Bacillus sp.
  ORGANISM  uncultured Bacillus sp.
            Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae;
            Bacillus;environmental samples.
REFERENCE   1  (bases 1 to 1341)
  AUTHORS   Chahal,N.K., Rajor,A. and Siddique,R.
  TITLE     Isolation and identification of ureolytic bacteria
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1341)
  AUTHORS   Chahal,N.K., Rajor,A. and Siddique,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-MAY-2012) Department of Biotechnology and
            Environmental Sciences, Thapar University, Patiala, Punjab
            147004,India
COMMENT     Sequences were screened for chimeras by the submitter using
            Bellerophon version 3.
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /isolation_source="soil"
                     /db_xref="taxon:83428"
                     /clone="NK2"
                     /environmental_sample
                     /country="India"
                     /collection_date="11-Jul-2009"
     rRNA            <1..>1341
                     /product="16S ribosomal RNA"
ORIGIN
        1 atgttagcgg cggacgggtg agtaacacgt gggtaacctg cctgtaagac tgggataact
       61 ccgggaaacc ggggctaata ccggatggtt gtttgaaccg catggttcaa acataaaagg
      121 tggcttcggc taccacttac agatggaccc gcggcgcatt agctagttgg tgaggtaacg
      181 gctcaccaag gcgacgatgc gtagccgacc tgagagggtg atcggccaca ctgggactga
      241 gacacggccc agactcctac gggaggcagc agtagggaat cttccgcaat ggacgaaagt
      301 ctgacggagc aacgccgcgt gagtgatgaa ggttttcgga tcgtaaagct ctgttgttag
      361 ggaagaacaa gtaccgttcg aatagggcgg taccttgacg gtacctaacc agaaagccac
      421 ggctaactac gtgccagcag ccgcggtaat acgtaggtgg caagcgttgt ccggaattat
      481 tgggcgtaaa gggctcgcag gcggtttctt aagtctgatg tgaaagcccc cggctcaacc
      541 ggggagggtc attggaaact ggggaacttg agtgcagaag aggagagtgg aattccacgt
      601 gtagcggtga aatgcgtaga gatgtggagg aacaccagtg gcgaaggcga ctctctggtc
      661 tgtaactgac gctgaggagc gaaagcgtgg ggagcgaaca ggattagata ccctggtagt
      721 ccacgccgta aacgatgagt gctaagtgtt aggggtttc cgccccttag tgctgcagct
      781 aacgcattaa gcactccgcc tggggagtac ggtcgcaaga ctgaaactca aaggaattga
      841 cgggggcccg cacaagcggt ggagcatgtg gtttaattcg aagcaacgcg aagaacctta
      901 ccaggtcttg acatcctctg acaatcctag agataggacg tcccccttcgg gggcagagtg
      961 acaggtggtg catggttgtc gtcagctcgt gtcgtgagat gttgggttaa gtcccgcaac
     1021 gagcgcaacc cttgatctta gttgccagca ttcagttggg cactctaagg tgactgccgg
     1081 tgacaaaccg gaggaaggtg gggatgacgt caaatcatca tgccccttat gacctgggct
     1141 acacacgtgc tacaatggac agaacaaagg gcagcgaaac cgcgaggtta agccaatccc
     1201 acaaatctgt tctcagttcg gatcgcagtc tgcaactcga ctgcgtgaag ctggaatcgc
     1261 tagtaatcgc ggatcagcat gccgcggtga atacgttccc gggccttgta cacaccgccc
     1321 gtcacaccac gagagtttgt a
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Uncultured Bacillus sp. clone NKC8 16S ribosomal RNA gene, partial sequence

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LOCUS       JX081256         983 bp    DNA     linear   ENV 01-OCT-2012
DEFINITION  Uncultured Bacillus sp. clone NKC8 16S ribosomal RNA gene,
            partial sequence.
ACCESSION   JX081256
VERSION     JX081256.1  GI:407020965
KEYWORDS    ENV.
SOURCE      uncultured Bacillus sp.
  ORGANISM  uncultured Bacillus sp.
            Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae;
            Bacillus;environmental samples.
REFERENCE   1 (bases 1 to 983)
  AUTHORS   Chahal,N.K., Rajor,A. and Siddique,R.
  TITLE     Isolation and identification of calcite producing bacteria
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 983)
  AUTHORS   Chahal,N.K., Rajor,A. and Siddique,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (17-MAY-2012) Department of Biotechnology and
            Environmental Sciences, Thapar University, Patiala, Punjab
            147004,India
COMMENT     Sequences were screened for chimeras by the submitter using
            Bellerophon version 3.
FEATURES             Location/Qualifiers
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                     /mol_type="genomic DNA"
                     /isolation_source="alkaline soil"
                     /db_xref="taxon:83428"
                     /clone="NKC8"
                     /environmental_sample
     rRNA            <1..>983
                     /product="16S ribosomal RNA"
ORIGIN
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       61 aaggaagttt tgttcgaata gctcgatatc atatagcggt acctcaccca gatagtccgg
      121 ggcccgtact tgccagcaga cgttggtttt cgttgcgtgg ctagccttgt cgagaattat
      181 agggcgtaaa gcgcgcgctg gcggtttgtt aagttttgtt ttgaagcccc cggctctacc
      241 ggggagggtc attggagact ggggaacttg agtgcagcag aggagagtgg atttccacgt
      301 tgagcggtga aatgcgtata gatttggcgg aacacctgtg gcgaaggcgt ctctcttttc
      361 tgtaatcgac ggggagtccc gatagcttgg ggagcgacca ggttcacata tcccggtttt
      421 ccctccgtaa tcgctgagtg ctaagtgttt gagggtttgc gcccttagtg ctgcagcaaa
      481 cgccttaaac tctcggcaag gggagtacgg tcgcaagatt gaaagtcaag gaatggaggg
      541 gggcccgcac aagcggtgga gcatgtggtt taattcgcac caacgcgaag aaccttacca
      601 ggtcttgaca tcctatgaca accctagaga aaggttttcc cttcgggggc agagtgacag
      661 gtggtgcatg gtccttgtca agtttccttt gggttttggg ttaagtcccg caacgagctc
      721 aaccttccca tttgttgcca gcattcagtt gggcactcta aggtgactgc cgttgacaaa
      781 ccggaggaag gtggggatga cgtcaaatca tcatcccctt tttacttggg ctacacacct
      841 cctacaatgg gcagaccaaa gggcacagaa gccgtgaggg taaaccaatc ccacacatca
      901 ttctcagttt ggatcgcagt ctgcaactcg cgtgcgtgaa gctgtaatcg ctagtaatcc
      961 cgaacagcat cccgcccccg ccc
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