

INFLUENCE OF BACTERIA ON COMPRESSIVE STRENGTH AND DURABILITY OF CONCRETE MADE WITH CEMENT BY-PASS DUST

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

**MASTERS OF ENGINEERING
IN
STRUCTURAL ENGINEERING**

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JULY 2015

DECLARATION

The author hereby declare that this dissertation entitled “**INFLUENCE OF BACTERIA ON COMPRESSIVE STRENGTH AND DURABILITY OF CONCRETE MADE WITH CEMENT BY-PASS DUST.**”, in whole or part has not been used to obtain any degree in this, or any other, institute, except where references have been given in text, it is entirely the author own work. The author conform that the library may lend or copy this upon request for academic purposes.

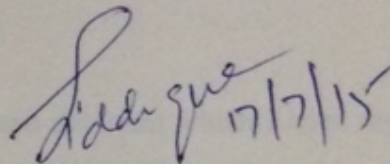
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CERTIFICATE

This is to certify that the work presented in dissertation entitled "INFLUENCE OF BACTERIA ON COMPRESSIVE STRENGTH AND DURABILITY OF CONCRETE MADE WITH CEMENT BY-PASS DUST" submitted by Mr. Vasu Nanda in partial fulfilment of the requirements for the award of degree of **Master of Engineering** in Structural Engineering at Thapar University, Patiala, is a bonafide work carried out by the student under my supervision and guidance. The matter embodied in this report has not been submitted anywhere for award of any other degree.



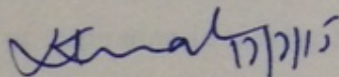
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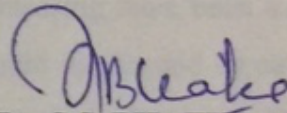


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ABSTRACT

Rapid industrialization and urbanization increases the demand of building and construction material for infrastructure development. Generation of cement by-pass dust (CBPD) during cement clinker manufacturing has become one of the major environmental and economical issues. Globally, cement industries generate tons of cement by-pass dust every year as a measure to control product quality and to ensure uninterrupted operation of the plant. In order to reduce disposal costs utilization of CBPD as construction or land fill material has become an alternative to its disposal. Cement by-pass can be used as partial replacement in concrete. This study presents the utilization of cement by-pass dust in control and bacterial concrete, cement by-pass dust as partial replacement of Portland cement (0, 10, 20 and 30%) on the normal consistency, setting times and hydration process of blended cement pastes, and on compressive strength at 28 and 56 days of curing of control and bacterial concrete was investigated. Several properties such as compressive strength, water absorption and porosity, RCPT and sorptivity of control and bacterial concrete was also studied at the age of 28 and 56 days. Calcite producing bacteria AKKR5 was mixed in water during concrete mixing. SEM and XRD analysis confirmed formation of calcite. Control and bacterial Concrete with 0% CBPD achieved best results for compressive strength of 32.89 N/mm² and 34.66 N/mm², respectively as the percentage of CBPD increased the compressive strength decreased. Water absorption was significantly reduced in bacterial concrete. The chloride permeability of concrete specimens containing control and bacterial concrete CBPD showed “moderate” to “low” permeability with increasing curing period from 28 to 56 days except 30% CBPD concrete which falls in “High” chloride permeability range. Scanning electron microscopy (SEM) results exhibits formation of non-expansive ettringite in pores and increased calcium silicate hydrate (CSH) which dense the mortar and concrete structure, and thus, increases the compressive strength in bacterial concrete CBPD concrete. X-ray diffraction (XRD) analysis confirmed the formation of CSH and non- expansive ettringite within the matrix of bacterial concrete specimens responsible for improvement in the strength of the material. It was also found that CBPD alone is not cementitious in nature and replacing it with cement will reduce cement content. But upto 10 % CBPD replacement there was 14 % decrease (27.23 N/mm²) in strength which is acceptable in accordance to design target strength (26 N/mm²) obtained for M20 grade concrete.

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CHAPTER 1

INTRODUCTION

1.1 GENERAL

Concrete is a combined building material, which contains of a binder (cement), coarse and fine aggregate. In building construction concrete discovers effective usage. Cement by-pass dust can be benefited in concrete blend because of its small particle size and lightweight. Lately, different types of building materials have found their way in building construction. These incorporate metals (for the structural framework of bigger buildings), plastics, asbestos and fabrics. Tar-based waterproof materials, polyvinyl chloride clay, paper linoleum, and solvent coatings for internal wall are additional building materials. Cement, tiles and bricks are some major building materials employed in the construction of buildings.

1.2 DURABILITY OF CONCRETE

Durability is described as the competence of concrete to oppose chemical attack, weathering action, and abrasion while preserving its much needed engineering properties (compressive strength, modulus of elasticity, creep, shrinkage, coefficient of thermal expansion, fire resistance). It usually concerns about the extent of trouble-free performance. Concrete require different degrees of durability depending on the exposure environment and properties desired.

Concrete remains durable under these conditions:

- Cement paste structure is dense and of low permeability.
- Under extreme weather conditions, air trapped in concrete 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 alkalies, chlorides, sulphates and silt.

1.2.1 Factors Affecting Durability of Concrete

Cement Content

Concrete blend should be designed to make sure it is cohesive and does not permit segregation and bleeding. If cement content is reduced, then at fixed w/c ratio the workability will be reduced, causing poor compaction. However, if water is added to improve workability, water /cement ratio rises and subsequent in highly permeable material. If cement is replaced then some supplementary cementitious material must be used to ensure less permeable concrete structure.

Compaction

The concrete as a whole is comprised of voids that are produced by poor compaction. Usually it is being governed by the compaction equipment used, type of form works and density of the steel work.

Curing

It is very important to permit good strength development, aid moisture retention and to ensure hydration process take place completely.

Permeability

It is considered the most important factor for durability. It can be noticed that higher permeability is usually caused by higher porosity that affects the durability.

1.2.2 Methods to Improve Durability

Physical Methods

A pozzolan is a siliceous and aluminous material which, in itself, Contains little or no cementitious value but which would, in finely divided form and in the presence of water, react chemically with calcium hydroxide at room temperature to form compounds possessing cementitious properties. Therefore improving durability of concrete.

Development of Self-healing Bacterial Concrete (Biological Method)

An innovative technique for the remediation of deteriorated structural formations has been developed by employing a selective bacterial plugging process, in which metabolic activities encourage precipitation of calcium carbonate in the form of calcite. Bio-mineralisation of calcium carbonate is the biological strategy to heal cracks in building materials.

Here we will be investigating the effect of physical and biological approaches on concrete .In the physical method, supplementary cementitious material cement by-pass

dust will be used and the durability effect on replacing cement with CBPD will be studied. Also calcite producing bacteria will be benefited to study the durability properties.

1.3 SUPPLEMENTARY CEMENTITIOUS MATERIALS

Supplementary cementitious materials (SCMs) are frequently used in concrete mixes to decrease cement contents, improve workability, increase strength and improve durability through hydraulic or pozzolanic activity. Utilization of these by-products in cement/concrete not only stops them from being land-filled but also enhances the properties of concrete in the fresh and hardened states. In addition, the usage of SCMs conserves energy and has environmental benefits because of reduction in carbon dioxide release as a consequence of reduction in manufacture of Portland cement. Severe air pollution controls and regulations have produced richness of industrial by-products 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 Cement by-pass dust which can be used in concrete as addition or as partial cement replacement. Sharman, et al. (2013) studied self-healing properties of cementitious composites combining different supplementary cementitious materials like fly-ash and slag, and discovered improved durability of concrete structure.

1.4 CEMENT BY-PASS DUST

Cement by-pass dust (CBPD), is a fine-grained by-product left at some stage in cement manufacturing and thus is considered as an industrial waste. CBPD is different from cement kiln dust (CKD). Cement kiln dust is the dust which passes out of the top of the pre-heater with the exhaust gases, or additionally out of the back of a long wet or long dry kiln. By-pass dust is the dust that is drawn out of the kiln inlet when some kiln exit gases are extracted between the kiln and pre-heater to disruption the cycle of volatile species between the kiln and pre-heater. The by-pass is therefore located between the kiln inlet and the pre-heater and the dust gathered from the by-pass is known as cement by-pass dust (Fig 1.1). Taha et al. (2002) reported that CBPD is a by-product produced during the production of Portland cement. As the raw materials are heated in the kiln, dust particles are produced and then passed out with the exhaust gases at the higher end of the kiln. These gases are cooled and the accompanying dust particles are drawn by

effective dust collection systems. The composition of CBPD is quite variable from source to source due to variation in raw materials and process variations. It is mainly made up of a variable amount of fine calcined and uncalcined feed materials, fine cement clinker, fuel combustion by-products, and condensed alkali compounds (Emery et al., 1992). The main component of CBPD is lime (CaO). Other compounds include SiO_2 , Al_2O_3 , Fe_2O_3 , K_2O , Na_2O , Cl, etc.

Quite a few research works has been published by using cement by-pass dust in concrete as supplementary replacement material. With the rising need to recycle industrial by-products and to protect the environment, there is an evolving need to provide technical data about the performance of concrete containing supplementary cementitious material cement by-pass dust.

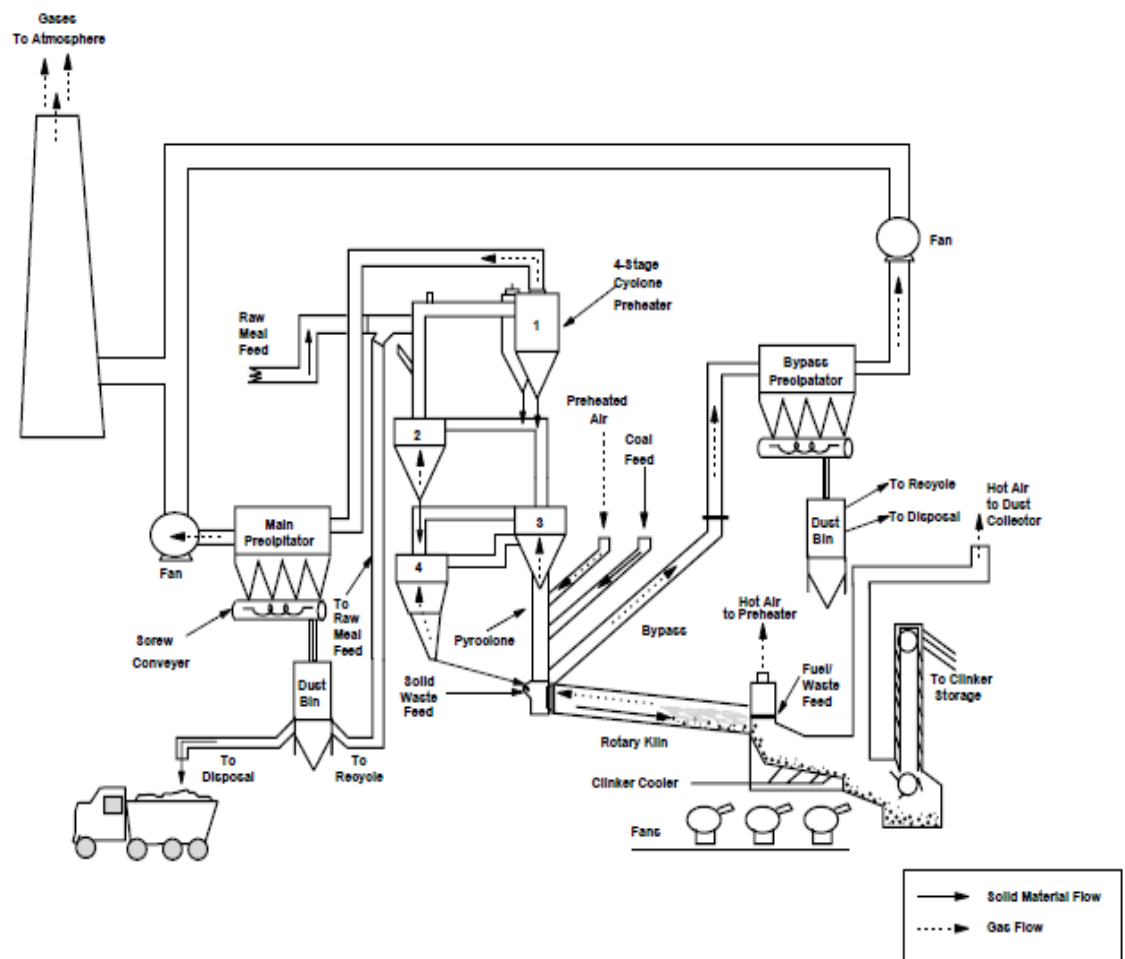


Fig 1.1: Cement by-pass dust production during cement manufacturing process. (Peray, 1986)

1.4.1 Advantages of Using Cement By-pass Dust

- **Agriculture** : Potash/lime source and animal feed
- **Civil Engineering** : Soil stabilization, fly ash stabilization, and blacktop filler
- **Building materials** : Lightweight aggregate, blocks, low-strength concrete, and masonry cement
- **Pollution control** : sulphur absorbent, waste treatment and solidification

1.4.2 Physical Properties

CBPD is fine powdered material of tan to pink in colour and relatively uniform in size. The particle size distribution of CBPD depends on the process technology, method of dust collection, chemical composition of CBPD. Table 1.1 represents the physical properties of the ordinary Portland cement and cement by-pass dust (CBPD). The data signifies that CBPD has greater fineness and greater surface area than OPC. The specific gravity of OPC is higher than CBPD.

Table 1.1 Physical characteristics of OPC and CBPD (Al-Jabri et al., 2005)

Test Type	OPC	CBPD
Specific Gravity	3.15	2.40
Fineness (cm ² /g)	3357	4824
Initial Setting (min)	110	150

1.4.3 Chemical Properties

Cement by-pass dust is drawn from the similar raw materials as ordinary Portland clinker, and despite the fact that it has alike chemical composition to that of ordinary Portland cement, significant difference in physical and chemical composition of CBPD obtained from various cement plants has been observed. Compounds of lime, silica and alumina comprise the major chemical composition of CBPD, Table 1.2 gives the typical chemical composition for CBPD and OPC, respectively.

Table 1.2 Chemical composition of cement by-pass dust. Al-Jabri et al. (2005)

Parameter (%)	OPC	CBPD
CaO	63.06	63.76
SiO ₂	20.85	15.84
Al ₂ O ₃	4.78	3.57
MgO	2.32	1.93
Na ₂ O	0.24	0.33
K ₂ O	0.55	2.99
Fe ₂ O ₃	3.51	2.76
SO ₃	2.48	1.65
LOI	1.75	5.38

1.5 BACTERIAL CONCRETE

1.5.1 Introduction

Microorganisms are microscopic, minute living things which are very small to be seen with the naked eye. These microorganisms are extremely diverse in nature. There are numerous diverse microbial species that contribute in the precipitation of mineral carbonates in numerous natural environments. The environment might include soils, geological formations, freshwater biofilms, oceans and saline lakes. Microorganisms can influence the carbonate precipitation both through affecting local geochemical conditions and by serving as possible, nucleation sites for mineral formation. Siddique and Chahal (2011) reported that selective microbial plugging process has been advanced and employed as a new technique for the remediation of damaged structure in which metabolic activities leads to precipitation of calcium carbonate in the form of calcite.

1.5.2 Bacteria

Bacteria are unicellular organisms. Bacteria have broad range of shapes and are a few micrometres in size. Bacteria are abundantly found in soil, water, as well as in organic

matter and the living bodies of plants and animals. There are typically 40 million bacterial cells in a gram of soil and a million bacterial cells in a millilitre of fresh water and in all, there are about five nonillion (Quintillion) (5×10^{30}) bacteria on Earth, forming much of the world's biomass. The weight of the cells is 30.2×10^{-6} g in 1g of wet soil while the weight in 1 g of dry soil is 40×10^{-6} g. 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} g. Bacteria help in recycling nutrients by course of fixation of nitrogen from the atmosphere. Lately, it has been found that bacteria are used in concrete as a self-healing agent. (De Muynck et al., 2008a).

1.5.3 Morphology of Bacteria

Morphology of bacteria talk about the shapes and sizes which are exhibited by bacteria. Spherical 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 somewhat 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 observed in some bacterial species.

1.5.4 Factors Affecting Bacterial Activity

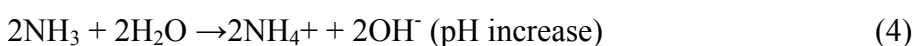
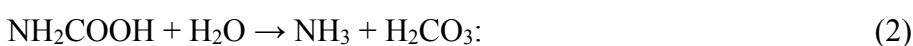
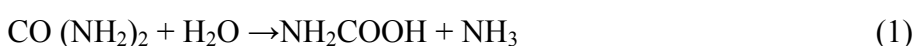
The factors which affect the growth of bacteria and the production of calcite include nutrients, water, pH, temperature, presentation of the organic contaminants and heavy metals, space of solids, the concentration of dissolved organic carbon, concentration of calcium ions and presence of nucleolus sites (Mitchell and Ferris, 2005; De Jong et al, 2006). Urea hydrolysis generates carbonate ions at a 1:1 molar ratio, hence controlling one of the key parameters for microbial induced cemetitious process (MICP) of dissolved inorganic carbon concentration. To sustain life the nutrients are energy source for the bacteria and the growth of bacteria is effected by the available type and amount of nutrient in system. Carbon (C), nitrogen (N), phosphorous (P), potassium (K) and Magnesium (Mg) are some of the nutrients helpful in the growth of bacteria (Mitchell and Ferris, 2005). The activity of bacteria also depends upon water which has different type and amount of soluble materials, different pH, aeration control and thermal stability. It was studied that calcium carbonate precipitation reached peak at pH level of 8 (Stocks

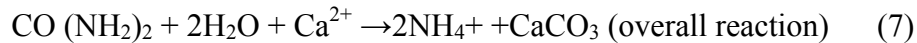
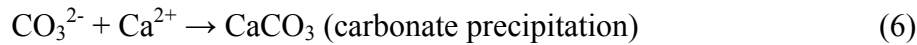
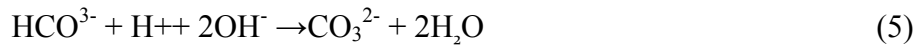
et al., 1999). The production of CaCO₃ was improved with lower concentration of urease enzyme (0.03 g/l) and an increase of temperature from 20 to 50°C (Nemati et al., 2005).

1.6 UREOLYTIC AND CARBONATE BIOMINERALIZATION

A biologically induced precipitation in which an organism creates a local micro-environment with conditions allowing optimal extracellular chemical precipitation of mineral phases is called biomineralization (Hamilton, 2003). Various natural environments have number of diverse microbial species which participate in the precipitation of carbonates. While the precise role of the microbes in the carbonate precipitation process is still not clear but almost all bacteria are capable of calcium carbonate precipitation (Boquet et al., 1973) and precipitation occurs as a by-product of common metabolic processes such as photosynthesis, sulphate reduction and urea hydrolysis (Hammes et al., 2003). The hydrolysis of urea generates carbonate ions without production of protons. When hydrolysis occurs in calcium rich environment, calcite precipitates are formed. The rate of carbonate formation has an important role to play in the strength of precipitated crystals and under suitable conditions it is possible to control the reaction to generate hard binding bio-cement.

The urease enzyme 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 gives 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 CaCO₃ 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 (Chahal et al., 2013)





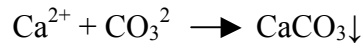
Calcium carbonate is an appropriate mineral to use for the reduction of porosity of underground formations for many reasons. Calcium ions (Ca^{2+}) is one of the most abundant cations and carbonate ions (HCO_3^- and CO_3^{2-}) are some of the most abundant anions in most subsurface waters. In order to produce the most mineral mass, utilizing elements already present in the subsurface is a more efficient method than adding another chemical. Injection of supercritical CO_2 into the underground formations will also make more carbonate ions by the dissolution and disassociation of CO_2 , which in turn will be used to precipitate more mineral.

Bacterial calcium carbonate precipitation results from both passive and active nucleation. Passive carbonate nucleation occurs from metabolically driven changes in the bulk fluid environment surrounding the bacterial cells. This increases the mineral saturation and induces nucleation (Schultze et al., 1996). In the ureolysis driven system, this occurs from an increase in pH due to ammonification (Stocks et al., 1999). Active carbonate nucleation occurs when the bacterial cell surface is utilized as the nucleation site. The cell clusters exhibit a net electronegative charge which favours 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. It has already been noted that cell walls have an inherent electronegative charge that affect the binding of certain ions (Beveridge, 1988), but the extracellular polymeric substance (EPS) associated with biofilms may also be involved. Biofilm cells are contained in the EPS matrix and may use it as an attachment device, for structural support, and/or protection (Ghannoum and O'Toole, 2004). The EPS matrix is composed primarily of polysaccharides and, depending on the side chains attached to the polysaccharides (e.g. carboxyl groups, pyruvate, phosphate, or sulfate), the matrix can exhibit an overall negative charge. This negative charge is important in trapping metal ions within the EPS

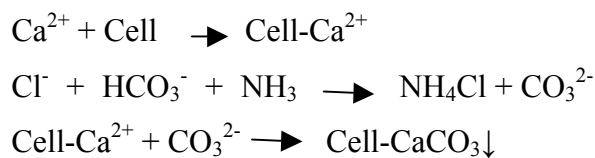
matrix (Kawaguchi and Decho, 1999). One of the primary applications of biomineralization is the plugging of porous media with applications leaning toward bioremediation (Mitchell and Ferris, 2005). Because plugging of porous media can occur in many different environmental locations and involve many different factors, such as soil alkalinity, temperature, and pressure, it is important to monitor the effectiveness of the bacteria's ability to precipitate out calcium carbonate in each different environmental situation.

Ferris et al. (2003) reported that the hydrolysis of urea by *Bacillus pasteurii* (now reclassified as *Sporosarcina pasteurii*) 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, and emphasized the impact of environmental conditions on calcite precipitation that were previously noticed. Members of the genus *Bacillus* are Gram-positive, rod-shaped, endosporeforming bacteria commonly found in soil (Todar and Kenneth, 2005). *Bacillus pasteurii*, a member of this genus, converts urea to ammonium carbonate more actively than any other known bacterium. Therefore, *B. pasteurii* and other members of the *Bacillus* genus are incorporated into studies to determine their influence on calcium carbonate precipitation in various environments. Experiments performed indicated that urease activity at high pH in *B. pasteurii* favored calcium carbonate precipitation (Stocks et al., 1999). Upon examination of the sand grains from columns used in the experiment, bacterial cells were shown encased in calcite crystals, which indicated that the bacteria acted as a nucleation site for the mineralization process, an example of active nucleation. Microbiologically induced (also called "bacteriogenic") calcium carbonate precipitation is comprised of a series of complex biochemical reactions, including concomitant participations of a bacterium like *Bacillus pasteurii*, urease (urea amidohydrolase; EC 3.5.1.5), and high pH. In this process, an alkalophilic soil microorganism, like *B. 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)



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

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



1.7 APPLICATIONS

1.7.1 Bacterial Concrete as an Alternative Surface Treatment

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

1.7.2 Bacterial Concrete as Concrete Crack Healing.

When cracks appear in the concrete, the possibility for corrosion of the embedded steel arises which could eventually ruin the integrity of the structure. Without immediate attention, the cracks can expand and cause extensive damage. Current forms of concrete crack remediation are structural epoxy, resins, epoxy mortar, and other synthetic filler agents. These synthetic solutions often need to be applied more than once as the cracks expand. Clearly there is a need for an effective, long-term, environmentally safe method to repair cracks in concrete structures.

Several research groups have investigated the possibility of bio-mineralization as an effective method to remediate cracks and fissures in concrete structures. Cracks filled with a mixture of *B. pasteurii* (now reclassified as *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). Other groups have noted that biomineralization can be used in the conservation of ornamental limestone statues or carvings, similar to its use in concrete remediation. *Myxococcus xanthus* is capable of precipitating calcium carbonate. The CaCO₃ cements pre-existing calcite grains on the pore walls of the limestone without completely plugging the pore. The resulting crystals are strongly attached and more resistant to stress than were the pre-existing calcite grains (Rodriguez et al., 2003).

1.8 OBJECTIVE

Cement by-pass dust does not possess binding properties thus replacing cement with cement by-pass dust will lead to less cement content, therefore bacteria is added to the mix to enhance concrete properties

CHAPTER 2

LITERATURE REVIEW

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. Calcium carbonate precipitation adopts two different mechanisms which involves both biological and controlled or induced. 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 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 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 CO₂ 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.

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. 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 *B. megaterium* induced carbonate bio-mineralization by producing a long term effect on calcium carbonate precipitation. 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).

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; Oriol et al., 2002).

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

B. pasteurii creates a local environment with conditions that allow optimal extracellular chemical precipitation of mineral phases (Hamilton, 2003; De Muynck et al. 2008a; Bachmeier et al., 2002.) 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). When weathered concrete samples were treated

with *Thiobacillus* bacteria a dense layer of calcite and vaterite crystals were observed by SEM and XRD analysis.

Chahal et al. (2012) presented the effect of the ureolytic bacteria (*Sporosarcina pasteurii*) on the compressive strength. Three bacterial concentrations were optimized (10^3 , 10^5 , 10^7 cells/ml) and further designing of concrete mixes was done. In making concrete, cement was replaced with 5% and 10% of silica fume by weight. Compressive test at the age of 28 and 91 days. Test results indicated that inclusion of *S. pasteurii* in concrete enhanced the compressive strength. Maximum increase of 38.2 N/mm^2 and 44 N/mm^2 in compressive strength at 28 and 91 days was observed.

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 (bio-deposition) 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.

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” (1000-2000) whereas for control concrete specimens the class changed to “moderate”. The average charge passed was 3177 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 1019 and 1185 C, respectively.

Chahal et al. (2012) found influence of bacteria on the water absorption of fly ash concrete as water absorption test at 7-days was conducted as per ASTM C 642 (2006). It

was observed that the inclusion of bacteria, water absorption capacity of fly ash concrete decreased with increase in the range of bacteria concentration. Maximum reduction in water absorption was observed with 10^5 cells/ml for all fly ash concretes; however, concrete with 10% fly ash concrete gave minimum 3.25% water absorption.

2.3.3 Rapid Chloride Permeability Test

Siddique and Chahal (2011) reported decrease in gas permeability due to the bio-deposition treatments resulted in an increased resistance towards carbonation. Except for the water repellents, similar tendencies were observed between the gas permeability and carbonation rate results. The rate of carbonation and the performance of the surface treatment were correlated to the water–cement ratio. Carbonation was shown to be related to the nature and connectivity of the pores, with larger pores giving rise to higher carbonation depths. Significant differences in carbonation depth between treated and untreated specimens were already noticeable after 2 weeks of accelerated carbonation. The protective effect of the bio-deposition treatment towards carbonation could be improved by additional treatments with bacteria and a calcium source or an increased concentration of calcium ions.

2.3.4 Sorptivity

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, where bacteria served as nucleation sites in a metal. (Ferris et al., 1987).

2.4 EFFECT OF CEMENT BY-PASS DUST ON CONCRETE PROPERTIES

2.4.1 Compressive Strength

Al-Jabri et al. (2006) studied the effect of copper slag (CS) and cement by-pass dust (CBPD) addition on concrete properties. In addition to the control mixture. Two different trial mixtures were prepared using different proportions of CS and CBPD. Cement by-pass dust was primarily used as an activator. One mixture consisted of 5% copper slag

substitution for Portland cement. The other mixture consisted of 13.5% CS, 1.5% CBPD and 85% Portland cement. Three water to binder (w/b) ratios were studied: 0.5, 0.6 and 0.7. Concrete cubes, cylinders and prisms were prepared and tested for strength after 7 and 28 days of curing. The modulus of elasticity of these mixtures was also evaluated. Results showed that 5% copper slag substitution for Portland cement gave a similar strength performance as the control mixture, especially at low w/b ratios (0.5 and 0.6). Higher copper slag (13.5%) replacement yielded lower strength values. Results also demonstrated that the use of CS and CBPD as partial replacements of Portland cement has no significant effect on the modulus of elasticity of concrete.

Dhir, et al. (2002) discussed compressive strength of concrete partially replaced with CBPD, 0, 5, 10, 15, 20, 25 and 30 % at different water-to-cement ratio of 0.5, 0.6 and 0.7, respectively. For all the mix the control mixture (0%CBPD) had highest compressive strength, it was observed that there is decrease in compressive strength in mixes with higher water cement ratios ($w/c = 0.70$) than in those mixes with low water to cement ratio ($w/c=0.5$). CBPD alone is not cementitious and replacement of cement by CBPD will lead to less cement content and therefore less strength. However small amount of replacement up to 10% does not seem to have any significant adverse effect on strength especially at low w/c ratios.

Al-Jabri et al. (2002) studied copper slag (CS) and cement by-pass dust (CBPD) are by-products of the production of copper and cement, respectively. The main objective of this research is to investigate the potential use of copper slag and cement by-pass dust in concrete as partial replacements for Portland cement. The physical and chemical properties of both slag and cement by-pass dust were determined. Mortar samples were prepared using different proportions of slag, cement by-pass dust and lime, which was used as an activating material. Proportions up to 15% of Portland cement replacement were used. In addition, a control mixture containing 100% Portland cement was prepared for comparison. Results obtained indicated that the increase in the proportions of copper slag and cement by-pass dust alone resulted in a decrease in the compressive strength of mortars compared with the control mix. The highest compressive strength was achieved in control 44 N/mm^2 , samples containing 5% CBPD + 95% cement, which was 41.7 N/mm^2 after 90 days. The optimum copper slag and cement by-pass dust to be used is

5%. In addition, it was determined that using cement bypass dust as an activating material will work better than using lime.

Al-Harthy et al. (2003) investigated the effects of CKD on the compressive strength of concrete mixtures at the ages of 3, 7, and 28 days. Seven different concrete mixtures were made by replacing cement with 0% (control), 5%, 10%, 15%, 20%, 25% and 30% CKD by total weight of the cement. For each of the mixtures, three water-to-binder ratios of 0.50, 0.60 and 0.70, by weight were used. Based on the investigation they reported that (i) there was generally a decrease in compressive strength with an increase in CKD replacement for cement. More reduction in compressive strength was observed in mixtures with higher water-to-binder ratio (0.7) than in mixtures with low water-to-binder ratio (0.5). For all blends, the control mixture (0% CKD) generally produced the highest compressive strength. At the age of 28 days, control mixtures (0% CKD) with water-to-binder ratio of 0.70, 0.60, and 0.50 achieved compressive strengths of 34.4, 37.5 and 55 N/mm², respectively. However, small amounts of replacements up to 5% did not seem to have an appreciable adverse effect on the compressive strength, especially at low water-to-binder ratio (0.50). At 5% and 10% CKD substitution for Portland cement, the reductions in the 28-day compressive strength values were 1.8% and 4.5%, respectively. At water-to-binder ratio of 0.60, 28-day strength reductions were 12.4% and 18% for 5% and 10% CKD replacement for Portland cement, whereas at water-to-binder ratio (0.70), strength reductions were 8% and 13% for 5% and 10% CKD replacement. At maximum level (30%) of CKD substitution, the reductions in strengths were 31%, 29%, and 22% for water to binder ratios of 0.70, 0.60, and 0.50, respectively; and (ii) concrete mixtures containing lower percentages (5%) of CKD produced close compressive strength values to the control mix, especially at a water-to-binder ratio of 0.50.

Kunal et al. (2014) found in his study when cement is replaced with different percentages of untreated CKD, the alkalinity of concrete (5%, 10% and 15%) goes on decreasing instead of increasing. After addition of bacterial treated CKD in concrete, 64%, 62.8% and 60% alkalinity was observed in 5%, 10% and 15% treated CKD concrete compared to control concrete mix. After casting at the age of 7 days, the control mix (0% CKD) showed compressive strength of 23.23 N/mm², whereas 5%, 10% and 15% CKD (untreated) concrete showed compressive strength of 23.78, 24.31 and 23.03 N/mm²,

respectively. The compressive strength of the CKD control (untreated) concrete increased with increase in curing period. At the age of 28 days, the compressive strength of the control CKD concrete (0% CKD) was 34.82 N/mm² whereas of 5%, 10% and 15% control CKD concrete was 35.78, 36.29 and 34.53 N/mm², respectively. Similarly at 91 days of curing period, there was 40.44, 41.89, 44.12 and 40.03 N/mm² of compressive strength in 0%, 5%, 10% and 15% CKD control concrete.

2.4.2 Water Absorption and Porosity

Udoeyo and Ridnap (2002) reported the results of a study on the properties of hollow sandcrete blocks with CKD as an additive and as a replacement of OPC. When CKD was used as a replacement for cement, the percentage water absorption of blocks increased with higher replacement levels. The density of blocks also increased with higher CKD content as an additive, while the water absorption of blocks showed a reverse trend.

Kunal et al. (2013) during cement manufacturing, cement kiln dust (CKD) is generated which represents significant environment concern related to its emission, disposal and reuse due to high alkalinity. This study presents the effect of bacterial treated cement kiln dust on the compressive strength, water absorption and porosity (at 7, 28 and 91 days) of concrete after reducing the alkalinity. Concrete specimens were prepared with 0%, 5%, 10% and 15% untreated and treated CKD replacing cement. Test results indicated that 7.15% and 26.6% increase in strength of concrete was achieved at 28 and 91 days, respectively, with the addition of bacterial treated 10% CKD whereas reduction in water absorption (20%) and porosity (12.35%) was observed at 91 days.

2.4.3 Rapid Chloride Permeability Test

Maslehuddin et al. (2009) reports results of a study conducted to assess the properties of cement kiln dust (CKD) blended cement concretes. Cement concrete specimens were prepared with 0%, 5%, 10%, and 15% CKD, replacing ASTM C 150 Type I and Type V. The durability characteristics were assessed by evaluating chloride permeability and electrical resistivity. The compressive strength of concrete specimens decreased with the quantity of CKD. However, there was no significant difference in the compressive strength of 0% and 5% CKD cement concretes. A similar trend was noted in the drying shrinkage strain. The chloride permeability increased and the electrical resistivity decreased due to the incorporation of CKD. The performance of concrete with 5% CKD

was almost similar to that of concrete without CKD. Therefore, it was suggested to limit the amount of CKD in concrete to 5% since the chloride permeability and electrical resistivity data indicated that the chances of reinforcement corrosion would increase with 10% and 15% CKD.

Rukzon and chindaprasirt (2008) observed that the reduced chloride permeation is due to reduced average pore size and the improved interfacial zone. Studies reported that as more and more CKD is added, more free chloride ions are penetrated due to large pore size and thus increase the chloride permeability.

2.4.4 Sorptivity

Al-Harthy et al. (2003) used sorptivity and initial surface absorption test (ISAT) to measure the absorption characteristics of different mortar samples containing CKD. They concluded that sorptivity of mortar decreased with the incorporation of CKD (up to 30%) in the mixtures. Without affecting strength, addition of CKD improved absorption properties of mortars and thus, can enhance their durability. The mortar mixtures containing CKD produced lower sorptivity values than the mixture containing no CKD (control). This was attributed to the very fine particle sizes of CKD.

Siddique (2013) reported that properties of self-compacting concrete (SCC) made with coal bottom ash. The mixes were prepared with three percentages (0, 10, 20 and 30) of coal bottom ash as partial replacement of fine aggregates. Properties investigated were; slump flow, J-ring, V-funnel, L-box and U-box, compressive strength, abrasion resistance, rapid chloride permeability, water absorption, and sorptivity. Tests for compressive strength, abrasion resistance, chloride permeability were conducted up to the age of 365 days whereas water absorption and sorptivity tests were conducted up to the age of 28 days. The effect of bottom ash on the sorptivity of SCC mixes cured for age of 28 days 0% bottom ash was $0.05 \text{ mm/s}^{1/2}$ increased with the increase in bottom ash content.

2.4.5 Summary

Literature on the use of bacteria in concrete containing CBPD as partial replacement is not extensive. Therefore, present study is to check the strength and durability properties which is important and its findings will be useful in concrete technology area.

CHAPTER 3

EXPERIMENTAL STUDY

This chapter describes the details of experimental programs for the measurements of fresh properties, strength properties (compressive strength) and durability properties such as water absorption, porosity, sorptivity and rapid chloride permeability of concrete mixes made with varying percentages of cement by-pass dust as partial replacement of cement and along with that studying the effect of bacteria on its properties.

3.1 BACTERIA

3.1.1 Isolation of Bacteria

Alkaliphilic and/or alkalitolerant bacteria (that tolerate high pH) was isolated from rhizospheric (near to root of plant) soil and from marble sludge. The samples were suspended in sterile saline solution (0.85% NaCl), diluted properly and plated on enrichment medium containing glucose (10 g/l), peptone (10 g/l), yeast extract (5 g/l), KH_2PO_4 (1 g/l), agar (15 g/l) and pH was adjusted to 10.5 with 1 N.

3.1.2 Urease Test

For preparation of Urea agar medium, following ingredients were used Peptone 1.0 g/l , Sodium Chloride 5.0g/l , Potassium di Hydrogen Phosphate 2.0g/l, Agar 20.0g/l, 0.2% phenol red and Distilled Water 1000ml. All the above ingredients were dissolved and the pH was adjusted to 6.8 and autoclaved at 121⁰C for 15 minutes and cooled later 1g of glucose and steamed for one hour, finally 20% aqueous 100ml of urea was added and sterilized by filtration and poured into the test tube and slants were prepared. The organisms isolated were streaked on the surface of the media and incubated at 37⁰C and observed for the change of the color of the media from yellow to pink.

The colonies of bacteria which uses urea as the source of energy were used for calcite precipitation in concrete mix.

Table 3.1 Measurement conditions for isolation of bacteria

Data Set	Values
Start Position (2θ)	10.0066
End Position (2θ)	59.9916
Step Size (2θ)	0.0130
Scan Step Time (s)	34.1700
Scan Type	Continuous
PSD Mode	Scanning
PSD Length (2θ)	3.35
Offset (2θ)	0.0
Divergence Slit Type	Automatic
Irradiated Length (mm)	10.00
Specimen Length (mm)	10.00
Measurement Temperature ($^{\circ}\text{C}$)	25.00
Anode Material	cu
k-Alpha (\AA)	1.54060
Generator Settings	40 mA, 45 kV
Diffraction Type	0000000011059259
Diffraction Number	0
Goniometer Radius (mm)	240.00
Dist. Focus-Diverg. Slit (mm)	100.00
Incident Beam Monochromete	No
Spinning	Yes

3.2 MATERIALS

3.2.1 Cement

Ordinary Portland cement was used in this study. The cement was tested as per Indian standard specification (BIS-1489 part 1:1991). The physical and chemical properties of cement are shown in table 3.2 and Table 3.3, respectively.

Table 3.2: Physical properties of ordinary Portland cement (OPC)

Physical Properties	Obtained Values	Standard Values
Standard Consistency (%)	28	-
Initial Setting Time (min)	123	Not to be less than 30 minutes.
Final Setting Time (min)	270	Not to be greater than 600 minutes.
Specific Gravity	3.1	-

Table 3.3: Chemical properties of ordinary Portland cement (OPC)

Constituent	Constituent %
SiO ₂	21.25
Al ₂ O ₃	4.74
Fe ₂ O ₃	4.30
CaO	63.49
MgO	1.02
K ₂ O	0.78
Na ₂ O	0.30
SO ₃	2.93
TiO ₂	0.36

3.2.2 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 sieve analysis and physical properties are given in Tables 3.4 and 3.5, respectively.

Table 3.4: Sieve analysis of fine aggregates

Sieve Size	Retained (g)	Retained (%)	Passing (%)	%age Retained Cumulative
4.75 (mm)	10	1	99	1
2.36 (mm)	60	6	93	7
1.18 (mm)	200	20	73	27
600 (micron)	190	19	54	46
300 (micron)	350	35	19	81
150 (micron)	150	15	4	96
Pan	40	4	0	100
			Σ % Retained = 258	

Fineness Modulus = $258/100 = 2.58$

Table 3.5: Physical properties of fine aggregate

Properties	Observed values
Specific gravity	2.68
Moisture content (%)	0.16
Water absorption (%)	0.86
Fineness modulus	2.58

3.2.3 Coarse Aggregates

Crushed stone with maximum of 12.5mm graded aggregates (nominal size) were used in this study. Physical properties and the sieve analysis results are mentioned in Tables 3.6 and Table 3.7, respectively.

Table 3.6: Physical properties of coarse aggregates

Properties	Observed Value
Maximum Size(mm)	12.5
Specific gravity	2.7
Water Absorption (%)	1.14
Moisure Content (%)	nil

Table 3.7: Sieve analysis of coarse aggregates

Sieve Size (mm)	Weight (g)	Retained (%)	Cumulative (%)	Passing (%)
20	0.0	0.0	0.0	100
12.5	0.97	4.8	4.8	95.2
10	624	32.1	36.9	63.1
4.75	1184	59.2	96.1	3.9
Pan	77	3.85	100	0.0
		Cumulative % Retained = 237.8		

Weight of the sample taken = 2.0 kg.

Fineness modulus = $(237.8+500)/100 = 7.37$

3.2.4 Cement By-Pass Dust

Cement by-pass dust was collected from Village at Himachal Pradesh. Cement by-pass dust has a very high content of calcium oxide CaO_2 followed by SiO_2 , Al_2O_3 , Fe_2O_3 and MgO . It consists of fine rhombus particles. Physical and chemical properties results are given in Tables 3.8 and 3.9, respectively. The fineness of CBPD was determined on the basis of percentage retained in $90\mu\text{m}$ sieve. It was observed that fineness of CBPD is 10 %. Fig 3.1 shows SEM image of CBPD.

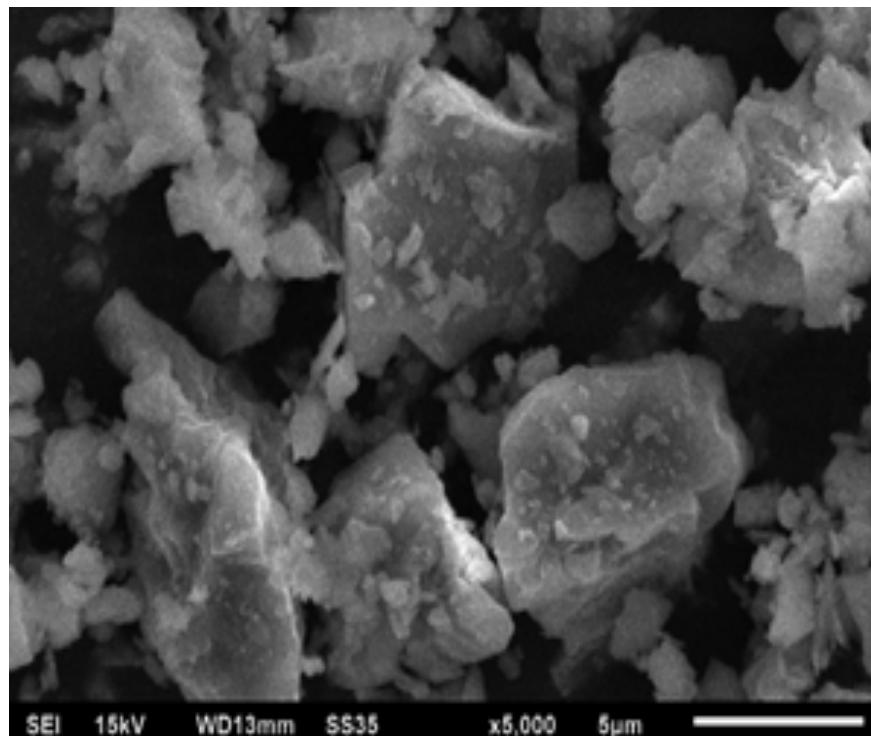


Fig 3.1 SEM image of cement by-pass dust.

Table 3.8: Physical properties of cement by-pass dust

Test Type	CBPD
Specific Gravity	2.54
Fineness	10 % retained in 90 µm Sieve
Initial Setting Time (min)	140

Table 3.9: Chemical properties of cement by-pass dust

COMPONENT	CBPD
CaO	56.07
SiO ₂	14.92
Al ₂ O ₃	4.34
Fe ₂ O ₃	2.96
SO ₃	0.20
MgO	1.07
K ₂ O	1.22
TiO ₂	0.32
BaO	0.04
Na ₂ O	0.03
P ₂ O ₅	0.19
Cl	0.12
MnO	0.03
SrO	0.05
ZrO ₂	0.02
LOI	35.33

3.2.5 Concrete Mix Design

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: 10262-2009. 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 (compressive strength of 20 N/mm² at 28 days) grade concrete is done according to BIS: 10262-2009.

3.3 TESTING PROCEDURE FOR CONCRETE

The test as shown in table were performed in this study

Table 3.10 Test and codes

Test	Code
Compressive Strength	BIS: 516 -1959 (1959)
Water absorption and Porosity	ASTM C 642 (2006)
Rapid chloride permeability test	ASTM C 1202 (2012)
Sorptivity	ASTM C 1585 – 04 (1989)

3.3.1 Compressive Strength

The casting of concrete cubes, containing CBPD as partial replacement of control concrete and bacterial concrete, of size 150 mm³ prepared for compressive strength was in accordance with Indian Standards BIS: 516 -1959 (1959). The cubes were cured at 28 and 56 days and studied in triplicates. Specimen were tested for compressive strength using compression testing machine (CTM: AIMIL Ltd.) with load bearing capacity of 3000 kN. The compressive strength was calculated by the formula given below and then compared with the concrete with CBPD.

$$\text{Compressive strength} = \frac{\text{Load in Newton(N)}}{\text{Area in mm}^2}$$

3.3.2 Water Absorption and Porosity

The water absorption test was conducted as per ASTM C 642 (2006) in order to determine the increase in resistance towards water penetration in concrete. The cube moulds of 150mm for concrete were prepared both with and without bacteria and partially replaced with CBPD. The concrete specimens were cured for 28 days and 56 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:

$$\text{Volume of permeable voids \%} = \frac{(C-A)}{(C-D)} * 100$$

Where in; A = mass of the oven dried sample in air, in 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 is based on Archimedes Principle. The total porosity can be calculated from water saturated surface dry mass (m_{sat}), mass suspended in water (m_{water}) and oven dry mass

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

3.3.3 Rapid Chloride Permeability Test

Rapid chloride permeability test (RCPT) has been developed as a quick method to measure the rate of transport of chloride ions in concrete as per ASTM C 1202 (2012) method for determining the durability of concrete structure to prevent corrosion. Cylindrical samples of 100 mm diameter and 200 mm height were prepared. After cutting for 28 and 56 days, the samples were cut into 50 mm thick slices and control and

bacterial concrete with CBPD as partial replacement were tested with RCPT (PROOVE'it, German Instrument) by applying 60 V. The current passing through the specimen in 6 hours was measured and interpreted on the basis of total charge (Coulomb number) transferred from NaCl (3%) to NaOH (0.3N) in the form of chloride ions. The relationship between chloride penetrating rate and the charge passed in coulomb is given in Table 3.11

Table 3.11 Chloride ion permeability based on charge passed (ASTM C 1202-12)

Charge Passed (Coulombs)	Chloride ion permeability
>4000	High
2000-4000	Moderate
1000-2000	Low
10-1000	Very Low
<100	Negligible

3.3.4 Sorptivity

Sorptivity is a measure of the capillary forces exerted by the pore structure causing fluids to be drawn into the body of the material. As per ASTM C 1585 – 04 (1989) Cylindrical concrete specimens of 50mm width and 100 mm diameter were preconditioned by drying in an oven at 50°C for four days and then allowed to cool in a sealed container for three days. The sides of the concrete specimens were coated with transparent epoxy resin in order to allow the flow in one direction. The initial mass of the sample was taken and at time 0, the specimen was kept partially immersed to a depth of 5 mm in the water. At selected times (typically 1, 5, 10, 20, 30, 60, 120....up to 360min for initial sorption and every 24 hours for 7 days), the sample was removed from the water, excess water blotted off with a damp paper towel and then the sample was weighed. It was then replaced in the water for the selected time period. The gain in mass per unit area over the density of water was plotted versus the square root of the elapsed time. The slope of the line of best fit of these points was taken as the sorptivity value. The sorptivity values of CBPD blended concrete specimens after 28 and 56 days of moisture curing were calculated by the following formula,

$$I = \frac{m_t}{a/d}$$

where I = water absorption (mm),

m_t = change in specimen mass in gram, at the same time t ,

a = exposed area of the specimen in mm^2

d = density of water in g/mm^3

The initial rate of water absorption ($\text{mm}/\text{s}^{1/2}$) is defined as the slope of the line that is the best fit to I plotted against the square root of time ($\text{s}^{1/2}$). Obtain this slope by using least squares, linear regression analysis of the plot of I versus $\text{time}^{1/2}$. For the regression analysis, use all the points from 1 min to 6 h.

Initial Absorption:

$I = S_i = t + b$ (points measured up to 6 h are used)

The secondary rate of water absorption ($\text{mm}/\text{s}^{1/2}$) is defined as the slope of the line that is the best fit to I plotted against the square root of time ($\text{s}^{1/2}$) using all the points from 1 d to 7 d. Use least-square linear regression to determine the slope.

Secondary Absorption:

$I = S_s = t + b$ (points measured after the first day are used).

3.4 MICROSTRUCTURE ANALYSIS

Concrete specimens were subjected to microstructure analysis which includes scanning electron microscopy (SEM) and X-ray diffraction studies (XRD).

3.4.1 SEM Analysis

Scanning electron microscopy (SEM) has been developed for the analysis of complex microstructure of cement and concrete, and verifies the mechanism predicted by compressive strength. After each curing for 28 and 56 days, the broken concrete specimen were taken from inner of the matrix after compressive strength testing, and were dried at 80°C for 24 hours. Before SEM analysis the samples were placed in desiccator for over-night for removal of moisture. The small broken specimen of control and bacterial concrete were mounted on brass stub using carbon tape, gold coating and then microscopic structure was analysed at 15 kV by scanning electron microscope (JEOL JSM 6510 LV, USA).

3.4.2 XRD Analysis

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 unravelling the structure of the materials in bulk and thin film forms. XRD spectra were obtained using an XRD; (PAN analytical X'Pro diffractometer) with a Cu anode (40 kV and 30 mA) and scanning from 5° 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 Joint Committee on Powder Diffraction Standards (JCPDS) data file and published literature. 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.

CHAPTER 4

RESULTS AND DISCUSSIONS

4.1 BACTERIAL PROPERTIES

4.1.1 Isolation of Bacteria

Presence of urea in the media (composition of media: urea (20 g/l), NaHCO₃ (2.12 g/l), NH₄Cl (10 g/l), Nutrient broth (3 g/l), CaCl₂·2H₂O (25 g/l) hydrolyzes the media (due to the occurrence of the urease enzyme) which in turn increases the pH, as it (bacteria) utilizes urea as a nitrogen source and also as a source of energy. The addition of urea and calcium chloride in the medium supports the microbial growth. In this study, out of 3 strains AKKR5 was found to be best on the basis of calcite formation.

4.1.2 Urease Test

The isolate AKKR5 was studied for urease activity. XRD Analysis revealed formation of calcite.

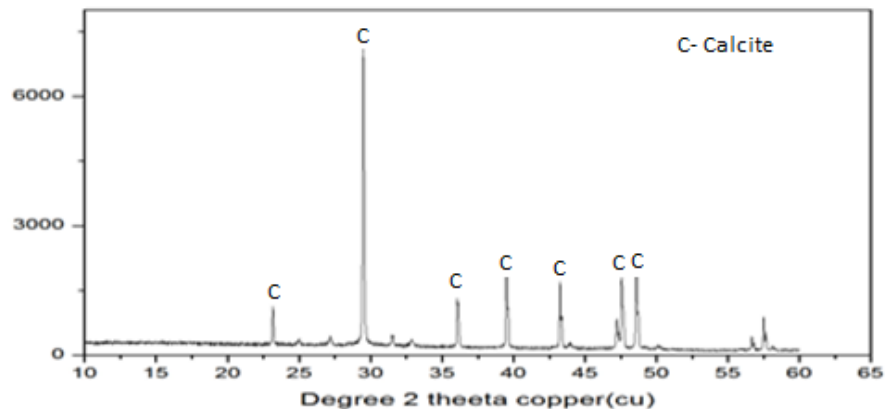


Fig 4.1 Graphic analyse view

4.2 STUDY OF CONCRETE PROPERTIES

4.2.1 Compressive Strength

The compressive strength of CBPD control concrete increased with increase in curing period. The compressive strength of control (0%) CBPD at the age of 28 and 56 days was 32.89 N/mm² and 36.33 N/mm², there was an increase of 10% strength in bacterial concrete. At day 28 of curing, cell walls of *B. subtilis* amended at 3.3 mg/ml (10×) and 0.33 mg/ml (1×) concentration caused significant increase of compressive strength by

15.6% and 14.8%, respectively, in comparison with the control specimens. The increase in compressive strength was due to accelerating CaCO_3 formation in $\text{Ca}(\text{OH})_2$ (Pei et al., 2013). Compressive strength of concrete specimens cast with *B. megaterium* is 16.1% more than control concrete specimens (Krishnapriya et al., 2015).

At the age of 28 days 10, 20 and 30% CBPD (control) concrete showed compressive strength of 27.23 N/mm^2 , 24.64 N/mm^2 , and 20.53 N/mm^2 , respectively. whereas bacterial concrete with 10, 20 and 30% CBPD showed compressive strength of 29.28 N/mm^2 , 25.02 N/mm^2 , 21.18 N/mm^2 . After curing for the age of 56 days the compressive strength of control concrete with 10, 20 and 30% control CBPD (control) was 30.36 N/mm^2 , 27.23 N/mm^2 , 22.68 N/mm^2 , respectively. Whereas 10, 20 and 30% bacterial CBPD was 34.96 N/mm^2 , 28.56 N/mm^2 , 23.56 N/mm^2 , respectively. Replacement of cement with CBPD will lead to less cement content and therefore less compressive strength (Dhir et al., 2002).

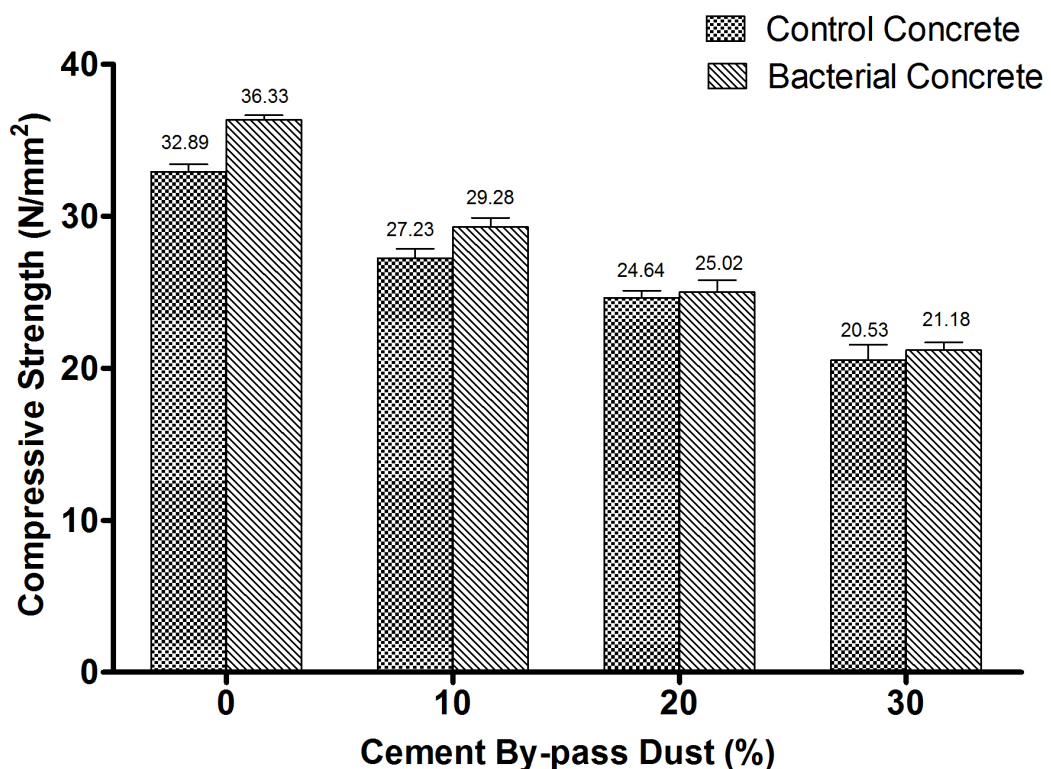


Fig 4.2 Compressive strength at age of 28 days

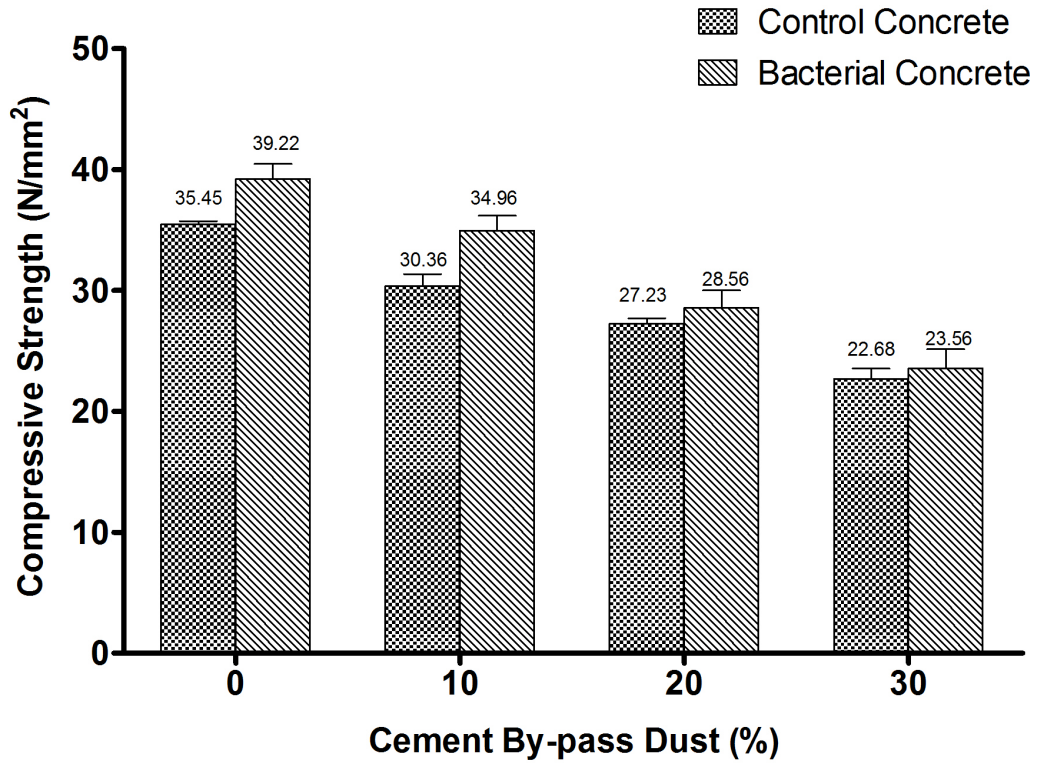


Fig 4.3 Compressive strength at age of 56 days

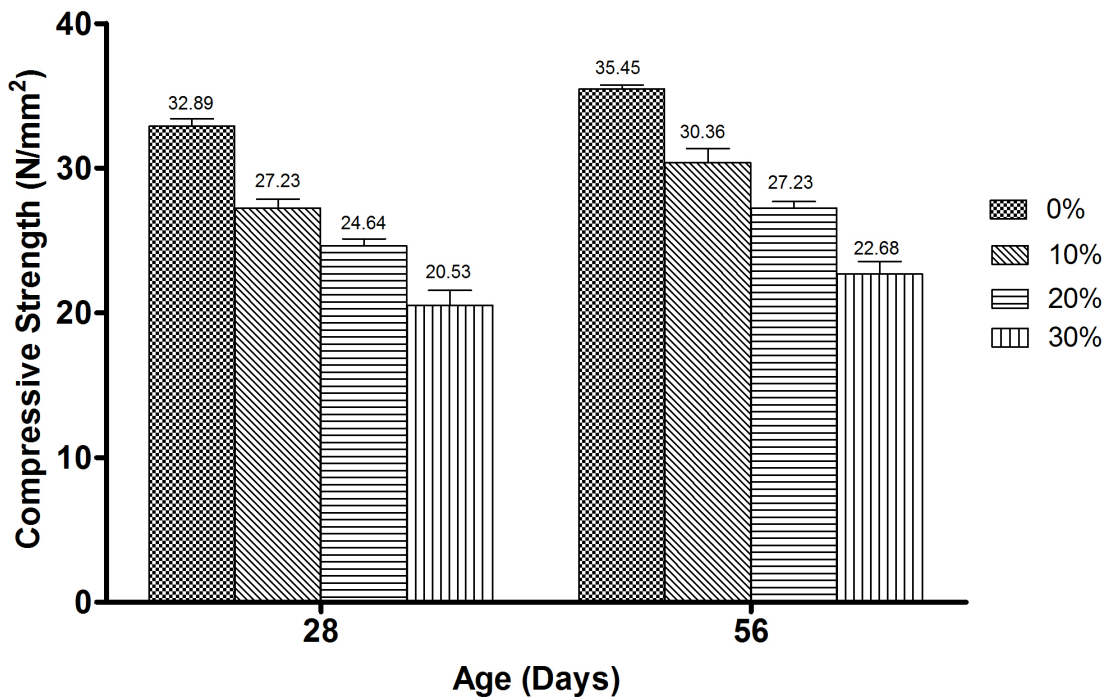


Fig 4.4 Compressive strength of control concrete at age of 28 and 56 days

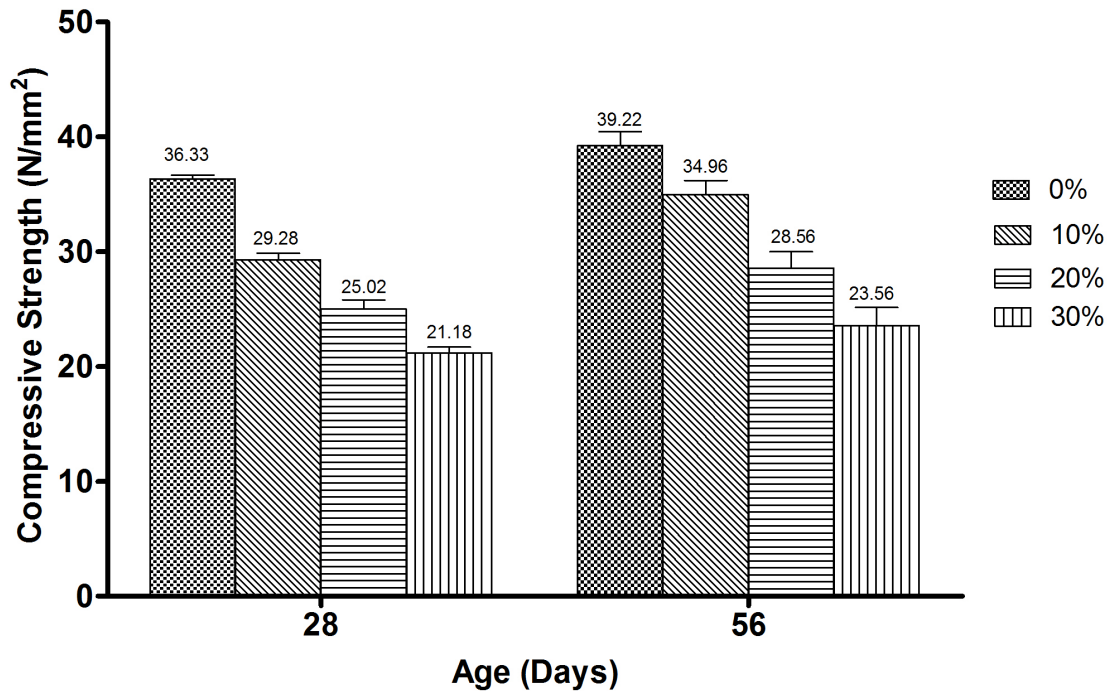


Fig 4.5 Compressive strength of bacterial concrete at age of 28 and 56 days

4.2.2 Water Absorption and Porosity

Water absorption of concrete specimen containing control and bacterial concrete was determined using ASTM C642-06 method at the age of 28 and 56 days. Water absorption and porosity has direct relation with the compressive strength of concrete. Increased water absorption and pore size leads to decrease in compressive strength of concrete, whereas decreased water absorption and porosity leads to increase in compressive strength of concrete. Control concrete specimen follows an increasing trend with increase in CBPD partial replacement percentage for 28 days and 56 days curing. The water absorption of control concrete (0% CBPD) cured for 28 days was 1.56%, whereas 10%, 20% and 30% were 2.58%, 3.15% and 3.36%, respectively., similar trend was observed for control concrete specimen cured for 56 days, it was observed that control (0% CBPD) was 1.24%, whereas 10%, 20% and 30% was 1.56%, 1.84% and 2.18%, respectively. The calcium carbonate generated by the MICP in the samples not only improved the strength by bonding the particles together, but also lowered the porosity and water absorption ratio by filling the pores efficiently (Zhang et al. 2015).

In bacterial concrete specimens cured for 28 days, for bacterial concrete (0% CBPD) absorption was observed 1.2%. Water absorption increased but very insignificantly with

increase in CBPD partial replacement percentage. Water absorption for 10%, 20% and 30% was 1.55%, 1.70% and 1.78%, respectively. In bacterial concrete specimens cured for 56 days, 0% CBPD had minimum water absorption 1.03%. Water absorption increased but very insignificantly with increase in CBPD partial replacement percentage. Water absorption for 10%, 20% and 30% was 1.20%, 1.38% and 1.52%, respectively. Inclusion of bacteria in concrete, water absorption capacity of fly ash concrete decreased with increase in the range of bacteria concentration. Maximum reduction in water absorption was observed with 10^5 cells/ml for fly ash concrete (Chahal et al., 2012).

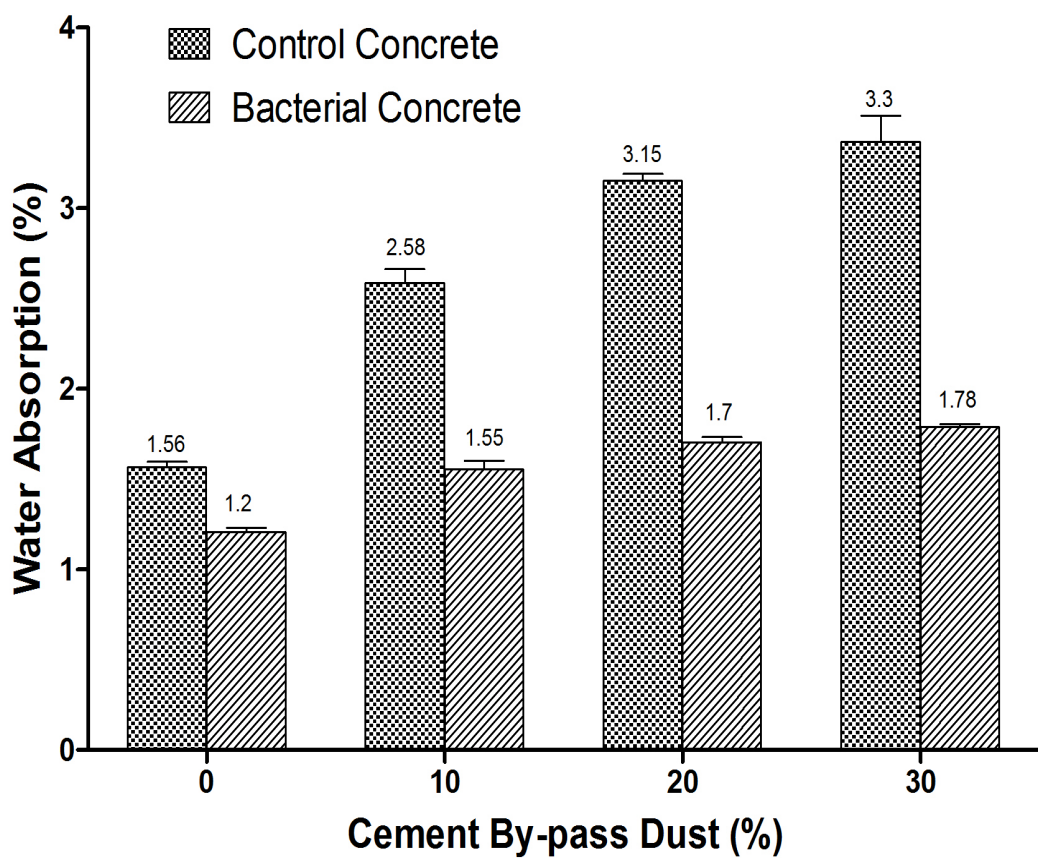


Fig 4.6: Water absorption at age of 28 days

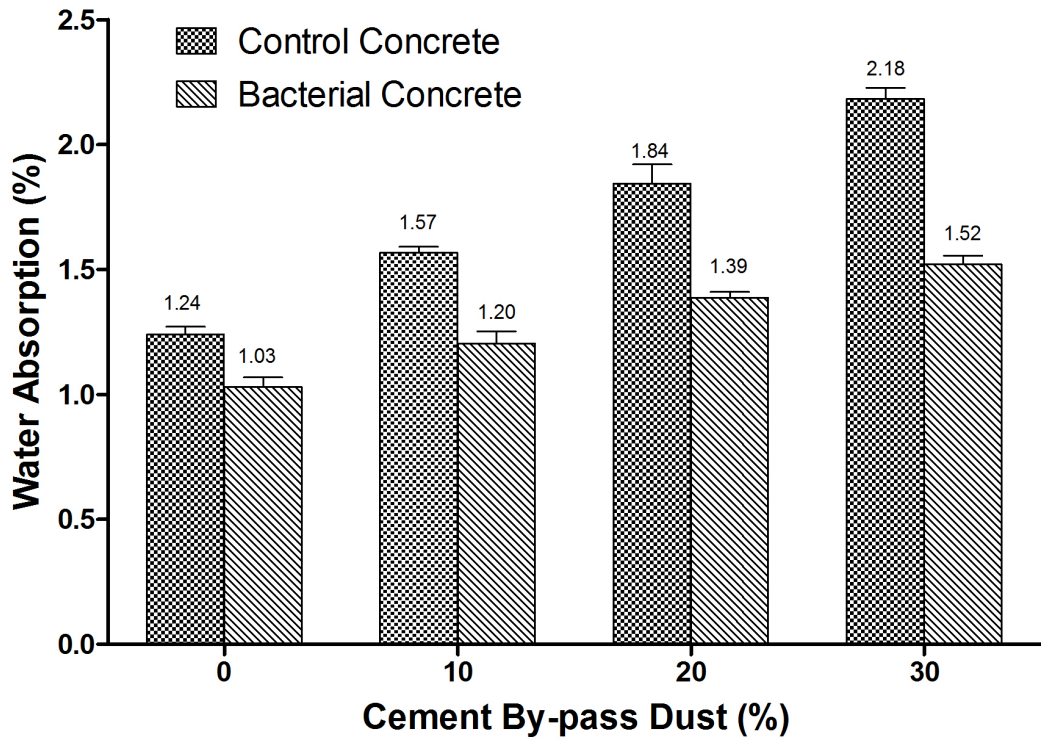


Fig 4.7: Water absorption at age of 56 days

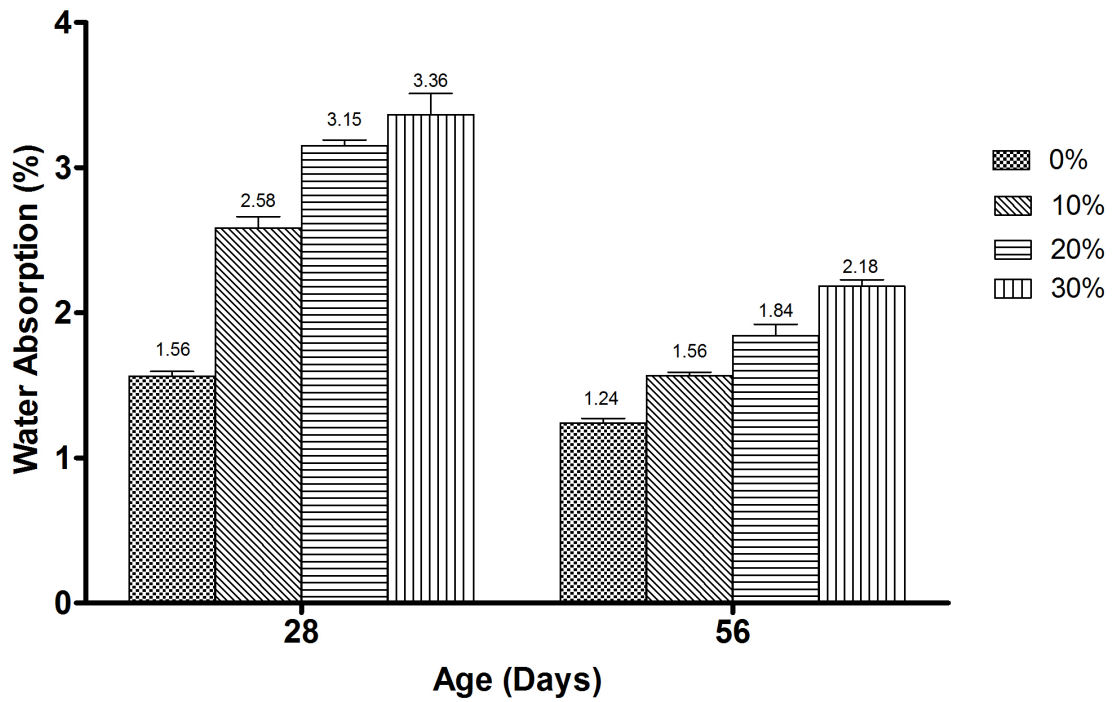


Fig 4.8: Water absorption of control concrete at age of 28 and 56 days

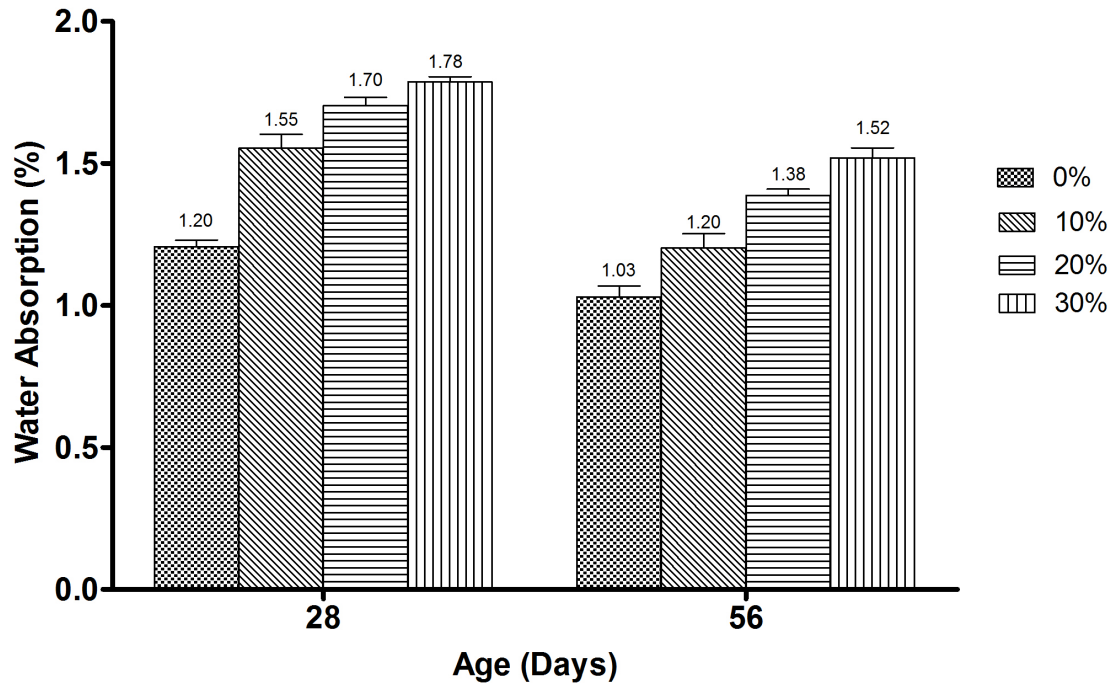


Fig 4.9: Water absorption of bacterial concrete at age of 28 and 56 days

Porosity of concrete is the measure of the voids or empty spaces in concrete, decrease in porosity was observed in concrete at the age of 28 and 56 days, respectively. The maximum reduction in porosity was observed in bacterial concrete (0% CBPD) specimen at the age of 28 (2.8%), 56 (2.44%) days compared to 0% CBPD control concrete 28 (3.37%), 56 (2.94%), respectively. Bacteria present in concrete helps fill the voids by accelerating CaCO_3 . A number of potential mechanisms are proposed for formation of CaCO_3 , by converting $\text{Ca}(\text{OH})_2$, which is the most important source of Ca^{2+} in concrete but plays a weak structural role, but when converted into solid CaCO_3 it shows strong structural role. Formation of CaCO_3 increases the compressive strength and resulted in decreased porosity (Pie et al., 2013).

Porosity for control concrete after curing for 28 days follows an increasing trend for 10%, 20% and 30% was 5.01%, 6.4% and 7.4%, respectively. In bacterial concrete curing for 28 days porosity for 10%, 20% and 30% was 3.7%, 4.03% and 4.22%, respectively. Similarly for control concrete after curing for 56 days follows an increasing trend for 10%, 20% and 30% was 3.7%, 4.35% and 5.15%, respectively. In bacterial concrete curing for 56 days porosity for 10%, 20% and 30% was 2.8%, 3.44% and 3.98%, respectively.

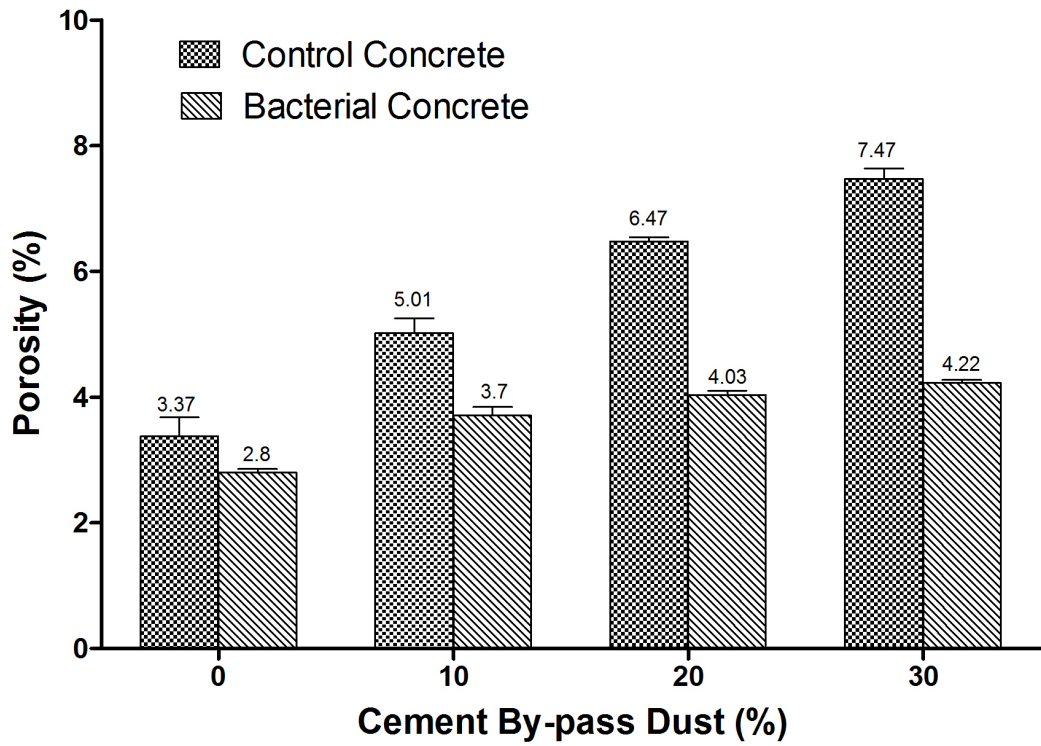


Fig 4.10: Porosity at age of 28 days

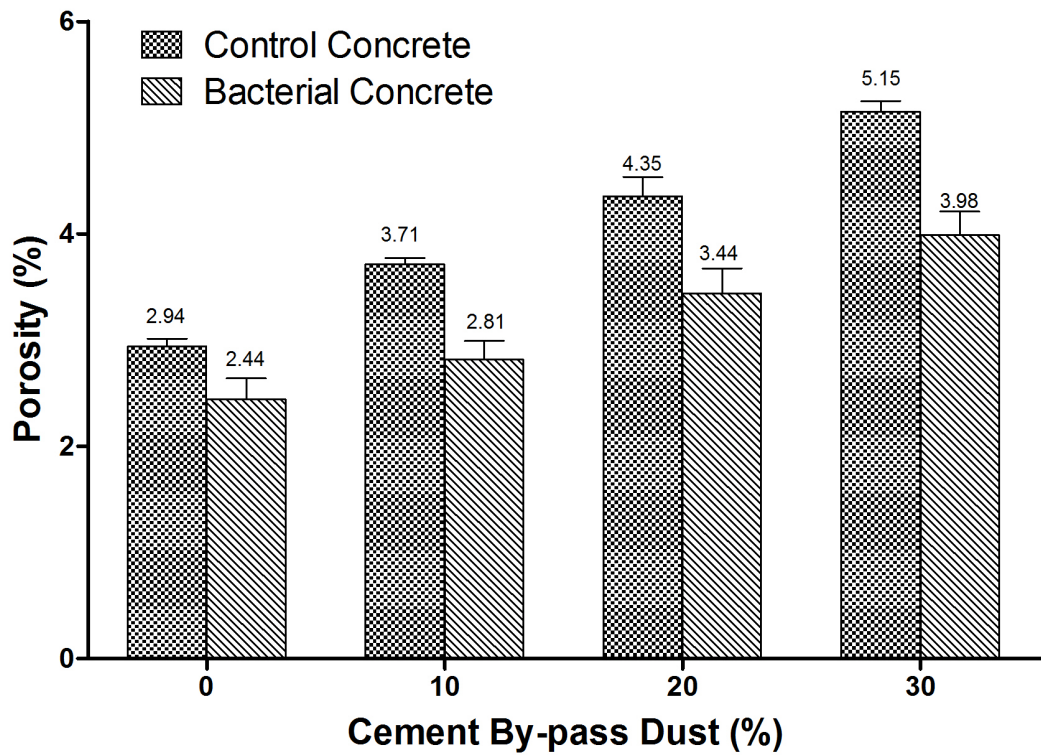


Fig 4.11: Porosity at age of 56 days

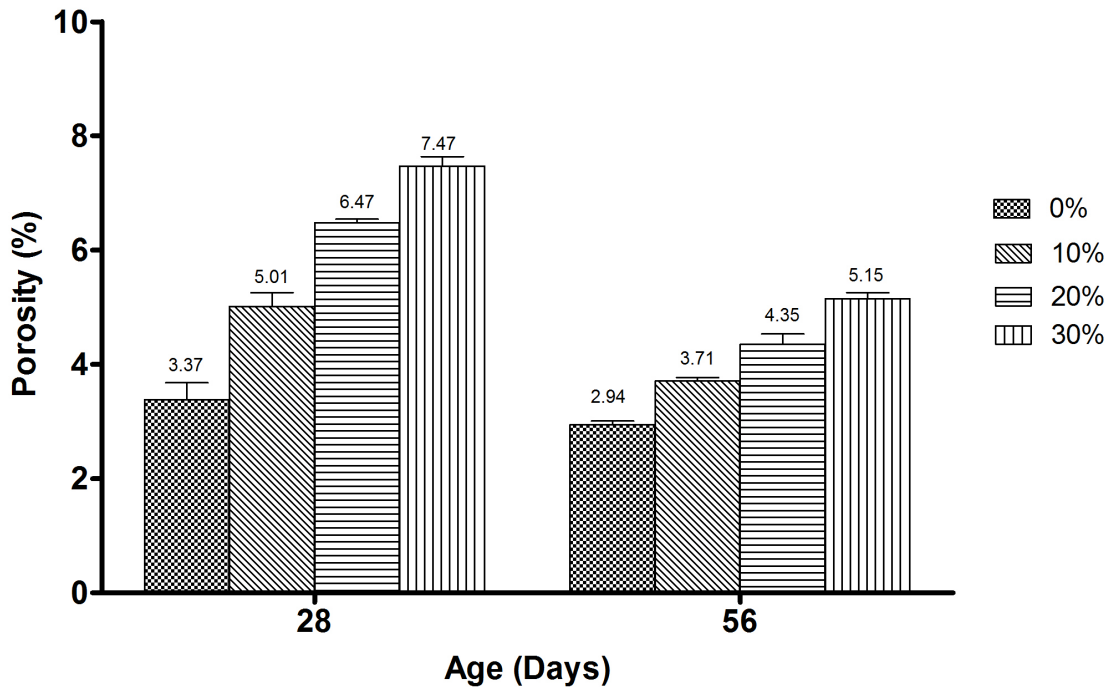


Fig 4.12: Porosity of control concrete at age of 28 and 56 days

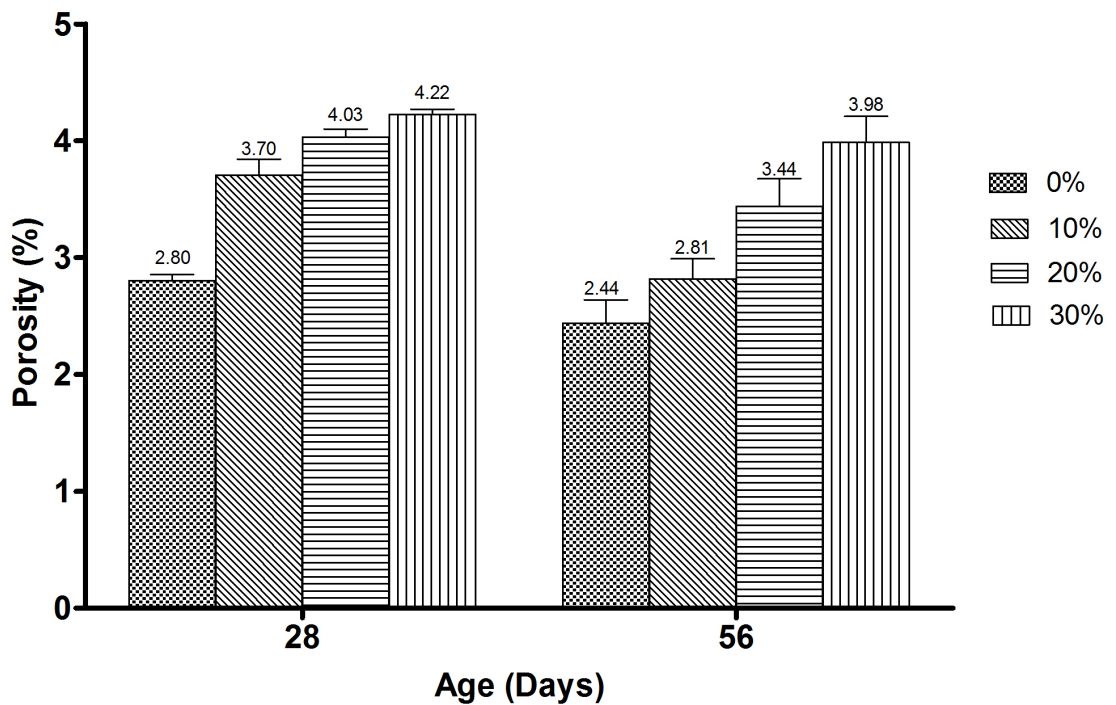


Fig 4.13: Porosity of bacterial concrete at age of 28 and 56 days

4.2.3 Rapid Chloride Permeability Test

One major problem associated with crack formation is that the process results in a drastic increase in material permeability increasing the risk of matrix and embedded

reinforcement degradation by ingress water and other aggressive chemicals. Active bacterially mediated mineral precipitation could result in crack-plugging and concomitant decrease in material permeability (Jonkers, H.M. et al., 2015). The chloride permeability test of concrete specimen containing control and bacterial tested CBPD showed decrease in chloride permeability with increasing curing period from 28 to 56 days (Table 4.1) the relationship between chloride penetrating rate and the charge passed in coulomb is already given in table 3.10. At 28 days, all control CBPD concrete specimen showed permeability from “high” to “moderate” range, whereas bacterial concrete specimens showed “high” to “low” range. In 28 day specimens, sample with 30% CBPD was observed to have “high” range, whereas 10 and 20% were in “moderate” range. But control concrete was observed to lie in “moderate” range while bacterial concrete control sample had “low” chloride permeability. In bacterial specimen SEM confirmed on closer observations, rod-shaped bacteria associated with calcite crystals were found. This deposition serves as a barrier to harmful substances from entering the sample, and thus reduces its permeability (De-Muyneck et al. 2010).

Similar trend was observed after 56 days of curing, sample with 30% CBPD was observed to have “moderate” range, whereas samples with 20 % control and bacterial concrete were in “moderate” range while 10 % and 0% Control and bacterial concrete was observed to possess “low” chloride permeability. The chloride permeability increased and the electrical resistivity decreased due to the incorporation of CKD (Maslehuddin et al., 2009).

Table 4.1: Rapid chloride permeability test of control and bacterial concrete at age of 28 and 56 days.

% CBPD in Concrete	Rapid Chloride Permeability Test (Coulomb or C)			
	28 Day Curing		56 Day Curing	
	Control	Bacterial	Control	Bacterial
0%	2366±21 C	1998±11 C	1488±20 C	1066±30 C
10%	2575±15 C	2222±13 C	1599±26 C	1444±15 C
20%	2845±10 C	2824±8 C	2217±28 C	2223±21 C
30%	4636±10 C	4310±24 C	2982±21 C	2338±26 C

4.2.4 Sorptivity

Sorptivity of control Concrete specimen 0% CBPD cured for the age of 28 days was 1×10^{-7} mm/sec^{1/2} while 10, 20 and 30% showed increasing trend with increase in the percentage of CBPD. The absorption of water through capillary action depends on the porosity of the concrete structure. The sorptivity trend was found similar to that of porosity and water absorption. Over a period of 168 h (7 days), the cubes amended with fly ash (0%, 10% and 20%) with bacterial cells absorbed nearly 3.5 times less water than the control cubes. The presence of bacteria resulted in a significant decrease of the water uptake compared to control specimens (Achal et al., 2011).

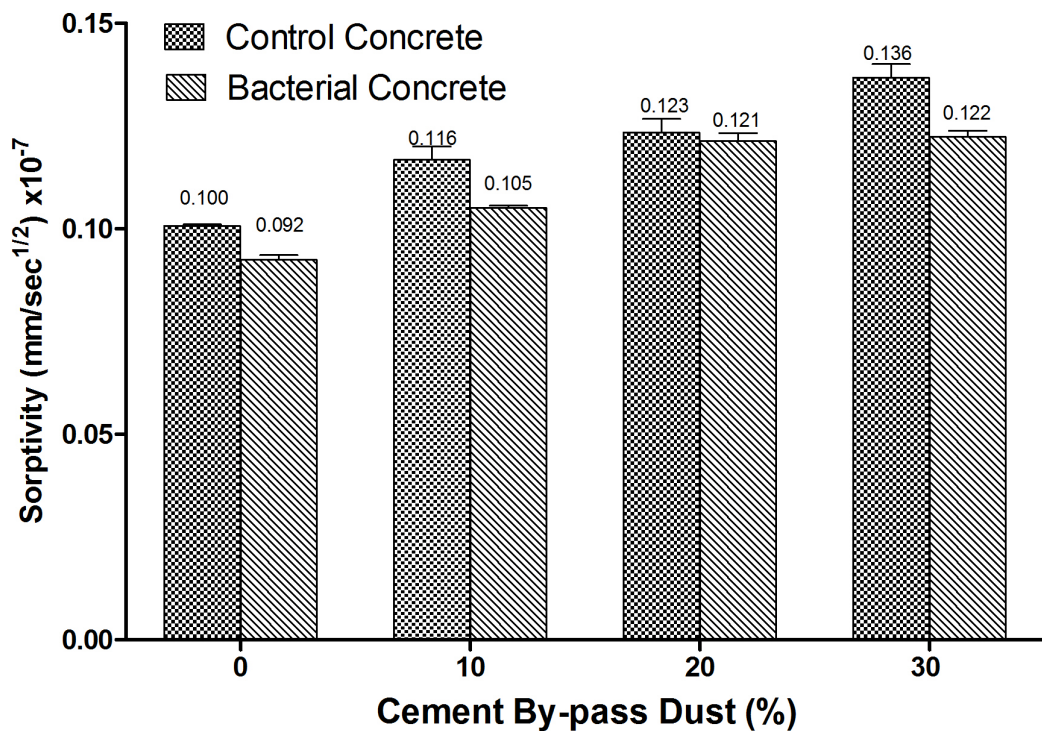


Fig 4.14 Sorptivity at age of 28 days.

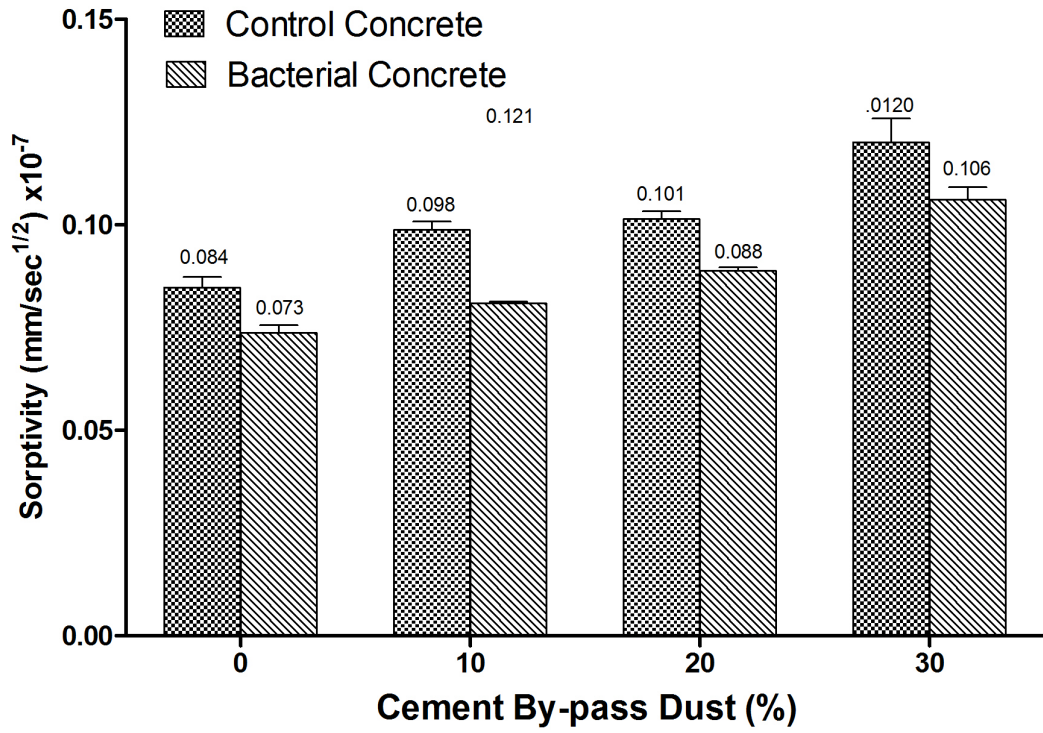


Fig 4.15 Sorptivity at age of 56 days

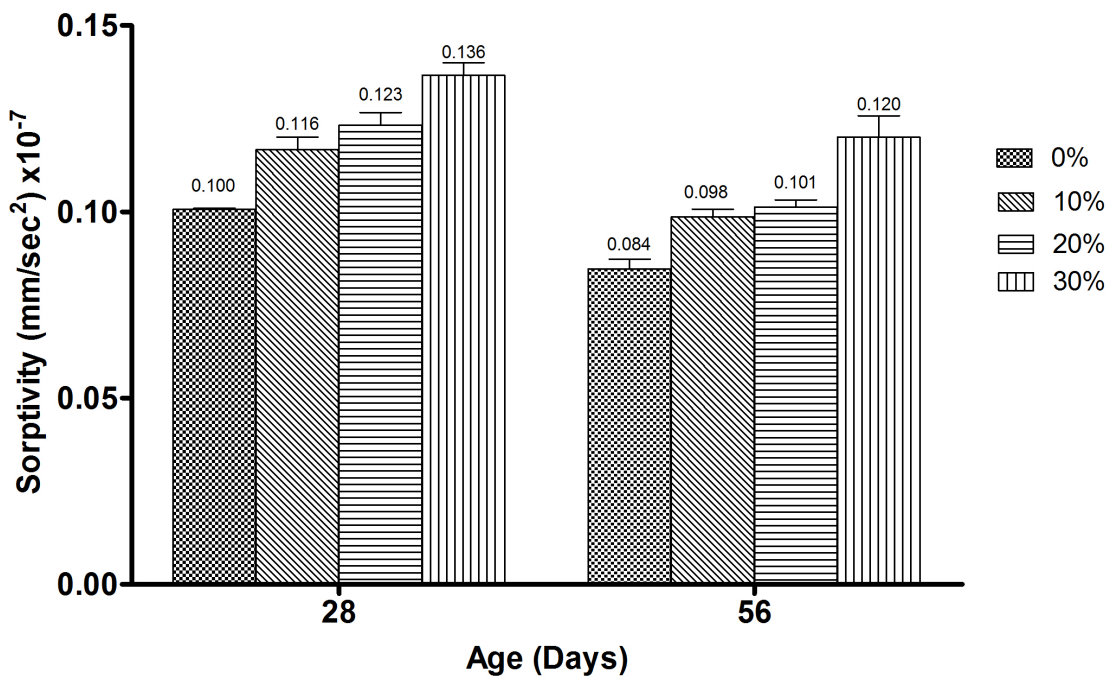


Fig 4.16 Sorptivity of control concrete at age of 28 and 56 days

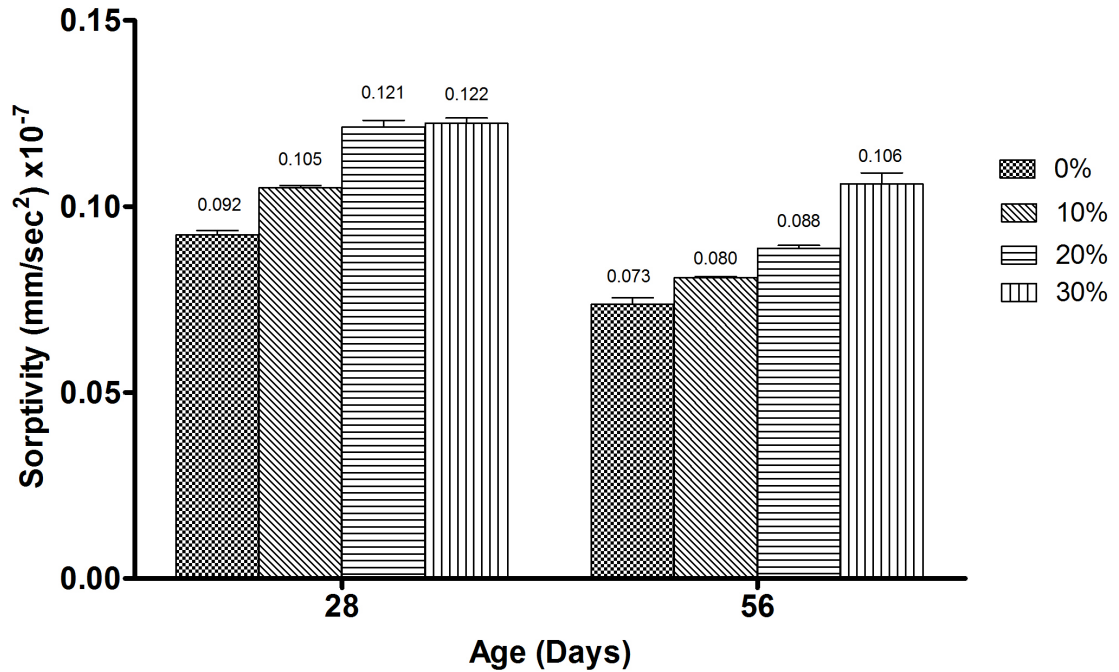


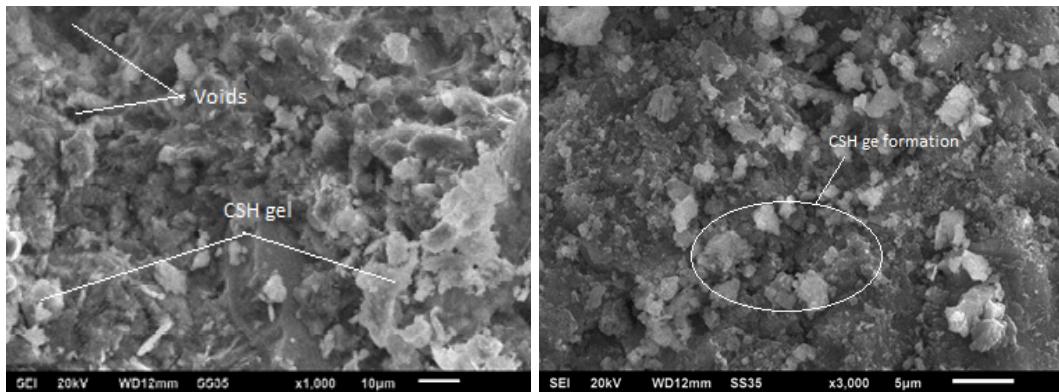
Fig 4.17: Sorptivity of bacterial concrete at age of 28 and 56 days

4.3 MICROSTRUCTURE ANALYSIS

4.3.1 SEM Analysis

Figs. 4.18-4.21 shows the scanning electron microscope (SEM) analysis of control and bacterial concrete containing 0%, 10%, 20% and 30% CBPD. SEM images shows the formation of calcium silicate hydrate (CSH) and hydration reaction formed dense structure resulted in compressive strength in the concrete specimen. In control (0%CBPD) specimen at 28 days of curing CSH gel was observed similar observations was made for bacterial concrete (0% CBPD) showed CSH gel formation, the dense matrix in control and bacterial (0% CBPD) showed highest compressive strength. While in control 10%, 20% and 30% CBPD replacement CSH gel formation was observed with voids and bacterial concrete, 10%, 20% and 30% CBPD concrete specimen showed non-expansive needle shaped ettringites which densified the matrix and lead to increase in strength as compressive strength as compared to control 10 %, 20% and 30% CBPD. (Lee et al., 2005) Micro-cracks that extend from ettringites filled voids instead they were associated with rim ettringite that developed outside the voids i.e. on the surface. Famy et al. (2000) indicating the expansive nature of ettringite on the outer surface of calcium silicate hydrate surface at the interface of cement paste-aggregate and non-expansive nature of ettringite in the voids of the concrete i.e. micro-porous zones in the cement

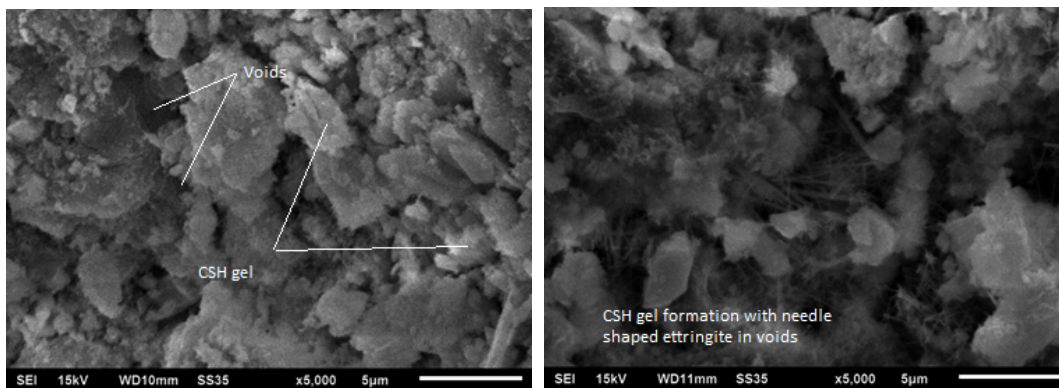
paste, pores or bubbles. The expansive ettringite exerts pressure to the aggregates forming gaps at the interface resulting in increased porosity and reduced strength.



28 Day Control

Bacterial

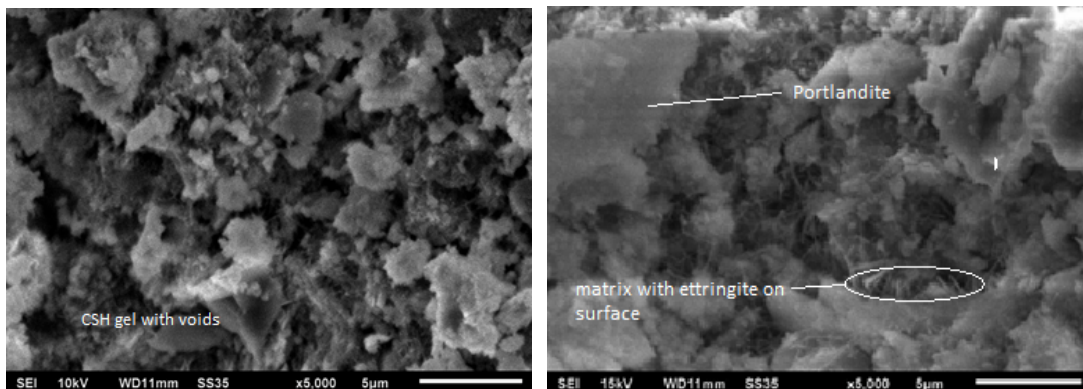
Fig 4.18 SEM image shows control and bacterial concrete (0% CBPD) at age of 28 days



28 Day Control

Bacterial

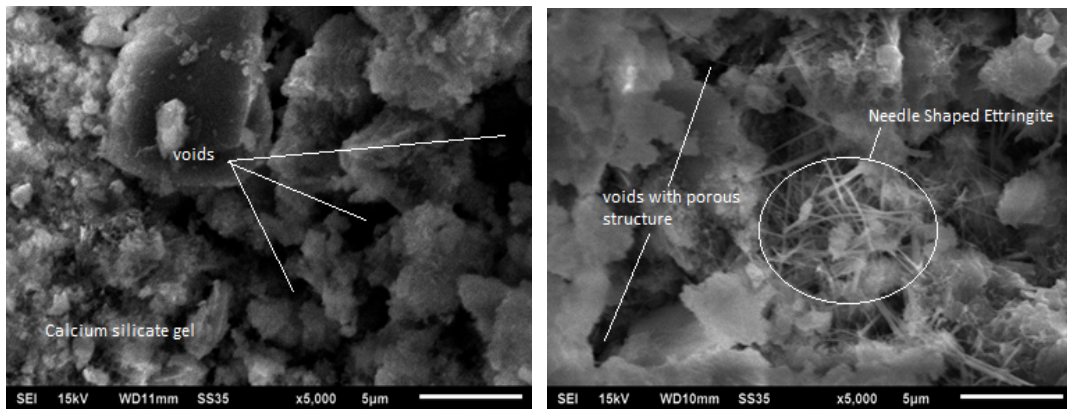
Fig 4.19 SEM image shows control and bacterial concrete (10% CBPD) at age of 28 days



28 Day Control

Bacterial

Fig 4.20 SEM image shows control and bacterial concrete (20% CBPD) at age of 28 days



28 Day Control

Bacterial

Fig 4.21 SEM image shows control and bacterial concrete (30% CBPD) at age of 28 days

4.3.2 XRD Analysis

X-ray diffraction (XRD) analysis of concrete samples control and bacterial concrete samples shows peaks of quartz (Q), calcium silicate hydrate (CSH), calcite (C), larnite (L) and ettringite (E) phases of comparing the value of $2\theta/d/I/I$ of the peaks by JCPDS data file (Fig 4.18-4.25). Peaks of different phases in treatment shows the intensity corresponding to the strength of concrete. Hydration and hydrolysis reaction of C_3S and C_2S mineral components produce calcium silicate hydrate gels and later the solid phase develops crystals during curing period leading to strengthening of the cement-concrete mixes. (Neville, 2003; Molnar et al., 2010; Jumate and Manea, 2011).

In 0%, 10%, 20% and 30% bacterial concrete (Fig: 4.23, 4.25, 4.27 and 4.29) the increased formation of CSH and calcite resulted in increased strength compared to 0%, 10%, 20% and 30% control concrete (Fig: 4.22, 4.24, 4.26 and 4.28). Ettringite formation in bacterial concrete is non expansive due to less alkali content and filled the pore structure in concrete resulted in dense structure and increased compressive strength. XRD results shoes increased intensity of CSH (21, 26, 29 degree 2θ) and non-expansive ettringite (17, 34 degree 2θ) in bacterial concrete, responsible for high strength development in concrete.

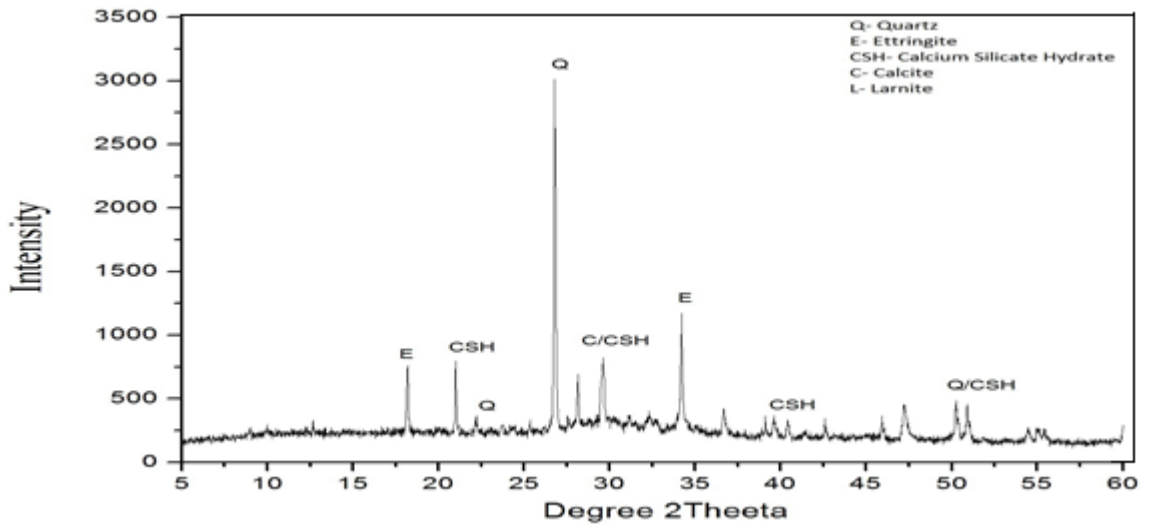


Fig 4.22 X-ray diffraction shows control concrete (0% CBPD) at age of 28 days

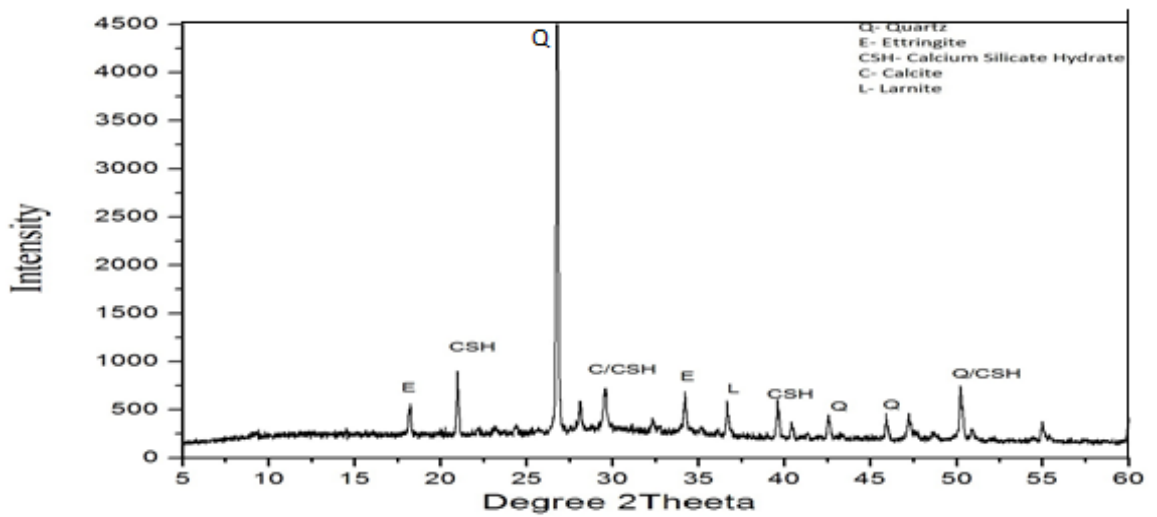


Fig 4.23 X-ray diffraction shows bacterial concrete (0% CBPD) at age of 28 days

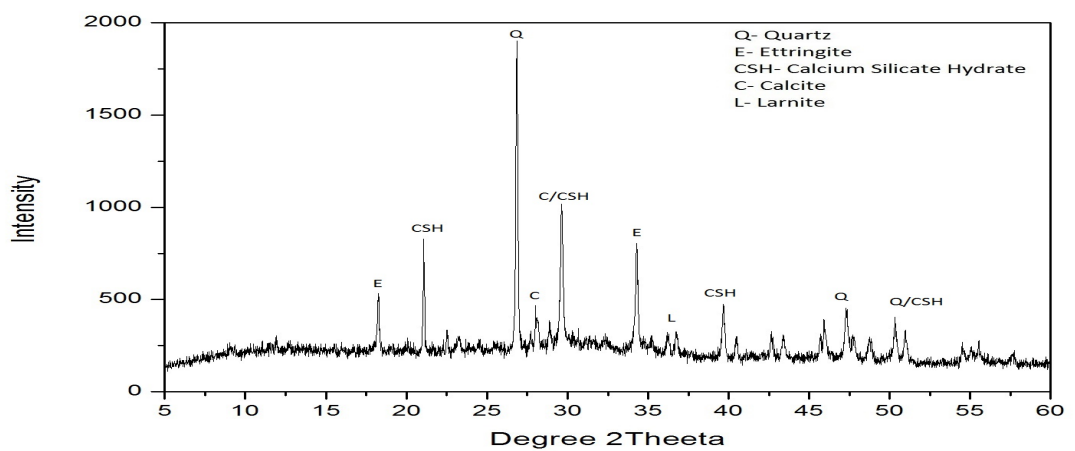


Fig 4.24 X-ray diffraction shows control concrete (10% CBPD) at age of 28 days

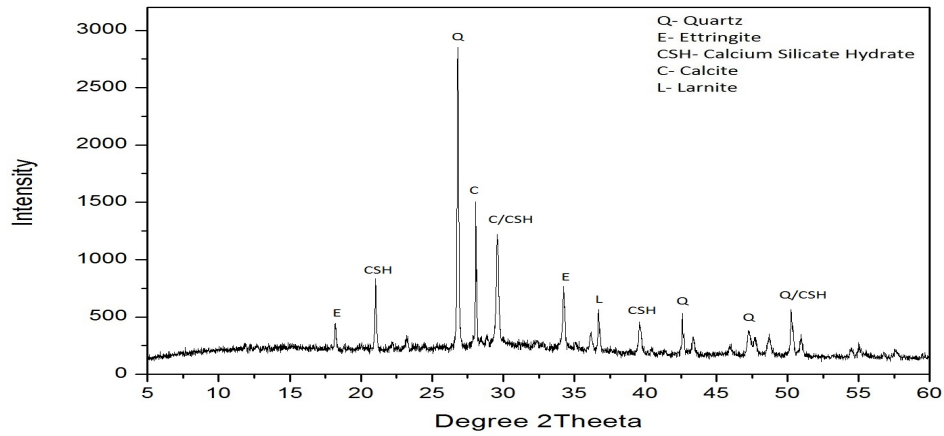


Fig 4.25 X-ray diffraction shows bacterial concrete (10% CBPD) at age of 28 days

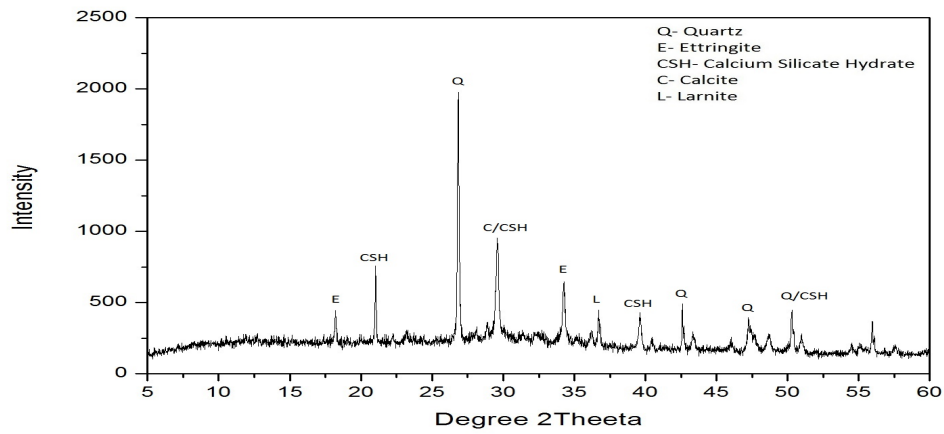


Fig 4.26 X-ray diffraction shows control concrete (20% CBPD) at age of 28 days

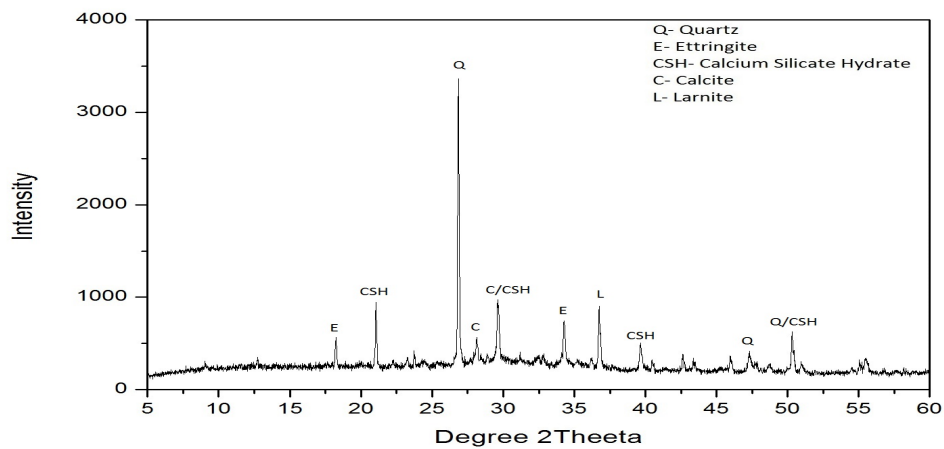


Fig 4.27 X-ray diffraction shows bacterial concrete (20% CBPD) at age of 28 days

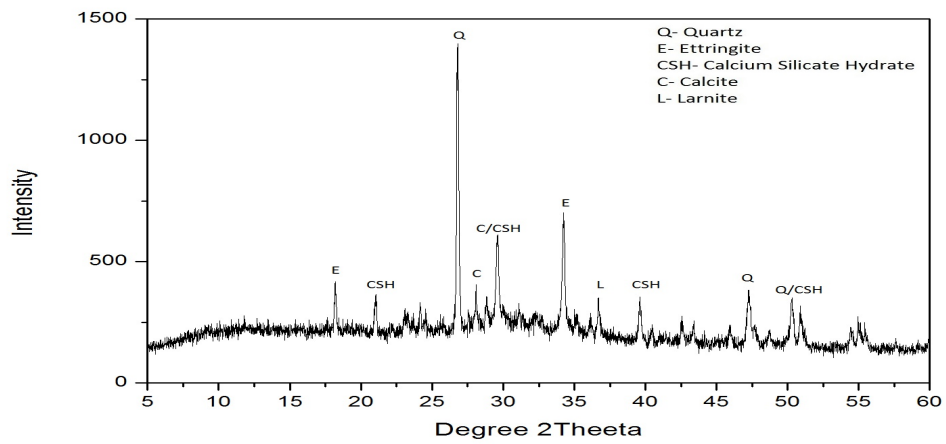


Fig 4.28 X-ray diffraction shows control concrete (30% CBPD) at age of 28 days

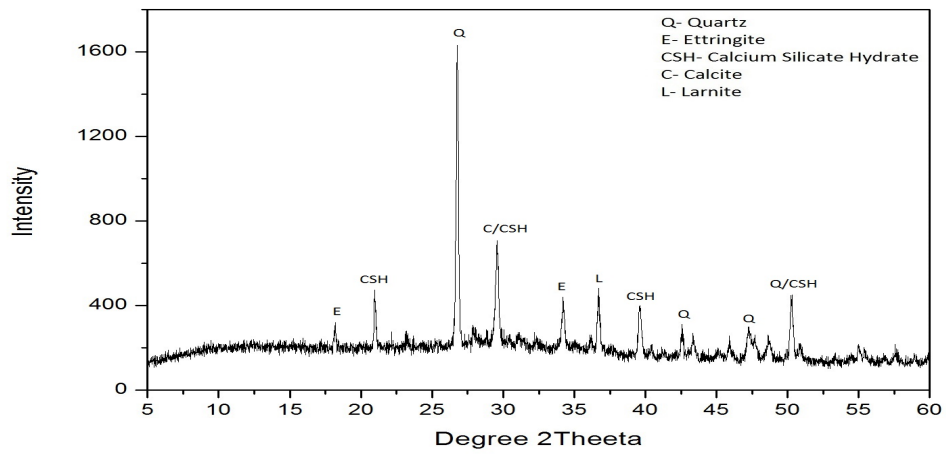


Fig 4.29 X-ray diffraction shows bacterial concrete (30% CBPD) at age of 28 days

CHAPTER 5

CONCLUSIONS

This study investigated the results for the compressive strength, water absorption and porosity, rapid chloride permeability test, sorptivity and microstructural analysis using SEM and XRD techniques of control and bacterial concrete containing cement by-pass dust with different percentage (0, 10, 20 and 30%) were analysed. On basis of results from present studies, following conclusions are drawn.

5.1 STUDY OF CONCRETE PROPERTIES

5.1.1 Compressive Strength

- i. The compressive Strength of control concrete (0% CBPD) at the age of 28 days was 32.89 N/mm², whereas 10, 20 and 30% control concrete was 27.23, 24.64 and 20.53 N/mm², respectively. Similar trend of decrease in strength was observed at 56 days of curing for all the percentages.
- ii. The compressive strength of bacterial concrete at the age of 28 days curing was more than that of control concrete at the age of 28 days curing. Bacterial concrete (0% CBPD) had highest compressive strength of 34.66 N/mm² and 39.23 N/mm² at the age of 28 and 56 days curing.
- iii. CBPD alone is not cementitious and replacement of cement by CBPD will lead to less cement content and therefore less strength.
- iv. In control concrete replacement of CBPD up to 10% reduced strength by 14% (30.37 N/mm²), but still achieving more strength than target strength (26 N/mm²) and thus can be used as partial replacement up to 10 %.

5.1.2 Water Absorption and Porosity

- i. Increased water absorption and pore size decreased the compressive strength and vice-versa. The water absorption of control concrete 0% CBPD at the age of 28 days was 1.56%, whereas 10, 20 and 30% control CBPD showed water absorption of 2.58, 3.15 and 3.37%, respectively. In bacterial concrete minimum water absorption (1.21%) was observed in 0% CBPD concrete.
- ii. Bacterial concrete seemed to have reduced water absorption as compared to control concrete, at 56 days of curing bacterial concrete had minimum water absorption (1.03%) for 0% CBPD.

- iii. Increased percentage of CBPD lead to increase in water absorption as CBPD reduced cement and caused bleeding and made concrete structure more porous.

5.1.3 Rapid chloride permeability test

- i. The chloride permeability of control and bacterial concrete specimen showed reduction in chloride permeability with increasing curing period from 28 to 56 days.
- ii. At 28 days of curing control concrete with 0% CBPD was characterized in “moderate” range (2366 C), whereas bacterial concrete with 0% CBPD was in “low” range (1998).
- iii. At the age of 56 days control and bacterial concrete with 0 and 10 % CBPD were under “low” range, where was 20 and 30% showed “moderate” range.
- iv. Permeability was reduced in bacterial concrete with all percentages as compared to control concrete, which leads to the conclusion that calcite was formed pores in bacterial concrete.

5.1.4 Sorptivity

- i. In control concrete cured for 28 days 0% CBPD has least sorption 1×10^{-7} mm/sec^{1/2}. Bacterial concrete showed least sorption of 0.092×10^{-7} mm/sec^{1/2}.
- ii. Sorptivity depends on the porosity of concrete structure, the sorption of control and bacterial concrete specimen was similar to that of porosity and water absorption.

5.2 MICROSTRUCTURE ANALYSIS

- i. SEM analysis revealed the increased formation of CSH gel and non-expansive ettringite formation in the matrix of bacterial concrete which supports the increase in compressive strength as compared to control concrete.
- ii. X-ray diffraction (XRD) analysis of concrete samples showed the peaks of quartz (Q), calcium silicate hydrate (CSH), calcite (C), larnite (L) and ettringite (E) phases. The peak of larnite (Ca₂SiO₄) or C₂S or Belite was observed at 37 degree 2θ responsible for late development of strength in concrete.
- iii. XRD analysis confirmed the formation on CSH (21, 26, 29 degree 2θ) and non-expansive ettringite (17, 34 degree 2θ) within the matrix of control and bacterial concrete specimen, which causes an improvement in the strength of material.

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