BACTERIAL CONCRETE - A REMEDY FOR MICRO CRACKS

Submitted in partial fulfillment of the requirements

For the degree of

Bachelor of Engineering

By

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CERTIFICATE

This is to certify that the project entitled **“Bacterial Concrete -A remedy for Micro Cracks”**is a bonafide work of**AnsariMohd.ParvezAlam(13CE74), KadriAasad Anwar(13CE86), MakandarImtiyazMehboob (13CE80)** submitted to the University of Mumbai in partial fulfilment of the requirement for the award of the degree of “Bachelor of Engineering” in Department of Civil Engineering.

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Declaration

We declare that this written submission represents my ideas in our own words and where others ideas or words have been included; we have adequately cited and referenced the original sources. We also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in our submission. We understand that any violation of the above will be cause for disciplinary action by the Institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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Abstract

Carbonate-producing bacteria have attracted lots of interest as a promising, natural, environmental friendly novel technique to improvement of concrete characteristics. Considerable research has been conducted on utilizing microbial-induced carbonate precipitation to mitigate several concrete problems such as crack repair, reduction and modification of porosity and permeability. Furthermore, bacterial carbonate precipitation (bio deposition) has shown positive influences on compressive strength improvement of concrete. In the meantime, it seems that the study related to the optimum dosage of bacterial solution and its effect on the durability of concrete has not been comprehensively investigated. Therefore, it is decided to carry out an investigation of determining optimum dosages of bacterial solution required for concrete by forming various concrete cube samples having variations of bacterial solution viz. 15 ml, 30 ml, 45 ml, 60 ml and 75 ml. Further these various samples are tested under various laboratory methods viz. slump cone test, compressive strength testing machine, ultrasonic pulse velocity test, plate count cells and scanning electron microscopes thereby an optimum dosage required is computed. Bacterial concrete is found to be superior as compare to that of conventional concrete in all the aspects of durability. Among the different specimen incorporated it shows that bacterial concrete containing 45ml solution is the optimum dosage required, after which the strength found to be stable or decreased.

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Chapter 1

Introduction

1.1 Background

Carbonate-producing bacteria have attracted lots of interest as a promising, natural, environmental friendly novel technique to improvement of concrete characteristics. Considerable research has been conducted on utilizing microbial-induced carbonate precipitation to mitigate several concrete problems such as crack repair(Van Tittelboom et al. 2010;Wiktor and Jonkers 2011), reduction and modification of porosity (Ghosh et al. 2005, 2009), and permeability(De Muynck et al. 2007a; Jonkers and Schlangen 2008;Nemati and Voordouw 2003). Furthermore, bacterial carbonate precipitation (bio deposition) has shown positive influences on compressive strength improvement of concrete (Bang et al. 2001;Ghosh et al. 2005, 2009; Jonkers and Schlangen 2008; Jonkers et al.2010; Reddy et al. 2010) and also, it also reduces water absorptionand carbonation of concrete as an alternative surface treatment(De Muynck et al. 2007a, b, 2008a, b).As part of metabolism, some bacteria produces enzyme urease whichcatalyzes the hydrolysis of urea to generate carbonate ions without an associated production of protons which leads to CaCO3 precipitation in presence of calcium ions (Chahal et al. 2012;Okwadha and Li 2011; Siddique and Chahal 2011). Therefore, bacteria cells not only provide a nucleation site for CaCO3 precipitation due to their negatively charged cell walls, but also create an alkaline environment inducing further growth of CaCO3 crystals (Ferris et al. 1987; Stocks-Fischer et al. 1999). One ml of urea is hydrolyzedintracellularly to 1 ml of ammonia and 1 ml of carbonate, which is presented in Eq.

(1). According to Eq. (2), carbonate hydrolyzes to ammonia and carbonic acid.Eqs. (3) and (4) demonstrate former products subsequently equilibrate in water to form bicarbonate, ammonium, and hydroxide ions. The latter causes pH increase resulting in the formation of carbonate ions [Eq. (5)], which in the presence of soluble calcium ions precipitate as CaCO3 [Eq. (6)]. Eq. (7) is the overall reaction, which demonstrates that ammonium and calcium carbonate are the products of added urea and calcium to the system (Siddique and Chahal 2011; Van Tittelboom et al. 2010).

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

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

$$H_2CO_3 \rightarrow HCO_3 + H^+. \quad \ldots\ldots (3)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_{4+} + 2OH^- \quad \ldots\ldots (4)$$

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

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

$$CO(NH_2)_2 + 2H_2O + Ca^{2+} \rightarrow 2NH_{4+} + CaCO_3 \quad \ldots\ldots (7)$$

Effect of the application of ureolytic bacteria such as Bacillus pasteurii [now reclassified as Sporosarcina pasteurii (Siddique and Chahal 2011), B. subtilis, and B. sphaericus on concrete characteristics has been extensively studied. Stocks-Fischer et al. observed CaCO3 crystals in sand specimens containing B. pasteurii cells accompanied with urea and CaCl2. Possible biochemical reactions in urea-CaCl2 medium to precipitate CaCO3 at the cell surface can be summarized as follows (Stocks-Fischer et al. 1999):

$$Ca^{2+} + Cell \rightarrow Cell - Ca^2 \quad \ldots\ldots (1)$$

$$Cl^- + HNO_{3-} + NH_3 \rightarrow NH_4Cl + CO_3^{2-} \quad \ldots\ldots (2)$$

$$Cell - Ca^2 + CO_3^{2-} \rightarrow Cell - CaCO_3 \downarrow \quad \ldots\ldots (3)$$

Reddy et al. have reported the addition of B. subtilis bacteria increases the compressive strength of standard grade concrete up to about 15% at 28 days, and also shows a significant improvement in split tensile strength compared to conventional concrete (Reddy et al. 2010). Chahal et al. have prepared fly ash concrete specimens employing S. pasteurii, and attributed significant 28-day compressive strength increment of fly ash concrete to

consolidation of the pores inside the concrete cubes with bacterial induced calcium carbonate precipitation (Chahal et al. 2012).

De Muynck et al. have applied bio deposition treatment on the surface of mortar specimens using B. sphaericus. They demonstrated the presence of a layer of calcium carbonate crystals, as was confirmed by XRD analyses, resulted in a decrease of the permeation properties of cementitious materials. As a result, an increased resistance toward carbonation and chloride migration has been noticed (De Muynck et al. 2007b, 2008b).In the meantime, it seems that the study related to the optimum dosage of bacterial solution and its effect on the durability of concrete has not been comprehensively investigated. Therefore, it is decided to carry out an investigation of determining optimum dosages of bacterial solution required for concrete by forming various concrete cube samples having variations of bacterial solution viz. 15 ml, 30 ml, 45 ml, 60 ml and 75 ml. Further these various samples are tested under various laboratory methods and thereby an optimum dosage required is computed.

1.2 Aim **ofthe Project work**

The aim of this project is to

- Develop a bacterial concrete by introducing the bacteria's of bacillus family (Bacillus subtilis).
- To find the optimum dosage of bacteria required for bacterial concrete.
- To determine the viable bacterial cells by serial dilution method.
- To know the presence of voids by Ultrasonic pulse velocity test.
- To know the presence of voids in the internal structure of concrete by SEM.
- Observe the behavior of bacteria chemically.
- Observe the change in the properties of concrete such as compressive strength & permeability.

1.3 Future Scope of Investigation

- To study the effect of bacteria on High Strength concrete.
- To study the durability of concrete under various weathering conditions.
- To determine the maximum width of crack healing using bacterial concrete under various environmental condition.

Chapter 2

Review of Literature

In order to carry out the project work various literatures were studied and findings obtained by them were used to identify the research area, summarizations of literatures are as follows:-

- A method of strength improvement of cement–sand mortar by the microbiologically induced mineral precipitation was described by **P.Ghosh et al. (2005)**. A thermophilic anaerobic microorganism is incorporated at different cell concentrations with the mixing water. The study showed that a 25% increase in 28 day compressive strength of cement mortar was achieved with the addition of about 10^5 cell/ml of mixing water. The strength improvement is due to growth of filler material within the pores of the cement–sand matrix as shown by the scanning electron microscopy. The modification in pore size distribution and total pore volume of cement–sand mortar due to such growth is also noted. *E. coli* microorganisms were also used in the cement mortar for comparison, but no improvement in strength was observed**.-"Use of microorganism to improve the strength of cement mortar".**

- As synthetic polymers, used for concrete repair, may be harmful to the environment, the use of a biological repair technique was investigated by **K. Van Tittelboom et al. (2010)**. Ureolytic bacteria such as *Bacillus sphaericus* were able to precipitate $CaCO_3$ in their micro-environment by conversion of urea into ammonium and carbonate. The bacterial degradation

of urea locally increases the pH and promotes the microbial deposition of carbonate as calcium carbonate in a calcium rich environment. These precipitated crystals can thus fill the cracks. The crack healing potential of bacteria and traditional repair techniques were compared in this research by means of water permeability tests, ultrasound transmission measurements and visual examination. Thermo gravimetric analysis showed that bacteria were able to precipitate $CaCO_3$ crystals inside the cracks. It was seen that pure bacteria cultures were not able to bridge the cracks. However, when bacteria were protected in silica gel, cracks were filled completely**.-"Use of bacteria to repair cracks in concrete."**

- Microbially enhanced calcite precipitation on concrete or mortar had become an important area of research regarding construction materials. Study examined by **V.Achal et al. (2011)** stated the effect of calcite precipitation induced by *Sporosarcina pasteurii* (Bp M-3) on parameters affecting the durability of concrete or mortar. An inexpensive industrial waste, corn steep liquor (CSL), from starch industry was used as nutrient source for the growth of bacteria and calcite production, and the results obtained with CSL were compared with those of the standard commercial medium. Bacterial deposition of a layer of calcite on the surface of the specimens resulted in substantial decrease of water uptake, permeability, and chloride penetration compared with control specimens without bacteria. The results obtained with CSL medium were comparable to those obtained with standard medium, indicating the economization of the biocalcification process. The results suggest that calcifying bacteria play an important role in enhancing the durability of concrete structures**.-** **"Effect of calcifying bacteria on permeation properties of concrete structures,"**

- Fly ash acts as a partial replacement material for both Portland cement and fine aggregate. An innovative approach of microbial calcite precipitation in fly ash-amended concrete had been investigated. This is the first report by **V.Achal et al. (2011)** to discuss the role of microbial calcite precipitation in enhancing the durability of fly ash-amended concrete. The present study investigated the effects of *Bacillus megaterium* ATCC 14581 on compressive strength, water absorption and water impermeability of fly ash-amended mortar and concrete. Mortar specimens were used for compressive strength and water absorption tests, while concrete specimens were used for water impermeability tests. At the fly ash concentrations of 10%, 20% and 40% in mortars, bacterial cell enhanced mortar compressive strength by 19%, 14% and 10%, respectively, compared to control specimens. Treated mortar

cubes absorbed more than three times less water than control cubes as a result of microbial calcite deposition. Microbial deposition of a layer of calcite on the surface of the concrete specimens resulted in substantial decrease of water uptake and permeability compared to control specimens without bacteria. Microbial cells also prevented ingress of water effectively in different concentrations of fly ash-amended concrete. Scanning Electron Micrography (SEM) analyses evidenced the direct involvement of bacteria in calcite precipitation. The approach of the present study gives us dual environment friendly advantages. First, use of fly ash-a recovered resource reduces depletion of natural resources and also reduces the energy-intensive manufacturing of other concrete ingredients, leading to savings in both energy usage and emissions of greenhouse gases. And second, use of bacterial cells to improve strength and durability of fly ash-amended concrete further provides greener and economic options**.- "Improved strength and durability of fly ash amended concrete by microbial calcite precipitation."**

- Microorganism is a unique living element and has the ability to precipitate minerals through the process of biomineralization. The precipitation process occurred naturally and most of the precipitated products are very important compound composed of such as carbon, nitrogen, oxygen, sulphur, phosphorus and silica. So far, concrete incorporated with microorganism that able to precipitate calcium carbonate (calcite) was reported. However, little information on silica precipitation and its effect on concrete properties had been revealed. The concrete specimens were incorporated with Bacillus subtilis silica adsorbed in their cell wall by **H Afifudin et al. (2011)** -. Concrete specimens with five different concentrations of Bacillus subtilis cell with 104, 105, 106 and 107 cell/ml and control (without Bacillus subtilis) were cast. The experimental investigation made to prove that the silica precipitated by this microorganism can enhance the concrete properties namely its compressive strength and resistance to carbonation. The microstructure of the concrete contained Bacillus subtilis was also examined. It was found that the inclusion of Bacillus subtilis into the concrete enhanced the compressive strength. The concentration of 106 cell/ml was found to be the optimum concentration to give most enhanced effect to the compressive strength. However the effect of including Bacillus subtilis to the resistance to carbonation of the concrete specimen is found to be insignificant**.-"Microorganism precipitation in enhancing concrete properties."**

- Cracks in concrete are the main reason for a decreased service life of concrete structures. It is therefore more advisable and economical to restrict the development of early age small cracks the moment they appear, than to repair them after they have developed to large cracks. A promising way is to pre-add healing agents to the concrete to heal early age cracks when they appear, i.e. the so-called self-healing approach was described by **J. wang et al. (2012)**. In addition to the more commonly studied polymeric healing materials, bacterial $CaCO_3$precipitation also has the potential to be used for self-healing. It is more compatible with the concrete matrix and it is environment friendly. However, bacterial activity decreases a lot in the high pH (>12) environment inside concrete. In this research, the possibility to use silica gel or polyurethane as the carrier for protecting the bacteria was investigated. Experimental results show that silica gel immobilized bacteria exhibited a higher activity than polyurethane immobilized bacteria, and hence, more $CaCO_3$ precipitated in silica gel (25% by mass) than in polyurethane (11% by mass) based on thermo gravimetric analysis. However, cracked mortar specimens healed by polyurethane immobilized bacteria had a higher strength regain (60%) and lower water permeability coefficient (10^{-10}–10^{-11} m/s), compared with specimens healed by silica gel immobilized bacteria which showed a strength regain of only 5% and a water permeability coefficient of 10^{-7}–10^{-9} m/s. The results indicated that polyurethane has more potential to be used as a bacterial carrier for self-healing of concrete cracks**.-"Use of silica gel or polyurethane immobilized bacteria for self-healing concrete."**

- The relevant experiments were designed by **A.vahabi et al. (2013)** to determine the ability of indigenous bacterial strains isolated from limestone caves, mineral springs, and loamy soils to induce calcium carbonate precipitation. Among all isolates examined an efficient carbonate-precipitating soil bacterium was selected from among the isolates and identified by 16S r RNA gene sequences as *Bacillus licheniformis* AK01. The ureolytic isolate was able to grow well on alkaline carbonate-precipitation medium and precipitate calcium carbonate more than $1\,g\,L^{-1}$. Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD) analyses, and scanning electron microscopy (SEM)/energy-dispersive X-ray spectroscopy (EDX) examinations were performed in order to confirm the presence of calcium carbonate in the precipitate and to determine which polymorphs were present. The selected isolate was determined to be an appropriate candidate for application in a surface treatment of cement-based material to improve the properties of the mortar. Bio deposition of a layer of calcite on the surface of cement specimens resulted in filling in pore spaces. This

could be an alternative method to improve the durability of the mortar. The kind of bacterial culture and medium composition had a profound impact on the resultant $CaCO_3$ crystal morphology**.-"Calcium carbonate precipitation by strain Bacillus licheniformis AK01, newly isolated from loamy soil: a promising alternative for sealing cement-based materials".**

- The role of bacterial cell walls of *Bacillus subtilis* as a concrete admixture to improve the mechanical performance of concrete. The bacterial cell walls are known to mediate microbial induced carbonate precipitation, a process in which $CaCO_3$ is formed from Ca^{2+} ions and dissolved CO_2. Consistent with such knowledge, incorporation of bacterial cell walls increased carbonation of $Ca(OH)_2$ and formation of $CaCO_3$ in concrete. Furthermore, the bacterial cell walls significantly increased compressive strengths of concrete by 15% while also decreased porosity at 28 days of curing as described by **R.Pei et al. (2013)**. Assay for $CaCO_3$ precipitation *in vitro* indicated that bacterial cell walls, but not dead cells, accelerated carbonation of Ca^{2+} ions in $Ca(OH)_2$ solution. Since $CaCO_3$formed can fill up the void, decrease the porosity and increase the compressive strength in concrete, bacterial cell walls could act as a promising concrete admixture with benefits in enhancing mechanical performance and improving other carbonation-related properties**.-"Use of bacterial cell walls to improve the mechanical performance of concrete".**

- The well-known fact that concrete structures are very susceptible to cracking which allows chemicals and water to enter and degrade the concrete, reducing the performance of the structure and also requires expensive maintenance in the form of repairs. Cracking in the surface layer of concrete mainly reduces its durability, since cracks were responsible for the transport of liquids and gasses that could potentially contain deleterious substances. When micro cracks growth reaches the reinforcement, not only the concrete itself may be damaged, but also corrosion occurred in the reinforcement due to exposure to water and oxygen, and possibly CO2 and chlorides too. Micro-cracks are therefore the main cause to structural failure. One way to circumvent costly manual maintenance and repair is to incorporate an autonomous self -healing mechanism in concrete. One such an alternative repair mechanism is currently being studied by **M.V.S.Rao et al. (2013)** i.e. a novel technique based on the application of bio mineralization of bacteria in concrete. The applicability of specifically calcite mineral precipitating bacteria for concrete repair and plugging of pores and cracks in concrete had been recently investigated and studies on the possibility of using specific

bacteria as a sustainable and concrete -embedded self-healing agent was studied and results from ongoing studies were discussed. Synthetic polymers such as epoxy treatment etc were currently being used for repair of concrete are harmful to the environment, hence the use of a biological repair technique in concrete is focused. In the present paper, an attempt is made to incorporate dormant but viable bacteria in the concrete matrix which will contribute to the strength and durability of the concrete. Water which enters the concrete will activate the dormant bacteria which in turn will give strength to the concrete through the process of metabolically mediated calcium carbonate precipitation. Concrete, due to its high internal pH, relative dryness and lack of nutrients needed for growth, is a rather hostile environment for common bacteria, but there are some extremophiles spore forming bacteria may be able to survive in this environment and increase the strength and durability of cement concrete. Overview of development of bioengineered concrete using bacterial strain Bacillus subtilis JC3 and its enhanced mechanical and durability characteristics was briefly described in this paper.-"**A sustainable self-healing construction material."**

- World widely, concrete is one of the most popular construction materials because of its strong, durable and inexpensive material. It has specialty of being cast in any desirable shape but plain concrete however is porous, possesses very low tensile strength, limited ductility and little resistance to cracking. These problems become more complicated in various environmental conditionsto which concrete is exposed. Conventionally, a variety of sealing agent namely, latex emulsions suffer from serious limitations of incompatible interfaces, susceptible to ultraviolet radiations, unstable molecular structure and high cost. Therefore, a novel and more environmental friendly technique was proposed for treating concrete material in structure by employing bacteria induced calcium carbonate precipitation in the form of calcite by **J.M Irwan et al. (2013)-"Concrete Repair, Rehabilitation & Retrofitting II".**

- Study was carried out by **A. Talaiekhozani et al. (2013)** to investigate two indigenous micro-organisms that can be isolated from soil. The isolated micro-organisms could precipitate calcium carbonate. These micro-organisms were applied to design self-healing concretes. Concrete is one of the most important materials which are used to build structures. Strength and durability of concrete is very important. Hence, a lot of research in this field is being conducted. Although a few reports can be found on the use of different micro-organism to design self-healing concretes, no research had been carried out to isolate suitable

indigenous micro-organisms in Malaysia. In this study two strains of microorganisms were isolated from soil. Broken concrete was treated by a medium culture (MC) containing micro-organisms. Results of this study showed that, cracked concrete could be filled by calcium carbonate after treating by a MC containing micro-organisms. However, this treatment is not very effective on the strength of concrete. Results of this study can be used to have a better grasp of biological self-healing concrete, it is extremely important to have cheap and durable materials to build concrete structures in future**.-"Application of Proteus mirabilis and Proteus vulgaris mixture to design self-healing concrete."**

- The applications of concrete are rapidly increasing worldwide and therefore the development of sustainable concrete is urgently needed for environmental reasons. As presently about 7% of the total anthropogenic atmospheric CO_2 emission was due to cement production, mechanisms that would contribute to a longer service life of concrete structures would make the material not only more durable but also more sustainable. One such mechanism that receives increasing attention in recent years is the ability for self-repair, i.e. the autonomous healing of cracks in concrete. In this study we investigated the potential of bacteria to act as self-healing agent in concrete, i.e. their ability to repair occurring cracks. A specific group of alkali-resistant spore-forming bacteria related to the genus *Bacillus* was selected by **H.M. Jonkers et al. (2013)** for this purpose. Bacterial spores directly added to the cement paste mixture remained viable for a period up to 4 months. A continuous decrease in pore size diameter during cement stone setting probably limited life span of spores as pore widths decreased below 1 μm, the typical size of *Bacillus*spores. However, as bacterial cement stone specimens appeared to produce substantially more crack-plugging minerals than control specimens, the potential application of bacterial spores as self-healing agent appeared promising.-**"A two component bacteria-based self-healing concrete."**

- Microbially induced calcium carbonate precipitation (MICCP) is a novel method for the protection of cement-based materials. This paper produced by**Senthilkumar et al. (2014)**deals the comparative studies on strength characteristics in microbial cement mortars which were treated by Enterobacter sp. M2 microorganism in different calcium source (calcium hydroxide, calcium acetate, calcium chloride and calcium oxide) with various curing process. The crystalline phases of calcium carbonate ($CaCO_3$) crystals formation and the surface morphology of cement mortar were investigated by X-ray diffraction (XRD) and

scanning electron microscope (SEM). Cement mortar specimens with and without addition of bacterial species were casted and ~ 44% increase in compressive strength, ~56% in tensile strength was noticed while compared to control specimen (without bacteria). Surface treatment of specimen with bacteria resulted around ~40% decrease of water absorption and increases the resistance to water and hazard material penetration, mainly attributed to its pore blocking effects. This biological surface treatment shows promising prospect for increasing durability aspects of concrete/cement mortar**.-"Comparative studies on strength characteristics of microbial cement mortars".**

- Shortcomings of conventional treatments have drawn the attention to alternative techniques for the improvement of the compressive strength. This paper reported by **V. Senthilkumar et al. (2014)** reports the effects of bacterial carbonate precipitation on the compressive strength of cement mortar specimens. The method of microbial mineral plugging in porous media was common in nature. Physical and biochemical properties of $CaCO_3$ precipitation induced by Enterococcus sp. microorganism into cement mortar specimen was studied and analyzed. X-ray diffraction is used to identify the calcium carbonate ($CaCO_3$) crystal as calcite, vaterite, aragonite and scanning electron microscope (SEM) was used to verify these formations as white precipitation ($CaCO_3$) in the microbial cement mortars. In the present study a noteworthy enhancement of compressive strength 45% is observed in the Enterococcus sp. treated bio curing specimen while compared to control.- **"Fortification of compressive strength in enterococcus microorganism incorporated microbial cement mortar."**

- -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. **R. Siddique et al. (2014)** studied the effect of bacterial (*Bacillus halodurans* strain KG1) 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. X-ray diffraction (XRD) and scanning electron microscopy (SEM) results suggested that in bacterial treated

10% CKD concrete increased calcium silicate hydrate and formation of non-expansive ettringite in pores dense the concrete structure resulted in increased compressive strength.-**" Influence of bacterial treated cement kiln dust on the properties of concrete."**

- **A** paper by **R. Andalib et al. (2014)** provided an insight into a new biotechnological method based on calcite precipitation for achieving high strength bio-concrete durability. It was very clear that mineral precipitation has the potential to enhance construction material resistance towards degradation procedures. The appropriate microbial cell concentration ($30 * 10^5$ cells/ml) was introduced onto different structural concrete grades (40, 45 and 50 MPa) by mixing water. In order to study the durability of structural concrete against aggressive agents, specimens were immersed in different types of acids solution (5% H_2SO_4 and HCl) to compare their effects on 60^{th}, 90^{th} and 120^{th} day. In general, sulphuric acid and hydrochloric acid are known to be the most aggressive natural threats from industrial waters which can penetrate concrete to transfer the soluble calcium salts away from the cement matrix. The experimental results demonstrated that bio-concrete has less weight and strength losses when compared to the ordinary Portland cement concrete without microorganism. It were also found that maximum compressive strength and weight loss occurred during H_2SO_4 acid immersion as compared to HCl immersion. The density and uniformity of bio-concrete were examined using ultrasonic pulse velocity (UPV) test. Microstructure chemical analysis was also quantified by energy dispersive spectrometer (EDS) to justify the durability improvement in bacterial concrete. It was observed that less sulphur and chloride were noticed in bacterial concrete against H_2SO_4 and HCl, respectively in comparison to the ordinary Portland cement concrete due to calcite deposition.-**"Durability improvement assessment in differenthigh strength bacterial structural concrete grades against different types of acids."**

- Cracks increase permeability affecting the durability of concrete. As they develop gradually, it is difficult to determine when to repair them. Self-healing materials can repair themselves gradually as cracks form. A study carried out by **C. Stuckrath et al. (2014)** shows, the isolated and combined effect of two self-healing agents for concrete, both based on calcium carbonate precipitation, was studied. Lightweight aggregates were impregnated with chemical and biological solution to be added as healing agents in concrete mixtures. The influence of two common chemical admixtures on the performance of the self-healing agents was also studied. All self-healing agents were able to seal cracks between 0.08 and 0.22 mm

in width. The estimated effect of chemical agents on the mean healing was higher than that of biological agents. In addition, thermo gravimetric analysis suggests the precipitates were different. Admixtures had no significant influence on the performance of self-healing agents**.-"performance of self-healing in reinforced mortar containing chemical admixture."**

- Microcapsules were applied to encapsulate bacterial spores for self-healing concrete. The viability of encapsulated spores and the influence of microcapsules on mortar specimens were investigated by **J.Y. Wang et al. (2014)** firstly. Breakage of the microcapsules upon cracking was verified by Scanning Electron Microscopy. Self-healing capacity was evaluated by crack healing ratio and the water permeability. The results indicated that the healing ratio in the specimens with bio-microcapsules was higher (48%–80%) than in those without bacteria (18%–50%). The maximum crack width healed in the specimens of the bacteria series was 970 μm, about 4 times that of the non-bacteria series (max 250 μm). The overall water permeability in the bacteria series was about 10 times lower than that in non-bacteria series. Wet–dry cycles were found to stimulate self-healing in mortar specimens with encapsulated bacteria. No self-healing was observed in all specimens stored at 95%RH, indicating that the presence of liquid water is an essential component for self-healing.-**"Self-healing concrete by use of microencapsulated bacterial spores."**

- Bacterial-based self-healing is a promising solution for sustainable concrete maintenance. A study carried out by **J.Y. Wang et al. (2014)** shows, bacterial spores were first encapsulated into hydrogels and then were incorporated into specimens to investigate their healing efficiency. The precipitation of $CaCO_3$ by hydrogel-encapsulated spores was demonstrated by Thermo gravimetric analysis (TGA). The mortar specimens with hydrogel-encapsulated spores, showed a distinct self-healing superiority: the maximum healed crack width was about 0.5 mm and the water permeability was decreased by 68% in average. Other specimens in non-bacterial series had maximum healed crack width of 0–0.3 mm and the average water permeability was decreased by 15–55% only**.-"Application of hydro gel encapsulated carbonate precipitating bacteria for approaching a realistic self-healing in concrete."**

- In this paper published by **C.X. Qian et al. (2014),** three bio-mineralization mechanisms were proposed to repair cement-based materials cracks. The common feature was

that the three are all induced by bacterial. A type of bacterial which can decompose urea and release carbonate ions could be applied to repair micro cracks on concrete surface when combining calcium ions. But what need to be noted is that the way of repairing cracks is passive. Some alkaliphilic bacterial spores could be added to concrete when casted and two different types of bacterial were used to realize the function of self-healing. The sources of carbonate ions made them different; the one release carbonate dioxide through its own cellular respiration, the other could transfer carbon dioxide in air to bicarbonate. Coefficient of capillary suction, apparent water permeation coefficient and area repairing rate were applied to characterize the repairing effectiveness. The tests results were that all three bio-mineralization mechanisms showed excellent repair effect to small cracks formed at early ages. When the bacteria were immobilized by ceramsite, the self-healing effect could be improved for the cracks formed at late ages**.-"Self-healing and repairing concrete cracks based on bio-mineralization."**

- Using Carbonate producing bacteria was a promising novel technique by **F. Nosouhian, et al. (2015)** for the improvement of concrete characteristics. Durability of concrete in harsh environment such as sulphate exposure has been constantly an important issue. The intention of the current study was evaluation h mixing water, the effect of sulfate solution exposure on durability properties of tested specimens including mass variation, volume variation, and water absorption of durability improvement of concrete containing bacteria exposed to sulfate environment. To do so, seven groups of 70-mm concrete prisms were made using two different bacterial strains accompanied with mixing water, the effect of sulfate solution exposure on durability properties of tested specimens-**"Concrete Durability Improvement in Sulfate Environment Using Bacteria".**

- Concrete structures are often reinforced with steel. In order for the reinforcement to take over tensile forces, concrete has to crack. Through such cracks, water and compounds that are harmful to concrete can enter. This can cause durability issues like leakage, concrete degradation and reinforcement corrosion. In situ repair of cracks was often labor-intensive and inefficient.

Preferentially, cracks were autonomously healed from the inside out in an early stage, preventing the ingress of water. This can be achieved by incorporating healing agent particles composed of nutrients and bacterial spores into the concrete matrix. The bacteria will

germinate when water is available, plugging cracks with calcium carbonate. However, a coating is needed to protect the water-soluble healing agent from water during mixing. In order to allow the bacteria access to water for activation after the concrete has hardened, such a coating should break whenever a crack occurs in the concrete. Therefore, it should adhere well to the concrete matrix. It is possible to achieve this by protecting the particles with a brittle geopolymer coating.

For this study, healing agent particles were coated with geopolymers following different mixture recipes. Metakaolin is used as an aluminosilicate source and sodium silicate as well as sodium aluminate is used as activator liquids. The particles are coated by granulation in a low-shear granulator. In order to improve the coating process, the operating window and the granulation mechanism were determined for all activator liquids used. Leaching and strength tests were performed by **S.A.L. De Koster et al.(2015)**and coated particles are incorporated in cement paste in order to determine the feasibility of application of the particles in concrete.

Results show that the prepared particles are better protected from leaching than untreated particles. Using a high pressure single-fluid nozzle to improve nebulisation when coating produces more particles of the desired size than coating with a low pressure single-fluid nozzle with poor nebulisation. Furthermore, particles prepared with a high pressure nozzle sprayer perform better when incorporated into cement paste than particles prepared with a low pressure nozzle sprayer.**-"Geopolymer coating of bacteria-containing granules for use in self-healing concrete."**

- The objective of this study by **E. Mostavi et al.(2015)** was to evaluate a new generation of self-healing materials that hold promise for better durability and performance. The in situ polymerization method was used to develop double-walled microcapsules. The microcapsules were prepared in a single batch process containing sodium silicate as the healing agent encapsulated in double-walled polyurethane/urea-formaldehyde (PU/UF) microcapsules. Double-walled microcapsules provide enhanced durability at high temperatures compared with single-walled microcapsules while preserving adequate interfacial bonding of microcapsules. A parametric study was carried out to investigate the effect of different parameters such as agitation rate, pH, and temperature on the performance of the microcapsules and to determine the optimum microencapsulation procedure. The prepared microcapsules were then incorporated into self-healing concrete beams. To monitor the healing process of the cracks, micro cracks were created by imposing a certain magnitude

of displacement in the middle of the beams. The healing process of concrete specimens was monitored and quantified using portable ultrasonic nondestructive digital indicating tester (PUNDIT). Results showed that lower pH and higher agitation rate and curing temperature improve the formation of microcapsule shells. Measurements of ultrasonic wave transmission time through the concrete specimens containing different contents of microcapsules were analyzed to quantify the healing rate. It was found that the healing rate in concrete beams with 5% microcapsules was higher in the first week in comparison with specimen containing 2.5% of microcapsules**.-"Evaluation of self-healing mechanisms in concrete with double-walled sodium silicate microcapsules."**

- Using carbonate-producing bacteria was a promising novel technique by **F. Nosouhian et al. (2016)** for the improvement of concrete characteristics. Durability of concrete in harsh environments such as sulfate exposure has been constantly an important issue. The intention of the current study is evaluation of durability improvement of concrete containing bacteria exposed to sulfate environment. To do so, seven groups of 70-mm concrete prisms were made using two different bacterial strains accompanied with mixing water; the effects of sulfate solution exposure on durability properties of tested specimens including mass variation, volume variation, water absorption, and compressive strength were then determined. Furthermore, seven groups of concrete discs with 100 mm diameter and thickness of 50 mm were prepared from the aforementioned batches to investigate the chloride permeability of bacterial concrete by rapid chloride permeability test (RCPT). The results indicated that bacteria incorporation in concrete reduces mass variation, volume variation (in higher ages), and water absorption; it also increases the compressive strength of the specimens. The results also showed that the 28-day compressive strength of the bacteria-containing concretes is about 20% more than that of the control specimens. Moreover, bacterial concrete have lower chloride penetration in comparison with the control specimens**.-"Concrete durability improvement in a sulfate environment using bacteria."**

- Crack formation and progression under tensile stress was a major weakness of concrete. These cracks also make concrete vulnerable to deleterious environment due to ingress of harmful compounds. Crack healing in concrete can be helpful in mitigation of development and propagation of cracks in concrete. This paper published by **W. Khaliq et al. (2016)**, presents the process of crack healing phenomenon in concrete by microbial activity of

Bacteria, *Bacillus subtilis*. Bacteria were introduced in concrete by direct incorporation, and thorough various carrier compounds namely light weight aggregate and graphite Nano platelets. In all the techniques, calcium lactate was used as an organic precursor. Specimens were made for each mix to quantify crack healing and to compare changes in compressive strength of concrete. Results showed that bacteria immobilized in graphite nano platelets gave better results in specimens pre-cracked at 3 and 7 days while bacteria immobilized in light weight aggregates were more effective in samples pre-cracked at 14 and 28 days. In addition, concrete incorporated with bacteria immobilized in light weight aggregate, also exhibited significant enhancement in compressive strength of concrete.-**"Crack healing in concrete using various bio influenced self-healing techniques."**

2.1 Summary of literature review-

Based on the above literature study it was found that various work has been conducted in regards with comparison between conventional and bacterial concrete. Also some literature express the maximum width of cracks healed by the bacterial concrete, some of them highlited the effect on bacterial concrete in various environmental conditions. But no literature so far indicate the optimum dosage value of bacterial solution required after the insertion in concrete, therefore it needs to be addressed.

Chapter 3

Bacterial Conditions & Physical Materials

3.1 Selection of Bacteria:-

There are various types of Bacterias that can be used in the concrete such as B. Subtilis, B. Pasteurii, B. Cohnii, B. Licheniformis etc. We have selected ***Bacillus Subtilis*** since this bacteria produces Calcium Carbonate and due to ease of availability from pharmacy department of AIKTC, we have used it for our furture investigation. It is also formally known as **Hay bacillus** or **grass bacillus,** is a Gram-positive, catalane-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. A member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and can form a tough, protective endo-spores, allowing it to tolerate extreme environmental conditions. *B. subtilis* has historically been classified as an obligate aerobe, though evidence exists that it is a facultative aerobe. *B. subtilis* is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation. It is one of the bacterial champions in secreted enzyme production and used on an industrial scale by biotechnology companies.
The microphotograph of strains of Bacillus Subtilis is shown in fig. 1 below.

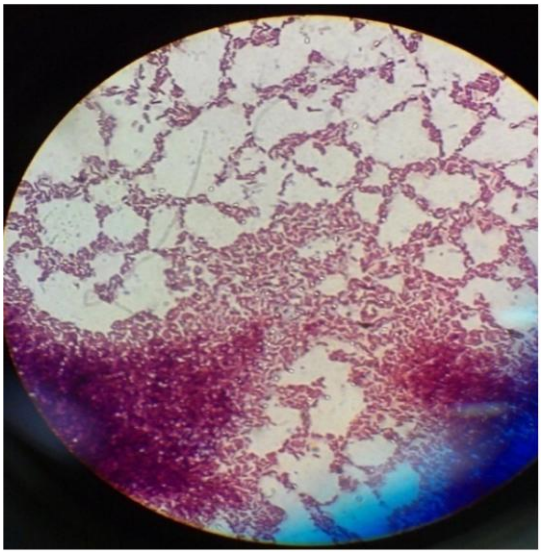

Fig 1: Microphotograph of strains of Bacillus Subtilis

3.2 Cultivation of Bacteria:-

The pure culture of bacteria i.e. Bacillus Subtilis is preserved on nutrient agar slants. It forms irregular dry white colonies on nutrient agar slants. Two colonies of the bacteria are inoculated into nutrient both of 350 ml in 500ml conical flask and incubated at the temperature of 37 degree Celsius and 150 rpm orbital shaker incubator.

The medium composition used for growth of bacterial culture consists of Peptone, NaCl, yeast extract.

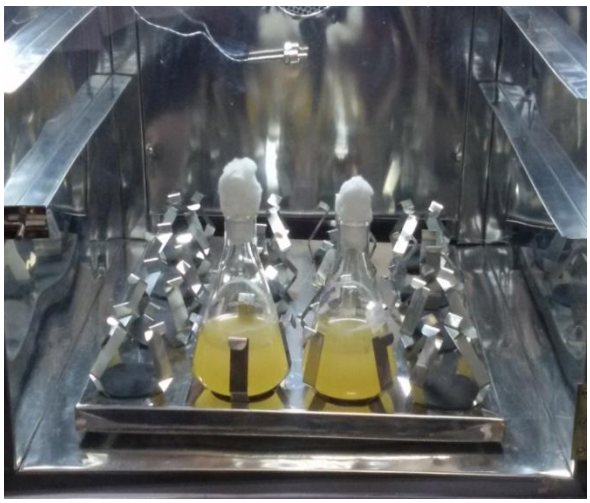

Fig 2: Bacterial solution in incubator.

3.2.1 Experimental Procedure for Cultural Growth of Bacteria:-

S. pasteurii PTCC 1645 (DSM 33, ATCC 11859; CCM 2056; NCIB 8841; NCTC 4822) and B. subtilis PTCC 1715 (BGSC 1A747) prepared from the Persian type culture collection were used throughout the study. S. pasteurii formerly known as B. pasteurii is a bacterium with the ability to precipitate calcite and solidify sand given a calcium source and urea, through the process of biological cementation. B. subtilis is a common soil bacterium, which can produce calcite precipitates on suitable media supplemented with a calcium source (Reddy et al. 2010). The bacteria were cultured in liquid medium according to the suppliers' recommendations. The medium used to grow bacteria consisted of 5.0 g peptone, 3.0 g meat extract, per liter of distilled water; to which 1.5% agar was added to obtain a solid medium for the stock culture. This medium was supplemented with 0.01 g $MnSO4 \cdot H2O$ to enhance sporulation and pH was adjusted to 7.0 using 1 N HCl. The mixture was first sterilized by autoclaving for 20 min at 121°C, allowed to cool to room temperature (25°C). According to supplier's recommendation for culturing of S. pasteurii strain, 10 mL filter-sterilized20% urea solution through a sterile 0.22 μm filter (Jet Biofil) was added aseptically post autoclaving to 100 ml cooled molten peptone/meat extract medium. For the first experiments, B. subtilis and S. pasteurii cultures were obtained through activation of lyophilized bacteria whereas for all later experiments cultures were obtained through sub culturing. Note that the whole culturing process was performed under sterile condition. Then, cultures were incubated at 30°C on a shaker incubator at 130 rpm for 72 h. Afterward, bacterial cells were harvested by centrifuging (5,000 r=min, 10 min) the 72 h-old grown culture and the cells were washed twice in saline solution (NaCl, 8.5 g=L).

3.2.2 Safety Measures for Bacterial Solution:-

Bacteria are harmful for the health and it may lead to diseases, therefore precautions must be taken. It is compulsory to use gloves while dealing with the bacterial solution. The flask must be heated before pouring the bacterial solution. The whole procedure must be done between the two candles so that the bacterium doesn't get contaminated by the interference of the other bacteria's present in the environment.

3.3 Material:-

3.3.1. Cement-Ordinary Portland cement of 53 Grade available in local market is used in the investigation. The cement used has been tested for various properties as per IS: 4031-1988 and found to be confirming to various specifications of IS: 12269-1987 having specific gravity of 3.0.

Fig 3: Cement.

3.3.2. Sand-In our investigation we had used the Gujarat sand confirming the zone III according to IS- 383.Specific gravity of sand was found out to be 2.60.

Fig 4: Sand.

3.3.3. Coarse Aggregate-The coarse aggregate is strongest and porous component of concrete. Presence of coarse aggregate reduces the drying shrinkage and other dimensional changes occurring on account of movement of moisture. In our investigation we had used the aggregate passing through 20mm IS-Sieve and retaining on 12.5mm sieve. The specific gravity of aggregate was found out to be 2.50.

Fig 5: Coarse Aggregate.

3.3.4. Cube Moulds- The cube Moulds (150x150 mm) was placed in position on an even surface. All the interior faces and sides were coated with mud oil to prevent the sticking of concrete to the Moulds.

Fig 6: Cube Moulds.

Chapter 4

Experimental Methods & Test

4.1 Preparation of concrete Mix, Cubesand samples labeling

Mix design can be defined as the process of selecting suitable ingredients of concrete and determining their relative proportions with the object of producing concrete of certain minimum strength and durability as economically as possible. In our investigation we have made M 30 grade of concrete. The mix ratio obtained after the mix design as per IS 456: was M30(1:1.92:2.89).Further, we have poured the concrete in the cube Moulds and six different samples were made which are as follows

a. Conventional Concrete of grade M 30.
b. Concrete with 15 ml bacterial solution.
c. Concrete with 30 ml bacterial solution.
d. Concrete with 45 ml bacterial solution.
e. Concrete with 60 ml bacterial solution.
f. Concrete with 75 ml bacterial solution.

.

Fig 7: Mixing of Concrete.

Fig 8: Labeling of Cube samples

4.2 PH of concrete-

The term pH refers to the measure of hydrogen ion concentration in solution and defined as the negative log of H+ ions concentration materials. The values of PH 0 to a little less than 7 are termed as acidic and the values of PH a little above 7 to 14 are termed as basic. When the concentration of H+ and OH- ions is equal then its termed as neutral PH.

In our investigation we find out the PH value of concrete by using the litmus paper. Once the concrete was completely mixed the litmus paper was touched to the concrete, the

color of the litmus paper changed to darkish green with reading between 7-8.Therefore it gives the basis that bacterial solution will survive and further concreting work proceeded.

Fig 9: Litmus paper indicating the pH value of Concrete.

4.3 Methods of mixing bacterial solution into concrete-

There are different methods of mixing the bacterial solution in the concrete which are viz.

(a) Direct Mixing

(b) Indirect Mixing

(c) Injection method

In our investigation we have adopted the direct method in which, firstly the measuring jars were sterilized in oven for a temperature of about $100^0 C$for 5 min. After 5 min once it gets slightly cooled, the bacterial solution is poured form the flask in the measuring jar. The flask is firstly heated under the candle before pouring it into the jar, so that the bacterium doesn't get contaminated by the other bacteria's present in the environment.

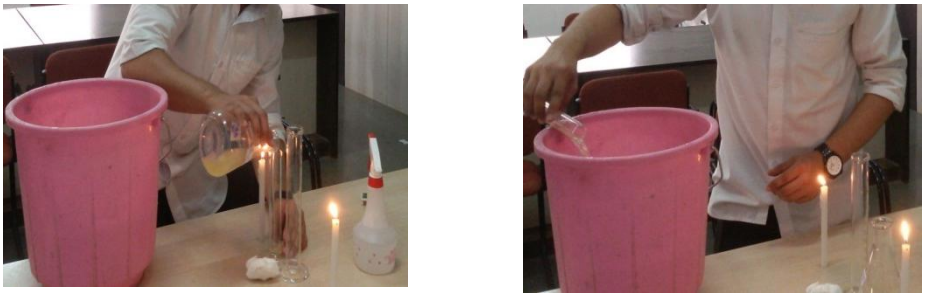

Fig 10: Method Adopted before mixing the Bacterial solution in the Mixing water.

Once the bacterial solution is mixed in the water, the water is properly stirred and then it is used for immersion in the concrete.

4.4 Casting of cubes and curing of different specimens of bacterial concrete-

Once the concrete is completely mixed the concrete is poured in the cube, compaction is been done by the vibration machine. Concrete cubes were removed from the Moulds after 24 hrs. And they were put into the curing tank. Curing was done for 7, 14 and 28 days for all samples viz. Conventional, 15 ml, 30 ml, 45 ml, 60 ml and 75 ml.

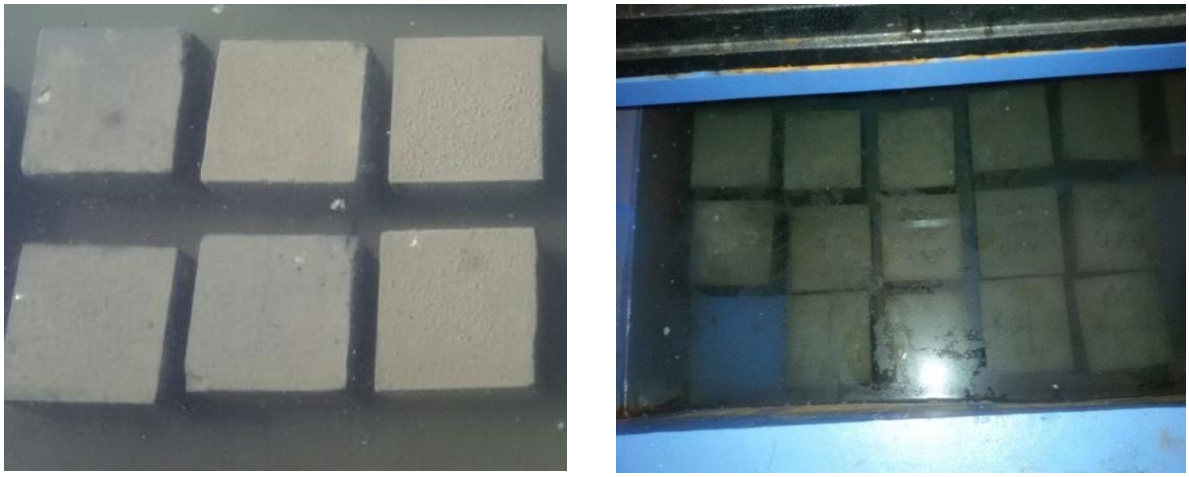

Fig 11: Curing Tank of concrete cube.

4.5 Experimental test on bacterial concrete-

Various test are performed on bacterial concrete in order to get the results in various forms these experimental methods are summarized below-

4.5.1. Slump cone test-

The **concrete slump test** is an empirical test that measures workability of fresh concrete. The slump cone test indicates the behavior of a compacted concrete cone under the action of gravitational forces. The test is carried out with a Moulds called as slump cone. The slump cone is placed on a horizontal and a non-absorbent surface and filled in three layers of fresh concrete, each layer being tamped 25 times with a standard tamping rod. The

test is suitable only for concretes of medium to high workability's (i.e. having slump values of 25mm to 125mm).

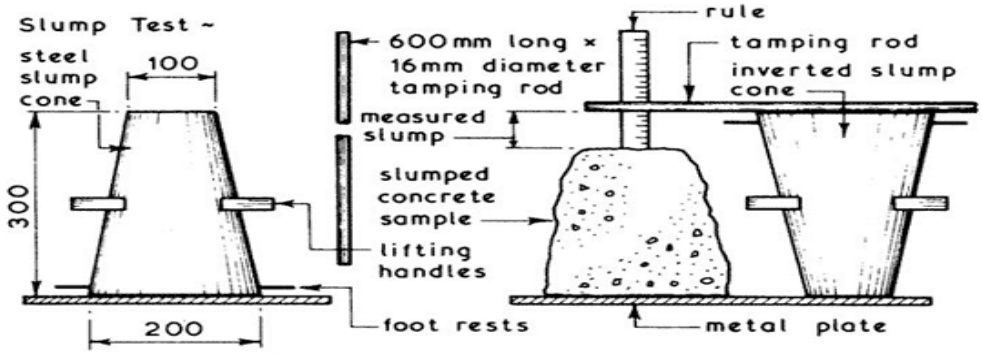

Fig 12: Showing the Slump Height.

The slump cone test results are used to observe the behavior of the concrete as shown in the below fig.11

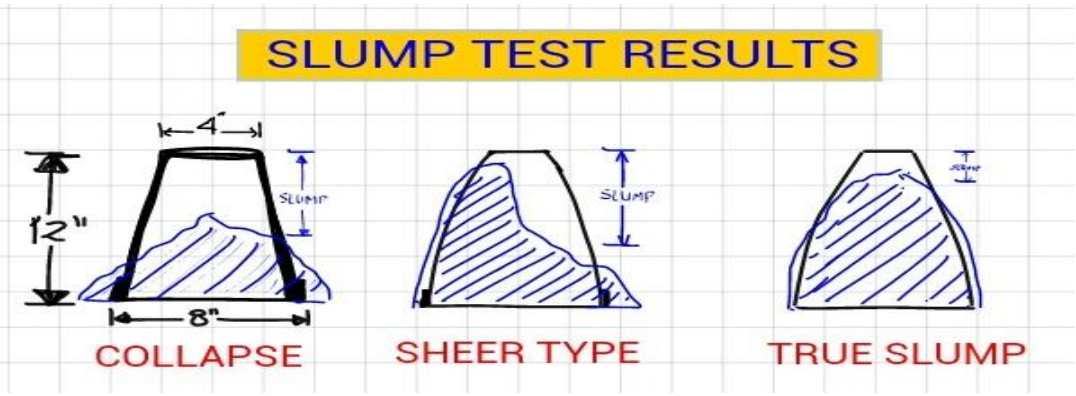

Fig 13: Slump Test Result.

The metal plate i.e. base is placed on a smooth surface and the container is filled with bacterial concrete in three layers, whose workability is to be tested. Each layer is temped 25 times with a standard 16 mm (5/8 in) diameter steel rod, rounded at the end. When the mould is completely filled with bacterial concrete, the top surface is struck off (leveled with Moulds top opening) by means of screening and rolling motion of the temping rod. The Moulds firmly held against its base during the entire operation so that it could not move due to the pouring of concrete by means of handles or foot - rests. Immediately after filling is completed and the concrete is leveled, the cone is slowly and carefully lifted vertically, an unsupported bacterial concrete will now slump. The slump is measured by placing the cone

just besides the slump concrete and the temping rod is placed over the cone so that it should also come over the area of slumped concrete. The decrease in height of concrete to that of Moulds is noted with scale which is found to be 110mm for conventional concrete and 50mm for bacterial concrete. Figure shows the performance of slump cone test.

Fig 14: Slump Cone Test

4.5.2. Compressive strength test-

The concrete cubes were removed from the tank after their respective days of curing. The cubes were allowed to dry under the Laboratory condition. Once the cube were completely dried, placed under the compressive testing machine with an intention to get the compressive strength of concrete. The entire sample specimen tested under compressive testing machine.

Fig 15: CTM Machine.

After removing the specimen from water over specified curing time and wiped out excess water from the surface. Cleaning out the bearing surface of the testing machine. The various sample specimens were placed one after another in the machine in such a manner that the load shall be applied to the opposite sides of the cube cast. The specimen centrally aligned on the base plate of the machine. The load gradually applied without shock and continuously at the rate of 5.2 KN/sec till the specimen fails. The maximum load recorded and any unusual features in the type of failure noted down. Concrete cubes placed in the CTM machine before crushing and after crushing shown in fig. 16 below. Readings of each bacterial concrete sample viz. conventional, 15ml, 30ml, 45ml, 60ml and 75ml were taken each time after curing interval of 7days, 14 days and 28 days

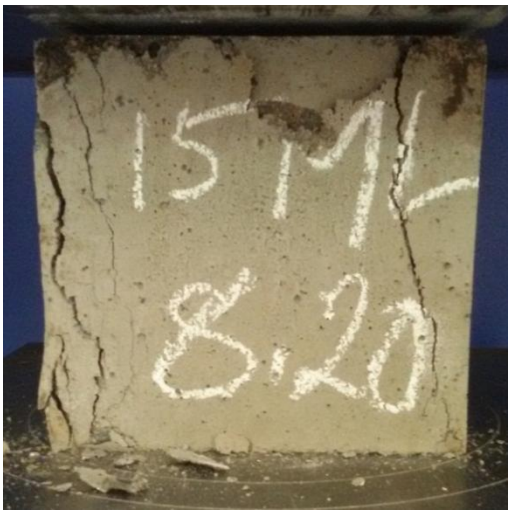

Fig 16: Before and After Crushing of Cubes.

4.5.3. Ultra sonic pulse velocity-

This method consists of producing an ultrasonic longitudinal pulse by an electro acoustical transducer which is held in contact with one surface of the freshly placed concrete member under test. After traversing a known distance in the concrete, the pulse to be measured from which the pulse velocity timing circuit enables the transit time of the pulse to be measured from which the pulse velocity is calculated. This procedure is called the "**Ultrasonic method**." Ultrasonic pulse velocity test is generally carried out to determine the presence of voids in the internal structure of concrete by means of passing the ultrasonic rays through the body on concrete and also to know the denseness of the concrete structure. All the respective bacterial concrete samples viz. conventional, 15ml, 30ml, 45ml, 60ml and 75ml were tested at Core Engineers and Consultancy Services Kharghar shown in fig.17 below. The corresponding readings were obtained in the form of trouble time and velocity.

Fig 17: Test of bacterial concrete samples using Ultrasonic Pulse Velocity Machine.

4.5.4. Plate count test-

The plate count test was conducted to determine total viable cells in a bacterial culture by plate count method.This method is used for determination of the number of cells that multiply under define conditions. It requires culture viz. Liquid culture of bacillus subtilis, water, and milk. Further the media taken is 20ml nutrient agar deep tubes (3 in nos.), also the apparatus used were test tubes, pipettes, petri plates , glass marking pencil and spreader.The plate count method is most commonly used for enumeration of viable cells in water, milk, food, and many other pharmaceutical substances.. A major amount of bacterial suspension is introduced into an agar medium (liquid form at 45 degree Celsius) and after mixing, added into the patri plate. All organisms grow, reproducing a visible mass of microorganism called colony. After testing the bacterial concrete cubes in CTM machine, small part of all the samples were tested result shows the formation of visible mass as shown in fig.

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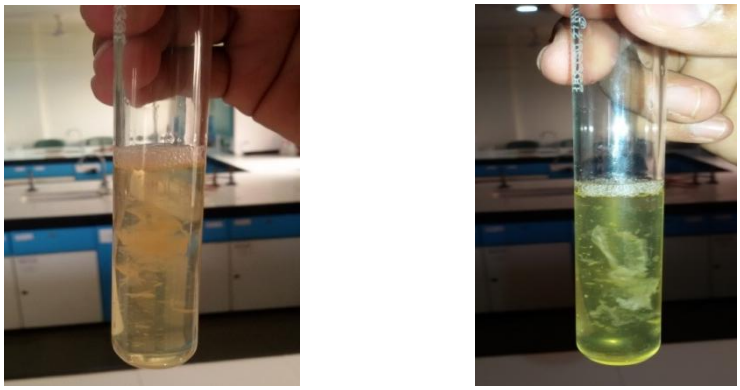


Fig. formation of visible mass

The development of one colony from one microorganism can occur when the bacterial suspension is homogenous. If microorganism have a tendency to aggregate (e.g- Staphylococci, streptococci, diplococcic) that resulting counts will be lower than the actual no. of individual cells. Hence, counts of microorganism are often reported as colony forming units/ml rather than no. of bacteria/ml. the original sample is usually diluted so that the no of colonies developing on the plate will be in the range of 30-300. Within this range the count can be accurate and the possibility of mixing of the growth of one organism with other is minimized. The total count of microbial suspension is obtained by multiplying the no. of cells per plate by the dilution factor. 1 g of concrete material from concrete block which was kept for curing for 14 days from different concrete block (containing 15, 30, 45, 60 and 75 ml of bacterial suspension) collected to study number of viable bacteria by serial dilution method.

4.5.4.1 Experimental procedure to obtain plate count test of bacterial solution

First mixing of 24hr. Incubated 1 g concrete material from each block was done by rolling the test tube between the palms to ensure even dispersion of cell in the culture. By using sterile pipette, aseptically transfer of 0.1ml bacterial suspension to the test tube containing 10 ml waterfall injection was done. The test tubes were labeled as tube A, tube B and tube C. The content of test tube A mixed and transfer with a sterile pipette into the test tube B containing 10 ml waterfall injection. Again mixing of content of test tube B and transfer 0.1ml to the test tube C containing 10 ml waterfall injection with a sterile pipette was made. Further additions of approx. 10 ml nutrient agar medium into these three separate test tubes labeled as A, B, C and sterilize by autoclave at 121 degree Celsius for 15min and cooled to 45 degree Celsius. After which mixing of all dilutions and transfer 0.1ml from each dilution to test tube containing sterile nutrient agar i.e. from test tube A to A, Test tube B to B and test tube C to C. by rolling the test tube between the palms to ensure even dispersion of culture medium for mixing bacterial suspension thereby immediately pouring the media containing three test tube (A, B and C) into three sterilize petri plate labeled as A, B and C as shown in fig. , allow the plates to solidify. Incubating the plates in an inverted position for 24 hours at 37 degree Celsius in an incubator. Numbers of viable bacteria are proportional to the number of bacterial colonies.Numbers of bacterial colonies are counted by using colony counter.

Advantages

1. Only viable cells are counted
2. It is easy to perform and this technique is routinely used for the estimation of bacterial populations in milk, water, food, and many other pharmaceutical substances.
3. It is a very sensitive method. Hence, very small no. of microorganisms can be counted.
4. It allows isolation of discrete colonies that can be sub cultured in pure cultures, easily studied and identified.

4.5.5 Scanning Electron Microscope (SEM)-

The Morphology and mineralogical composition of the deposited calcium carbonate crystals were investigated using scanning electron microscope (SEM). SEM micrographs were obtained using a jeol JSM 5600 LV model Philips XL 30 attached with EDX unit, with accelerating voltage 30K.V., magnification 10x upto 400000x and resolution for W.(3.5 nm). Samples surface were first coated with carbon then with gold.

Fig 18: Scanning Electron Microscope Machine

Chapter 5

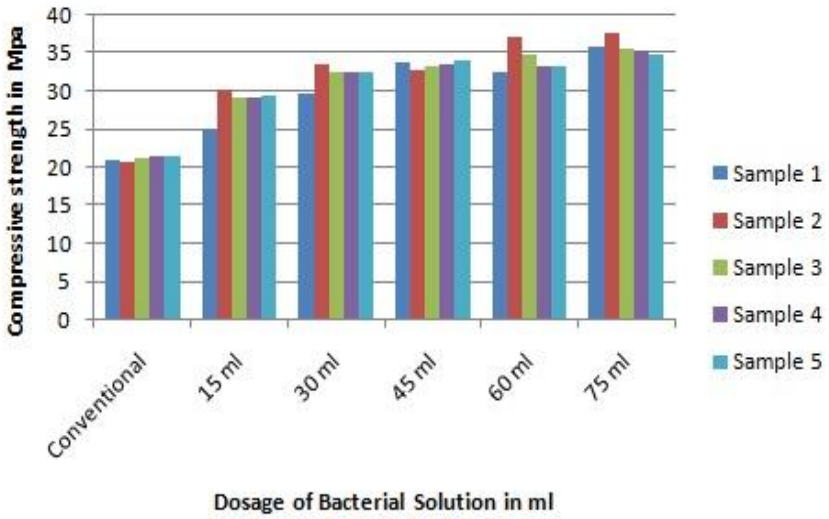
Experimental Results and Discussion

Various tests were conducted to know the characteristics of the concrete cube. The test was conducted to investigate the optimum dosage of the bacterial solution under which the cube attains its maximum strength.

5.1 Compressive Strength:-

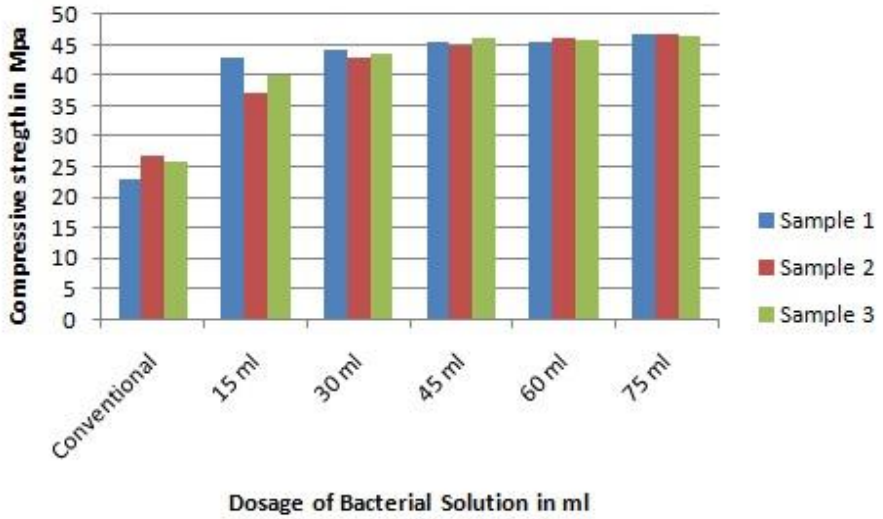
Compressive strength of concrete cube was carried out after curing period of 7, 14 and 28 days. The results so obtained are tabulated below with their respective graph.

Type Of concrete	**Compressive strength of concrete after 7 days**				
	Sample 1	**Sample 2**	**Sample 3**	**Sample 4**	**Sample 5**
Conventional	20.8	20.7	21.1	21.4	21.5
15 ml	25	30.2	29.1	29.2	29.4
30 ml	29.7	33.6	32.4	32.5	32.6
45 ml	33.8	32.74	33.27	33.54	34.1
60 ml	32.5	37.2	34.85	33.3	33.2
75 ml	35.8	37.6	35.7	35.3	34.9



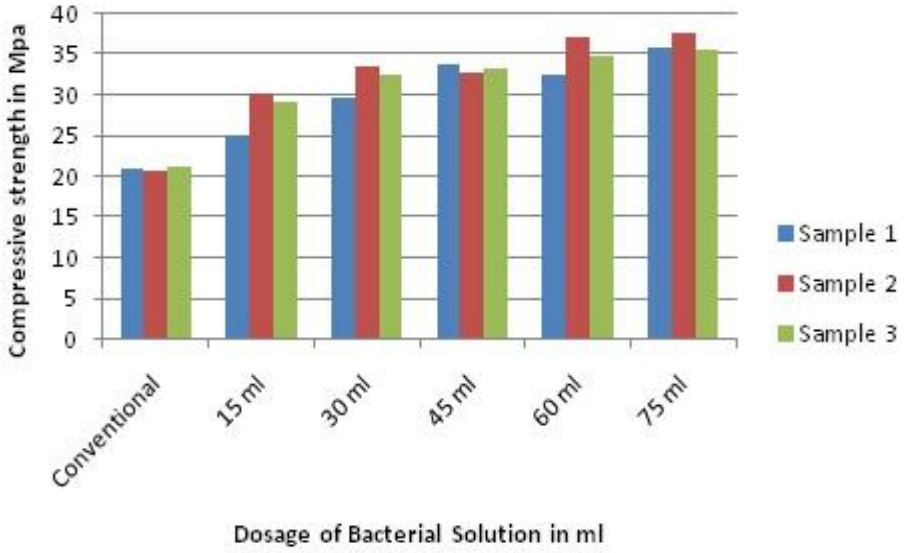
Type of concrete	Compressive strength of Concrete after 14 Days		
	Sample 1	**Sample 2**	**Sample 3**
Conventional	22.8	26.7	25.83
15 ml	43.0	37.1	40.05
30 ml	44.2	42.9	43.55
45 ml	45.4	44.8	46.1
60 ml	45.6	46.2	45.7
75 ml	46.9	46.7	46.5

Table No 2: Compressive strength for 14 days



Type of concrete	Compressive strength of Concrete after 28 Days		
	Sample 1	Sample 2	Sample 3
Conventional	31.45	33.9	36.81
15 ml	42.6	48.3	46.9
30 ml	54.5	51.9	53.2
45 ml	51.6	55.4	53.7
60 ml	53.8	54.1	53.95
75 ml	49.7	52.9	51.0

Table No 3: Compressive strength for 28 Days



5.2 Ultrasonic Pulse Velocity:-

Ultra sonic pulse velocity test was carried out to know the presence of voids in the internal structure of the concrete cubes. The results so obtained after conducting the test are tabulated below in table no.4. This results shows that of all the samples tested the trouble time of 30ml and 45ml bacterial concrete found to be much lesser, again velocity is also higher.

Table No 4: Ultrasonic Pulse Velocity Reading

SR No.	Property of Concrete	RCC Member	Prob. Distance mm	Time Micro sec	Velocity Km/sec	Probing Method
1	**Conventional concrete**	Cube	150	29.3	5.12	Direct
2	**Bacterial concrete**					
	15 ml	Cube	150	29.8	5.03	Direct
	30 ml	Cube	150	28.3	5.30	Direct
	45 ml	Cube	150	29	5.17	Direct
	60 ml	Cube	150	30.2	4.97	Direct
	75 ml	Cube	150	29.2	5.14	Direct

5.3 Plate Count Method:-

Table No 5: Plate Count Test Result.

Sr. No.	ml of bacterial suspension	Number of viable bacteria
1.	15	68×10^3
2.	30	77×10^3
3.	45	89×10^3
4.	60	48×10^3
5.	75	32×10^3

Chapter 6

Conclusion

Chapter 7

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