Effect of pH on Bacterial Concrete

Submitted in partial fulfillment of the requirements of the degree of
Bachelor of Engineering

by

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CERTIFICATE

This is to certify that the project entitled **"*Effect of pH on Bacterial Concrete*"** is a bonafide work of ***SIDDIQUI TANVEER AHMAD (13CE60), KHAN AYUB MOHD YAKUB (13CE22), SAYED SHARJEEL AHMED (13CE47), SHAIKH REHAN KALANDAR (13CE57)***. Submitted to the University of Mumbai in partial fulfillment of the requirement for the award of the degree of "Bachelor of Engineering" in Department of Civil Engineering.

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We declare that this written submission represents our 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 we have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my 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 and novel technique for 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 Sustainability of bacteria at various pH levels and its effect on the durability of concrete has not been comprehensively investigated. Therefore, it is decided to carry out an investigation of determining the optimum pH value required for cement mortar by forming various cement mortar cube samples having different pH value wiz. 6.25, 6.5, 6.75, 7.0, 7.25, 7.5, 7.75, 8.0, 8.25, 8.5, 8.75. Further these various samples were tested under various laboratory methods viz. Compressive strength testing machine, Moisture content, Acid test on bacterial residue, Rebound hammer test, Colony forming unit/plate test and Scanning electron microscopes thereby an optimum pH value 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 with pH value equals to 7.0 is the optimum value.

LIST OF CONTENT

LIST OF FIGURES

Chapter 1

Introduction

1.1 Background

Concrete which forms major components in the construction industries as it is cheap, easily available and convenient to cast. But drawback of these materials is it is weak in tension so, it fails under sustained loading and due to aggressive environmental agents which ultimately reduce the life of the structure which are built using these materials. Synthetic materials like epoxies are used for remediation but they are not compatible, costly, reduces aesthetic appearance and need constant maintenance. Therefore bacterial induced Calcium Carbonate (calcite) precipitation has been proposed as an alternative and environment friendly crack remediation and hence improvement of strength of building materials.

A novel technique is adopted in remediating cracks and fissures in calcium concrete by utilizing Microbiologically Induced Calcite Or Carbonate ($CaCO_3$) Precipitation (MICP). It is a technique that comes under a broader category of science called biomineralization. MICP is highly desirable because the Calcite precipitation induced as a result of microbial activities is pollution free and natural. The technique can be used to improve the compressive strength and stiffness of cracked concrete specimens. Often bacterial activities simply trigger a change in solution chemistry that leads to over saturation and mineral precipitation. Use of these Bio mineralogy concepts in concrete leads to potential invention of new material called —Bacterial Concrete. 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 and also, it also reduces water absorption and carbonation of concrete as an alternative surface treatment. As part of metabolism, some bacteria produces enzyme urease which catalyzes 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).

When the concrete is mixed with bacteria (bacillus subtilis), the bacteria go into a dormant state, a lot like seeds. All the bacteria need is exposure to the air to activate their functions. Any cracks that should occur provide the necessary exposure. When the cracks form, bacteria very close proximity to the crack, starts precipitating calcite crystals. When a concrete structure is damaged and water starts to seep through the cracks that appear in the concrete, the spores of the bacteria germinate on contact with the water and nutrients. Having been activated, the bacteria start to feed on the calcium lactate nutrient. Such spores have extremely thick cell walls that enable them to remain intact for up to 200 years while waiting for a better environment to germinate. As the bacteria feeds oxygen is consumed and the soluble calcium lactate is converted to insoluble limestone. The limestone solidifies on the cracked surface, thereby sealing it up. Oxygen is an essential element in the process of corrosion of steel and when the bacterial activity has consumed it all it increases the durability of steel reinforced concrete constructions. Tests all show that bacteria embedded concrete has lower water and chloride permeability and higher strength regain than the surface application of bacteria. The last, but certainly not least, key component of the self-healing concrete formula is the bacteria themselves. The most promising bacteria to use for self-healing purposes are alkaliphilic (alkali- resistant) spore-forming bacteria. The bacteria, from the genus Bacillus, subtilus is adopted for present study. It is of great concern to the construction industry whether or not these bacteria are "smart" enough to know when their task is complete because of safety concerns. Bacillus Subtilis which is a soil bacterium (isolated from JNTUH soil) is harmless to humans as it is non-pathogenic microorganism.

Chemistry of the Process Microorganisms (cell surface charge is negative) draw cations including $Ca2+$ from the environment to deposit on the cell surface. The following equations summarize the role of bacterial cell as a nucleation

Ca^{2+} + Cell --------> Cell- Ca^{2+}……………….(i)

Cell- Ca^{2+} +CO_3^{2-}-----> Cell-$CaCO_3$…………(ii)

The bacteria can thus act as a nucleation site which facilitates in the precipitation of calcite which can eventually plug the pores and cracks in the concrete. This micro biologically induced calcium carbonate precipitation (MICCP) comprises of a series of complex biochemical reactions. As part of metabolism, B.Subtilis produces urease, which catalyzes urea to produce CO_2 and ammonia, resulting in an increase of pH in the surroundings where ions Ca^{2+} and CO_3^{2-} precipitate as $CaCO_3$. These create calcium carbonate crystals that further expand and grow as the bacteria devour the calcium lactate food. The crystals expand until the entire gap is filled. In any place where standard concrete is currently being used, there is potential for the use of bacterial self-healing concrete instead. The advantage of having self- healing properties is that the perpetual and expected cracking that occurs in every concrete structure due to its brittle nature can be controlled, reduced, and repaired without a human work crew. Bacterial self-healing concrete also prevents the exposure of the internal reinforcements. This form of self-healing concrete was created to continuously heal any damage done on or in the concrete structure. It was made to extend the life span of a concrete structure of any size, shape, or project and to add extra protection to the steel reinforcements from the elements.

1.2 Aim of the Project Work

The aim of this project is:

- To develop a bacterial concrete by introducing the bacteria's of bacillus family (Bacillus subtilis).
- To determine the effect of PH on bacterial concrete.
- To know the presence of voids in the internal structure of concrete by SEM.
- To observe the behavior of bacteria chemically.

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.

Chapter 2

Review of Literature

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

Permeability Study of Cracked Concrete.

Cracks in concrete generally interconnect flow paths and increase concrete permeability. The increase in concrete permeability due to the progression of cracks allows more water or aggressive chemical ions to penetrate into the concrete, facilitating deterioration. The present work studies the relationship between crack characteristics and concrete permeability. In this study **By Kejin Wang, Daniel C. Jansen, Surendra P. Shah, Alan F. Karr (1997).** Feedback controlled splitting tests were introduced to generate crack width-controlled concrete specimens. Sequential crack patterns with different crack widths were viewed under a microscope. The permeability of cracked concrete was evaluated by water permeability tests. The preliminary results indicated that crack openings generally accelerate water flow rate in concrete. When a specimen was loaded to have a crack opening displacement smaller than 50 microns prior to unloading, the crack opening had little effect on concrete permeability. When the crack opening displacement increased from 50 microns to about 200 microns, concrete permeability also increased rapidly. After the crack opening displacement reached 200 microns, the rate of water permeability increased steadily.

Microbiological Precipitation of CaCO3.

The process of microbial mineral plugging in porous media is common in nature. We examined physical and biochemical properties of CaCO3 precipitation induced by Bacillus pasteurii, an alkalophilic soil microorganism. X-ray diffraction analysis quantified the composition of the mineral deposited in sand and identified the CaCO3 crystal as calcite. Examination by scanning electron microscopy identified bacteria in the middle of calcite crystals, which acted as nucleation sites. The rate of microbiological CaCO3 precipitation correlated with cell growth and was significantly faster than that of chemical precipitation. Biochemical properties of urease (urea amidohydrolase, E.C. 3.5.1.5) from B. pasteurii that was indirectly involved in CaCO3 precipitation were examined **By Shannon Stocks-Fischer, Johnna K. Galinat, Sookie S. Bang (1999)** to understand the kinetics of the microbiological process. Urease from B. pasteurii exhibited a relatively low affinity for urea at pH 7.0 with a Km of 41.6 mM and Vmax of 3.55 mM min−1 mg−1 protein and increased affinity at pH 7.7 with a Km of 26.2 mM and Vmax of 1.72 mM min−1 mg−1 protein. Results of kinetic studies indicate that urease activity and its affinity to urea are significantly high at the pH where calcite precipitation is favorable. The findings further suggest a potential use of the microbial calcite precipitation process in remediation of the surface and subsurface of porous media.

Calcite precipitation induced by polyurethane-immobilized Bacillus pasteurii.

Polyurethane (PU) foam was used to immobilize the whole cell of bacillus pasteurii. The immobilized cells exhibited the rates of calcite precipitation and ammonia production as high as those of the free cells. Scanning electron micrographs identified the cells embedded in calcite crystals throughout PU matrices. Calcite in PU showed little effect on the elastic modulus and tensile strength of the polymer, but increased the compressive strengths of concrete cubes, whose cracks were remediated with PU-immobilized cells. These observations **By S.S Bang, Galinat, J,K., Ramakrishnan (2001)** led us to believe that the calcite may remain as a form of precipitation, not as a bonding material within the matrices.

Remediation of concrete using micro-organisms.

This paper **By S.K. Ramachandra,Venkataswamy Ramakrishnan, S.S. Bang(2001)** describes an innovative biotechnology utilizing microbiologically-induced mineral precipitation for concrete remediation. Calcite precipitation induced by Bacillus pasteurii was studied in two types of portland cement mortar specimens: one prepared from mixing with micro-organisms, and the other with simulated cracks filled with microbial mixtures. The study showed that there was a significant increase in compressive strength of the portland cement mortar cubes containing lower concentrations of live cells. Compressive strengths of the cubes containing live or dead cell mass, however, decreased as cell concentrations and curing time increased, suggesting the interference of mortar integrity by biomass. Cracks filled with bacteria and sand demonstrated a significant increase in compressive strength and stiffness values when compared with those without cells. Scanning electron micro-graphs identified that microbiological calcite precipitation occurred mainly close to the surface areas of the crack, where a dense growth of calcite crystals embedded with cells was observed.

Improvement of concrete durability by bacterial mineral precipitation.

A novel technique in remediating cracks and fissures in concrete by utilizing microbiologically induced calcite ($CaCo3$) precipitation was discussed. Microbiologically induced calcite precipitation (MICP) is a technique that comes under a broader category of science called biomineralization. It is a process by which living organisms form inorganic solids. Bacillus Pasteruii, a common soil bacterium can induce the precipitation of calcite. As a microbial sealant, $CaCO_3$ exhibited its positive potential in selectively consolidating simulated fractures and surface fissures in granites and in the consolidation of sand. MICP is highly desirable because the calcite precipitation induced as a result of microbial activities, is pollution free and natural. The technique can be used to improve the compressive strength and stiffness of cracked concrete specimens. A durability study on concrete beams treated with bacteria, exposed to alkaline, sulfate and freeze-thaw environments were also studied **By Venkataswamy Ramakrishnan, Ramesh K. Panchalan, Sookie S. Bang (2001).** The effect of different concentrations of bacteria on the durability of concrete was also studied. It was found that all the beams with bacteria performed better than the control beams (without bacteria). The durability performance increased with increase in the concentration of bacteria. Microbial calcite precipitation was quantified by X-ray diffraction (XRD) analysis and visualized by SEM. The unique imaging and microanalysis capabilities of SEM established the presence of calcite precipitation inside cracks, bacterial impressions and a new calcite layer on the surface of concrete. This calcite layer improves the impermeability of the specimen, thus increasing its resistance to alkaline, sulfate and freeze-thaw attack.

Conservation of Ornamental Stone by Myxococcus xanthus-Induced Carbonate Biomineralization.

Increasing environmental pollution in urban areas has been endangering the survival of carbonate stones in monuments and statuary for many decades. Numerous conservation treatments have been applied for the protection and consolidation of these works of art. Most of them, however, either release dangerous gases during curing or show very little efficacy. Bacterially induced carbonate mineralization has been proposed **By Carlos Rodriguez-Navarro, Manuel Rodriguez-Gallego, Koutar Ben Chekroun, Maria Teresa Gonzalez-Muñoz (2003)** as a novel and environmentally friendly strategy for the conservation of deteriorated ornamental stone. However, the method appeared to display insufficient consolidation and plugging of pores. Here we report that Myxococcus xanthus-induced calcium carbonate precipitation efficiently protects and consolidates porous ornamental limestone. The newly formed carbonate cements calcite grains by depositing on the walls of the pores without plugging them. Sonication tests demonstrate that these new carbonate crystals are strongly attached to the substratum, mostly due to epitaxial growth on pre-existing calcite grains. The new crystals are more stress resistant than the calcite grains of the original stone because they are organic-inorganic composites. Variations in the phosphate concentrations of the culture medium lead to changes in local pH and bacterial productivity. These affect the structure of the new cement and the type of precipitated $CaCO_3$ polymorph. The manipulation of culture medium composition creates new ways of controlling bacterial biomineralization that in the future could be applied to the conservation of ornamental stone.

Bio-deposition of a calcium carbonate layer on degraded limestone by Bacillus species.

To obtain a restoring and protective calcite layer on degraded limestone, five different strains of the bacillus sphaericus group and one strain of bacillus lentus were tested for their ureolytic driven calcium carbonate precipitation. Although all the bacillus strains were capable of depositing calcium carbonate, differences occurred in the amount of precipitated calcium carbonate on agar plate colonies. Seven parameters involved in the process were examined **By Jan Dick, Wim De Windt, Bernard De Graef, Willy Verstraete (2006)** ie calcite deposition on limestone cubes, pH increase, urea degrading capacity, extracellular polymeric substances (EPS)-production, biofilm formation, zeta-potential and deposition of dense crystal layers. The strain selection for optimal deposition of a dense CaCO(3) layer on limestone, was based on decrease in water absorption rate by treated limestone. Not all of the bacterial strains were effective in the restoration of deteriorated Euville limestone. The best calcite precipitating strains were characterised by high ureolytic efficiency, homogeneous calcite deposition on limestone cubes and a very negative zeta-potential.

Life cycle, sustainability and the transcendent quality of building materials.

This paper explores the relationship between the life cycle of engineering works and their sustainable and transcendent qualities, and considers the possibility of creating durable works with ephemeral materials. This paper **By Eduardo peris mora (2007)** also studies the impact of urban growth and its infrastructures on the environment through the consumption of raw materials and energy. City metabolism is one of the main causes of environmental deterioration, and present-day tendencies make it foreseeable that both urban and infrastructure development shall continue to increase. Although the expression sustainable construction is being used more and more, it is necessary to distinguish between the sustainability of the construction activity and the sustainability of works constructed. Both the materials and technologies used since ancient times have allowed many past works to have lasted thousands of years. Some were made out of permanent materials such as stone while others were made out of more ephemeral materials such as adobe bricks or cob walls. Structures built with Roman cement are still standing after 20 centuries. The overall durability of built structures depends on the durability of their materials.

Transcendent construction was made possible either using permanent materials or more ephemeral materials, providing the project had taken the need for maintenance into consideration. The development of building works in a modular fashion makes the repairing action of modifying materials or parts of works possible without destroying its basic structure. With our present-day knowledge, plain concrete permits to create transcendent structures that could last several centuries.

Use of bacteria to repair cracks in concrete.

Ureolytic bacteria such as Bacillus sphaericus were able to precipitate CaCO3 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 K. Van tittelboom (2010)** by means of water permeability tests, ultrasound transmission measurements and visual examination. Thermo gravimetric analysis showed that bacteria were able to precipitate CaCO3 crystals inside the cracks. It was seen that pure bacteria cultures were not able to repair the cracks. However, when bacteria were protected in silica gel, cracks were filled completely.

Micro-organism precipitation inenhancing concrete properties.

Microorganism is a unique living element and has the ability to precipitate minerals through the process of bio mineralization. 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. 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 **By H Afifudin et al(2011)** proves 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.

Use of silica gel or polyurethane immobilized bacteria for self-healing concrete.

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 etal. (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.

A two component bacteria-based 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 investigated **By H.M. Jonkers et al. (2013)** shows 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 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.

Concrete Repair, Rehabilitation & Retrofitting II.

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 conditions to 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).**

Use of bacterial cell wallsto improve the mechanical performance of concrete.

The role of bacterial cell walls of *Bacillus subtilis* as a concrete admixture to improve the mechanical performance of concrete. The study was carried out **By R.Pei et al. (2013)** states that 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.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.

Bacterial carbonate precipitation as an alternative surface treatment for concrete.

Surface treatments play an important role in the protection of construction materials from the ingress of water and other deleterious substances. Due to the negative side-effects of some of the conventional techniques, bacterial induced carbonate mineralization has been proposed as a novel and environmental friendly strategy for the protection of stone and mortar. This paper **By Willem De Muynck, Kathelijn Cox, Nele De Belie, Willy Verstraete (2013)** reports the effects of bacterial CaCO3 precipitation on parameters affecting the durability of concrete and mortar. Pure and mixed cultures of ureolytic bacteria were compared for their effectiveness in relation to conventional surface treatments. Bacterial deposition of a layer of calcite on the surface of the specimens resulted in a decrease of capillary water uptake and permeability towards gas. This bacterial treatment resulted in a limited change of the chromatic aspect of mortar and concrete surfaces. The type of bacterial culture and medium composition had a profound impact on CaCO3 crystal morphology. The use of pure cultures resulted in a more pronounced decrease in uptake of water, respectively less pronounced change in the chromatic aspect, compared to the use of mixed ureolytic cultures as a paste. The results obtained with cultures of the species Bacillus sphaericus were comparable to the ones obtained with conventional water repellants.

A sustainable self-healing construction material.

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 J.Y. Wang et al. (2014)** 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 thepossibility 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 bio engineered concrete using bacterial strain Bacillus subtilis JC3 and its enhanced mechanical and durability characteristics was briefly described in this paper.

Self-healing concrete byuse of microencapsulated bacterial spores.

Microcapsules were applied to encapsulate bacterial sporesfor 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.

Fortification of compressive strength in enterococcus microorganism incorporated microbial cement mortar.

Shortcomings of conventional treatments have drawn the attention to alternative techniques for the improvement of the compressive strength. This paper **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.

Influence of bacterial treated cement kiln dust on the properties of concrete.

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. Study carried out **By R. Siddique et al. (2014)** shows 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 treated10% CKD concrete increased calcium silicate hydrate and formation of non-expansiveettringite in pores dense the concrete structure resulted in increased compressive strength.

Comparative studies on strength characteristics of microbial cement mortars.

Microbially induced calcium carbonate precipitation (MICCP) is a novel method for the protection of cement-based materials. This paper produced **V. Senthilkumar et al. (2014)** deals with 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.

Performance of self-healing in reinforced mortar containing chemical admixture.

Performance of 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)** in which 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 self-healing agents.

Self-healing and repairing concrete cracks based on bio-mineralization.

In this paper published **ByC.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.

Concrete DurabilityImprovement in Sulfate Environment Using Bacteria.

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 a sulfate environment using bacteria.

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.

Crack healing in concrete using various bio influenced self-healing techniques.

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 ofBacteria, *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 Performance of PVA-Coated Granules Made of Cement, CSA, and Na2CO3 in the Cement Matrix.

Various self-healing methods for concrete, such as the use of supplementary cementitious materials, adhesive agents, mineral admixtures, and bacteria, have been suggested to date, and each of these has merits and demerits. Among these, however, the use of cementitious materials may be appropriate due to their good healing efficiency, low cost, and compatibility with the cement matrix. In the paper published **By Yong-Soo Lee, Jae-Suk Ryou (2016)** states that granulation and coating methods were applied to a new cementitious composite material. The self-healing property of these materials was controlled by the polyvinyl alcohol (PVA) coating until cracks were created. Water dissolved the PVA coating after entering through the cracks, and reacted with the healing materials to generate healing products. The self-healing performance was evaluated at various elapsed times through the measurement of the crack widths, visual observation, and examination of the microscopic images. Simultaneously, a water permeability test was performed and the dynamic modulus of elasticity was measured to verify the recovery of the cracks. In addition, the healing products that had been formed in the cracks were analyzed via X-ray diffraction (XRD) and scanning electron microscopy (SEM).

2.1 Summary of literature review:

On the basis of the above literature study it was found that various works has been done in relation to comparison between conventional and bacterial concrete. From the above studies we get to know the effect of various environmental conditions on the bacterial concrete and also the optimum dosage of bacterial solution for the bacterial concrete. But no literature so far indicate the effect of different pH of Water on the bacterial concrete after the concrete has been cast, therefore it needs to be addressed.

Chapter 3

Experimental Program & Tests

3.1 Selection of Bacteria:-

The various types of Bacteria that can be used in the concrete are B. Subtilis, B. Pasteurii, B. Cohnii, B. Licheniformis etc.***Bacillus Subtilis***, known also as the **hay bacillus** or **grass bacillus**, is a Gram-positive, catalase-positive bacterium, found in soil and the gastrointestinal tract of ruminants and humans. We have selected ***Bacillus Subtilis*** since this bacteria produces Calcium Carbonate and due to ease of availability from pharmacy department of AIKTC for our project work. A member of the genus *Bacillus*, *B. subtilis* is rod-shaped, and can form a tough, protective endospore, 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 micro-photograph of strains of Bacillus Subtilis is shown in fig. 1 below.

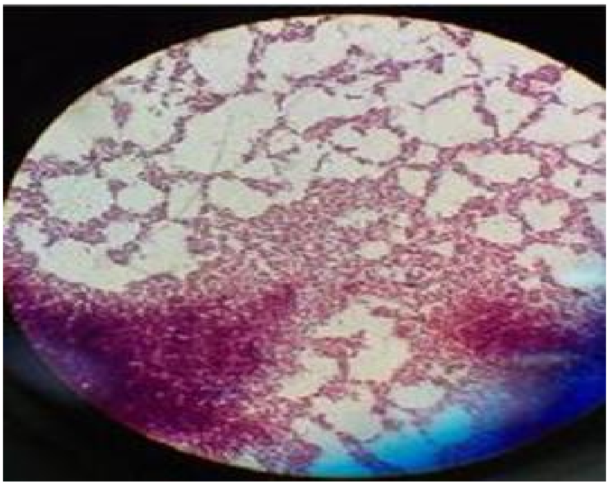

Fig 1: Microphotograph of strains of Bacillus Subtilis.

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. The cultivation of bacteria is shown in fig 2 below.

Fig 2: Bacterial solution.

3.2.1 Experimental Procedure for Cultural Growth of Bacteria:-

S. pasteurii PTCC 1645 and B. Subtilis 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 · 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 for the 72 h-old grown culture and the cells were washed twice in saline solution.

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 Materials to be used:

3.3.1 Cement:

Portland Pozzolana cement is the common type of cement in general usage. It is a basic ingredient of concrete, mortar and many plasters. Portland cement and similar materials are made by heating limestone (a source of calcium) with clay and/or shale (a source of silicon, aluminium and iron) and grinding this product(called *clinker*) with a source of sulfate (most commonly gypsum). In our investigation Portland pozzolana cement of 53 Grade available in local market is used. 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:

Sand is a naturally occurring granular material composed of finely divided rock and mineral particles. In our work we have 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. Cube Moulds:

The cube Moulds (70x70mm) 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 5: Cube Moulds.

Chapter 4

Experimental Methods & Test

4.1 Preparation of Cement Mortar Mix, Cubes and 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 M53 grade of concrete. The mix ratio obtained after the mix design as per IS 456: was M53(1:1.92:2.89). After 24 hours remove the cubes from the mould and immediately submerge in clean water till testing. Take out the cubes from water just before testing. Testing should be done on their sides without any packing. The rate of loading should be 500 kg/cm^2/minute and uniform. Test should be conducted for 5 cubes and report the average value as the test result for both 14 days and 28 days compressive strength. The preparation of cement mortar mix and labeling of moulds is shown in fig 6 & 7 below.

Fig 6: Mixing of cement mortar

Fig 7: Labeling of Cement Moulds

4.2 PH of Cement mortar mix-

pH is a critical factor in the chemistry of concrete. The values of pH 0 to less than 7 are termed as acidic and the values of pH 7 and above are termed as basic. Portland cement, the "binding" component in concrete, has a pH approaching 11, which is very alkaline. In our investigation, we prepared water samples of different pH ranging from 6.25 to 8.75 with an interval of 0.25. Thus we prepared a total of 11 different pH samples (500 ml each). We first checked the pH of water which was to be used in the process and it worked out to be 6.75. Thus, we used Hydrochloric acid for attaining the lower pH values and Potassium Hydroxide base to get the different higher pH values. We used the Digital pH Meter to obtain the desired pH values (as per intervals). We followed the trial and error method i.e. drop by drop addition of acid or base to get the desired pH values as there is no standard procedure available. Preparation of pH samples is shown in fig 8 below.

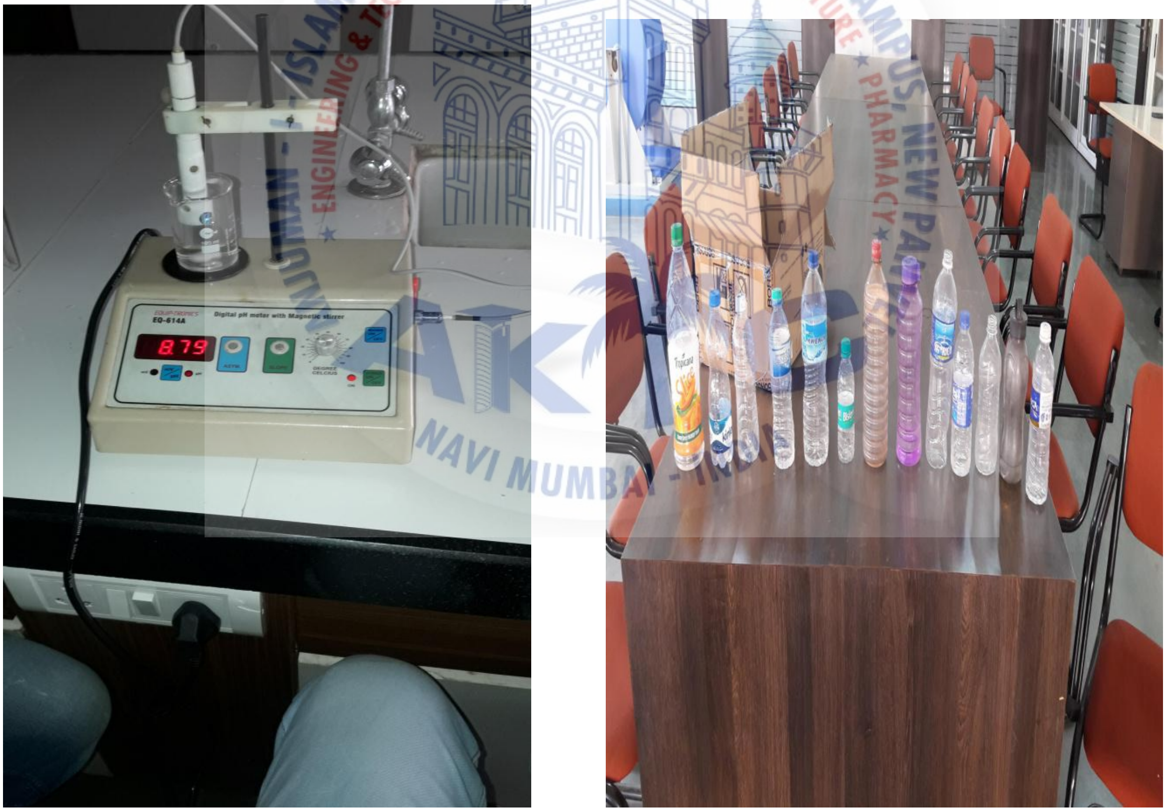

Fig 8: Different pH samples.

4.3 Methods of mixing bacterial solution into cement mortar-

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 we directly poured bacterial solution into 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 cement mortar-

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 14 and 28 days for all samples of different pH. The curing of cubes is shown in fig 9 below.

Fig 9: Curing of cube moulds.

4.5 Experimental Tests-

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

4.5.1 Compressive Strength Test:

Compressive strength is one of the most important engineering property of concrete which designers are concerned of. It is a standard industrial practice that the concrete is classified based on grades. This grade is nothing but the Compressive Strength of the concrete cube or cylinder. Cube or cylinder samples are usually tested under a compression testing machine to obtain the compressive strength of concrete. In our investigation cement mortar cubes were removed from the tank after respective days of curing and the cubes were allowed to dry. Once the cubes were completely dried, they were then placed under the compressive testing machine with an intention to get the compressive strength of each sample.

Fig 10: CTM Machine.

After removing the cubes from water after respective curing time the cement mortar cubes were subjected to loading under CTM 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 specimens were centrally aligned on the base plate of the machine. The load gradually applied without shock and continuously at the rate of 5.0 KN/sec till the specimen fails. The maximum load recorded and any unusual features in the type of failure were noted down. Cubes placed in the CTM machine before crushing and after crushing shown in fig. 11 below. Readings of each bacterial cement mortar made up of different pH sample were noted after curing interval of 14 and 28 days.

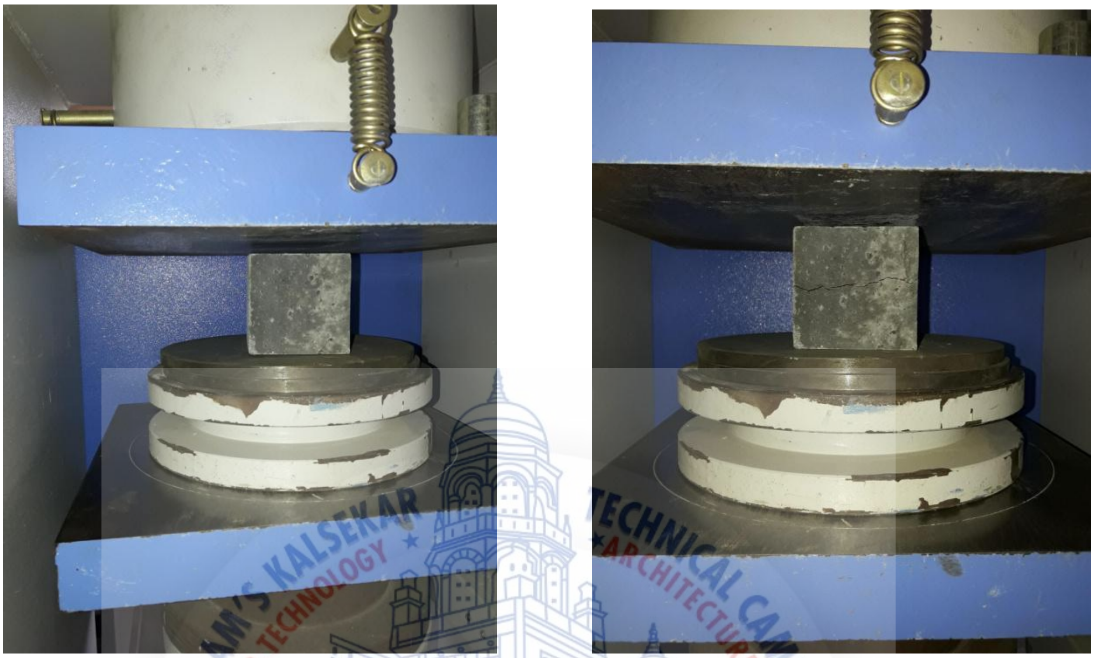

Fig 11: Before and After crushing of Cubes.

4,5,2. Scanning Electron Microscope Test:

SEM is especially useful in studies of deleterious processes such as sulfate attack, including the particular form of sulfate attack known as delayed ettringite formation (DEF), alkali-silica reaction (ASR), alkali carbonate reaction and any other situation where the micro-structural or microcompositional characteristics of the concrete need to be examined. Typically, a large number of analyses is carried out of the paste in a polished section of concrete or mortar. The data is represented graphically in the form of atomic ratios of selected elements to show the compositions of the phases present; these plots can show subtle changes in composition and can be used in many ways 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 up to 400000x and resolution for W.(3.5 nm). Samples surface were first coated with carbon then with gold.

. **Fig 12: Scanning Electron Microscope Machine**

4.5.3. Colony Forming Unit/ Plate Count Test-

The purpose of plate counting is to estimate the number of cells present based on their ability to give rise to colonies under specific conditions used, and the d of nutrient medium, temperature and time. Theoretically, one viable cell can give rise to a colony through replication. However, solitary cells are the exception in nature, and most likely the progenitor of the colony was a mass of cells deposited together. In addition, many bacteria grow in chains or clumps. Estimation of microbial numbers by CFU will, in most cases, under-count the number of living cells present in a sample for these reasons. This is because the counting of CFU assumes that every colony is separate and founded by a single viable microbial cell.

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. 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.

4.5.4. Determination of Moisture Content-

Water content or moisture content is the quantity of water contained in a material, such as soil, ceramic, and wood etc. Water content is used in a wide range of scientific and technical areas, and is expressed as a ratio, which can range from 0 to the value of the materials' porosity at saturation. It can be given on a volumetric or mass basis. In our investigation the moisture content of cement mortar samples of different pH were calculated. Firstly the cubes were removed from curing tank were being weighed. The cubes were then allowed to dry and weighed again thus giving us the dry weight of cement mortar cubes. The moisture content of different samples was then determined by following formula:

Moisture content: ((wet weight - dry weight)/(wet weight))*100.

Fig 13: Moisture Content Machine

4.5.5. Acid test on bacterial residue-

Acid test means placing a drop of dilute 10% hydrochloric acid on a rock or mineral and watching for bubbles of carbon dioxide gas to be released. The bubbles signal the presence of carbonate minerals such as calcite. The bubbling release of carbon dioxide gas can be so weak that you need a hard lens to observe single bubbles slowly growing in the drop of hydrochloric acid - or so vigorous that a flash of effervescence is produced. These variations in effervescence vigor are a result of the type of carbonate minerals present, the amount of carbonate present, the particle size of the carbonate, and the temperature of the acid. The acid test on bacterial residue is shown in fig 14 below.

Fig 14: Acid Test on Bacterial Residue

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4.5.6. Rebound Hammer Test-

Rebound hammer test is done to find out the compressive strength of concrete by using rebound hammer as per IS: 13311 (Part 2) – 1992. The rebound hammer is held at right angles to the surface of the concrete structure for taking the readings. The test thus can be conducted horizontally on vertical surface and vertically upwards or downwards on horizontal surfaces as shown in figure below. Procedure to determine strength of hardened concrete by rebound hammer.

- Before commencement of a test, the rebound hammer should be tested against the test anvil, to get reliable results, for which the manufacturer of the rebound hammer indicates the range of readings on the anvil suitable for different types of rebound hammer.
- We first applied light pressure on the plunger – till it got released from the locked position and allowed it to extend to the ready position for the test.
- Then we pressed the plunger against the surface of the concrete, keeping the instrument perpendicular to the test surface. Thus we kept applying a gradual increase in pressure until the hammer did impact.
- The readings obtained were thus noted down.

Fig 15: Rebound Hammer Test on Cement mortar cubes.

Chapter 5

Experimental Results and Discussion

Various tests were conducted to know the characteristics of the cement mortar cubes. The tests were conducted to observe the effect of pH on the cement mortar cubes and to obtain optimum value of the pH in which bacteria can sustain.

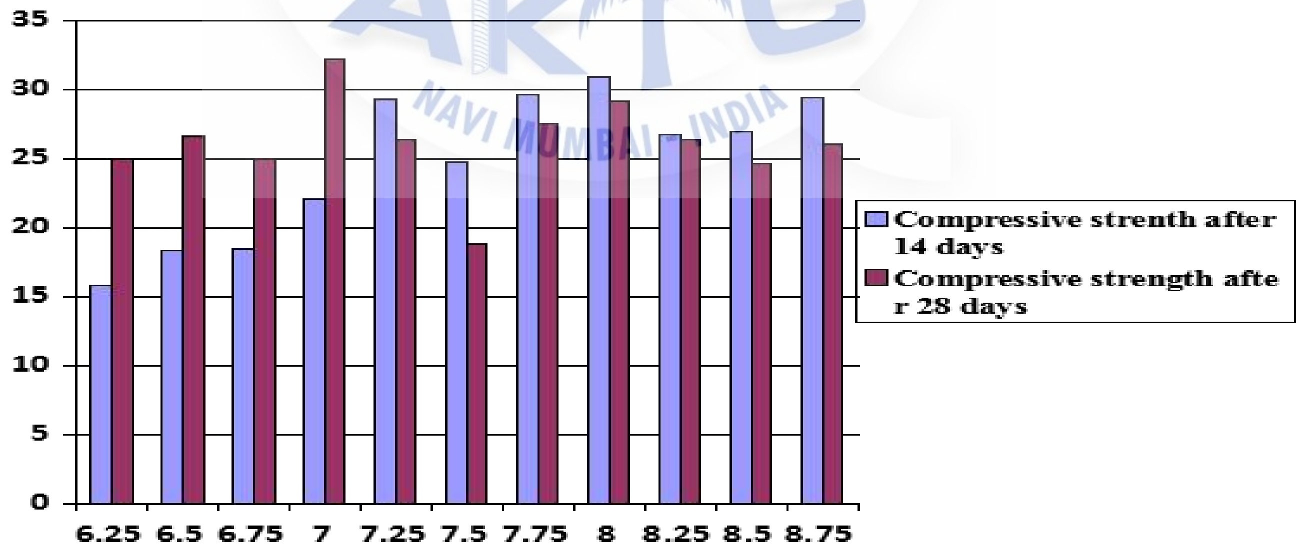
5.1 Compressive Strength:-

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 specimens were centrally aligned on the base plate of the machine. The load gradually applied without shock and continuously at the rate of 5.0 KN/sec till the specimen fails. The maximum load recorded and any unusual features in the type of failure were noted down.

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

pH of Cement Mortar cube	Avg. Compressive Strength after 28 days	Avg. Compressive Strength after 14 days
6.25	24.9	15.78
6.5	26.53	18.35
6.75	25	18.45
7.0	32.1	22
7.25	26.33	29.26
7.5	18.73	24.7
7.75	27.56	29.65
8.0	29.13	30.9
8.25	26.4	26.75
8.5	24.56	26.95
8.75	25.96	29.4

Table No: 1.



Graph of Compressive Strength Test Readings after 14 & 28 days

5.2 Rebound Hammer-

Rebound hammer test was done to find out the compressive strength of concrete by using rebound hammer. The following readings were obtained:

pH of Cement Mortar Cubes	Avg. Compressive strength after 28 days.
6.25	26
6.5	27.67
6.75	29.7
7.0	30.9
7.25	27
7.5	24.33
7.75	25
8.0	23.67
8.25	24.33
8.5	25.3
8.75	24.7

Table No: 2.

5.3 Moisture Content Test-

Since sand contain some porosity, water can be absorbed into the body of the particles or retained on the surface of the particle as a film of moisture. The following readings were obtained:

pH of Cement Mortar Cubes	Moisture Content
6.25	6.17
6.5	4.93
6.75	4.7
7.0	3.5
7.25	4.2
7.5	4.5
7.75	3.8
8.0	3.79
8.25	4.93
8.5	5.5
8.75	5.70

Table No: 3.

Chapter 6

Conclusion

Based on the above experimental investigations, the following conclusions are drawn:

- The addition of Bacillus subtilis bacteria increases the compressive strength of cement mortar cubes. The compressive strength is increased nearly 23% at 28 days for ordinary, standard and high grades of cement mortar cubes when compared to conventional cubes.
- The study showed that there was a significant increase in compressive strength of the Portland cement mortar cubes containing pH water within the range of 6.75 to 7.25.
- The 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 with pH value equals to 7.0. is the optimum value.
- From the above investigations, it can be concluded that Bacillus subtilis can be safely used in crack remediation of concrete structure. Bacillus subtilis which can be produced in the laboratory is proved to be a safe, non pathogenic and cost effective.

Chapter 7

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