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Review Paper

Bioengineered Concrete - A Sustainable Self-Healing Construction Material

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Abstract

It is a 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 are responsible for the transport of liquids and gasses that could potentially contain deleterious substances. When microcracks growth reaches the reinforcement, not only the concrete itself may be damaged, but also corrosion occurs in the reinforcement due to exposure to water and oxygen, and possibly CO_2 *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, i.e. a novel technique based on the application of biominerilization of bacteria in concrete. The applicability of specifically calcite mineral precipitating bacteria for concrete repair and plugging of pores and cracks in concrete has 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 are discussed. Synthetic polymers such as epoxy treatment etc are 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 extremophilic 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 will be briefly described in this paper.*

Keywords: Bacterial concrete, bacillus subtilis, self-healing, biominerlization.

Introduction

Bioengineered Self-repairing concrete biologically produces calcium carbonate crystals to seal cracks that appear on the surface of the concrete structures[1]. Specific spore forming alkaliphilic bacteria genus Bacillus, supplied with a calcium-based nutrient are incorporated in to the concrete suspended in mixing water. This bacteria based self healing agent is believed to remain hibernated within the concrete for up to 200 years[2]. When cracks appear in a concrete structure and water starts to seep in through, the spores of the bacteria starts microbial activities on contact with the water and oxygen. In the process of precipitating calcite crystals through nitrogen cycle the soluble nutrients are is converted to insoluble $CaCO_3$. The $CaCO_3$ solidifies on the cracked surface, thereby sealing it up. It mimics the process by which bone fractures in the human body are naturally healed by osteoblast cells that mineralize to reform the bone[3]. The consumption of oxygen during the metabolic biochemical reactions to form $CaCO_3$ helps in arresting corrosion of steel because the oxygen is responsible to initiate the process of corrosion thereby increasing the durability of steel reinforced concrete structures.

Using bacteria to self-heal cracks in concrete

Bacteria added to concrete mix in suspension state must meet certain criteria. Concrete is a highly alkaline building material , so bacteria used as self healing agent should be able to survive in this high alkaline environment for long durations and be able to form spores (highly resistant structures) withstanding mechanical forces during concrete mixing. In the concrete technology laboratory, a bacterial concrete mix is prepared using alkali-resistant soil bacteria Bacillus subtilis JC3 , along with nutrients from which the bacteria could potentially produce calcite based bio-minerals . 28 days cured bacterial specimens are examined visually by Scanning Electron Microscope to establish evidence for the precipitation of calcite crystals in concrete[4]. It was found that strains of the bacteria genus Bacillus were found to thrive in this high-alkaline environment. Such gram positive bacteria have extremely thick outer cell membrane that enables them to remain viable until a suitable environment is available to grow. They would become lively when the cracks form on concrete surface allowing water to ingress into the structure. This phenomenon will reduce the pH of the concrete environment where the bacteria incorporated become activated[5]. A peptone based nutrients supplied along with bacteria in suspension helps in producing calcite crystals. It is found that this biomineralisation process will not interfere with the setting time of the concrete. The most expensive ingredient in developing bacterial concrete is nutrients. So any inexpensive alternative for laboratory growth media would potentially bring down the cost of the bacteria based self-healing sustainable concrete. Only factor need to be checked is the effect of nutrients media on the setting time of cement.

Microbiologically Induced $CaCO_3$ Precipitation

Three main groups of microorganism that can induce the carbonate precipitation in nature: i. photosynthetic microorganism such as cyanobacteria and micro-algae that can remove CO_2; ii. sulphate reducing bacteria that are responsible for dissimilatory reduction of sulphates; and iii. some species of microorganism participate in nitrogen cycle by any one of the methods such as oxidative deamination of amino acids, nitrate reduction or hydrolysis of urea.

The evidence of microorganism involvement in calcium carbonate precipitation, has lead the development of bioprocess technology in the field of construction material. The involvement of microorganism in $CaCO_3$ precipitation can be described in three type of mechanism: i. spontaneous mechanism, usually by photosynthetic microorganism; ii. through nitrogen cycle; iii. through sulfur cycle.

Currently urease enzyme activity in most of microorganism metabolism process has been used as a tool to induce the precipitation of calcite crystals. Theoretically, calcium carbonate precipitation occur in nature following several process such as: i abiotic chemical precipitation from saturated solution due to evaporation, increase in temperature and/or decrease in pressure; ii. production of external and internal skeleton by eukaryotes; iii CO_2 pressure derivation under effect of autotrophic processes (photosynthesis, methanogenesis); iv fungal mediation; v heterotrophic bacterial mediation. Most of the mentioned processes above are mediated by microorganism. Both photosynthetic and heterotrophic microorganisms have natural ability to induce the precipitation of calcite crystals. The selected microorganism should meet the criteria such as: i. have a high urease enzyme activity; ii. ammonium and calcium ion tolerable; iii. not pathogenic[6].

Bacillus subtilis JC3

This strain isolated from soil has characteristics of high level urease activity, incessant precipitation of dense insoluble calcite crystals and has high negative zeta-potential. Potential applications of biological mineral precipitation are wide such as in sand consolidation and stabilization, remediation of cracks in concrete, preservation and restoration of historic heritage structures, areas where it is not possible to shut down the plant or hazardous for human beings to reach for repair work such as nuclear power plants, repair of waste water sewage pipes etc.

Mechanism of self-healing using bacteria

The microorganism used for manufacturing of microbial concrete should able to possess long-term effective crack sealing mechanism during its lifetime serviceability. The principle behind bacterial crack healing mechanism is that the bacteria should able to transform soluble organic nutrients into insoluble inorganic calcite crystals which seals the cracks. For effective crack healing, both bacteria and nutrients incorporated into concrete should not disturb the integrity of cement sand matrix and also should not negatively affect other important fresh and hardened properties of concrete. Only spore-forming gram positive strain bacteria can survive in high pH environment of concrete sustaining various stresses. It was reported that when bacteria is added directly to the concrete mix in suspension, their life-time is limited due to two reasons; one is continuing cement hydration resulting in reduction of cement sand matrix pore -diameter and other is due to insufficient nutrients to precipitate calcite crystals. However, a novel method of protecting the bacterial spores by immobilization before addition to the concrete mixture appeared to substantially prolong their life-time[7].

Working principle of Self-healing Process

In concrete the cracks up to 0.2 mm wide are healed autogenously. Such micro cracks are acceptable as these do not directly influence the safety and strength of the concrete. Research has shown that autogeneous healing happens due to hydration of non-reacted cement particles present in the concrete matrix when comes in contact with ingress water resulting in closure of micro cracks. However, because of the variability of autonomous crack healing of concrete micro cracks can still occur. The inbuilt bacteria-based self-healing process was found to heal cracks completely up to 0.5 mm width. On the surface of control concrete, calcium carbonate will be formed due to the reaction of CO_2 present with calcium hydroxide present in the concrete matrix according to the following reaction: $CO_2 + Ca(OH)_2 \rightarrow CaCO_3 + H_2O$

The calcium carbonate production in this case is due to the limited amount of CO_2 present. As $Ca(OH)_2$ is a soluble mineral gets dissolved in ingress water and diffuse out of the crack in the form of leaching.

The self healing process in bacteria incorporated concrete is much more efficient due to the active metabolic conversion of calcium nutrients by the bacteria present in concrete: $Ca(C_3H_5O_2)_2 + 7O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O$

This process does not only produce calcium carbonate directly due to microbial metabolic process but also indirectly due to autogeneous healing. This process results in efficient bio based crack sealing technique. Ureolytic bacteria such as Bacillus subtilis JC3 can able to precipitate $CaCO_3$ in the high alkaline environment by converting urea into ammonium and carbonate. The ammonia degradation of urea locally increases the pH and promotes the microbial deposition of carbonate as calcite crystals in a calcium rich environment along with maintaining the pH of concrete. These precipitated crystals can thus seal the cracks. The enhancement of strength and durability properties of concrete due to bacteria induction is studied in this research by conducting water permeability tests, ultrasound transmission measurements and visual examination.

The microbial precipitation of calcite crystals is determined by factors such as the concentration of dissolved inorganic carbon in the form of available nutrients, the pH of the environment, availability of calcium ions and the presence of nucleation sites. The first three factors are based on the metabolic process of the bacteria species added while the last factor is based on the cell membrane of the bacteria.

The bacteria used for study exhibits urease activity which catalyzes the hydrolysis of urea ($CO(NH_2)_2$) into ammonium (NH_4^+) and carbonate (CO_3^{2-}). First, urea is hydrolyzed to carbamate and ammonia. Carbamate then hydrolyses to form additionally ammonia and carbonic acid. These products subsequently form bicarbonate and ammonium and hydroxide ions. The ammonia is responsible for pH increase, which in turn shifts the bicarbonate equilibrium, resulting in the formation of calcium carbonate ions. Since the cell membrane of the bacteria is negatively charged, the bacteria draw cations from the environment, including Ca^{2+}, to deposit on their cell surface. The Ca^{2+} ions subsequently react with the CO_3^{2-} ions, leading to the precipitation of $CaCO_3$ at the cell surface that serves as a nucleation site.

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

Traditional crack repair systems

Repair of cracks in concrete structures usually involves applying a concrete mortar which is bonded to the damaged surface. Sometimes, the mortar needs to be keyed into the existing structure with metal pins to ensure that it does not fall away. Repairs can be particularly be time consuming and expensive because it is often very difficult to gain access to the structure to make repairs, especially if they are underground or at a great height. For crack repair, a variety of techniques is available but traditional repair systems have a number of disadvantageous aspects such as different thermal expansion coefficient compared to concrete and also have impact on environment and health. Therefore, bio based calcite precipitation has been proposed as an alternative and sustainable environmental friendly crack repair technique[8].

Viability of bacteria in concrete

Growth of bacteria in concrete is a most questionable factor because of concrete s high alkalinity which is a restricting aspect for the survival of the bacteria. Only specific alkaliphilic bacteria can survive in such hostile environment of concrete. Therefore, it is necessary to immobilize the bacterial cells and to protect them from the high pH in concrete[9]. Polyurethane (PU) has been widely for immobilization of nutrients and bacterial cells even silica gel was used to protect the bacteria against the high pH in concrete[10].

Calcium carbonate ($CaCO_3$)

Calcium carbonate ($CaCO_3$) is one of the most naturally precipitated minerals on earth in the form of natural rocks and exists in environments such as marine water, fresh water, and soils[11,12]. The variability of the concentration in the solubility of the calcium or carbonate in solution causes the natural precipitation of $CaCO_3$. Even Abiotic change or biotic action (microbial action) results in the natural precipitation of $CaCO_3$. It was found that $CaCO_3$ precipitation due to biotic process exceeds the abiotic in most environments on earth[13]. Stocks-Fischer et al.[14] have found that this microbial rate of precipitation was significantly faster than that of chemical precipitation. Hammes and Verstraete[15] suggested that the chemical $CaCO_3$ precipitation is controlled by the calcium ions concentration, carbonate concentration, pH and presence of nucleation sites. In microbial $CaCO_3$ precipitation, the presence of nucleation site is not a key factor for $CaCO_3$ precipitation because the bacteria themselves behave as nucleation sites. Bacterial $CaCO_3$ precipitation is a biologically mediated mineralization process leading to mineral nucleation and growth of mineral products[16]. Using Thermo-gravimetric analysis (TGA), the presence of $CaCO_3$ in the bacterial concrete specimens is determined.

Morphology of Naturally Precipitated Calcium Carbonate Crystals

$CaCO_3$ is available in three different crystal structures but with same chemical formula in the form of Calcite, aragonite and vaterite. Most stable form of $CaCO_3$, Calcite is rhombohedra in shape[17]. It is formed due to the presence of magnesium, manganese and orthophosphate ions.

Aragonite is a orthorhombic shaped crystal form of $CaCO_3$ and geologically changes into calcite over time at high temperature [18,19] or low temperature in the presence of magnesium ions and pH less than 11[20].

Vaterite mineral is rarely found in nature[21]. It is produced in the pH range from 8.5 to 10^{22}, low Ca^{2+} concentration or low temperature and high Ca^{2+} concentration[23]. The morphology of vaterite depends on the pH value and temperature. It was morphology of the crystals does not depend on calcium source only. Using SEM analysis it was proved that the rhombohedra shape to calcite crystals is due to the presence of chloride ions where as spherical shape to crystals is due to the presence of acetate ions.

Role of Urease Activity in Biomineralization

Urea is naturally available in the environment as a detoxification product. Urease activity (hydrolysis of urea) is mostly found in microorganisms from soil and aquatic environments. Biotic urease activity is widespread in the environment that includes the action of bacteria, yeasts, fungi etc. Urease hydrolyses the urea generating ammonia and carbamate. Carbamate decomposes to ammonia and carbonic acid. The ammonia and carbonic acid subsequently equilibrate in water with their deprotonated and protonated forms, resulting in an increase in the pH[24]

There are a number of species of $CaCO_3$ minerals associated with bacteria, for example calcite by bacillus pasturii , vaterite formation by Acinobacter sp., aragonitic sherulites by Deleyahlophila [25], calcite by Bacillus subtilus [26] and magnesium calcite spherulites and dumbbells by the slime-producing bacteria, Myxococcus xanthus[27,28]).

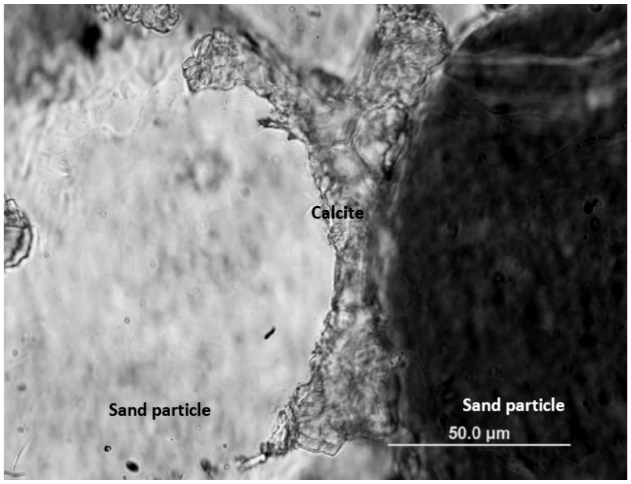

Figure-1
Calcite crystals produced by bacteria binding two sand particles

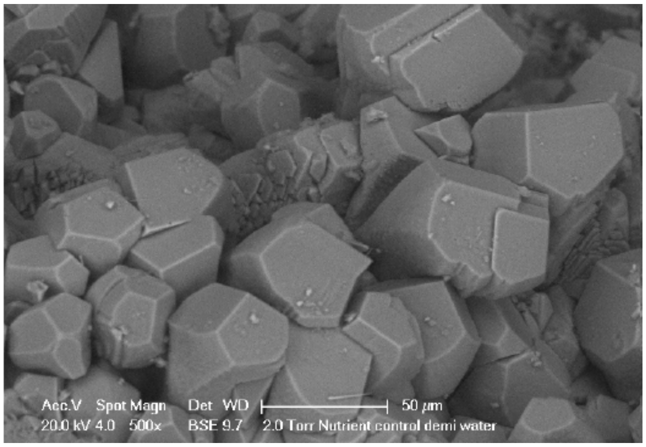

Figure-2
Bio-minerals observed by SEM

One of the most robust ureolytic bacteria is Bacillus subtilis which is an aerobic, spore forming, rod shaped and gram positive bacterium. It uses urea as an energy source and produces ammonia which increases the pH in the environment and generates carbonate, causing Ca^{2+} and CO_3^{2-} to be precipitated as $CaCO_3$. Alkaline pH is the primary requirement for microorganisms for calcite precipitation .In bacteria incorporated mortar samples, the live cells of optimum concentration were added directly in suspension along with mixing water to the cement sand mixture to study the application of microbiologically induced calcite precipitation in cement based materials.

The more $CaCO_3$ precipitates, the better the self-healing effect will be. The concentrations of bacteria, urea and Ca^{2+} will greatly affect the amount of precipitated $CaCO_3$.

Conclusion

Microbial mineral precipitation resulting from metabolic activities of some specific microorganisms in concrete to improve the overall behaviour of concrete has become an important area of research. The following are the summary of research outcomes done at JNTU Hyderabad by Dr M V Seshagiri Rao et al. on Bioengineered concrete:

An alkaliphilic aerobic microorganism bacillus subtilis JC3 is induced into cement mortar samples at various cell concentrations in suspension along with the mixing water. The greatest improvement in compressive strength occurs at cell concentrations of 10^5 cells/ml for all ages. The study showed that a 25% increase in 28 day compressive strength of cement mortar was achieved. 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. Scanning Electron Microscopy (SEM) also confirmed the role of microbiologically induced precipitation within the mortar matrix. The modification in pore size distribution and total pore volume of cement–sand mortar due to such growth is also noted. The cell concentration of the microorganism was determined from the standard curve made by observing optical density at 620 nm vs. bacterial cell numbers counted in haemocytometrically under microscope. Different cell concentrations were derived from the bacterial growth culture by serial dilution method. Nitrogen adsorption confirms the modification in pore size distribution due to the addition of microorganisms, and shows that a cell concentration of 10^5 cells/ml generates the greatest reduction in porosity. The extra cellular growth produced by the microorganism is expected to contribute more to the strength of concrete. Even the dead cells may simply remain in the matrix as organic fibers. Water permeability tests, ultrasound transmission measurements shows that bacterial concrete specimens are highly impermeable and have excellent quality. Pore size and structure studied through nitrogen adsorption shows bacterial specimens have modified dense pore structure due to calcite precipitation. Chloride penetrability studies done using Rapid Chloride penetration test shows that charged passed is very low to negligible in bacterial concrete specimens. Bacterial concrete has improved microstructure and permeation properties than controlled concrete. Studies showed that bacterial concrete has better acid resistance in aggressive environments.

Table-1
Strength Studies on Bacteria incorporated Concrete

Strength Studies				
@ 28 days age	**Controlled Concrete**		**Bio Concrete**	
	M20	**M40**	**M20**	**M40**
Compressive Strength (MPa)	28.18	52.01	32.74	61.06
Split Tensile Strength (MPa)	3.26	4.51	3.73	5.13
Flexural Strength (MPa)	4.68	6.11	6.11	7.73

Table-2
Overview of various Construction Materials made using MICP

Application	**Microorganism**	**Metabolism**	**Nutrients**	***Reference***
Biological mortar	Bacillus cereus	oxidative deamination of amino acids	Growth media (peptone, extract yeast, KNO3, NaCl) + CaCl2.2H2O, Actical, Natamycine	2
Crack in concrete remediation	Bacillus subtilis	Hydrolysis of urea	Nutrient broth, urea, CaCl2.2H2O, NH4Cl, NaHCO3	1
Crack in concrete remediation	Bacillus sphaericus	Hydrolysis of urea	Extract yeast, urea, CaCl2.2H2O	7
Bacterial concrete	Bacillus subtilis	Hydrolysis of urea	Nutrient broth, urea, CaCl2.2H2O, NH4Cl, NaHCO3	1
Bacterial concrete	Bacillus subtilis	oxidative deamination of amino acids	Peptone: 5 g/lit., NaCl: 5 g/lit., Yeast extract: 3 g/lit.	**27**

Table-3
Microorganism used for Calcium Carbonate Precipitation in Concrete

Type of microorganism	**System**	**Crystal type**	***Reference***
Photosynthetic organism : Synechococcus GL24	Meromictic lake	Calcite ($CaCO_3$)	**18**
Photosynthetic organism : Chlorella	Lurcene Lake	Calcite ($CaCO_3$)	**21**
Sulfate reducing bacteria: Isolate SRB LVform6	Anoxic hypersaline lagoon	Dolomite ($Ca(Mg) CO_3$)	26
Nitrogen cycle Bacillus subtilis	Urea degradation in synthetic medium	Calcite ($CaCO_3$)	13
Nitrogen cycle Bacillus cereus	Ammonification and nitrate reduction	Calcite ($CaCO_3$)	12
Nitrogen cycle Bacillus subtilis JC3	Ammonification (Ammo acid degradation)	Calcite ($CaCO_3$)	**27**

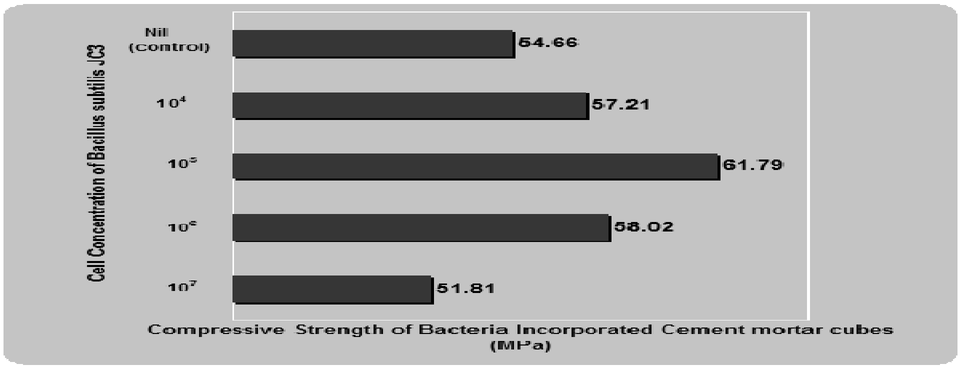

Figure-3
Compressive Strengths at different Cell concentrations of Bacillus subtilis JC3

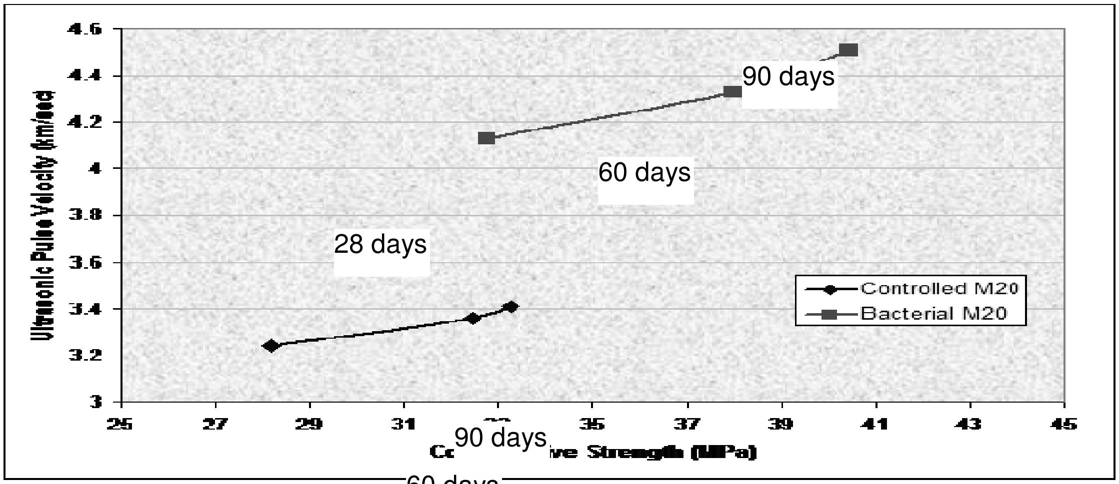

Figure-4
Correlation curve betwee 28 days **and compressive strength of Bacterial and controlled concrete specimens of M20 Grade**

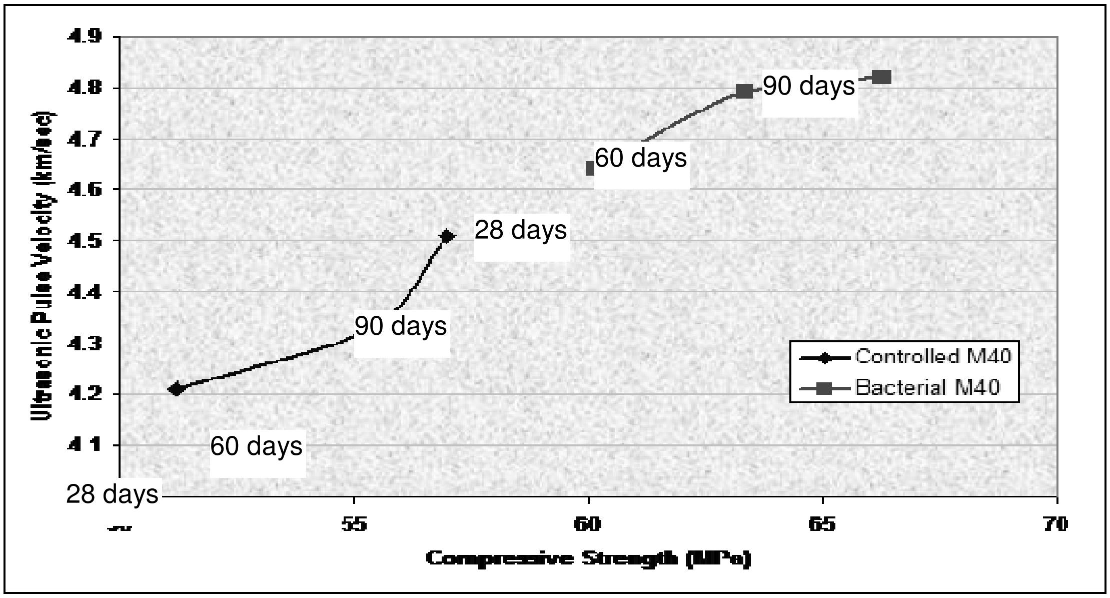

Figure-5
Correlation curve between UPSV and compressive strength of Bacterial and controlled concrete specimens of M40Grade

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