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An Eco-Sustainable Approach to Increase Compressive Strength of Cement Mortar by the Addition of Ureolytic Bacteria

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ABSTRACT:

In today's world, where development is on the rise, the necessity for auxiliary cementing substance that improves strength while having less environmental implications is critical. In present urea and a calcium supply, ureolytic bacteria could improving the strengthening of C.M. by precipitating calcium carbonate. Bacillus sphaericus is employed in this investigation to see if it may be used for this purpose. To determine the influence of bacterial solution on cement, various tests such as consistency and first setting time are performed. To determine the variance in the mechanical characteristics of cement mortar, compression strengthening and sorptivity are performed in this study. XRD and FESEM analyses are used to determine mineralogy and morphology of CaCo3 precipitated by bacteria. The strengthening of cube mortar increased with the rise in bacteria content up to 107 cells/ml (at 7 and 28 days). Over the control specimen, the optimum dosages of bacteria increased average strengthening by 58 percent (at 7 days) and 23 percent (at 28 days). The higher rise in strengthening later than 7th days of cured sample could be recognized to presence of nutritional medium, which depletes as time passes, causing bacteria to die. For a cell concentration of 109 cells/ml, the minimal cumulative water absorption is reached.

Keywords: Bacterial Concrete, Strengthening, Calcium Carbonate, Calcite Precipitation, FESEM, Cement Mortar.

INTODUCTION:

Concrete is widely regarded is more significant building materials in the world's construction industry. Concrete technology could be improving by adopting pollution-free and natural methods to increase the strength and longevity of the material. The use of cement has expanded tremendously as the construction industry progresses, as we seek stronger and more durable constructions. This raises worldwide cement productivity and, as a result, increases carbon dioxide emissions into the environment. For a brighter future, we need to identify a technique that can raise the strengthening and durabilities of structures without increasing the consumption of cement.

Through hydraulic or pozzolanic activity, supplementary cementing materials (SCMs) are frequently employed in concrete mixes to reduce cement content, improve workability, increase strength, and improve durability. As a partial cement replacement, silica fume and fly ash are often used in concrete. All the constructional material are permeable. The porosity of the construction material, combined with moisture penetration and other risky essentials likes acids, chlorides, and sulphates, has a negative impact on the concrete, reducing its strength and longevity. An additive that closes holes and fissures, reducing the structure's permeability, would greatly extend its life. Traditionally, a range of sealing agents, such as latex emulsions, have major drawbacks such as unsuitable interfaces, UV sensitivity, an unstable molecular structure, and a high cost.

Soil-thriving microorganisms perform one of nature's exciting biomimetic processes. Sand is transformed into sandstone. Bacillus pasteurii, a calcite precipitating microbe, was later discovered to be responsible for the binding agent generation in this conversion. This mineral deposition process can replace the natural approach of closing concrete and mortar pores and fissures. Biomineralization is a biologically produced precipitation in which organism establishes locally micro- environment with ideal extracellular chemical precipitation of mineral phases. Many biological organisms having natural settings, including soil, geological formations, fresh water biofilms, hot springs, saline lakes, and oceans. To far, no exact mechanism for microbial calcium carbonate precipitation has been discovered.

The motive is to see how adding Ureolytic bacteria to the microstructure affects the compressive strength and sorptivity of cement mortar.

LITERATURE REVIEW:

Bacteria are prokaryotic microorganisms. They are a small number of micrometres long and come in a variety of shapes, including spheres, rods, and spirals. Bacteria found approximately all habitat in the cosmos. Soils, water, acidic hot springs, radioactive wastes, and the deepest parts of the Earth's crust are all home to them. We may see them interacting with plants and animals in symbiotic and parasitic partnerships. On Earth, there are roughly 51030 bacteria, generating a biomass that dwarfs that of all combined. And its an essential part in nutrient recycling, such as in nutritional cycles, nitrogen fixation from the atmosphere, and putrefaction. Bacteria give nutrition to biological populations surrounding hydrothermal vents and cold seeps by converting dissolved chemicals such as hydrogen sulphide and methane to energy for biological communities. All the bacteria living in the earth has not been characterised. The bacteria grow in laboratories will be only short of phyla of bacterial of bacterial species. The branch of microbiology that studies bacteria is called bacteriology.

Its showing wider diversity of shapes and sizes, called morphologies. Typical size of Bacterial cells are 0.5–5.0 mm in length. Some of having spherical shape, called cocci (sing. coccus, from Greek kókkos, grain, seed), or rod-shaped, called bacilli (sing. bacillus, from Latin baculus, stick). Some bacteria, known as vibrio, are slightly bent rods or comma-shaped; others, known as spirilla or spirochaetes, are spiral-shaped or tightly coiled. Some species are even tetrahedral or cuboidal in form. Bacteria that develop as branching filamentous kinds with star-shaped have recently been identified deep beneath the Earth's crust. This morphology's having huge surface area to vol. ratio may provide benefit in nutrient-poor conditions in the earth's crust.

2.3 MECHANISM OF CALCIUM CARBONATE PRODUCTION

Biomineralization is a biologically produced precipitation in which an organism establishes a local micro-environment with ideal extracellular chemical precipitation of mineral phases. It's still unclear what role bacteria play in the carbonate precipitation process. Calcium carbonate precipitation is possible in almost all microorganisms. Urease is used by bacteria to hydrolyze urea for the following purposes:

(1) Increment ph ambient,

(2) Utilization as nitrogen source,

(3) Utilizing as source of energy.

Recently, in concrete the microbial precipitation of calcium carbonate for the improvement of properties has become a new area of research. The microbial precipitation of CaCO3 is determined by several factors including: concentration of dissolved inorganic carbon, the pH, and the concentration of calcium ions, presence of nucleation sites. The first three ingredients produced by bacteria's metabolism, while the bacteria's cell wall serves as a nucleation site. Urease is produced by the bacteria utilised in this study, and it catalyses the hydrolysis of urea (CO(NH2)2) into ammonium (NH4+) and carbonate (CO3 2). 1 mol urea is first hydrolyzed intracellularly into 1 mol carbamate and one mole ammonia (Eq. 1). Carbamate hydrolyzes spontaneously to produce one mole of ammonia and one mole of carbonic acid (Eq.2). These products then create 1 mol bicarbonate, 2 mol ammonium, and 2 mol hydroxide ions (Eqs.3 and 4). The last two reactions result in an increase in pH, which alters the bicarbonate balance, causing carbonate ions to form (Eq. 5)

Bang et al. (2001) Bacillus pasteurii immobilised in polyurethane is used in the study. The polyurethane provide protection to the bacterial cells from the extremely alkaline environment of concrete, while serving as nucleation sites for calcite crystals The variation in the amount of precipitation of calcite by free cells and immobilised cells is little. The strengthening of mortar cubes are increased considerably after 7th days of curing period in urea calcium chloride medium. Calcite in polyurethane showing smaller effects on improvement of E and strengthening of polymer. This can be accounted for the deterioration of chemical interaction between the calcite and the form after immersion on the medium.

Bachmeier et al. (2002) uses urease enzyme produced by Bacillus pasteurii immobilised in polyurethane (PU) for calcium carbonate production. Here also recombinant E.coli are also used for calcite production. It is suggested that use of immobilised enzyme is more recommended than using bacterial cells. The viability of enzyme immobilised in the PU is to be studied further. SEM images show well organised calcite crystals.

De Muynck et al. (2007) the improvement of resistance of mortar specimens to degradation processes is studied here. Bacillus sphaericus is used here. The resistance to carbonation and chloride infiltration has been measured to determine the durabilities of treated substrate. X-ray diffraction (XRD) analysis was used to determine calcite precipitation, while SEM was used to examine morphology. Mortar specimens, a newly produced coating of calcite can be seen. For the rapid carbonation testing and capillary water suction studies, mortar cubes were employed. For chloride migration and water vapour diffusion testing, cylindrical specimens were drilled out of mortar slabs. The presence of bacterial calcite reduced capillary water uptake and permeability to gas. The chromatic look of mortar and concrete surfaces changed only slightly as a result of the bacterial treatment.

De Muynck et al. (2008) Bacillus sphaericus uesd and mixed cultures of ureolytic bacteria is studied here. The effect of using various nutrients on treatment is also studied here. The capillary rise and gas permeability is decreased by the treatment. Pure culture of bacteria shows more decrease. The morphological variation is seen in the SEM images for calcite is accounted for usage of various cultures and hence the variation in the levels of urease enzyme. The capillary rise and gas permeability results for pure Bacillus sphaericus are comparable.

Achal et al. (2009) Studies the Improved calcite precipitation is studied by mixing bacterial medium with sand columns. Precipitated calcite is measured by EDTA titration method. The morphology and chemical onstituents of the bacteria and sand consolidated column is analysed with SEM-EDX and XRD.

Achal et al. (a) (2011) The study investigates the durability of concrete or mortar. The impact of Sporosarcina pasteurii- induced calcite precipitation is investigated. Corn steep liquor, a cheap industrial waste from the starch industry, is employed as a food source for bacteria growth and calcite synthesis, and the results are compared to those of a conventional commercial media. According to IS 4031-1988, 70.6 mm cement mortar cubes are used. Water absorption, permeability, and chloride permeability tests are performed on bacteria produced in CSL medium, and the findings are found to be comparable to those obtained from bacteria cultured in commercial medium, showing that the bio calcification process can be simplified.

Achal et al. (b) (2011) Bacillus sphaerius, isolated from commercially availability of cement is used in the study. Strengthening and W.A. test are done here and improvement of compressive strength by 36% and water absorption is reduced six times by the application of the bacteria in the mortar cubes. Nutrient broth-urea medium is used as curing solution in the study

Wiktor et al. (2011) The bio-chemical two-component self-healing compound was made up of calcium lactate and bacterial spores, which were both embedded in expanding clay particles. Spores from a bacterial isolate isolated from alkaline lake soil employed, with a homology of 98.7% to Bacillus alkalinitrilicus. The crack healing capacity of aged concrete specimen is studied here. The variation in the oxygen consumption rates of bacterial specimen and control specimen is observed and shows that active bacteria remain viable and functional several months after concrete casting. According to EDAX and FT-IR analyses, the material is a mix of morphologies.

Chahal et al. (2012) Sporosarcina pasteurii has been studied for its effect on strengthening, W.A., and fast chloride permeability of silica fume concrete (cement was substitute 5 percent and 10 percent of S.F. by weight).the bacterial concentrations used are 103, 105, 107 cells/ml. Compressive strength (BIS: 516-1959), Water absorption and porosity (ASTM 642), Rapid chloride permeability test (ASTM C 1202) are the tests done in the study and the results shows that there is significant increment in strengthening with 10% silica and the optimum dosage of 105 cell/ml of bacteria. It is said that when increment of bacterial concentration the matrix integrity is disrupted and hence the strength is decreased. XRD and EDAX data are given to support the observations.

Majumdar et al. (2012) bacterial protein produce by bacteria closely related with Thermoanaerobactor fermicutes is used in the study. This is directly mixed with the mortar and cubes of size 70.6x70.6x70.6 mm are casted. Crack repair test, sulphate resistance test, and water absorption test, flexural (The dimension of the standard beam was 200 mm × 50 mm × 50 mm. The beams were cured for 28th days under water and their flexural strength were determined in 4-point condition.) testing done in addition to the compression tests. Maximum increment in strengthening seen in bacterial concentration of 105 cells/ml and increment was 42.4% after 120 days of water curing. The max. Flexural strengthening upto 33% with 3 μg/g bioremediase protein incorporated samples.

Annamalai et al. (2012) introduces the use of Bio Caulk. Bacterial calcite produced by Bacillus sphaericus was separated and was used as sealing agent by itself and also by mixing with carbon nanotubes. This calcite produced was mixed with flyash and was used as sealing agent. The variation in compressive strength with conventionally used sealing agents, Bio Caulk, and Bio Caulk with carbon nanotubes are studied and the mortar cubes with carbon nanotubes with calcite showed max. increment in strengthening of 45%.

Achal et al. (2013) the bio cementation ability of a Bacillus sphaericus to seal cracking is demonstrated. The reduction in porosity and chloride permeability was studied with bacterial strain. The crack healing capability is also checked and visualisation of amount of calcite deposits is done by using scanning electron microscope. Cubes of size 70.6x70.6x70.6mm are used to evaluate by the application of bacterial strain. Cubes are cured in nutrient broth-urea medium. 40% increment in strengthening is observed in microbial remediated cubes and significant crack healing of 13.4mm wide ones are also seen. It results in the decrease in water and chloride ion.

Sujatha et al. (2014) An ant hill was used to obtain ureolytic bacteria, which was then grown for the study. Mortar cubes measuring 70.6x70.6x70.6mm were cast, and bacteria were added to the mortar via curing water. Control specimens and one batch of bacterial cubes were cured in tap water, while another batch was cured in 1g urea/L.

Maheswaran et al. (2014) This paper compares the ureolytic activity of Bacillus cereus with Bacillus pasteurii. The bacterial mortar cubes are made by substituting the full volume of water with suspended bacteria in phosphate buffered saline (PBS). The nutrition solution was used to cure the bacterial cubes. Bacillus cereus increased compressive strength by 38% of 0% C.M. at a bacterial cell concentration of 106 cells/ml, whereas Bacillus pasteurii increased compressive strength by 29% of 0% of C.M. at a bacterial cell concentration of 105 cells/ml. The chloride permeability of mortar cubes containing Bacillus cereus is significantly reduced. The bacterial calcite precipitation was confirmed by X-ray diffraction, scanning electron microscope, thermogravimetric analysis, and Fourier transform-infrared spectroscopy.

The use of cement is increasing exponentially, and the requirement of stronger and durable structures is also increasing. The compound annual growth rate of the cement industry is estimated to grow more than 9% by 2020 (Technavio.com). As the cement production increases, the emission of CO2, a major greenhouse gas, increases. In such a scenario, any technology that would reduce the consumption of cement can contribute significantly to the environment. Bacteria have been used to improve the strength and durability of the cement mortar (thereby reducing the consumption of cement) through biomineralization. Biomineralization is defined as a biologically induced mineral precipitation, in which an organism creates a local micro-environment with conditions that allow optimal extracellular chemical precipitation of mineral phases. This is induced by heterogeneous nucleation on bacterial cell walls (Bachmeier et al. 2002; Ramachandran et al. 2001; Stocks-Fischer et al. 1999; Bang et al. 2001). Biomineralization can be observed in many biological species living in various natural environments such as soil, geological formations, fresh water biofilms, hot springs, saline lakes, and oceans (Kaleyitch and Kefeli 2007). Biomineralization has been used for many years in several engineering applications. One encouraging biomimetic process in nature is the conversion of sand to sandstone by soil- thriving bacteria (Dick et al. 2006). Later it was found that this conversion was done by Bacillus pasteurii, which precipitate calcite that acts as a binding material for the limestone. Introducing a calciteprecipitating bacteria can therefore meet the need to improve the strength. The improvement of soil-bearing capacity by microbial calcite precipitation is reported by Whiffin et al. (2007).

Microbial mineral precipitation using ureolytic bacteria is reported to improve the overall behavior of concrete, including strength and durability (Bachmeier et al. 2002; De Muynck et al. 2008; Tittelboom et al. 2010; Achal et al. 2009, 2013; Siddique and Chahal 2011; Kim et al. 2012; Majumdar et al. 2012; Torgal and Labrincha 2013; Sujatha et al. 2014).

Ureolytic bacteria need urea to perform their function. Bacteria can be used externally as a healing agent on hardened concrete for sulphate treatment (Wiktor and Jonkers 2011). The microbial-induced precipitation can resist the carbonation and chloride ingress in concrete (De Muynck et al. 2008). Ureolytic bacteria has been used as an alternative and environmentally friendly crack repair technique (Achal et al. 2011a, b; Bang et al. 2001; De Muynck et al. 2007). Ureolytic bacteria have the ability to produce an urease enzyme, which hydrolyzes urea into ammonia and carbon dioxide. Previous studies have focused primarily on ureolytic bacteria.

A recent study pointed out that ureolytic bacteria produces ammonia gas, which may cause a health hazard. To avoid the possibility of a health hazard as a result of this mechanism, a nonureolytic bacteria is used to check the possibility of calcite precipitation through biomineralization. Jonkers et al. (2010) studied the effect of nonureolytic bacteria on compressive strength and durability of cement stones. Xu et al. (2014) attempted to use a nonureolytic bacteria for surface treatment of mortar prism to study its durability aspects. Most of the previous studies on the strength and durability aspects of concrete/mortar used ureolytic bacteria. The studies on the application of nonureolytic bacteria on the strength/ durability aspects of concrete/mortar are not available. Therefore, the present work focuses on the engineering properties of cement mortar using a nonureolytic bacteria, Bacillus cohnii. The engineering properties investigated in this study are setting time, soundness, compressive strength, capillary water absorption characteristics, drying shrinkage, and microstructure of cement mortar.

Behavior of Nonureolytic Bacteria on Concrete Like Environment Fresh concrete/mortar has a pH of 11.5 to 12.5, and will have a rise in temperature because of the heat of hydration. The behavior of bacteria in the alkaline concrete–like environment with a wide range of temperature and capability to produce calcium carbonate through the metabolism shall be studied. Bacillus cohnii is an alkaliphilic micro-organism that can survive up to 70°C (Ghorbel et al. 2009) and is capable of producing CaCO3 through biomineralization (Xu et al. 2014). Bacillus cohnii (MTCC3616) was purchased from the Microbial Type Culture Collection (MTCC), Chandigarh, India. It was cultured in liquid media according to the supplier's guidelines. To study the behavior of Bacillus cohnii for pH values, a pH tolerance test was conducted as presented in the following section.

SELECTION OF BACTERIAL SPECIES

A no. of bacterial species were reported in literature to improving various properties. Still, current study requires a bacteria which is non- contagious, that survive in the alkaline concrete like environment and that must be capable of producing calcium carbonate through metabolism. The single celled eukaryotes like bacteria and other microbes can live and reproduce only if they have some ecological circumstances. These are heat, pH, osmotic pressure, dissolved gases and water availability. The pH of the fresh concrete lies b/w 11.5 to 13 and there will be rise in temperature because of heat of hydration. The ureolytic bacteria used in this study should be alive in these alkaline environment and also have temperature tolerance. Two different non- contagious ureolytic bacteria (bacillus), namely, Bacillus cereus and Bacillus sphaericus are tested in this study to check its survival in a concrete-like environment.

TESTING OF SURVIVAL OF BACILLUS CEREUS IN CONCRETE-LIKE ENVIRONMENT

The following steps are involved in testing the tolerance of this bacteria in concrete-like environment.

(i). 200 ml of nutrient medium (Luria Bertani broth) was prepared for the culture of bacteria.

(ii). It was then transferred to 12 fresh clean test tubes and its volume was made to 10mlby adding NaOH to increase the pH in the test tubes. (pH of the medium was then found to be 8, 9, 10, 11, 12, and 12.5 in two sets of test tubes respectively).

(iii). After the preparation of media, tubes were preserved with cotton plug and thensterilized using autoclave.

(iv). Bacillus cereus bacteria from the mother culture were scraped and introduced to the test tubes after autoclaving, and thoroughly mixed.\

(v). It incubated at 37°C and 50°C for 24 hours.

(vi). The growth of the bacteria in each of the 12 test tubes was measured by turbidity of solution after a 24-hour incubation period at various temperatures and pH levels. The results of thistest are listed as follows:

(vii). Growth of bacteria wasn't experimental in the any of the six cultures incubated at 50ºC

(viii). At 37ºC, growth was observed only in cultures with pH 8 and 9

Table 3.1 presents the result of Bacillus cereus growth in different cultures. Table showing the bacteria could survived only in the cultures with pH 8 and 9. However, the pH of freshly prepared concrete or cement mortar lies b/w 11.5 to 13, which means that Bacillus cereus may not survive in concrete-like environment. Therefore some other species of Bacillus which have a pH tolerance of 11.5 to 13 should be used.

Table 3.1: Temperature and pH tolerance of Bacillus Cereus

pH	**Presence of Bacteria**	
	Cultures incubated at 37ºc	**Cultures incubated at 50ºc**
8	+	-
9	+	-
10	-	-
11	-	-
12	-	-
12.5	-	-
+ presence of bacteria; - Absence of bacteria		

Table 3.2: Temperature and pH tolerance of Bacillus sphaericus

pH	**Presence of Bacteria**	
	cultures incubated at 37ºC	**cultures incubated at 50ºC**
8	+	+
9	+	+
10	+	+
11	+	+
12	+	+
12.5	+	+
+ presence of bacteria; - Absence of bacteria		

TESTING OF SURVIVAL OF BACILLUS SPHAERICUS AT IN CONCRETE-LIKE ENVIRONMENT

As the trial of Bacillus cereus failed a different species of t h i s group. Bacillus sphaericus was considered next. Growth of bacteria was observing in cultures incubatedat both 37ºC and 50 ºC for all the pH value from 8 to 12.5. Table 3.2 presents the temperature and pH tolerance of Bacillus sphaericus. Bacteria could survive the pH lies of 8 to 12.5 at both 370C and 500C seeing in table.

Bacillus species can be suitable for fresh concrete (or cement mortar) which has pH about 11.5 to 13. The above result shows that, this species can survive the temperature in concrete (or cement mortar) arising out of the heat of hydration.

TEST FOR CACO3 PRECIPITATION IN AGAR PLATE STATE

Section 2.2 presents the mechanism of microbial precipitation of CaCO3 which is responsible for strength in concrete (or cement mortar). In order to confirm that selected bacterial species having capablity of producing CACO3, following standard test has been undertaken using Calcite Precipitation Agar (CPA). Its a solid medium for screening of bacterial precipitation of CACO3. Steps involved are as follows:

i. 0.6g of Nutrient broth, 5.7g of CaCl2; 0.424g of NaHCO3; 2.0g of NH4Cl; 3.0g of Agar, 190ml of distilled water was weighted and taken in a 200ml conical flask

ii. All media components were autoclaved

iii. After autoclaving urea is added to medium.

iv. 20µl of broth culture was inoculated in the center of a plate, and then incubated at 30°C for 6 days.

Fig. 3.1 presents the precipitation of some material on the plate at points A, B, C and D. To characterise this material, the precipitation is observed in FESEM.

Fig. 3.1: Calcite precipitation resting on plate

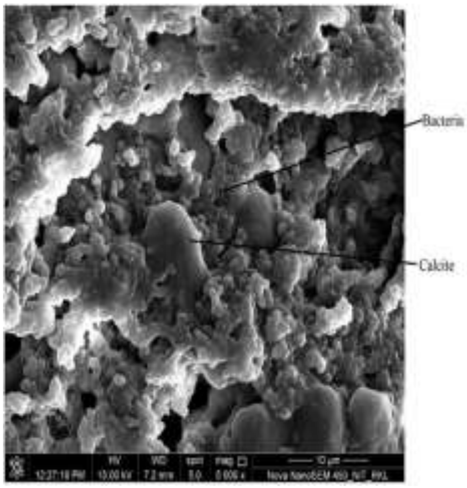

Fig 3.2 FESEM imagery of calcite precipitated

CONCLUSION

The objective of this study was defined for improving properties of normal strength cement mortar using a single bacterial species. There are many bacteria reported in literature which can improve the strength, durability and other mechanical properties of concrete and cement mortar. Bacillus cereus was first tried for this purpose. However, this species found not suitable as it has a less temperature and pH tolerance. Bacillus sphaericus then found to be suitable for concrete-like environment and selected for further studies. This bacterial species is also found to capable of producing CACO3 which is responsible for improved properties of concrete and cement mortar.

To investigate the change of compressive strength and capillary water absorption of cubes with varied bacteria concentrations, the mortar cubes were prepared using a cement to sand ratio of 1:6 and a water cement ratio of 0.55. As a result, different mortar cubes are made with bacterial concentrations of 0, 105, 106, 107, 108, and 109 cells/ml. The mortar cubes then cured in water, urea and calcium chloride. 2% of urea (in terms of volume of total water) was added in to solution to activate the urease enzyme used for the metabolism of bacteria. Calcium chloride of 20 g/lit was added to supply a source of calcium to the system in order bacteria can produce the desired CaCO3. Hardened specimens were tested after 7-days and 28-days of curing for Compressive Strength and capillary water absorption and the effect of bacteria (Bacillus sphaericus) on these two mechanical properties are studied.

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