Self-Healing Development AND Performance Assessment OF Bio Cementitious Concrete

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Abstract: A bio cementitious concrete is basically a construction material (concrete) which has been incorporated with bacteria and precursor compound as healing agent that awaken and seal cracks when they emerge. The bacteria act as a catalyst, and transform the precursor compound to limestone and this technique is based on bio mineralization of bacteria. In this research, an extensive work was undertaken to investigate self-healing development and performance assessment of bio cementitious concrete, and this was carried off by integrating Bacillus Subtilis into engineered cementitious composites (ECC), normal mortar, and concrete alongside with calcium lactate, urea and yeast extract as nutrient source for the bacteria. After carrying out all the tests of culturing, growing and checking pH and temperature resistivity of the bacteria. The bacterial cell solution was integrated into the concrete matrix by adopting, immobilization of bacterial cell in pumice solution, encapsulation of stiffen bacteria sand and immobilization of bacterial cell into porous expansive clay particles as techniques for adding and protecting the bacterial cell inside the concrete. During the mixing and specimen preparation stage, three bacterial cell solutions of concentrations (10^4,10^6,10^8)cells/ml were prepared and mixed with M25 mortar and normal concrete by using mix ratios 1:1:2 , 1:1.32:2.5 with water/cement ratio of 0.5. Again the bacterial cell solution was mixed with M40 ECC of water/binder ratio of 0.33 and sand/ binder ratio of 1: 0.84.The bacteria reacted with the concrete elements to form calcium carbonate precipitate ($CaCO_3$) or limestone which successfully sealed the created cracks. Performance assessment was done on the specimens and the following results were obtained; at a concentration of 10^4cell/ml of bacterial cells, the compressive strength of concrete begins to increase to an optimum value at 10^6cell/ml and declines at 10^8cell/ml. The specimens of stiffen sand capsules, pumice immobilized bacterial cell and loaded clay particle at 2% replacement by coarse aggregates weight increased the compressive strength of concrete by 35.20%, 31.59% and 0.20% respectively at the end of 28 curing days. Cracked Stiffen sand capsules and pumice immobilized bacterial cell ECC specimen regained flexural strength of 23.6% and 32.22% at the end of 56 self-healing days. Sorptivity at end of 56 days for self-healed stiffen sand capsules, pumice immobilized bacterial cell and loaded clay particle concrete specimens were reduced by 70.1%, 70% and 81.9% respectively. The bacterial incorporated concrete specimens with crack width of 0.015 mm, 0.018 mm and 0.02 mm respectively were completely sealed by $CaCO_3$ precipitate at the end of 56 self-healing days. The new material formed at crack areas was $CaCO_3$ according to the X-Ray Diffraction spectra obtained. Based on these factors, it is concluded that the bio cementitious concrete showed excellent performance assessments in terms of compressive strength, sorptivity reduction, flexural strength, crack repair and crack recovery strength at the end of 56 self-healing day.

Keywords : Bacillus subtilis, Bio mineralization, Calcium carbonate precipitate, Healing agents, Self-healing, Strength regain, and Precursor compound.

1. INTRODUCTION

Bio concrete is simply a dead concrete which has been infused with bacteria and precursor compound that awaken when cracks appear. The bacteria themselves act largely as a catalyst, and transform a precursor compound to a suitable filler material known as limestone that fills the cracks in a matter of weeks. The bacteria can last for 200 years and self-activates when crack occurs [1]. This technique is based on bio mineralization of bacteria in concrete, which involves the use of microbes in bacterial concrete known as microbial Induced Calcium Carbonate Precipitation (MICCP) or bio mineralization, a biological process whereby microorganism produce minerals to stiffen tissue. Bio concrete is an example of linking nature to construction. The bio concrete is mixed in the same way as that of regular concrete but extra ingredients i.e. the bacteria and precursor compound are added. The bacteria is added to the concrete in suspension state of the concrete and concrete being very base-forming in nature, the bacterium supplementary ought to slot in some special norms. The added bacteria should be able to withstand the harsh environmental conditions of concrete. Concrete is a dry material and the pH value of cement and water when mixed is up to 13 which becomes a confrontational problem since most of the organisms cannot survive in an environment having pH value higher than 10.1 [2]. The bacteria that can resist concrete matrix incorporation exist in nature, and they appear to be related to a specialized group of alkali-resistant spore-forming bacteria. These bacteria have spores that are viable but dormant cells and can withstand mechanical and chemical stresses and remain in dry state viable for over 50 years [1]. So to significantly increase the life and associated practicality of concrete incorporated bacterium, the effectiveness of microorganism reproductive structure and at the same time the required organic bio mineral precursor compound, the bacteria has to be immobilized or encapsulated in a bio degradable plastics before it is added into the concrete matrix [1],[2].

2. PROBLEM STATEMENT

India has already placed 55 - 60 billion m^3 of concrete for the past 50 years and now, India is at a pace of placing new concrete to a tune of approximately 1.75 – 2 billion m^3/annum (NBM&CW February 2019), and this has to be protected from cracks. when the cost of repair and maintenance of cubic tons of concrete placed is interpolated with regards to the distress and the health of inventory over the past 40 years, the annual cost to owners for repair, protection and strengthening could be estimated between US$ 40 to 45 billion (NBM&CW February 2019). This high cost involved for repairs and maintenance of concrete due cracking and other environmental factors has to be minimized. Due to these compelling issues, this research work seeks to investigate the development of self-healing crack repair concrete, their performances and how they can be utilized in concrete technology to reduce cost of maintenance at the same time increase the durability of structure as well as ensuring the avoidance of skin related problems in India.

3. OBJECTIVES OF STUDY

- Developing a self-healing normal mortar, concrete and engineered cementitious composites (ECC) by integrating Bacillus subtilis alongside with calcium lactate, urea and yeast extract as nutrient.

- Adopt immobilization of bacterial cell in pumice, immobilization of bacterial cell in porous expansive clay particle and encapsulation of bacteria stiffen sand particles as methods of adding and protecting the bacteria cell (healing agent) into the concrete matrix.
- Investigate the effect of healing agent additions on mechanical properties such as compressive strength and flexural strength.
- characterize mineral constituents in crack healing specimens using XRD studies
- Evaluate the efficiency of crack self-healing by measuring flexural strength regain and recovery of permeation properties such as sorptivity.

4. LITERATURE REVIEW

Bakhar sini (2016) investigated the development of self-healing mechanism in cementitious composites (ECC, FR and normal mortar) by incorporating Sporosarcina ureae, Sporosarcina pasteurii and Bacillus subtilis subsp. spizizenii into concrete along with pumice and zoelite as carrier materials(immobilization material) and calcium lactate, yeast extract and urea as nutrient. Three different bacterial cell concentrations, 10^4,10^6 and 10^8 cells/ml were used. He concluded that, significant amount of self-healing crack repair was achieved by all the three selected bacteria, out of which Sporosarcina pasteurii and Sporosarcina ureae, were also found to be promising choice besides Bacillus subtilis subsp. spizizenii. Both zeolite and pumice turned out to be effective protective vehicles. He also reported that optimum compressive strength was achieved by maintaining the amount of calcium lactate at 2% of cement mass weight. Mohammad et al. (2016) investigated the effect of Sporosarcina pasteurii inhabited in a soil. In their analysis, optimizing of enzyme protein created by Sporosarcina pasteurii bacterium was targeted to attach the sand particles and stiffen them by the assembly of $CaCO_3$ precipitation. Bacterium was cultivated aerobically in a culture medium containing yeast extract, lacto peptone and urea at the temperature of 25°C. Different conditions of cultivation with Urea concentration calcium chloride and pH were examined to obtain optimal results. The optimum condition for the stiffening of sand grains was obtained in the presence of urea (20 g/L), calcium chloride (30mM) and the pH of 9. Their test results of indicated that the sand calcification via urease enzyme produced by Sporosarcina pasteurii may well be utilized for the hardening of the sand only if the efficient factors for calcite precipitation could be optimized. Jing Xu and Wu (2014) carried a research on the mechanical behaviour of concrete by incorporating a non-ureolytic bacteria-based healing agent at multi scale levels. In their research, four-point bending and ultrasonic pulse velocity (UPV) was conducted. Macro scale mechanical measurements were performed to evaluate the mechanical properties of concrete during the processes of damaging and healing. In addition, nano indentation as a nano scale mechanical test was carried out to investigate the nano-mechanical properties of mineral precipitation and its bonding to concrete. Their results showed that the type of calcium source has a profound impact on healing effectiveness. Ultrasonic pulse velocity and four-point bending tests demonstrated that the highest healing ratio and recovery ratio of flexural strength and modulus were obtained by the two-component self-healing with calcium glutamate. 3.25% water absorption (minimum). Achal et al. (2011) carried a research work on bio concrete and observed that a considerable reduction in water permeability in cement mortar cubes by using Sporosarcina pasteurii. It is believed that this lower permeability of bacteria incorporated cubes may be due to the presence of a denser interfacial zone formed between the aggregate and the concrete matrix by calcite precipitation. Due to the better compaction and closing of pores at the top, they observed more water permeability at the sides than that at the top. This gives a more believable insight on the influence of microbial calcite on the permeability of concrete. Henk Jonkers (2010) conducted a research in bio concrete by integrating genus bacillus bacteria along with calcium lactate as nutrient in to concrete. The bacteria and precursor compound were immobilized into porous expanded clay and added into a wet concrete. This method of bacteria addition into concrete mass substantially prolonged the life-time. The viability experiments showed that after 6 months concrete incorporation, no loss of bacteria viability was observed. He concluded that the bacteria gave adequate crack repair healing ability since cracks of all six bacterial specimens were completely sealed resulting in no measurable permeability (percolation of 0 ml water / h).

5. MATERIALS USED

5.1 Bacteria

Bacillus subtilis with strain number MTCC 1427 was purchased from MTCC, (Chandigarh) and used as the bacteria for this research work.

5.2 Mineral Substrates

For the infused bacteria to precipitate limestone, a suitable mineral substrate has to be provided along with bacteria during casting of the concrete [1]. Therefore calcium lactate,urea and yeast extract were used as mineral substrate for the bacteria. They were purchased from Qualitech Fine Chemicals (India).

5.3 Protective Vehicles

In order to increase the bacterial activity and viability, the bacterial cells and the mineral substrates could not be directly added to concrete (Thijsse and Jonkers 2010). Therefore pumice, gelatin capsules and porous expansive clay were adopted as carrier materials.

5.4 Aggregation

Local granite of size 10 mm was used as coarse aggregate and river sand was used as fine aggregate passing 75µ sieve. They were obtained from RIMT University concrete laboratory.

5.5 Fibre

Polyvinyl Alcohol fiber (PVA) fibers with a length of 8 mm and a diameter of 40 mm was purchased from Yarn Guru India Inc (Rajasthan) and used for ECC mix. The density and tensile strength of the PVA fiber was 1,300 kg/m3 and 1600 MPa respectively. The fiber surface was coated with 1.2% oil by weight to reduce the fiber matrix chemical and friction bond.

5.6 Admixtures

ADVA® Cast 575 conforming to ASTM C 494 type F was obtained from geo concrete laboratory (Chandigarh) and used as High Range Water Reducing Admixture (HRWRA) in order to increase the workability of the ECC mix.

6. METHODOLOGY

6.1 Bacteria Culturing and Growth

A media consisting of Nutrient broth (Peptone: 5 g, yeast extract: 1.0 g, Urea: 15.0 g, $NaCl_2$: 5.0 g and Distilled water: 1.0 L) was prepared, and the freeze dried culture of Bacillus subtilis MTCC-1427 was grown at a pH 7 as recommended by MTCC. The yeast extract medium was first autoclaved for 20 min at 120°C and then the sterilized urea solution was added, which was obtained by means of filtration through a sterile 0.2-µm Millipore filter. The final concentrations of yeast extract and urea in the growth medium were 20g/L each. The media was supplemented with 10 mg/L of $MnSO_4$ x H_2O to enhance the sporulation. The culture was incubated aerobically at 30°C for 24hrs with shaking at 250 rpm. Growth and sporulation yield of bacteria was checked regularly and quantified by light microscopic analysis. The culture was streaked on nutrient agar plate and kept at room temperature and were re suspended in 9g/L $NaCl_2$ solution. The concentration of bacterial cells in the suspension was kept at 10^4, 10^6 and 10^8 cells/ml. In total 1.5 litres of bacterial solution was prepared. Figure1 is showing the characteristics of the culture.

Fig 1: *characteristics of the culture.*

6.2 Testing High Heat and pH Resistance of Bacteria

The high heat and pH resistance of the bacteria spores were tested by passing a heat of 65^0C and pH of 11 through the bacteria solutions for 65 minutes. The culture was incubated at 30°C on a shaker at 250 rpm for 48 hrs. Natural samples were suspended in 8 g of $NaCl_2$ / L and plated on an agar containing the required growth medium specified by the MTCC. The diluted cell was plated on the surface of nutrient agar plates of Bacillus subtilis to obtain viable count. The heat resistant spore counts were obtained by plating after heating at 60^0C for 15 min and 45 min.

6.3 Testing the CaCO3 Producing Capacity of Bacteria

The calcium carbonate precipitate formation ability of the bacteria was tested by suspending natural sample in a sterile solution of 9 g of $NaCl_2$ per litre and plated on an agar containing 3 g /l of Nutrient Broth; 20 g/l of Agar; 2.12 g/l of $NaHCO_3$; 10 g/l of NH4Cl and 30 mM $CaCl_2$ $2H_2O$. Crystal formation was observed after 7days and 16 days.

6.4 Techniques for Protecting and Adding the Bacteria cell (Healing Agent) into Concrete

In order to well increase the time period and associated practicality of concrete incorporated bacterium, the effectiveness of microorganism reproductive structure and the required organic bio mineral precursor compound (calcium lactate), three methods of adding and protecting the bacterial cell agents into the concrete was adopted to compare their effectiveness in self-healing of concrete. These methods are: immobilization of bacterial cell in pumice, immobilization of bacteria cell in porous expansive clay particles and stiffen sand encapsulation application.

6.41. Immobilization of Bacteria cells in Pumice

300 grams of sterilized pumice powder was poured into 500ml volumetric flasks and mixed with the three concentrations of bacterial solutions (10^4, 10 and 10^8 cells/ml). 50 ml of each the bacterial solution was mixed with 30 g of pumice. The flasks were placed on a shaker at 100 rpm for one hour. After that, the mixture (bacterial cells and pumice) was then incorporated into concrete mixes. Before this, nutrients such as calcium lactate was used as calcium carbonate precursor, urea as urease enzyme source and yeast extract was added as nutritional carbon and nitrogen source for bacteria. These ingredients were autoclaved separately and mixed afterwards to avoid precipitation. The final pH of the media was adjusted to 8 in order to avoid possible chemical precipitation of calcium carbonate. Part of these nutrient solutions was used as mixing water for the concrete. Figure 2 is showing bacterial cell solution immobilized in pumice.

Fig 2*: Bacterial cells immobilized in pumice.*

6.42 Stiffen Sand Encapsulation Application

This method was achieved by encapsulating stiffen bacterial incorporated sand into a gelatin capsules and mix with concrete mixtures. The healing agent present in these capsules will react with water and the calcium compound present in the concrete to form calcium carbonate precipitate to repair the cracks that will be developed in the concrete specimen. Firstly, 100 grams of washed sand passing 75µ sieve was poured into 250mL Erlenmeyer flasks and sterilized with autoclave 20 min at 120°C and mixed with 100 ml of bacteria solution at concentration of 10^6 cells/ml. The flask was placed on a shaker at 100 rpm for one hour and the mixture was allowed to stay for 30 minutes to dry. The dried mixture was then loaded into the gelatin capsules and infused into the concrete right after their preparation to avoid pre dissolving of gelatin capsule. Figure 3 is showing the encapsulation of stiffen sand into gelatin capsule. Sterilized nutrients solution such as calcium lactate was used as calcium carbonate precursor, urea as urease enzyme source and yeast extract was added as nutritional carbon and nitrogen source for bacteria.

Fig 3: *Encapsulation of stiffen sand immobilized bacteria cell into gelatin capsule.*

6.43 Immobilization of Bacteria cells in Porous Expansive Clay Particles

100g of oven-dried clay sample at 120oC for 48 hours was mixed 30 ml of distilled water and rolled to form partially wet round ball particles of average size of 10 mm and weight of 0.87 g. 100 ml of bacteria solution at concentration of 10^6 cells/ml was then loaded into the partially wet rounded ball particles by injection. Sterilized nutrients solution such as calcium lactate was used as calcium carbonate precursor, urea as urease enzyme source and yeast extract was added as nutritional carbon and nitrogen source for bacteria. They were used as part of the mixing water. Before applying into the concrete mixes, the loaded expanded clay particles were oven-dried until no further weight loss due to water evaporation was observed (one week at 40ºC). The average weight after oven dried was 0.80 g. The loaded particle served as a partial replacement of the coarse aggregate weight.

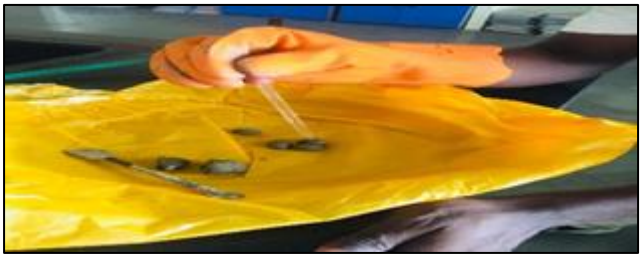

Fig 4: *Injecting bacterial solution (healing agent) into rounded expansive clay particles.*

Fig 5: *Oven dried loaded expansive clay particles with bacteria cell (healing Agent).*

6.5 Specimen Preparation and Testing

6.51 Preliminary Tests

Specific gravity and sieve analysis test on coarse and fine aggregate were carried out by the specifications conforming to IS: 1199-1959. 6.52 Mixing and Preparation of Specimens: The mortar and concrete specimen were prepared by using M25 grade with a mix ratio of 1:1:2 and 1:1.32:2.5 and water/cement ratio of 0.5 whiles the ECC specimens were prepared by using M40 concrete grade with water/binder ratio of 0.33 and sand/binder ratio of 1: 0.84. During the mixing stage the mixing water was fully and partially replaced by a nutrient solution. For all the bacteria specimens, the dry materials was mixed first, and after that 75% of the Nutrient solution and 100% of the bacterial solution was added and mixed 2 minutes. The remaining 25% of nutrient solution and admixtures were added until a consistent and uniform colour was observed. After the mixing, the specimens were casted by using mould size of 70.6 x 70.6 x 70.6 mm, 15 x 15 x15 cm and prism of dimension 10 x 10 x 50 cm and 50 mm x 75 mm x 360 mm . All the specimens were cured for 7, 14, and 28 days. Figure 6 to 9 describes how the bacterial solution and healing agents were mixed with the concrete and Table 1 to 3 represent details of the mix design by a cubic meter.

Table 1: *Mix Design by volume (Kg/m3) for mortar*

Mix	*Specimen*	*Conc. of BS (cell/ml)*	*Capsules*	*BS*	*NS*	*Pumice*	*Cement*	*Sand*	*Water*
1	*Control*	*-*	*-*	*-*	*-*	*-*	*554*	*554*	*277*
2	*BS + NS + pumice*	*10^4*	*-*	*46.2*	*138.5*	*92.3*	*544*	*544*	*-*
3	*BS + NS+ pumice*	*10^6*	*-*	*46.2*	*138.5*	*92.3*	*544*	*544*	*-*
4	*BS + NS+*	*10^8*	*-*	*46.2*	*138.5*	*92.3*	*544*	*544*	*-*

	pumice								
5	*Capsules + NS*	*10^6*	*163.2*	*-*	*138.5*	*-*	*544*	*380.8*	*138.5*

BS: bacteria cell solution
NS: nutrient solution, the amount of nutrient (yeast extract, urea, and calcium lactate) was taken as 0.2 %, 2%, 2% respectively of the cement weight.
Sand stiffen capsule were replaced by 30% of sand weight.
Grade: M25. Ratio 1:1. Water/cement ratio = 0.5.

Table 2: *Mix Design by volume for ECC (Kg/m^3)*

Mix	*Specimen*	*BS*	*NS*	*Capsule*	*Pumice*	*Cement*	*FA*	*Sand*	*PVA*	*HRWRA*	*Water*
1	*Control*	*-*	*-*	*-*	*-*	*324.17*	*138.93*	*552*	*24.72*	*9.26*	*152.8*
2	BS+NS + Pumice	25.47	76.4	-	50.93	324.17	138.93	552	24.72	9.26	-
3	Capsule + NS	-	76.4	165.6	-	324.17	138.93	386.4	24.72	9.26	76.4

BS: bacteria cell solution at 10^6 cells/ml.
FA: Fly Ash at 30% replacement by cement weight.
NS: nutrient solution, the amount of (yeast extract, urea, and calcium lactate) was taken as 0.2 %, 2%, and 2% respectively of the binder's weight.
PVA: Polyvinyl Alcohol Fibre.
HRWRA: High Range Water Reducing Admixture at 2% of binder's weight.
Grade: M40. Water/Binder ratio = 0.33 Sand : binder ratio = 1:0.84
Capsule = 30% of binders weight.

Table 6*: Mix Design by volume (Kg/m^3) for concrete specimens.*

1	*Control*	*-*	*-*	*-*	*-*	*-*	*440.96*	*-*	*584.3*	*1105.9*	*220.48*
2	*BS+NS + Pumice*	*36.75*	*10^4*	*110.24*	*73.49*	*-*	*440.96*	*-*	*584.3*	*1105.9*	*-*
3	*BS+NS + Pumice*	*36.75*	*10^6*	*110.24*	*73.49*	*-*	*440.96*	*-*	*584.3*	*1105.9*	*-*
4	*BS+NS + Pumice*	*36.75*	*10^8*	*110.24*	*73.49*	*-*	*440.96*	*-*	*584.3*	*1105.9*	*-*
5	*Capsule + NS*	*-*	*10^6*	*110.24*	*-*	*175.29*	*440.96*	*-*	*409.0*	*1105.9*	*110.24*
6	*LP + NS*	*-*	*10^6*	*110.24*	*-*	*-*	*440.96*	*22.12*	*584.3*	*1083.8*	*110.24*
7	*LP + NS*	*-*	*10^6*	*110.24*	*-*	*-*	*440.96*	*55.30*	*584.3*	*1050.6*	*110.24*

BS: bacteria cell solution
Water/Cement = 0.5. Ratio of cement/sand/ gravel = 1:1.32:2.5.
LP: loaded expansive clay particles, they were replaced by 5% and 2% of aggregate weight for mix 6 and 7 respectively.
NS: nutrient solution, the amount of (yeast extract, urea, and calcium lactate) was taken as 0.2 %, 2%, and 2% respectivelyof the cement weight.
Sand stiffen capsule were replaced by 30% of sand weight.

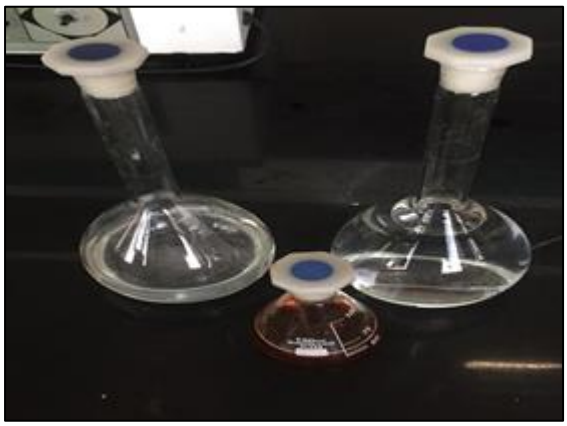

***Fig 6**: Nutrient solution used as mixing water.*

***Fig 7:** Pumice immobilized bacterial cell solution in sand and cement mixture.*

***Fig8:** Mixing stiffen sand capsules with sand and cement.*

***Fig 9:** mixing oven dried loaded expansive clay particles with concrete.*

6.53 Testing the effect of Healing Agents on the Compressive strength of Specimens

The effect of the healing agents on the compressive strength of the specimens (mortar, concrete and ECC) was done by carrying a compression test which conformed to IS: 516-1959 requirements. The compressive strength test for all the non-bacterial and bacterial incorporated hardened concrete of cube size 15x15x15 cm and 70.6x 70.6x70.6 mm for mortar, concrete and ECC were determined at the end of 7, 14 and 28 curing days. The test was conducted in the laboratory, as per IS 516-1959 on a digital compression testing machine of 3000 KN capacity. For each specimen, the peak load at the time of failure of specimen was recorded to compute the compressive strength.

6.54 Testing the effects of Healing Agents on Flexural Strength of concrete

The effects of the healing agents on the flexural strength of concrete was investigated by carrying out third point loading testing test which conformed to IS 516-1959 requirement on specimen by using the universal testing machine. The third point loading testing test for all the non bacterial and bacterial incorporated hardened concrete of cube size 10x 10 x 50 cm were determined at the end of 7, 14 and 28 curing days. During the test, the specimens were removed from the water tank and by using a marker pen, a center to center distance of 13.3 cm was marked on the specimen for the positioning of the rollers. After that, the wet specimen was placed and aligned to the loading machine. The machine was switched on and loading was applied at a rate of 180 kg/min. The load at which the specimen failed was recorded. The failed specimen was removed from the machine and the failure point depth and the distance between the line of fracture and nearer support measured from the center line was recorded to compute for modulus of rapture.

6.6 Investigating Self Healing Efficiency of Specimens

The one of the main objectives of this work was to investigate the efficiency of the incorporated bacteria self healed specimens. This was achieved by measuring the strength regain using four point bending test, Permeation properties such as Sorptivity test and characterizing mineral constituents of the material formed in the crack areas by using XRD test.

6.61 Investigating Self Healing Efficiency by using Sorptivity Test

The efficiency of the crack healed specimens due to the incorporated bacteria was tested by measuring the capillary rise absorption rate. The test was carried out based on ASTM C1585. 6 cylinder specimens were prepared with diameter and thickness of 100 mm and 50 mm. In the beginning, cylindrical specimens of diameter 100 mm and height 200 mm was prepared from which 50 mm thick discs were extracted by using a diamond blade saw from the central portion of the cylinder specimen. After 7 days of curing, all the specimens were pre-loaded by tensile splitting test so as to produce cracks. The non bacteria specimens served as control. Right after the crack preloading, Sorptivity test was conducted for first round by drying the specimens an oven at 50^0C for 72hrs and after that, their masses was recorded before each test. The sides of the specimen were sealed with an epoxy coating in order to guarantee one directional flow through the specimen and one surface of the specimen was allowed to be

in contact with water, with the depth of water between 1 and 3 mm. The increase in mass of a cylindrical specimen (100 x50 mm) when permitted to absorb water by capillary suction at a regular time interval of 1, 5, 10, 15, 20, 30 minutes for 7, 28 and 56 days was recorded. Immediately after the 7 days measurement, the test specimens were cured in water for 28 and 56 days for self healing for conducting the second and third round of test.

Sorptivity was determined as follows,

$I = S.t^{1/2}$ therefore $S = I/ t^{1/2}$

Where; S= sorptivity in mm, t= elapsed time ($sec^{-1/2}$).

$I = (\Delta w / A_0 D)$

Δw= change in weight = W2-W1

W1 = Oven dry weight of cylinder (g). W2 = Weight of cylinder after 30 minutes capillary suction of water (g).

A_0 = surface area of the specimen through which water penetrated.

D = density of water.

6.62 Investigating Mineral Constituent of Crack Healed Specimens by XRD Test

The element present in the cracked healed ECC specimen was investigated by carrying out a non-destructive X-ray powder diffraction technique to unraveling the structure of the specimen in thin film forms by using X Pert PRO apparatus. The test was carried out by collecting and grinding Calcium carbonate layer formed near the crack area and mounting them on to a glass fiber filter. XRD-spectra were obtained using an X'Pert PRO diffractometer with an X-ray tube of PW3373/10Cu LLF DK 400324 shutter (45 kV and 40 mA) and scanning from 3 to 60˚ 2 θ. The components of the sample were identified by comparing them with standards established by the International Centre for Diffraction data. X-ray diffraction is based on the fact that, in a mixture, the measured intensity of a diffraction peak is directly proportional to the content of the substance producing it. The samples for X-ray diffraction analysis were prepared in powdered form.

6.63 Measuring Flexural Strength Regain of crack Healed ECC

The flexural strength regain of ECC specimens were tested by carrying out a four point bending test on 28 days cured specimens. After that, the failed specimens were cured for 56 days for crack healing and testing of flexural strength regain. Four point bending test was conducted on ECC specimen with dimensions of 50 mm x 75 mm x 360 mm at a loading rate of 0.125 mm/min using MTS machine. The full span length was 300 mm with a middle span of 100 mm was marked for positioning of rollers. During the test, the load and the mid span deflection were recorded. Initially, all specimens were preloaded up to a deflection of 50% of the maximum deflection of the failed sample. When this deflection was reached the load was released, after which the specimens were removed and cured in water until 56 days to test the self-healing. The initial preloading which was done after 28 days was to deliberately introduce a number of micro-cracks. The crack width was measured by crack scope. Specimens with and without bacteria based agent were kept in separate water containers to avoid cross contamination. Reference sound specimens from each mixture were cured under the same conditions as the pre-loaded specimens and were tested after 56 days. After 56 days of healing in water, reloading of all specimens under four-point bending test was done to characterize residual mechanical behavior of bacteria based ECC after self-healing. In order to roughly estimate the preloading deflection, the reference sample was tested until final failure to derive the flexural stress-deflection relation. Deflection of 1 mm was selected since it is approximately equal to deflection corresponding to 50% of ultimate strength.

7. RESULTS AND DISCUSSIONS

7.1 Sieve Analysis

Grading test was done to determine whether the aggregates to be used for the concrete mixes were of nominal or variable sizes. Figure 10 and 11 represent the graph of the particle size distribution of coarse aggregates and fine aggregate and table 4 and 5 indicate grading test results. Aggregates with too much fines may affect the workability of concrete mixes. From fig. 10, the grading characteristic of the sand is that of a well graded soil with about 0.05% passing the 75μm sieve. The coarse aggregate is also uniformly graded as indicated figure 11. The specific gravity of the coarse aggregates and sand is 2.68 and 2.61 respectively.

7.2 Workability of the Mixes

The mixing water was partially and fully replaced by the nutrient solution in some of the mixes to ensure effective growth of bacteria cell inside concrete. The workability of fresh concrete was tested by taking measurement of the slumped concrete in centimetres. The test results in table 6 and 8 show that all the mixes for mortar and concrete specimens fall within a range of high workability, thus the mortar and the concrete mixes has an adequate mobility and stability. The concrete was easily placed into the steel moulds without any difficulties in terms of the mobility and placement. From table 7, the measured slump height for all the ECC mixes did not fall within the range of high workability, but the situation was rectified by the presence of HRWRA.

***Table 4**: Grading of the Fine aggregates*

SAMPLE	*BEFORE WASHING G.607.6*			
AGGT. SIZE	*AFTER WASHING G.585.7*			
SAMPLE: Sand				
Sieve Size (mm)	*Wt. Retained*	*% Retained*	*% Passing*	
13.2	*0*			
9.5	*0*		*100*	*100*
4	*30.4*	*5.19*	*94.81*	*95*
2	*69.6*	*11.88*	*82.93*	*83*
1	*150.2*	*25.64*	*57.28*	*57*
0.3	*261.7*	*44.68*	*12.60*	*13*
0.15	*63.8*	*10.89*	*1.71*	*2*
0.075	*9.7*	*1.66*	*0.05*	*0*
pan	*0.3*	*0.05*		
TOTAL	*585.7*			

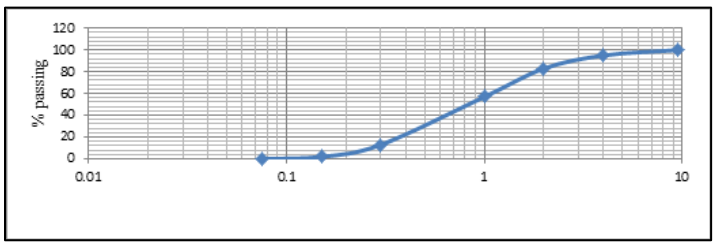

Fig 10: *A graph of particle size distribution of sand.*

Table 5: *Grading of the coarse aggregates*

Total Weight of sample 1166.5				
Weight retained on 75μ sieve after washing 967.3				
Weight passing 75 μ sieve				
Sieve Size No (mm)	*weight Retained*	*% retained*	*Total passing %*	
			100	*100*
14	*19*	*1.96*	*98.04*	*98*
10	*334.3*	*34.56*	*63.48*	*63*
6.3	*458.5*	*47.40*	*16.08*	*16*
4	*136.2*	*14.08*	*2.00*	*2*
2	*17.2*	*1.78*	*0.22*	*0*
1	*0.4*	*0.04*	*0.18*	*0*
0.3	*0.3*	*0.03*	*0.14*	*0*
0.15	*0.3*	*0.03*	*0.11*	*0*
0.075	*0.6*	*0.11*	*0.00*	*0*
Pan	*0.5*	*0.05*	*0.00*	*0*
Total	*967.3*			

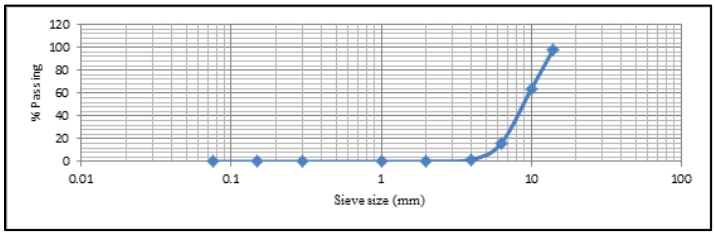

Fig 11: *A graph of particle size distribution of coarse aggregates.*

Table 6: *Testing Workability of Fresh Mortar*

M1 (Control)	*125*
M2 (Pumice + Bacteria + Nutrient)	*105*
M3 (Stiffen sand capsule +) Nutrient	*100*

Table 7: *Testing Workability of Fresh ECC.*

Mix	*Slump height (cm)*
M1 (Control)	*80*
M2 (Pumice + Bacteria + Nutrient)	*74*
M3 (Stiffen sand capsule + Nutrient)	*60*

Table 8: *Testing Workability of Fresh Concrete*

Mix	Slumpheight (cm)
Mix	*Slump height (cm)*
M1 (Control)	*130*

7.3 Effects of Healing Agents on Compressive strength

The effects of the healing agents, their concentrations (10^4, 10^6 and 10^8cells/ml) and methods of protecting and adding them into concrete on the compressive strength of the entire specimens (mortar, concrete and ECC) was investigated. Pumice immobilized bacterial cell at a concentration of 10^6 cells/ml attained optimum compressive strengths of 32.70 and 31.69 N/mm^2 for concrete and mortar at the end of 28 curing days, followed by 10^8cell/ml and 10^4cell/ml (see table 9 and 10, fig 12 and 13). From fig 12 and 13, it was realized that compressive strength start increasing from 10^4cell/ml to an optimum concentration at 10^6cell/ml and begins to decrease at 10^8cell/ml along all curing days. This indicates that, at higher concentration of bacterial cells, the compressive strength of concrete declines. So in order to obtain a higher compressive strength and without compromising the effectiveness of self-healing capacity of concrete, the concentration of bacterial cell has to be kept around 10^6cells/ml. The reduction in compressive strength at 10^8cell/ml along all the curing ages is due the high amount nutrient broth compounds and bacterial cells. Among all the three methods of protecting and adding the bacterial cell into the concrete (stiffen sand capsules application, pumice immobilized bacterial cell and loaded expansive clay particles), stiffen sand capsules application attained the highest compressive strength in all the specimens (mortar, concrete and ECC) at the end of 28 days of curing, followed by pumice immobilized bacterial cell and the least one, loaded expansive clay particles (See fig. 14, 16, 17 and table 9 to 15). Stiffen sand capsules application attained compressive strength of 32 N/mm^2, 33.60 N/mm^2, 60.10 N/mm^2 for mortar, concrete and ECC respectively at end of 28 days curing (see table 11, 12 and 15). This high compressive strength is due to the bacteria releasing urease enzyme which hydrolyses urea to ammonium and bicarbonate ions, in the presence of calcium ions, to produce calcium carbonate precipitate which connect sand particles to each other. This impacted some stiffens property to the concrete which eventually increased the compressive strength. Pumice immobilized bacterial cell attained compressive strength of 31.69 N/mm^2, 32.70 N/mm^2, 60.0 N/mm^2 for mortar, concrete and ECC respectively at the end of 28 days of curing (see table 9, 10 and 14). These values were very close to that of sand stiffen capsules and this due to the fact that, the pumice gave an optimal protection to bacteria cells inside the concrete to react with the calcium compound to form calcium carbonate precipitate which filled the voids inside the concrete and densified it. Loaded expansive clay particles attained the least compressive strength among the three methods of adding and protecting the bacterial cell inside the concrete in all the specimens (see table 13 and fig 16). Also the compressive

strength started reducing at 5% replacement of clay particles by the coarse aggregate weight (See fig15). The clay particles provided effective protective mechanism for bacteria cell inside the concrete but the clay content compromised the compressive strength.

7.4 Percentage Increase in Compressive Strength

The increase in compressive strength of each of the specimens was expressed in percentage by using their respective control specimens as the reference factor throughout all the curing days. Stiffen sand capsules attained highest increase in compressive strength of 35.2% and 29 % in concrete and mortar at the end of 28 days of curing whiles Pumice immobilized bacteria cells also increased the compressive strength of specimens by 31.59% and 27.78% for concrete and mortar at the end of 28 days curing (see table 9 to 13). In ECC mixes, stiffen sand capsule was able to increase the compressive strength by 18.11% by the end of 28 days curing and pumice immobilized bacterial also raised the compressive strength to 18.32% at the end of 28 days curing (see table 14 to 15). Loaded expansive clay particle had the least increase in compressive strength. 2% replacement of loaded clay particles by the coarse aggregate weight was able to raise the compressive strength of concrete by 0.20 whiles the 5% replacement of the loaded clay particles by the coarse aggregates weight actually decreased the compressive strength by 7.44% by end of 28 days of curing (see table 13).

Table 9: *Compressive strength of Pumice Immobilized Bacterial Mortar*

Specimen : Pumice + Bacterial Solution + Nutrient Solution								
Curing Age	*Compressive strength (N/mm^2)*				*% increase in compressive strength*			
	Control	*10^4 (cells/ml)*	*10^6 (cells/ml)*	*10^8 (cells/ml)*	*10^4 (cells/ml)*	*10^6 (cells/ml)*	*10^8 (cells/ml)*	
7 days	*16.77*	*18.50*	*20.80*	*19.89*	*10.32*	*24.03*	*18.60*	
14 days	*22.99*	*25.90*	*28.42*	*26.90*	*12.66*	*23.62*	*17.01*	
28 days	*24.80*	*26.80*	*31.69*	*29.89*	*8.10*	*27.78*	*20.52*	

Table 10: *Compressive strength of Pumice Immobilized Bacterial Concrete*

Specimen : Pumice + Bacterial Solution + Nutrient Solution							
Curing Age	*Compressive strength (N/mm^2)*				*% increase in compressive strength*		
	Control	*10^4 (cells/ml)*	*10^6 (cells/ml)*	*10^8 (cells/ml)*	*10^4 (cells/ml)*	*10^6 (cells/ml)*	*10^8 (cells/ml)*
7 days	*16.80*	*18.90*	*20.90*	*19.90*	*12.50*	*24.40*	*18.45*
14 days	*23.10*	*26.00*	*28.86*	*26.85*	*12.55*	*23.80*	*16.23*
28 days	*24.85*	*27.90*	*32.70*	*30.90*	*12.27*	*31.59*	*24.35*

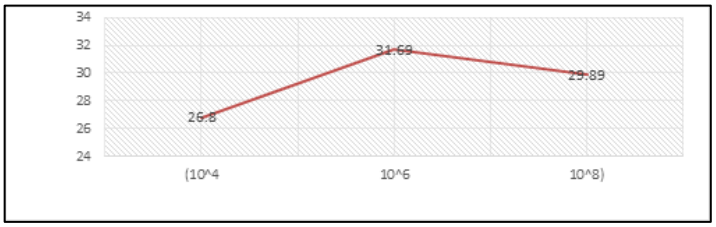

Fig 12: *Compressive strength of mortar specimens at 28 days* curing versus concentration.

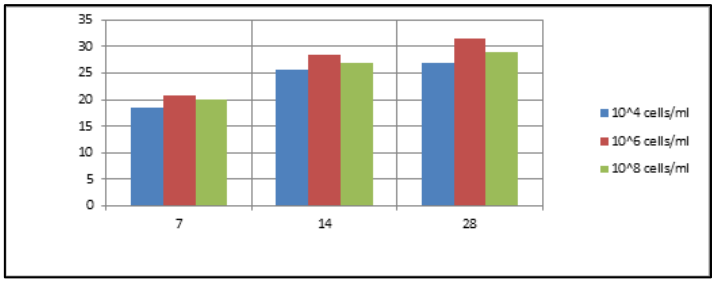

Fig 13: *Compressive strength of concentrations of bacterial cell in mortar versus curing age.*

Table 11: *Compressive strength of stiffen sand capsule Mortar*

Specimen : stiffen sand capsules + Bacterial Solution + Nutrient Solution			
Curing Age	*Compressive strength (N/mm^2)*		*% increase in Compressive strength*
	Control	*Bacteria*	*Bacteria*
7 days	*16.77*	*21.60*	*28.80*
14 days	*22.99*	*29.40*	*27.88*
28 days	*24.80*	*32.00*	*29.00*

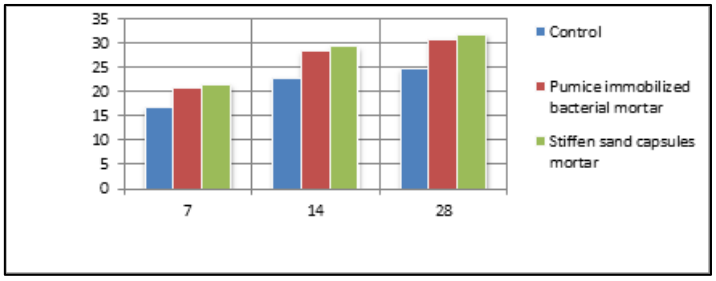

Fig 14: *Compressive strength of mortar specimens versus curing age.*

Table 12*: Compressive strength of stiffen sand capsule concrete*

Specimen : stiffen sand capsules + Bacterial Solution + Nutrient Solution			
Curing Age	*Compressive strength(N/mm^2)*		*% increase in compressive strength*
	Control	*Bacteria*	*Bacteria*
7 days	*16.80*	*22.00*	*30.95*
14 days	*23.10*	*28.90*	*25.11*
28 days	*24.85*	*33.60*	*35.20*

Table 13: *Compressive strength of loaded expansive clay particles concrete*

Specimen : loaded clay particles + Nutrient Solution				
Curing Age	Compressive strength(N/mm^2)			% increase in compressive strength
	% of clay particles			% of clay particles
	0	2	5	2
7 days	16.80	17.10	16.40	1.79
14 days	23.10	23.40	22.80	2.12
28 days	24.85	24.90	23.00	0.20

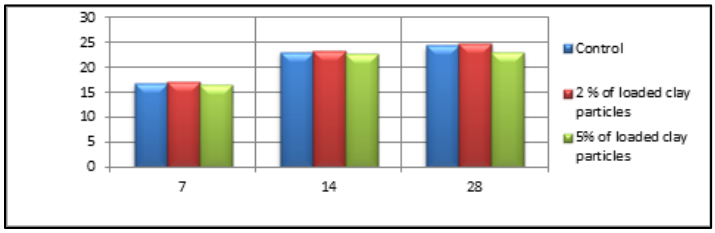

Fig 15: *Compressive strength of percentage of loaded clay particles concrete versus curing age.*

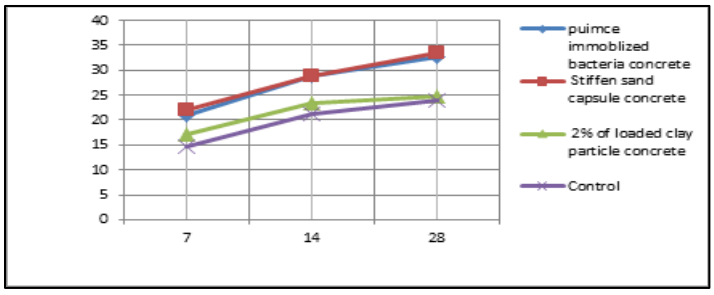

Fig 16: *Compressive strength versus curing age of concrete specimens.*

Table 14: *Compressive strength of pumice Immobilized Bacterial ECC*

Curing Age	Compressive strength (N/mm^2)		% increase in Compressive strength
	Control	Bacteria	
7 days	38.80	44.21	13.94
14 days	46.70	50.54	8.22
28 days	50.80	60.00	18.11

Table 15: *Compressive strength stiffen sand capsule ECC*

Curing Age	Compressive strength (N/mm^2)		% increase in Compressive strength
	Control	Bacteria	
7 days	38.80	45.00	15.98
14 days	46.70	52.80	13.06
28 days	50.80	60.10	18.32

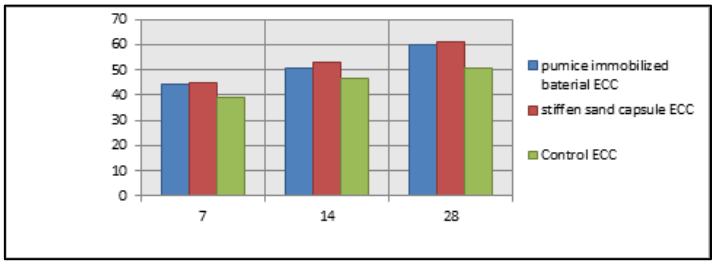

Fig 17: *Compressive strength of ECC specimens versus curing age.*

7.5 Effects of Healing Agents on Flexural strength of Concrete

The healing agents showed a positive reflection on the flexural property of the concrete. Stiffen sand capsule attained the highest flexural strength of 4.78 N/mm^2 at end of 28 days curing, followed by pumice immobilized bacterial cell which attained 4.00 N/mm^2 and the least, loaded clay particles which also attained a flexural strength of 3.36 N/mm^2 (see fig 18 and table 16 to 18). The increase in flexural strength in stiffen sand capsule is due to the fact that the urease enzyme from the bacteria in the presence of ammonia and calcium ion formed a calcium carbonate precipitate which is known as limestone in the sand particles which initiated confined structure with the concrete components to resist the bending stress for period of time. Similar situation occurred in the pumice immobilized bacteria cell. For the loaded expansive clay particles concrete specimens, the healing agent was protected to pave way for the strength property of the concrete to be improved but the clay component compromised the flexural strength. Stiffen sand capsules raised the flexural strength of concrete by 36.9% and 14.61% by the pumice immobilized bacteria at the end of 28 days curing (see table 16 to 18 and fig 19). Loaded expansive clay particles at 2% replacement by the coarse aggregate weight raised the flexural strength of concrete by 2.87 % by the end of 28 days of curing whiles the 5% replacement of clay particle by the coarse aggregate weight reduced the flexural strength by 3.72% (seetable18).

Table 16*: Flexural strength of stiffen sand capsule concrete*

Curing Age	Flexural strength (N/mm^2)		% increase in Flexural strength
	Control	Bacteria	Bacteria
7 days	2.89	3.56	23.20
14 days	3.37	3.96	17.50
28 days	3.49	4.78	36.96

Table 17: *Flexural strength of pumice Immobilized Bacterial concrete*

Curing Age	Flexural strength (N/mm^2)		% increase in Flexural strength
	Control	Bacteria	Bacteria
7 days	2.89	3.40	17.64
14 days	3.37	3.74	10.98
28 days	3.49	4.00	14.61

Table 18: *Flexural strength of loaded expansive clay particles concrete*

Flexural strength (N/mm^2)			% increase in Flexural strength	
% of clay particles			% of clay particles	
0	2	5	2	5
2.89	3.00	2.83	3.80	-0.02
3.37	3.40	3.34	0.89	-0.89
3.49	3.59	3.36	2.87	-3.72

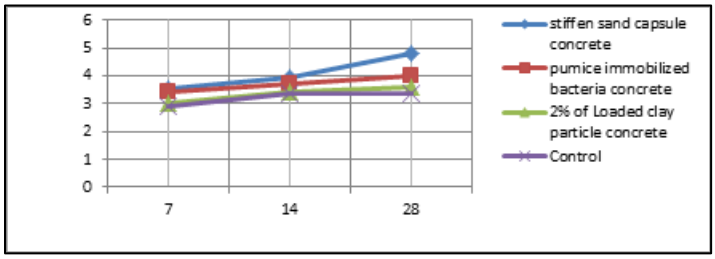

Fig 18: *Flexural strength versus curing age of concrete specimens.*

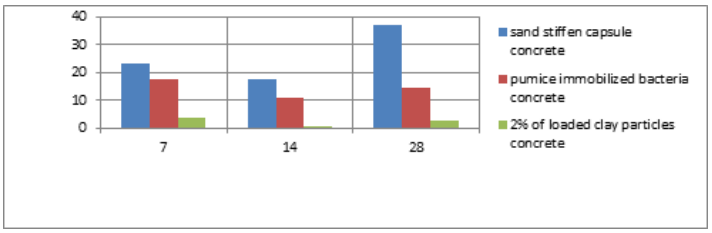

Fig 19: *Percentage increase in flexural strength versus curing age of concrete specimens.*

7.6 Flexural Strength Regain of cracked Self Healed ECC

One of the ways to investigate the efficiency of the self healed specimen was done by measuring the flexural strength regain (preloading at 28 days and reloading after 56 days self healing) using four point bending test. Table 19 to 21 and fig 21 to 23 describe the flexural behaviour at preload level and recovery after healing. All the ECC mixes showed good deflection capacity. Since the structural application of ECC requires high deformation and energy dissipation capacity, its deflection capacity is of major concern [2]. The deflection capacity is defined as the deflection that corresponds to the maximal flexural stress. Self healed pumice immobilized bacteria specimen at 56 healing days reloading test, attained a flexural strength of 11.9 N/mm^2 with a 32.22 % strength recovery to the reference 56 days sound specimen (see table 19 to 20 and fig 20). This result indicates that efficient self healing repair recovery has occurred to the specimen due to the production of $CaCO_3$ precipitate by the bacteria cell to seal the cracks. Also self healed stiffen sand capsule ECC specimen at 56 healing days reloading test, attained a flexural strength of 11 N/mm^2 with a 23.60% strength recovery to the reference 56 days healed sound specimen (see table 19 to 20 and fig 20). This indicates that the same situation in the pumice immobilized bacteria ECC also occurred here.The cracked control ECC specimen attained a flexural strength of 11.5 N/mm^2 at end of the 56 healing days reloading test and the reference sound specimen also attained 12.01 N/mm^2 at end of the 56 healing days reloading test (see table 19). This indicates that there was no strength gain recovery with respect to the reference sound specimen as compare to the bacteria based ECC specimens. Figures 21 to 23 show the flexural stress - deflection curve of the reloading tests (flexural test conducted after healing of the cracked specimen). The deflection capacity of the ECC mix with bacteria based healing agent appeared to be improving (see table 21). The improvement in deflection capacity might be due to the presence of fiber particles with bacteria based healing agent. It can be explained that, the $CaCO_3$ precipitate produced, improved the fiber bridging capacity providing extra bonding between ECC component and the fiber surface (Bakhar sini 2016 obtained similar outcome). However, self healed pumice immobilized bacteria specimen showed increase in deflection with the reference to it sound specimen. The control specimen at 56 healing days reloading test showed decrease in deflection in deflection as compare to the bacteria based ECC specimens. This is due to fact that t here was no bacteria cell to improve the fiber bridging capacity (see table 21)

Table 19: *Flexural strength of ECC at preloading stage and reloading after healing*

Mix	Specimen	Flexural strength at 28 Zwdays curing ($N/mm^{2)}$ (preloading)	Flexural strength at 56 healing days ($N/mm^{2)}$ (reloading)
Control	Preloading and Reloading	8.80	11.5
	Sound	-	12.01
Bacterial soln+ nutrient + capsules	Preloading and Reloading	7.20	11.00
	Sound	-	8.90
Bacterial soln+ nutrient+ pumice	Preloading and Reloading	7.21	11.90
	Sound	-	9.00

Table 20: *Flexural strength regain of ECC at 56 healing days* in percentage

Mix	Flexural strength regain at 56 healing days(%)
Control	-
Bacterial soln+ nutrient+ pumice	32.22
Bacterial soln+ nutrient + capsules	23.60

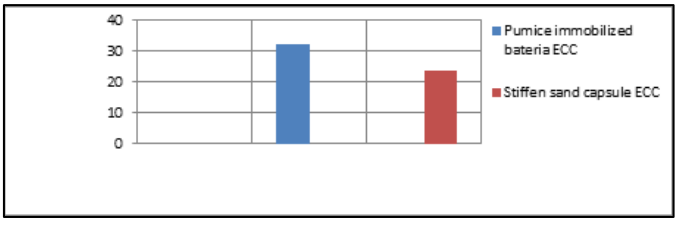

Fig 20: *flexural strength regain for crack ECC after 56 self healing days.*

Table 21: *Mid span deflection of ECC at preloading and reloading after healing*

Mix	Specimen	Mid span deflection at 24days curing (mm) (preloading)	Mid span deflection at 56 healing days(mm) (preloading)
Control	Preloading and Reloading	1.39	2.50
	Sound	-	3.19
Bacterial soln+ nutrient + capsules	Preloading and Reloading	1.42	3.85
	Sound	-	3.36
Bacterial soln+ nutrient+ pumice	Preloading and Reloading	1.6	3.76
	Sound	-	3.84

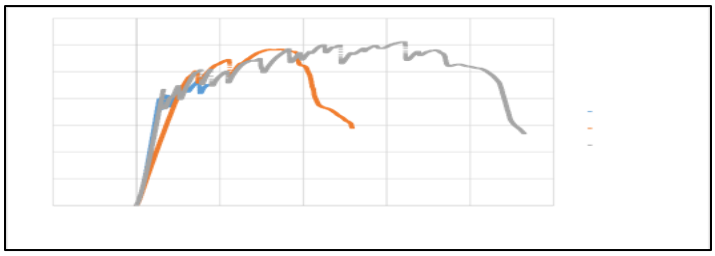

Fig 21: *Flexural stress versus deflection curve of ECC control specimen.*

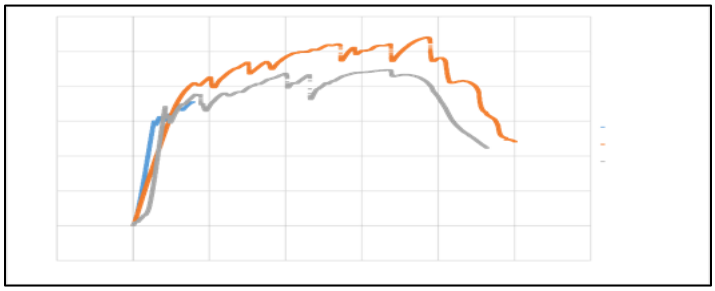

Fig 22: *Flexural stress versus deflection curve of stiffen sand capsule ECC specimen.*

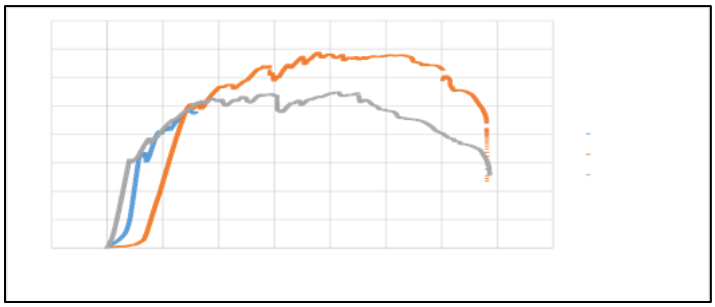

Fig 23: *Flexural stress versus deflection curve of pumice immobilized bacterial ECC specimen.*

7.7 Measuring Self Healing Efficiency by Sorptivity Test

The efficiency of crack self healed concrete specimen was also measured by considering permeation property such sorptivity test. Crack was induced into both control (no bacteria) and bacteria based concrete specimens after 7 days of curing and sorptivity test was carried on the 7th day and subject into water tank for 28 and 56 self healing days. The test was conducted for 2nd and 3rd on 28 and 56 self healing days. All the bacteria based specimens showed massive reduction in sorptivity than the control specimens (see table 22 and fig 24). Loaded expansive clay particle attained the highest reduction in sorptivity with 81.9% reduction in sorptivity of cracked specimen at 56 self healing days (see table 23). This massive reduction is due to the production $CaCO_3$ precipitate by the bacteria which sealed the crack area and formed a denser interfacial zone between the aggregate and the concrete matrix. Also the presence of clay content in the concrete specimen did not permit the capillary suction action for some time. Pumice immobilized bacteria cell and stiffen sand capsules also showed massive reduction in sorptivity with 70% and 70.1% reduction in sorptivity at the end of 56 self healing days (see table 23). This shows that there was massive formation of $CaCO_3$ precipitate to fill the cracked area by the bacteria and this suggest that self healing was efficient in concrete specimens.

Table 22: *sorptivity of crack healed concrete specimen after 7 days of curing*

Healing Age after 7 days of curing	Sorptivity of specimen at different Healing Ages ($mm/sec^{-1/2}$)			
	Control	Pumice+ nutrient+ Bacteria	Capsules+ nutrient+ Bacteria	Clay particles + nutrient+Bacteria
7	0.00720	0.00687	0.00691	0.00681
28	0.00700	0.00446	0.00482	0.00402
56	0.00690	0.00206	0.00206	0.00123

Table 23: *Reduction in sorptivity of crack healed concrete*

Specimen	% reduction in sorptivity at 28 and 56 Healing Days	
	28 Days	56 Days
Pumice+ nutrient+Bacteria	35	70.0
Capsules+ nutrient+Bacteria	30.2	70.1
Clay particles + nutrient+Bacteria	41.0	81.9

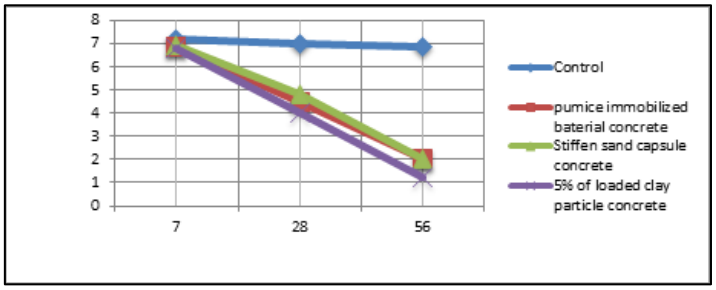

Fig 24: *Sorptivity of crack concrete versus self healing days.*

7.8 Investigating Mineral Constituent of Crack Healed Specimens

The main purpose of this work was to incorporate bacteria into a concrete and in the presence of calcium ion they will react to form calcium carbonate precipitate or calcite. This was confirmed by carrying XRD test on ECC specimen to determine nature of the crystalline material formed in the crack layer. Fig 24 to 26 represents the test results. The letter S on these figures represent silica whiles the C represent calcite. It can be observed that the crystalline material produced on the

cracked surface area was calcite in bacteria based ECC specimens. The obtained XRD spectra intensity were analyzed to get the specimen which gave maximum number of calcite peaks and out of that, excessive amount of calcite count of 1400 was observed in pumice immobilized bacterial cell ECC, while a calcite count of 1200 was also observed in stiffen sand capsules ECC (see fig 25 and 26). For the control ECC specimen (no bacteria), high amount of silicate was also detected (see fig 24). This suggest the there was no formation of calcium carbonate since no bacteria was incorporated into it.

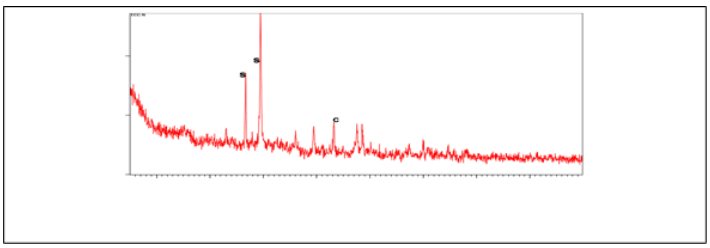

Fig 24: *XRD analysis of control ECC specimen.*

7.9 Visualization of Self Healed Specimens

Photograph all the self healed specimen and control (no bacterial) specimen that were subjected sorptivity and flexural strength regain test was taken. All the bacterial concrete showed massive crack self healing by sealing the cracks with calcium carbonate precipitate. Some of the cracks were completely healed from the crack tip however some of cracks were partially sealed (see fig 27 to 31). The control specimen did not show any visible crack sealing.

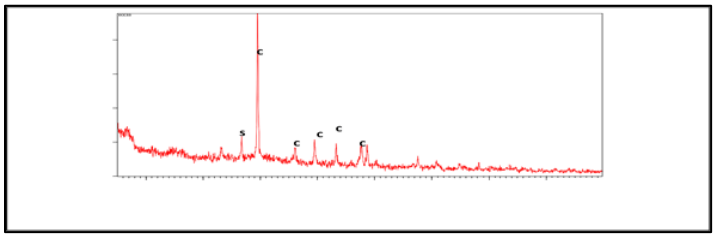

Fig 27: *Control concrete specimen with no visible crack healing.*

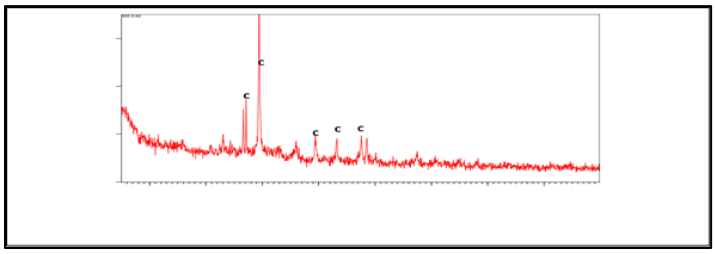

Fig 28: *loaded clay particles concrete with crack width of 0.02 mm completely sealed by $CaCO_3$ precipitate from the crack tip.*

Fig 29: *Pumice immobilized bacterial concrete with crack width of 0.015 mm completely sealed by $CaCO_3$ precipitate from the crack tip.*

Fig 30: *Stiffen sand capsule concrete with crack width of 0.017 mm completely sealed by $CaCO_3$ precipitate from the crack tip.*

Fig 31: *Stiffen sand capsule concrete with crack width of 0.018 mm partially sealed by $CaCO_3$ precipitate from the crack tip.*

8. CONCLUSION AND RECOMMENDATIONS

8.1 Conclusion

After going through all the processes of culturing and integrating bacillus subtilis into concrete, I found out that, the bacteria exhibited high capacity of producing limestone by releasing urease enzyme which hydrolyzes urea to ammonium and bicarbonate ions, in the presence of calcium to form calcium carbonate which also known as limestone which succefully healed cracks. This limestone, depending on the method of protecting and adding bacterial cell to concrete had significant effects on the mechanical properties as well strength regain after 56 self healing days of concrete. Out of these factors the following inferences were drawn.

1. At higher concentration of 10^8cells/ml of bacterial cells, the compressive strength of concrete declines. So in order to obtain an optimum compressive strength and without compromising the effectiveness of self-healing capacity of concrete, the concentration has to be kept around 10^6cells/ml.
2. Bacillus subtilis through pumice immobilized bacterial cell and stiffen sand application methods of protecting and adding the bacteria into concrete, increased the compressive strength of concrete by 31.59% and 35.20% respectively at the end of 28 curing days.
3. Loaded expansive clay particles showed effective protection of bacterial cell in the concrete but the clay content reduced the compressive strength by 7.44% at 5% replacement of clay particle by coarse aggregates weight and increased the compressive strength by 0.20% at 2% replacement by coarse aggregates weight at the end of 28 curing days.
4. Stiffen sand capsules and pumice immobilized bacterial cell increased the flexural strength of concrete by 36.9% and 14.61% respectively at end of 28 days curing.
5. Loaded expansive clay particles increased flexural strength of concrete by 2.87% at 2% replacement by coarse aggregates weight and reduced the flexural strength by 3.72% at 5% replacement by coarse aggregates weight at end of 28 curing days.
6. Pumice immobilized bacterial cell, stiffen sand capsules and loaded expansive clay particles concretes with crack width of 0.015 mm, 0.018 mm and 0.02 mm respectively were completely sealed by calcium carbonate precipitate at the end of 56 healing days.
7. The sorptivity of 56 days healed Pumice immobilized bacterial cell, stiffen sand capsules and loaded expansive clay particles concretes were reduced by 70%, 70.1% 81.9% respectively at the end of 56 self-healing days.
8. Self-healed ECC specimens, regained flexural strengths of 32.2% and 23.60% after 56 self-healing days for Pumice immobilized bacterial cell and stiffen sand capsules application.
9. The XRD spectra obtained suggested that the material formed in the cracked area of both stiffen sand capsule and pumice immobilized bacterial cell specimens were calcium carbonate precipitate.

Based on the above factors, it is concluded that, the bio concrete showed better performance assessments in terms of crack repair, strength and crack strength recovery after 56 self healing days. This suggest that, bio concrete can be utilize in construction sector to reduced the already estimated annual cost of US$ 40 to 45 billion to owners for repair, protection and strengthening of concrete structures in India over the past 40 years (NBM&CW February 2019).

8.2 Recommendations

1. The bacterial infused mortar and concrete in this research is recommended for low strength structures whiles the ECC is recommended for high strength structures such bridges, high rise buildings, dams etc.
2. I look forward to make extensive work on how bacteria concrete can produce in mass concrete.
3. I look forward to make studies on integrating bacteria into prestressed concrete.
4. I look forward to investigate other materials that can be used as carrier materials for protecting the bacterial cell inside the concrete.
5. To investigate other living organism that can be infused into concrete for self-healing activity.
6. I look forward to make extensive studies on how loaded expansive clay particles can be used as transport vehicles for bacterial cell without compromising the strength since it showed efficient protection of the bacterial cell and the massive reduction in sorptivity in this current research.

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