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ASSESSMENT OF PERFORMANCE OF BIO SELF-HEALING MORTAR USING DIATOMACEOUS EARTH AND SILICA FUME

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ABSTRACT

Cracking represents a major threat for the integrity and performance of structures. Self-healing concept was introduced to construction materials in order to enhance their performance and extend their service life with less repair. This study aims at assessing the performance of Portland cement mortar incorporating self-healing Bacillus *Pseudofirmus* bacteria using Diatomaceous earth (DE) to immobilize precursor and bacteria in mortar and lowering the pH level of mortar by using silica fume to provide a suitable growth environment for bacteria to generate limestone. The specimens were prepared at three different bacteria dosages and three DE dosages. Cracking of specimens was induced by load percent concept after 7 days and tests were performed at 14 and 28 days of curing. Micro analysis of the healed crack surface of the different specimens was performed and a parametric study was conducted to select the optimum dosage of bacteria, DE and mix design combination as well.

The testing scheme for the mortar included sporulation tests over bacteria inside mortar specimens, compression test, chemical soundness test and ultrasonic pulse velocity. Results demonstrate that self-healing bacteria is promising technique in minimizing cracking. It is recommended to expand this work to cover more dosages of bacteria, different types of self-healing as well as concrete specimens.

Keywords: Self-Healing, Bacteria, Diatomaceous earth, Silica Fumes, Mortar.

1. INTRODUCTION

1.1 Background

Cracks shape a huge deficiency of concrete as a material used in the construction industry. Cracks can occur due to many different factors such as mechanical, shrinkage, autogenous factors, etc. Cracks can differ in shape, depth, width and the negative severity on the strength of the concrete structure. Micro cracks are cracks that are of micrometer scale in width although they do not affect the strength of concrete or mortar severely, they highly affect the permeability of the material. As the permeability of concrete increases, it paves the way for ingress amount of water or harsh chemicals such as sulfate or chloride to inter the concrete structure that would result in concrete deterioration and steel corrosion and accordingly severely affect the integrity and the mechanical properties of the material. Due to the fact that there are many sources that induces cracks which cannot be all eliminated or it would increase the cost dramatically to eliminate most of them, repair for mortar or concrete becomes a must. However, repairing concrete is a time consuming, expensive and sometimes physically impossible; thus, an efficient way of healing shall be introduced. Self-healing of concrete is a new method that has a great potential in reducing the repair time as well as reducing the repair cost and reaching places of repair that are expensive or impossible to reach. Self-healing of concrete is a phenomenon introduced recently in the research which could be divided into two main sectors. The healing that occurs by regular concrete, which is due to the hydration of the cement particles that have not yet been hydrated, is referred to as autogenous healing of concrete or mortar which happens due to swelling effect, expansion effect, and re-crystallization. Another type of healing, which is due to adding special additives to concrete mix that results in healing is referred to as autonomous healing. The second approach of healing involves adding self-healing agents without compromising the initial properties of concrete during the mixing phase of

concrete or mortar. In this study, the material used is mortar and the self-healing agent is biological agent, a type of bacteria named Bacillus *Pseudofirmus*.

Bacillus *Pseudofirmus* Bacteria used in this study are the ones that have a high potential in living in a high alkali environment. Since mortar is a highly alkaline material, the bacteria had an optimum environment for living with a pH of 9.8 and has no adverse effect of the human health. Despite the fact that the mortar pH is 12 and higher, this specific kind of bacteria has the value of being alkali-resistant as it forms spores and is activated when exposed to water. The bacteria used in this study would work as a catalyst by feeding on calcium lactate and producing calcite, the sealing material of the crack.

1.2 Self-healing Mechanism

Mortar matrix seems a very aggressive environment for any life form to inhabit because of its' high alkalinity and dryness. A good analogy would be 'finding a life form on Mars'. However, this is not really the case as from the perspective of microbiology. Bacteria is found in extreme tough environments such as rocks, beds of oceans, ultra base environments and deep inside earths' crust (Jorgensen and D'Hondt 2006; Fajardo-Cavazos and Nicholson 2006; Dorn and Oberlander 1981; DelaTorre et al. 2003; Pedersen et al. 2004; Sleep et al. 2004). The Fact that bacteria can resist such high alkaline environments is due to its ability to form spores (Sagripanti and Bonifacino 1996). Such ability allows the bacteria to survive harsh conditions such as mechanical and chemical stresses by forming a thick surface layer around itself and reduction of the metabolic activity. This ultimately increases the livelihood of bacteria up to 200 years in certain types (Schlegel 1993).

Many researchers have utilised such potential of the bacteria to enhance the performance of concrete by healing cracks that appear on a micro level. Ghosh et al. have used Bacteria for increasing the compressive strength of mortar. Bacteria is used as a healing agent at the surface of the crack and has significantly decreased the permeability of concrete surfaces and sealing surface cracks.

The mechanism of self-healing is involved in forming calcium carbonate by using ureolytic bacteria of the genus Bacillus based on the enzymatic hydrolysis of urea to ammonia and carbon dioxide. Since the value of the equilibrium pK is constant, the reaction would integer the pH increase to alkaline conditions and accordingly forming bicarbonate and carbonate ions, which precipitate with calcium ions to form calcium carbonate minerals (Bang et al. 2001; Ramachandran et al. 2001; Rodriguez-Navarro et al. 2003; De Muynck et al. 2005; Dick et al. 2006). None the less, this mechanism involved a high risk of reinforcement corrosion due to production of massive amounts of ammonia (Neville, 1996).

Another mechanism used is to embed bacteria in the mix design inside the cement stone matrix and using a healing agent such as calcium lactate and using bacteria as a catalyst to produce calcite. Bacillus *Psydophirms* DSM 8715 and B. *cohnii* DSM 6307 were cultures, formed spores and tested for producing minerals and viability in cement stones. The results revealed that the bacteria was able to produce minerals when calcium lactate was used as a precursor in copious amounts at cracks surfaces and without decreasing the compressive strength of the cement stone unlike the other two procuress tested: calcium acetate and yeast extract. The viability of bacteria showed that the viable cells decreases drastically with time. (Jonkers et al. 2010, Jonkers and Schlangen, 2007, Jonkers, 2007, Jonkers and Schlangen, 2008). The main problem with this mechanism is mainly the massive decrease in the viable cell number as wells as calcium lactate, a relatively expensive material not used efficiently as an excess amounts of it reacts with the concrete matrix.

Physicochemically versatile PU has been used to immobilize Bacillus pasteurii which was successful in stabilizing the metabolic activity for a longer period of time by decreasing the rate of enzymatic activity. However, this reduced the rate of calcite precipitation and, accordingly, concluded that the performance, at the end, is equally effective whether the bacteria was immobilized or not (Bang et. al., 2001). Different studies have used several types of bacteria such as Bacillus *Sphaericus* (Van Tittelboom et al., 2010), Bacillus *Sphaericus* and Bacillus *Lentus* (Dick, 2006), B. *Subtilis* subsp. *globigii* ATCC 9372 (Sagripanti, 1996), Bacillus *Pasteurii* (Ramakrishnan, 2013) and Bacillus *Psydophirms* (Jonkers, 2010). The best of which to precipitate calcite in copious amounts was found to be Bacillus *Psydophirms*. Albeit, the immobilization factor for the bacteria was performed using several methods, such as capsules to immobilize healing agent (Fathy et al., 2014), cracking capsules have been a drawback of such method. Another method of immobilization that was investigated was by using expanded clay particles to

immobilize bacteria and a healing agent (Jonkers, 2007). However, since compressive strength of the specimens decreased by 50% of its control, such method proved to be inefficient.

DE was used in a study conducted on Bacillus *Sphaericus* as a protective immobilization technique to measure ureolytic activity in concrete matrix showed promising results, yet due to the ureolytic activity, the generation of ammonia indicated an adverse effect of such mechanism. Very limited studies have tackled the issue of lowering the pH level of mortar or concrete.

The present study builds further on the outcomes of previous studies, tackling the problem of mobilization by using DE and investigating lowering the pH level of mortar specimens by replacing a percent of cement with silica fume.

2. MATERIALS AND EXPERIMENTAL WORK

2.1 Materials

Cement used is Ordinary Portland Cement type I with a fineness modulus of 365 m2/kg. Cement will work as one of the two main binding materials in this experiment (Cement & Silica Fume). Silica fume is used in the mix design of the experiment with a specific surface of 20 m2/g. DE is used as in the mix design which is a porous sedimentary deposit formed from the fossil remains of diatom with a particle size of 23 µm and a pH level of 8.0-9.5, and specific gravity of 2.33. Fine aggregates used are well- graded sand passing sieve #8. Tap water was used for several purposes in the experiment including mixing, curing and a medium for spores' suspension. Bacillus *Pseudofirmus* bacteria is used as the bio catalysis for the generation of calcite. The precursor used for bacteria is calcium lactate. Chemical compounds were used for culturing and sporulation bacteria were used are nutrient broth, sodium carbonate, sodium bicarbonate and sodium chloride.

2.1 Experimental Work

2.2.1 Cultivation of Bacteria

Bacillus *Pseudofirmus* DSM 8715 strains were purchased form the DSMZ (German Collection of Microorganisms and Cell Cultures), Braunschweig, Germany and were cultivated based on the DSMZ recommendation using 0.8 g of nutrient broth in 0.9 liter of Mili-Q water and autoclaved. 4.2 g NaHCO3 and 5.3 g Na2CO3 (Sesqui Solution) were dissolved in a 0.1 Liter of Mili-Q water and filtered to ensure that no other bacteria would be interfered in the experiment. The Sesqui Solution was added to the autoclaved nutrient broth container until the pH level of the medium for bacteria cultivation was 9.2. Bacteria strains were extracted from their plates and suspended in four 50 ml falcon tubes containing only 15 ml of the alkaline nutrient medium prepared to and put in an incubator with a 37 °C and a rate of 150 rpm for 17 hours to grow. The growth was investigated with visual inspection when noticing that the medium turned turbid. The falcon pre-culture were poured in 4 1 liter containers continuing 0.25 L of the alkaline media each and left in the incubator for 16 hours to grow more. Centrifuging for 15 minutes on a rate of 10000 rpm was followed for the containers and bacteria pellets were extracted. Figure 1 shows the Cultured bacteria in a turbid situation.

Figure 1: Cultures of Bacillus *Psydophirms* before centrifuging

2.2.2 Sporulation of Bacteria

The bacteria pellets were extracted using a sporulation media which was prepared by autoclaving a 1 liter of Milli-Q water containing 0.9 g of sodium chloride. Containers that included bacteria and sporulation media were left in an incubator to form spores for two days then the bacteria spores were extracted by centrifuging for 15 minutes on a rate of 10000 rpm and the suspended in tab water. The viability of spores were tested on plates that contained a media of ajar and calcium lactate. Spores of bacteria were counted using hemocytometer and divided into three dosages of $3x\ 10^8$, $6\ x\ 10^8$ and 10^9 cm^{-3}.

2.2.3 Preparation of Mortar Specimen

Mortar specimens mix designs were prepared all at w/c ratio of 0.5 and 15% of silica fume of the Original Cement weight and cement and silica fume to fine aggregates ration of 1:3. 10 types of mortar mixes were prepared for this study to account for the comparison analysis of the study. Type I called (C1) consisted of above materials and ratios only; types II,III & IV called (B1a),(B1b) and (B1c) contained in addition to the Control Specimen materials ratios, 5% w/w fraction calcium lactate and a $3\ x\ 10^8$ cm^{-3} dosage of bacterial spores with 5,10 and 15% of DE respectively; types V,VI &VII called (B2a), (B2b) and (B2c) called consisted of the same mix as (B1a), (B1b) and (B1c), however, with a bacterial spores dosage of $6\ x\ 10^8$ cm^{-3} and type VIII, IX & X called (B3a),(B3b) and (B3c) consisted of the same mix as (B1a),(B1b) and (B1c), however, with a bacterial spores dosage of 10^9 cm^{-3}. Table 1 shows the mixtures proportions used in this study.

Specimens for compression test consisted of 21 cube specimens with dimensions of 5x5x5 cm and specimens for Ultrasonic test consisted of 21 specimens with a diameter of 10 cm and 5 cm in height which are to be used for rapid chloride permeability test in further research. Specimens for chemical soundness tests consisted of 21 cubical specimens with dimensions of 5x5x5 cm. Figure 2 shows a digitized image of the preparation process of mortar Specimens.

Table 1: Mixing proportions for all mixtures

Mixture	Mixture I.D.	Cement (g)	Silica Fume (g)	Water (g)	Fine Aggregate (g)	Diatomaceous earth (DE) (g)	Calcium lactate (w/w) ratio	Bacteria (cm-3)
Type I	C1	425	75	250	1375			
Type II	B1a	425	75	250	1306.25	68.75	0.05	3×10^8
Type III	B1b	425	75	250	1237.5	137.5	0.05	3×10^8
Type IV	B1c	425	75	250	1168.75	206.25	0.05	3×10^8
Type V	B2a	425	75	250	1306.25	68.75	0.05	6×10^8
Type VI	B2b	425	75	250	1237.5	137.5	0.05	6×10^8
Type VII	B2c	425	75	250	1168.75	206.25	0.05	6×10^8
Type VIII	B3a	425	75	250	1306.25	68.75	0.05	1×10^9
Type IX	B3b	425	75	250	1237.5	137.5	0.05	1×10^9
Type X	B3c	425	75	250	1168.75	206.25	0.05	1×10^9

Figure 2: Mortar Specimens with different DE concentrations cast

2.2.4 Cracking Specimens

A control cube from the compression test specimens was loaded until failure after 7 days of curing with water to determine its ultimate load then all the specimens of both the compression and chemical soundness tests batches were cracked at 80% of the ultimate load in order to induce micro cracks. Also, a control specimen of the rapid permeability test was as well loaded to failure after 7 days of curing with water to determine the ultimate load and all other 20 specimens were cracked at 80% of the ultimate load to induce the required micro cracks.

2.3 Tests

Sporulation test was conducted by visual inspection for the Agar and Calcium lactate plates that spores were put on them to monitor their viability. SEM Inspection was conducted on small samples that has been cut to view the precipitation of calcite at cracks. Compression test has been conducted in accordance with ASTM C109 procedures on 10 samples after 14 and 28 days of curing and records were tabled; Chemical soundness test was conducted by immersing 20 specimens in a saturated magnesium sulfate solution and subjected to cycles of wetting and drying for durations up to 28 days the loss in mass was calculated and tabulated. Ultrasonic pulse velocity was conducted in accordance with ASTM C 597 for 20 specimens that were used for rapid permeability test.

3. RESULTS

Spores were detected under microscope using the malachite green dye that was an enough proof for its ability to form spores using the sporulation method in this study. Testing whether spores will be able to transform to the vegetative form when the environment is suitable was tested and revealed that bacteria could truly shift from spores phase to vegetative phase at which it fed on the provided media and have showed growth rate and it calcite precipitation as figure 3 shows a digitized image of a culture plate at which spores have transformed into vegetative phase.

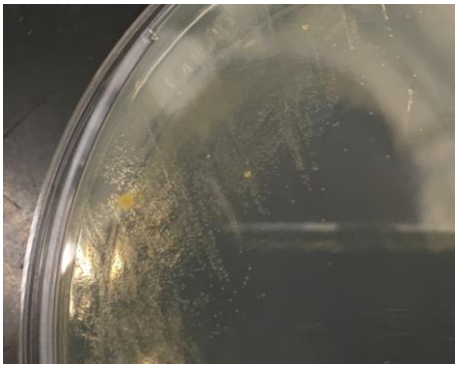

Figure 3: Bacillus *Psydophirms* cells growth after transforming from spores to vegetative stage.

SEM inspection was performed on mortar specimens used for compression test and it has revealed that the bacteria spores have actually transformed to vegetative form and calcite like crystals where noticed inside the mortar matrix as shown in figures 4 and 5.

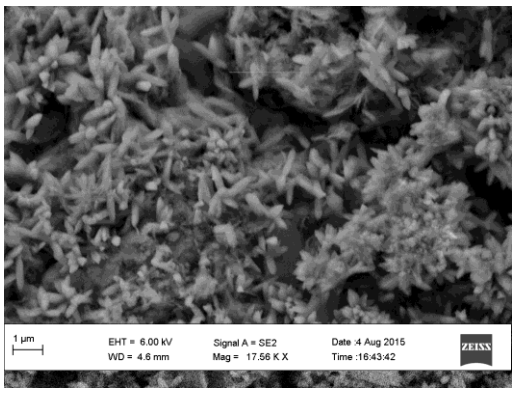

Figure 4: Bacteria cells in mortar matrix

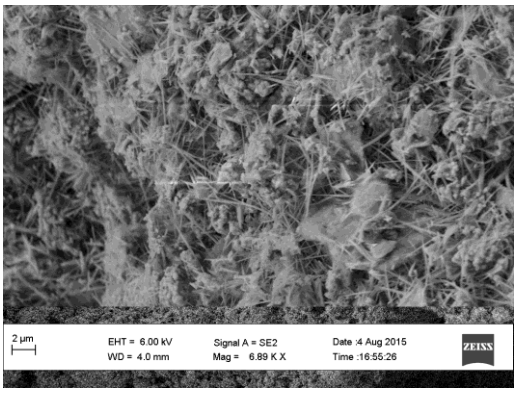

Figure 5: Calcite precipitation in mortar matrix.

Compression test results are shown in figure 6 after 14 and 28 days curing revealed that there is no fundamental difference between control and bacterial concrete with the three different doses of bacteria and DE.

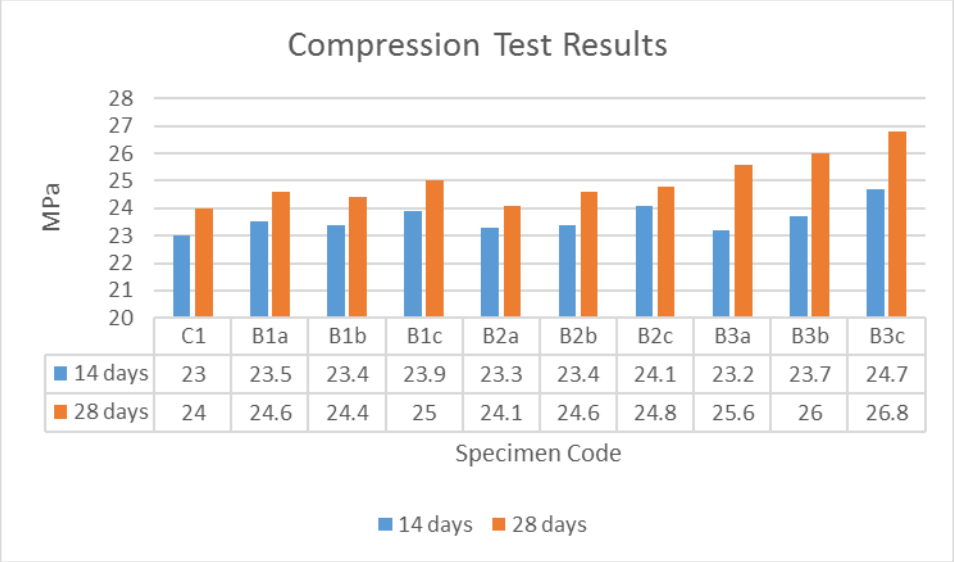

Figure 6: Compressive strength values at 14 and 28 days after curing for the different mortar mixes

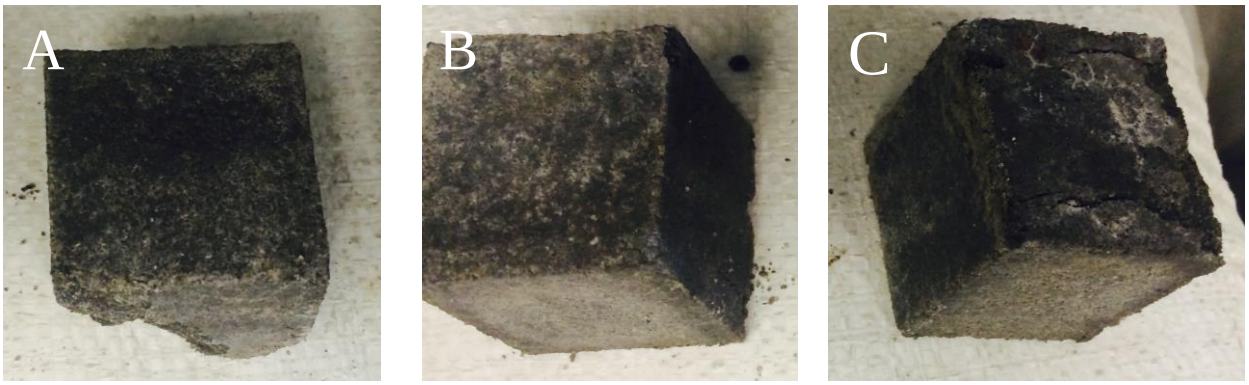

Figure 7: A: Specimen B1a, B: Specimen B2c presenting precipitation of calcite on the surface of the cube; C: Specimen B3c showing healing of few surface Cracks.

Chemical Soundness test results are clarified in Table 1, which is calculated in accordance to the original weight of each cube.

Table 2: Chemical Soundness Test Results

Test Day	C1	B1a	B1b	B1c	B2a	B2b	B2c	B3a	B3b	B3c
14 days	1.5	1.6	1.58	1.61	1.68	1.67	1.7	1.82	1.8	1.76
28 days	1.1	0.9	1.1	0.85	1.2	1.23	1.15	0.8	0.97	1.1

Ultrasonic Pulse velocity test results revealed increase in the velocity on all mortar specimens after 14 days of healing as Figure 8 shows

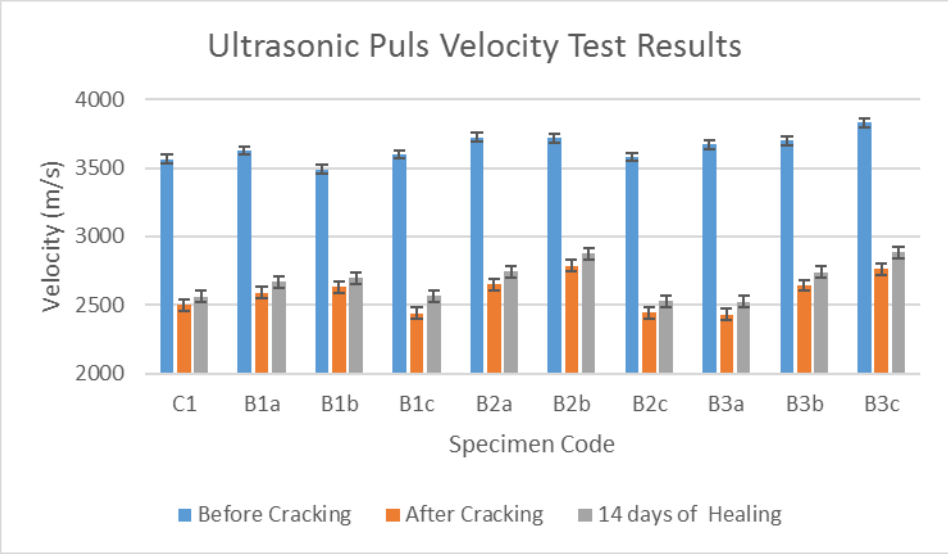

Figure 8: Ultrasonic Pulse Velocity for specimens before and after cracking and 14 days after cracking

4. DISCUSSION

According to the sporulation test, the fact that spores have turned into vegetative cells and started feeding on calcium lactate and generating calcite assures that the bacteria culturing and sporulation is successful. SEM inspection revealed that the mix design that has been prepared shaped a good environment for bacterial spores to transform to vegetative form easily when oxygen accessed the mortar matrix. Image (A), figure 7, shows that the effect of healing is minimal with low dosage of bacteria and low concentration of DE. While image (B) shows that specimen with medium dosage of bacteria and high concentration of DE healed the surface cracks and voids in the specimen which indicates that most of the bacterial concentration was at the surface of the specimen and, due to the abundance of oxygen, higher metabolic activity occurs. On the other hand image (C), figure 7, represents the higher dosage of bacteria and DE of the specimen's cracks fully healed, revealing that the higher the dosage and DE, the higher the rate and amount of healing.

Compression test revealed that compressive strength of the bacterial batches increases more than that of the control specimens, indicating that the healing process increases the compression strength of the material. It is clear from the compressive strength test results that batches with 15% of DE has a higher compressive strength than the other is due to the factor that more calcite precipitation was copious for those batches as inspected by the SEM. The fact that precipitation of calcite was more in batches containing 10% and 15% of DE is due to the fact that the DE provided a protective environment for the Bacterial Spores from mechanical stresses, heat of hydration and a nutrient storage for calcium lactate which increased the rate of its metabolic activity.

The rate of healing was relatively faster than that of previous studies and this is due to the improvement of the environmental conditions of the mortar matrix for the bacteria to grow and precipitate more calcite by decreasing the pH of the mortar matrix due to replacing 15% of the cement by Silica fume.

The compressive strength in the bacterial batches, containing 15% of DE, appears higher than that of the ones containing 5 and 10% in all bacterial dosages. However, the Calcite precipitation appears more copious in the batches containing 10% of DE. The higher compressive strength in B1c, B2c, and B3c, is due to the fact that DE has decreased the amount of water content on the mix design and accordingly increased the compressive strength. Thus, the compressive strength in this study was function with two variables due to the use of DE.

The Compressive strength appeared higher as the dosage of bacteria increases while pivoting the other variables of the mortar matrix environment. This is because more healing occurs with higher bacteria population.

The healing process was witnessed to improve with prolongation of time in the bacterial batches than the control specimens that are free of bacteria, indicating that the autogenous healing rate of mortar decreases with time, while autonomous healing rate increases with time.

Chemical soundness test presented that the percent loss of bacterial batches was less than the that of control specimens due to the fact that surface healing of bacterial mortar cubes prevent a high loss of weight for mortar specimen. However, when the samples of bacterial specimens were inspected by the SEM, the amounts of precipitated calcite was much less than that of the specimens used for compression test where they were left in a 95% humid environment. This observation is interpreted that the magnesium sulfate resulted in a tough environment for spores to transform and thus remained inactive and did not contribute to the self-healing process of the mortar specimen.

The ultrasonic pulse velocity results have revealed that the velocity increased after 14 days of healing; the results showed 50% higher in the bacterial specimens than that of the control specimens, roughly indicating that the autogenic healing using bacteria has the ability to close internal cracks. The ultrasonic pulse velocity also indicated that the increase in the dose of bacteria increases the velocity that points to a better healing for cracks and less voids in the bacterial specimens.

5. CONCLUSIONS

Based on the materials, equipment and other parameters associate with this study, the following can be concluded:

- The rate of healing using bacteria is higher than the one that is free of bacteria.
- Increase dosage of bacteria was found to have a positive effect on the Calcite precipitation of bacillus *Pseudofirmus* in the concrete matrix as more viable bacteria would have a higher cumulative effect on mortar specimens healing.
- Introducing the DE in the design mix has offered a protective environment for bacteria from the high pH of the cement mortar as well as from the heat of hydration and provided a storage for calcium lactate and prevented from wasting such a relatively expensive material that is vital for the healing process but not letting it react with matrix chemical compounds.
- Use of DE had an adverse effect in analyzing the results of the compression test comparison as the DE contributed indirectly to the increase of the compressive strength of mortar by decreasing the water cement ratio.
- Time is a dependent factor for self-healing using bacterial approach as its' potential increases with the prolongation of time.
- Decreasing the pH level of mortar specimens by replacing 15% of cement with silica fume enhanced the environmental conditions for higher percent of spores to transform to functioning cells.
- The analysis of chemical soundness test offered that self-healing using bacterial approach perform poorly as the chemical compounds toughen the environment for bacteria to transform and decrease its metabolism. Thus, decreasing the precipitation of calcite.

6. RECOMMENDATIONS

Similar to common research work, there are several recommendations that need to be considered by future research work as well as by the construction industry, this is incudes, but is not limited to the following:

- This work need to be expanded upon and further validated through investigations covering various types and higher dosages of bacteria, different precursor compounds and immobilization techniques
- Future studies should cover long term testing as well as exposure conditions.
- Rapid chloride permeability test should be avoided as it will have significant adverse effect on the transformation of spores to vegetative cells; permeability test using only water should be considered.
- Further studies should tackle the applicability of using self-healing mechanism for structures immersed in sea water.
- Investigating the rate of healing of bacteria using ultrasonic pulse velocity for higher number of samples.

- Experimenting on a larger models or prototypes is an essential step for the widespread and adoption of this relatively new technique.
- The construction industry needs to consider this new technique in structures of strategic nature as well as those where detection of damage, accessibility or repair effectiveness are in doubt.

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