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

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HIGHLIGHTS

- Experimental study was carried out in order to find optimum self-healing technique.
- Concrete crack healing was observed for various bacteria incorporation techniques.
- Graphite nanoplatelets emerged as good carrier compound for short period healing.
- Light weight aggregate depicted as good carrier compound for long period healing.
- Light weight aggregate incorporation improved compressive strength of concrete.

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ABSTRACT

Crack formation and progression under tensile stress is a major weakness of concrete. These cracks also make concrete vulnerable to deleterious environment due to ingress of harmful compounds. Crack healing in concrete can be helpful in mitigation of development and propagation of cracks in concrete. This paper presents the process of crack healing phenomenon in concrete by microbial activity of bacteria, *Bacillus subtilis*. Bacteria were introduced in concrete by direct incorporation, and thorough various carrier compounds namely light weight aggregate and graphite nano platelets. In all the techniques, calcium lactate was used as an organic precursor. Specimens were made for each mix to quantify crack healing and to compare changes in compressive strength of concrete. Results showed that bacteria immobilized in graphite nano platelets gave better results in specimens pre-cracked at 3 and 7 days while bacteria immobilized in light weight aggregates were more effective in samples pre-cracked at 14 and 28 days. In addition, concrete incorporated with bacteria immobilized in light weight aggregate, also exhibited significant enhancement in compressive strength of concrete.

1. Introduction

Concrete is most widely used engineering material in construction due to its strength, durability and low cost as compared to other construction materials. The major drawback of concrete is its low tensile strength which makes it susceptible to progression and coalescence in microcracks resulting in low strength and durability. These tensile stresses can be due to tensile loading, plastic shrinkage and expansive chemical reactions [1]. This liability to cracking not only results in strength reduction of concrete, but also makes concrete vulnerable to deleterious environment. Entry of harmful chemicals through these cracks may result in concrete deterioration through chemical attack and can also cause corrosion of steel reinforcement [2]. This corrosion leads to increase in crack damage resulting in loss of strength and stiffness of concrete structures [3]. This deterioration in reinforced concrete for both concrete and reinforcement results in high maintenance cost. According to report of Federal Highway Administration [4], United States of America spends 4 billion dollars annually in terms of direct cost of maintenance of concrete highway bridges. De Rooij, Van Tittelboom [5] stated that UK spends 45% of its annual construction cost on maintenance of existing concrete structures. With the capability of self-healing in concrete, the formation and propagation of cracks can be reduced and a concrete with dense microstructure can be obtained. As a result, more durable structural concrete, with reduced maintenance cost can be produced.

Different strategies are used to retard crack propagation and bridge cracks leading to increased durability of concrete. However, most of the strategies, such as epoxy systems, acrylic resins and sil-

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icone based polymers, involve the use of materials which are non-compatible with concrete, expensive and mostly hazardous to environment [6]. As a result of recent studies, bio concrete or bio influenced self-healing concrete is emerging as a viable solution for controlling crack propagation. Bio concrete is a product which involves healing of cracks by production of mineral compounds through microbial activity in the concrete. Autonomous healing through this process increases the structural durability through reduction in concrete cracks and on the other hand reduces the maintenance required for reinforced concrete structures. Bio mineralization is preferred as it is a natural process, environmental friendly and improves the compressive strength of cracked concrete [6].

The process of self-healing is directly related to the production of calcium carbonate which depends on many factors including pH of concrete, dissolved inorganic carbon, nucleation sites and presence of calcium ions throughout the mixture [7]. In addition, other variables such as type of bacteria, their varying concentrations, various curing procedures and material used for incorporation of bacteria also contribute towards efficient self-healing of concrete. For better action at depth in concrete matrix and to keep bacteria readily available, these bacteria along with organic mineral precursor compound are incorporated in the concrete during the mixing phase, instead of external application. Among the different bacteria capable of crack healing and its incorporation techniques in concrete used for self-healing purpose, there is need to identify the effectiveness of bacteria namely, "*Bacillus subtilis*", introduced in concrete by different incorporation techniques. The effects of these techniques on magnitude of crack healing and importance of influence on compressive strength of concrete is also envisaged necessary.

2. State-of-the-art review

Over the past few years many different types of bacteria have been used for crack remediation in concrete. However, it was noted that addition of bacteria not only effects the self-healing in concrete but also results in a change in compressive strength. Fig. 1 shows effect of different bacteria on the compressive strength of concrete and cement mortar. Results by Ramachandran, Ramakrishnan [8]show that using *Bacillus pasteurii*, 28 days compressive strength of concrete increased by 18% at concentration of 7.6×10^3 cells/cm^3. Whereas, the research work by Ghosh, Mandal [9] shows that, at the concentration of 10^5cells/cm^3, Shewanella results in 25% increase in 28 day compressive strength and *Escherichia coli* results in 2% increase in compressive strength. This improvement in compressive strength due to Shewanella is greater as compared to the 18% increase due to *B. pasteurii*, as reported by Ramachandran, Ramakrishnan [8]. In the case of *Bacillus pseudofirmus*, used by Jonkers, Thijssen [10] it can be seen that a concentration of 6×10^8 cells/cm^3 results in a 10% decrease in the strength of mortar. Research work done by Wang, Van Tittelboom [11] shows that *Bacillus sphaericus* decreased the 28 days compressive strength of mortar by 35% at the replacement level of 5%.

In addition to the type of bacteria, the use of carrier compound for protection of bacteria in the concrete matrix is also of prime importance. Introduction of bacteria without the use of carrier compound greatly decreases the viability of bacterial survival over the period of time Jonkers, Thijssen [10]. Therefore, researchers have used different carrier compounds to increase the viability of bacterial survival in concrete and increase the efficiency of self-healing process. De Belie and De Muynck [12] and Van Tittelboom, De Belie [13] used sol gel as mode of bacteria protection. On the other hand, Wang, Van Tittelboom [11] used polyurethane and Wang, Soens [14] used the technique of microencapsulation to provide bacteria with better cover for survival in concrete. In all of the above mentioned studies water permeability test was used as a measure of crack healing and the minimum value of water permeability was observed by the technique of micro-encapsulation. However, the process of microencapsulation, involving polycondensation reaction, is still quite novel and complex. Therefore, there is a need to determine more practical and conducive carrier technique that can be used at a large scale in concrete practices.

Carrier compounds are not only helpful in increasing the possibility of bacteria survival but they also have significant effect on the mechanical properties of the concrete. As mentioned above, the low tensile strength of concrete is a major cause of crack formation in concrete therefore, it is desirable to use a carrier compound which not only increases the possibility of bacteria survival but also increases the tensile strength of concrete.

Fig. 2 shows the effect of light weight aggregates (LWA), polyurethane (PU), graphite nano platelets (GNP) carrier compounds on flexural properties of concrete. Light weight aggregates (LWA), when used by Wiktor and Jonkers [15], as a carrier compound for bacteria in self-healing concrete, provided a better cover to bacteria but it also resulted decrease the flexural strength of concrete and made it more liable to cracking. Wang, Van Tittelboom [11] used both polyurethane (PU) and silica gel as a carrier compound for bacteria and observed that bacteria immobilized in polyurethane produced better self-healing. However, when studied by Gadea, Rodríguez [16] polyurethane foam wastes (PFW) in making lightweight cement based mortar it was found that polyurethane had a negative effect on the flexural strength of cement mortar. Therefore, PU is also undesirable for its use as a carrier compound and there is still a need of carrier compound which enhances the tensile strength of concrete. Sixuan [17] investigated the possibility of using graphite nano platelets (GNP) in cement based mortar

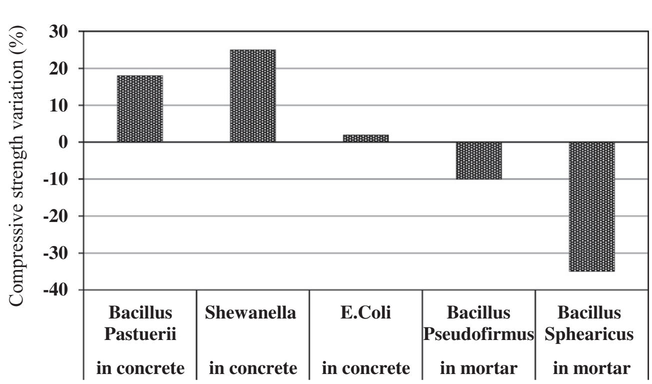

Fig. 1. Effect of various bacteria on compressive strength of concrete.

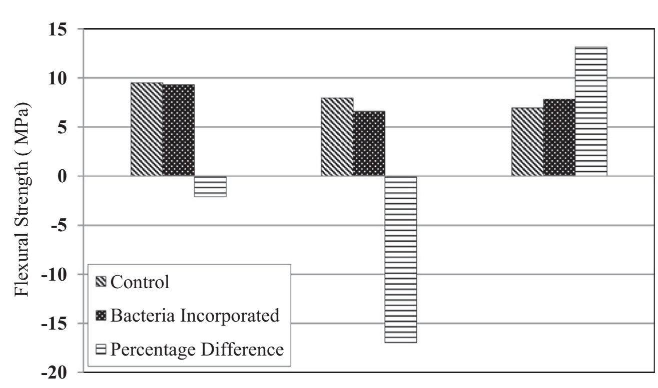

Fig. 2. Comparison of flexural strength in LWA, PU, and GNP incorporated concrete.

and studied its mechanical properties. GNP increased the flexural strength of mortar and at 1% addition of GNP maximum flexural strength was observed. It is evident out of three presented methods that only GNP increases the flexural strength of concrete, therefore GNP is considered helpful in reducing the crack formation in concrete.

The test data shows that there is large variation in the type of bacteria being used, its concentration, different carrier compounds and their effects on properties of concrete/mortar. This difference in properties requires further study to determine the impact of different bacteria and incorporation techniques on the self-healing efficiency and compressive strength of concrete. The objective of this study is to determine the effective self-healing technique in concrete by incorporating bacteria namely, "*B. subtilis*", introduced in concrete by different techniques, and comparing the effectiveness with controlled samples without any bacteria. Bacteria were incorporated both directly and by immobilization in carrier compounds such as LWA and GNP. The effectiveness of techniques was determined by quantification of healing of cracks in concrete samples developed at early age and determination of variation in compressive strength as a result of self-healing in concrete. Furthermore, microscopic analysis was also conducted through scanning electron microscope (SEM) to monitor and establish the amount of the mineral formation in concrete by bacteria through changes in microstructure of concrete. In order to determine the nature of healing compounds produced, specimen of said compounds were obtained from the samples and subjected to X-ray diffraction analysis (XRD).

3. Research significance

Self-healing in concrete is dependent on a number of factors such as type of bacteria, carrier compounds, life of bacteria, activation of bacteria and bacteria introduction techniques. There is limited data on comparative efficiency of type of bacteria, carrier compounds and techniques for introduction of bacteria into concrete. In this study, bacteria samples were introduced in the concrete matrix and effect of bacteria through different carriers on their self-healing capacity were investigated. These results are used to quantify different incorporation techniques to identify the optimum technique for bacteria introduction in self-healing of concrete.

4. Experimental program

An experimental program was planned to determine the self-healing in concrete through use of bacteria in concrete mix. This program included *B. subtilis* bacteria, different carrier compounds with three different introduction techniques in concrete mix to evaluate the self-healing performance.

4.1. Micro-organism

In order to be used as a healing agent in concrete, bacteria must fulfil some requirements. It must be able to adjust to alkaline atmosphere in concrete for the production of calcium carbonate, it should produce copious amount of calcium carbonate without being affected by calcium ion concentration, it must be able to withstand high pressure and should be oxygen brilliant to consume much oxygen and minimize corrosion of steel [10,18,19]. Bacteria namely, *B. subtilis*, was selected in this study as it fulfilled the necessary criteria for survival in harsh environment. It is gram-positive bacteria having an ability to form spores when subjected to unfavourable conditions. This spore formation provides its protection against high mechanical pressure and alkaline environment, making it ideal selection. Members of genus bacillus can produce spores which can lay dormant for over 200 years [20].

Bacterial solution (bacteria in nutrition bath), specifically prepared and treated to ensure spore formation in controlled microbiology laboratory, is usually used to introduce these bacteria in different incorporation techniques in concrete [10]. The quantity of solution required in the mix was calculated on the basis of concentration found by optical density test using a spectrophotometer. For this purpose, medium in which bacteria was growing in was selected as blank. This blank solution was used as a reference, on the basis of which optical density of bacterial solution was measured. A quantity of 0.5 ml of blank solution was placed in spectrophotometer with a selected wavelength of 600 nm. After the machine had read the blank solution, it was replaced by 0.5 ml of bacterial solution and again the same wavelength of 600 nm was used. On the basis of this test, concentration of bacteria in the solution measured using the expression $Y = 8.59 \times 10^7\ X^{1.3627}$ [8]. Where Y is the bacterial concentration per mL and X is the reading at OD_{600}. With spectrophotometer, the bacterial concentration was found to be 2.8×10^8 cells/ml. Based on these results, spore concentration in samples was kept equal to 3×10^8 cells/cm^3 of concrete mixture.

4.2. Mix proportions

Four different types of mix were used for the study. The mix proportion for these four different categories of specimens contained ordinary portland cement (OPC) type – I conforming to ASTM C 150-07 as 370 kg/m^3, fine aggregate as 840 kg/m^3, coarse aggregate as 990 kg/m^3 and calcium lactate of 18 kg/m^3 with a water to cement ratio of 0.4 for all the mixes for concrete. The mix was designed for a compressive strength of 4000 psi. Sikament®-520 set-retarding admixture was used as 1% by weight of cement for producing free-flowing concrete in hot climates. ASTM C 191-11 and ASTM C 187-11 codes were conformed for normal consistency test and initial and final setting time respectively for cement. Control specimens were named "Mix 1" in which no bacterial spore specimens were added. In "Mix 2" specimens, bacteria were incorporated directly by mixing the bacterial solution in water during mixing of concrete, without use of any protective carrier compounds. In the same way, those incorporated with bacteria by the use of LWA as protective carrier were labelled as "Mix 3". In order to incorporate bacteria, LWA were kept soaked in bacterial solution for 24 h till they were saturated prior to their mixing in concrete. Specimen containing GNP as a mean of bacteria introduction were termed as "Mix 4". GNP was also soaked with bacterial solution before mixing in concrete. However, in order to ensure uniform distribution of GNP in concrete, superplasticizer (Sikament®-520) was added to the GNP soaked bacterial solution. Addition of superplasticizer in GNP prior to mixing in concrete ensures uniform distribution of GNP particles throughout the concrete mix [17]. Table 1 shows the mix proportions for all four types of mixes.

4.3. Test specimens

Specimens were removed from moulds after 24 h of casting and were cured under controlled temperature and humidity conditions. For all mix types, samples for two different dimensions were made. For pre-cracking specimens of 150 mm dia and 100 mm height were prepared and five specimens pre-cracked at 3,7,14 and 28 days for each mix were studied for healing measurements. The effect of self-healing efficiency, achieved through different techniques, involve measuring compressive strength as per ASTM C 39. For compressive strength tests, standard sized specimens of 150 mm dia and 300 mm height were prepared and an average of three specimens were utilized to determine 3,7,14 and 28 days compressive strength. Moreover, samples were also subjected to scanning electron microscope (SEM) analysis to monitor microstructural changes due to mineral formation.

Table 1
Mix design of all sets of specimens.

Specimens		Mix 1	Mix 2	Mix 3	Mix 4
Cement	kg/m^3	370	370	370	370
Fine aggregate	kg/m^3	840	840	840	840
Coarse aggregate	kg/m^3	990	990	990	990
Water cement ratio		0.4	0.4	0.4	0.4
Super plasticizer	(%)	1	1	1	1
Calcium lactate	kg/m^3	18	18	18	18
Bacteria with spore concentration (2.8×10^8 cells/ml)	liter/m^3	0	6.33	6.33	6.33
Bacteria incorporation technique		None	Direct	By LWA	By GNP

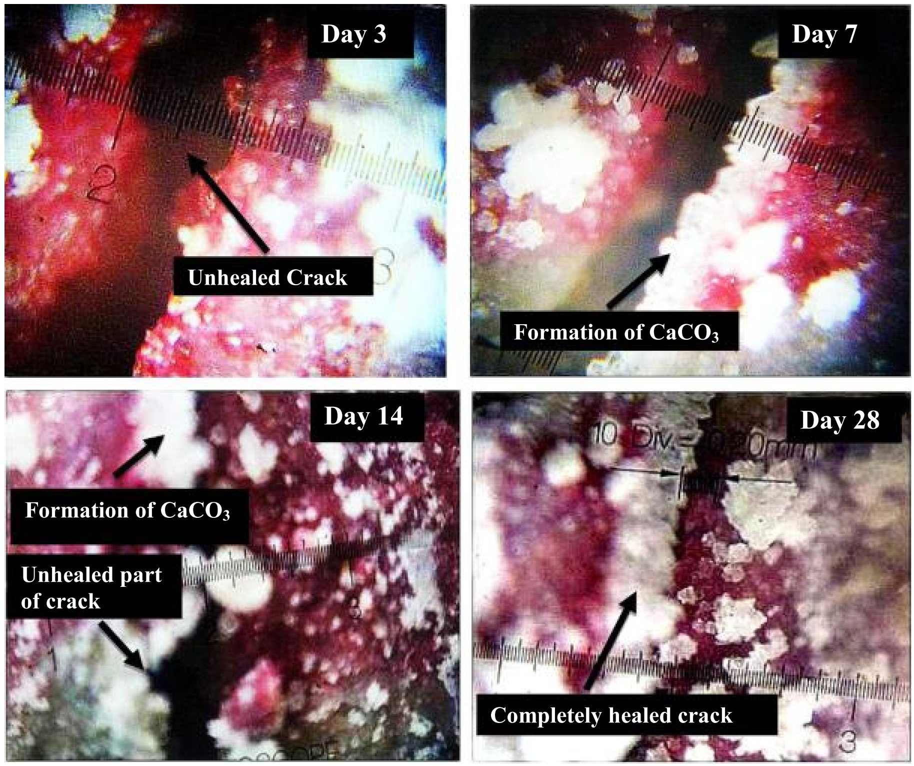

Fig. 3. Closer view of cracks showing self-healing process with calcium carbonate formation.

4.4. Test procedure

Compressive strength tests were performed on specimens at the age of 3,7,14 and 28 days of curing. The specimens prepared to monitor self-healing process were pre-cracked after 3,7,14 and 28 days of curing. The specimens were subjected to compressive test machine under controlled and careful compressive loading till visible cracks appeared on the surface. The crack widths were measured at different points on the specimens and the cracks with a width around 1 mm were selected and marked for further observations of self-healing. The pre-cracked specimens were continued to cure under controlled curing conditions. After pre-cracking, crack width was measured on regular intervals of 3,7,14 and 28 days and difference between the original crack width and that observed on later days was considered as a measure of self-healing as shown in Fig. 3. In addition to these tests, specimens were collected after a healing period of 28 days from the samples pre-cracked on 7 and 28 days. These specimens were then subjected to SEM and XRD analysis. ASTM C 39 test procedure was used to determine the compressive strength of concrete. An average of three tests on specimens was taken for compressive strength, while for self-healing measurement an average of five test specimens was accounted.

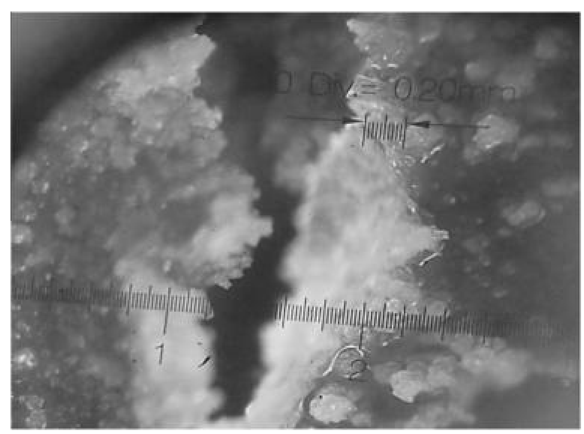

Fig. 4. Crack width measurement by crack measuring microscope.

5. Results and discussions

Results from tests are presented and discussed here to determine the efficiency of self-healing process of all mixes. These results include the crack width measurements, visual inspection of cracks, compressive strength of self-healing concrete samples, micro structural study through SEM images and mineral composition of healing compounds through XRD analysis.

5.1. Self-healing analysis

Pre-cracked specimens were observed at specified time of 3,7,14 and 28 days to determine the efficiency of self-healing process by use of crack measuring microscope. These cracks showed significant self-healing and production of calcium carbonate crystals ($CaCO_3$). The healing compound, $CaCO_3$ crystals, was clearly visible on samples surface incorporated with self-healing bacteria as shown in Fig. 4. This formation of healing compound was also observed and discussed by Jonkers, Thijssen [10] as a result of bacterial conversion of calcium lactate into calcium carbonate.

While observing specimens of all incorporated techniques pre-cracked on 3 days of curing, healing of cracks was prominent especially after 7 days of curing. However, as shown in Fig. 5 healing efficiency of Mix 4 samples with GNP as a carrier compound show the maximum healing as a function of time on all crack measuring days. These are followed by specimens containing LWA as a carrier material. This increase in healing in Mix 4 is due to the particle size of GNP. The small size of GNP enables it to act like a filler material [17] and assure its uniform distribution throughout the mixture. As GNP particles are saturated with bacteria medium, this enables the bacteria to spread uniformly through colloidal dispersion in the mix and become readily available at the crack site. On the other hand, LWA are not as small as GNP particles and therefore, cannot be distributed in the mix uniformly as GNP. This feature of LWA

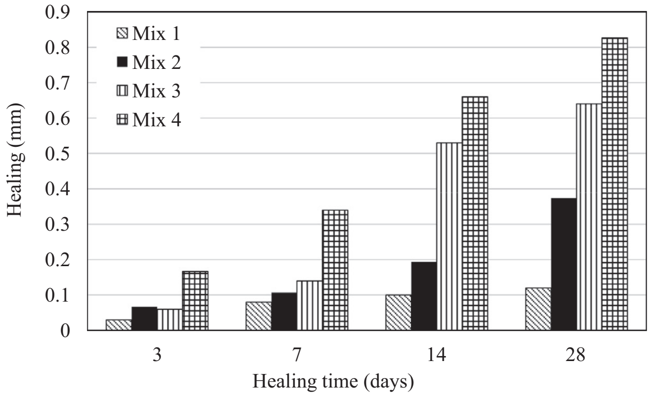

Fig. 5. Crack healing in specimens pre-cracked at 3 days.

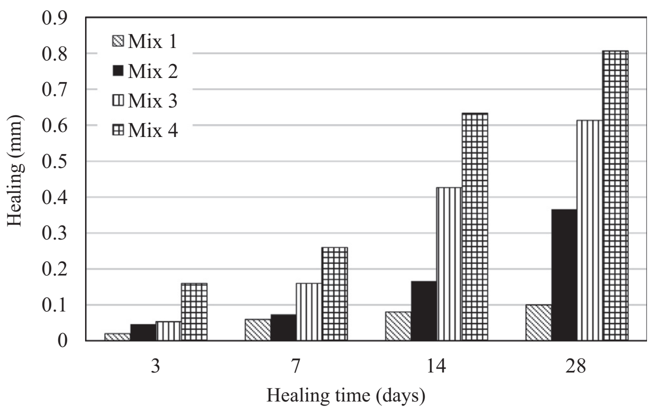

Fig. 6. Crack healing in specimens pre-cracked at 7 days.

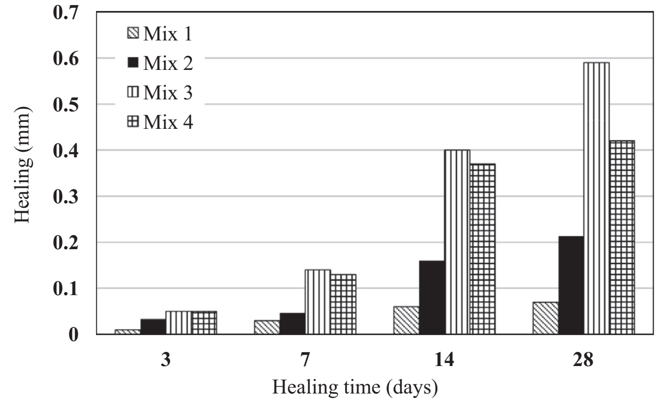

Fig. 7. Crack healing in specimens pre-cracked at 14 days.

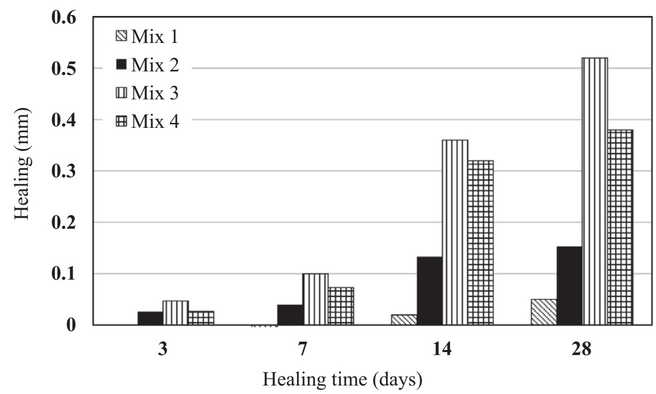

Fig. 8. Crack healing in specimens pre-cracked at 28 days.

hinders the equal and even distribution of bacteria in the mix hence decreasing the efficiency of self-healing process in concrete.

The measure of healing is obtained in millimetres, as the difference of initial crack and healed crack widths at different predefined times. Figs 5–8 illustrate the efficiency of crack healing of each mix in millimetres as a function of time. It can be seen from Fig. 5 that all the samples incorporating bacteria had better healing results as compared to control samples. Controlled concrete samples, without any bacteria, showed some crack healing as well which can be attributed to a number of reasons. It can be a result of continued hydration process of cement particles due to incomplete hydration process at early age and precipitation of calcium carbonate crystals due to carbonation of calcium hydroxide and can heal the crack completely provided the crack is of smaller width [15,21,22]. According to Ter Heide [23] it can also be attributed to the swelling of cement matrix as well.

Fig. 6 shows crack healing in specimens pre-cracked at 7 days of curing. All these specimens show a similar trend in magnitude of crack healing as observed in specimens pre-cracked at 3 days. It is observed that Mix 4 specimens incorporated with GNP as carrier compound, show higher extent of healing as compared to other techniques. As described earlier, this is due to the small size of GNP which makes through and even distribution of particles carrying bacteria in the mix. This small size allows GNP particles to enter the places between particles where LWA cannot penetrate and make the bacteria available for healing. The maximum healing of cracks observed in Mix 4 after 28 days was 0.81 mm. Whereas, crack healing of 0.61 mm was observed in Mix 3 specimens, comprising of LWA. Mix 2 samples, prepared by direct introduction of bacteria in the concrete mix, showed healing of 0.37 mm. This decreases in crack healing of Mix 2 samples is due to decrease in the viability of bacteria survival in concrete under the pressure applied during the mixing phase and that developed due to formation of dense micro structure [10]. Jonkers and Schlangen [21] also reported the disintegration of calcium lactate in the concrete mix as a reason of decrease in self-healing activity. However, Jonkers and Schlangen [21] used small amount of calcium lactate in his research (1% of cement weight) and in this research 4.86% of cement weight has been used to ensure enough availability of calcium lactate to the bacteria. Furthermore, this drastic decrease in bacterial activity was only observed in Mix 2 specimens, which shows that it is not a result of disintegration of calcium lactate.

Healing observed in specimens pre-cracked after 14 days of curing is expressed in the Fig. 7. Mix 3 specimens show maximum healing in samples pre-cracked at 14 days of curing. Mix 4, which displayed maximum healing in samples pre-cracked at 3 and 7 days of curing, proved less efficient than Mix 3 when pre-cracked at 14 days of curing. This change in behaviour of Mix 4 can be due to continued hydration reactions in concrete resulting in development of dense micro structure. This dense micro structure in concrete creates a pressure on the carrier compounds containing incorporated bacteria. Viability of bacteria decreases as GNP is weak when subjected to multi axial loading [17], resultantly GNP is unable to provide better cover to bacteria as compared to that provided by LWA. This leads to elimination and annihilation of bacteria in Mix 4 and therefore, healing process observed in Mix 4 was decreased than it was in samples pre-cracked at 3 and 7 days. This decline in self-healing can be attributed to underdeveloped microstructure which is not fully matured till only 7 days of casting and becomes more compact and mature at 28 days. The change in self-healing process can also be observed in the Mix 2 specimens, with directly incorporated bacteria, with the value of 14 days healing decreased from 0.37 mm to 0.21 mm. This variation in self-healing behaviour can be attributed to the loss of bacteria by elimination due to production of dense microstructure produced in the concrete after 14 days of hydration.

Fig. 8 depicts the results obtained through measurements of self-healing in samples pre-cracked after 28 days of curing. It can be seen that Mix 3, with LWA, is showing maximum healing of 0.52 mm, higher than all other mixes. Mix 4 specimens, with GNP, shows much less healing than it showed at 3 and 7 days of pre-cracking. The healing exhibited by Mix 4 specimens was 0.38 mm, which is higher than healing of 0.15 mm showed by Mix 2 specimens. This shows that Mix 4 still provides a significant improvement in healing with GNP incorporated bacteria but this improvement in healing is lower than that exhibited by Mix 3. This reduction in healing of Mix 4 specimens at 28 day pre-cracking can again be attributed to the dense microstructure formed in the concrete after 28 days of curing similar to samples pre-cracked at 14 days of curing.

5.2. Microstructure analysis

In addition to the results achieved by visual inspection of concrete samples, specimens of all four mixes were subjected to scanning electron microscopy (SEM) analysis to study changes in concrete microstructure due to self-healing. For comparison of self-healing process SEM analysis was conducted at 7 and 28 days of healing.

Production of calcium carbonate based crystals were the main focus of this study as it expresses the crack healing efficiency of respective mix. Calcium carbonate crystals are developed in three different forms which are named calcite, aragonite and vaterite [19]. Out of the three, calcite is most stable form of calcium carbonate. Fig. 9 shows SEM images of all four mixes at 7 days pre-cracked specimens with 2 μm resolution. Fig. shows the development of calcite crystals which are orthorhombic in nature [24]. Mix 4 containing GNP as carrier compound showed maximum calcium carbonate ($CaCO_3$) formation as compared to mixes with other techniques. This $CaCO_3$ formation results from presence of two component, bacteria and calcium lactate, based healing system.

Although $CaCO_3$ is also formed in controlled samples, however presence of bacteria along with calcium lactate catalysis the production of $CaCO_3$ crystals. $CaCO_3$ crystals formation in higher quantities and similarities in shape is identical and conform to those reported by Jonkers, Thijssen [10] and Wang, Van Tittelboom [11]. The chemical process of calcium carbonate formation by bacterial activity is presented in equation given below.

$$CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O \quad (1)$$

As stated earlier that production of $CaCO_3$ is not only limited to concrete with bacteria incorporated in them. The presence of $CaCO_3$ is also evident in controlled concrete specimens. However, the process of $CaCO_3$ crystals formation in controlled specimens is quite different to that in bacteria incorporated specimens. Formation of $CaCO_3$ in Mix 1 specimens is due to the carbonation of calcium hydroxide, which is one of the major hydration products of cement. The carbonation process of calcium hydroxide is expressed with the help of equation as under:

$$CO_2 + Ca(OH)_2 \rightarrow CaCO_3 + H_2O \quad (2)$$

However, this production of $CaCO_3$ crystals in Mix 1 due to carbonation process is very slow as compared to those produced by bacterial activity. In addition, as calcium carbonate production in controlled specimens is due to the availability of carbon dioxide (CO_2) dissolved in the permeated water, therefore, less amount of

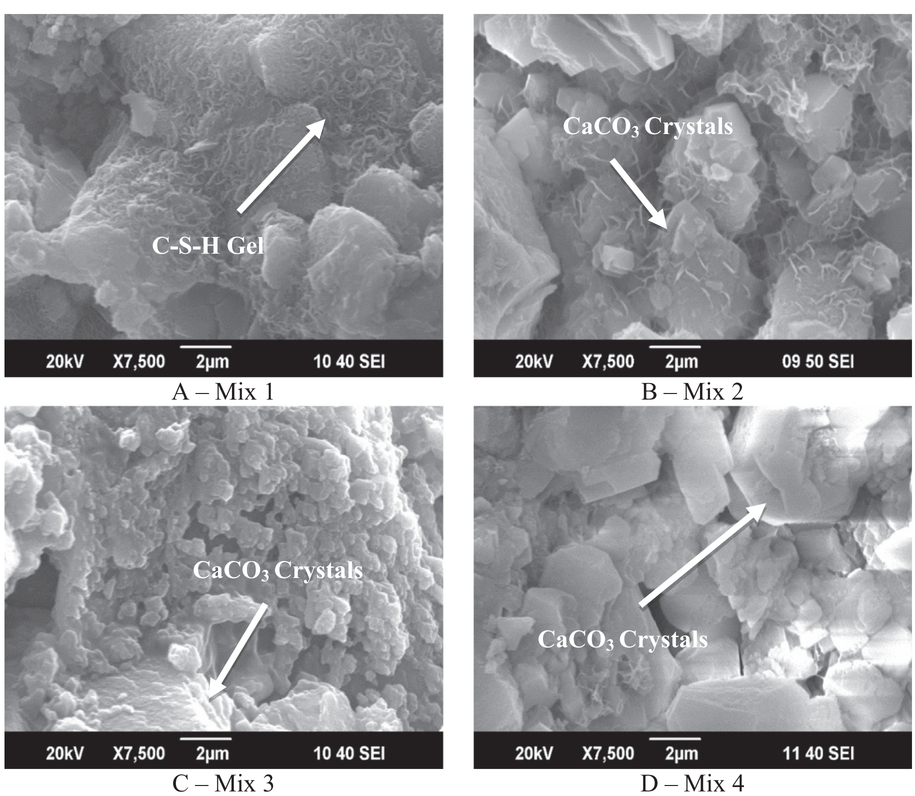

Fig. 9. Scanning electron microscope analysis of 7 days pre-cracked samples.

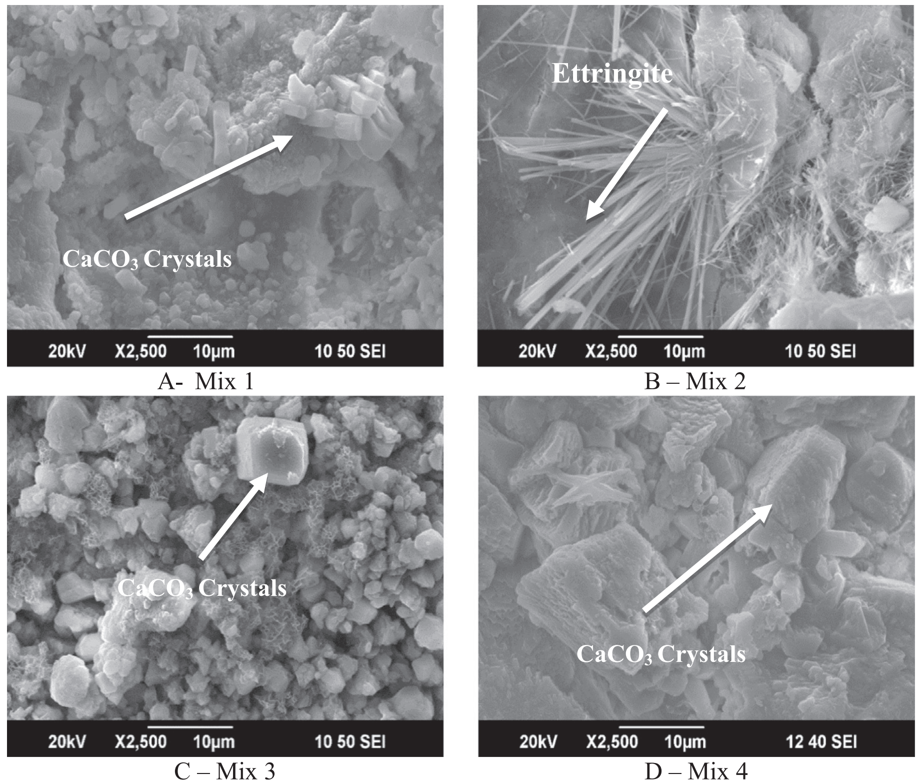

Fig. 10. Scanning electron microscope (SEM) analysis of 28 days pre-cracked samples.

CO_2 is available for carbonation process. Furthermore, as portlandite ($Ca(OH)_2$) is soluble in water so whenever it comes in contact with permeated water it gets mixed in it, leaving less calcium hydroxide on the contact surface to convert in $CaCO_3$. On the other hand, in bio concrete, the process is different due to the presence of calcium lactate and bacteria. Bacteria converts calcium lactate directly into calcium carbonate which is insoluble in water and as result of this metabolic reaction CO_2 is produced which reacts with calcium hydroxide on spot and does not let it wash away. Hence, resulting in production of more calcium carbonate [25].

Fig. 10 shows SEM analysis of specimens pre-cracked at 28 days. It can be seen that calcium carbonate crystal formation is higher in Mix 3, observed in specimens pre-cracked at 28 days compared to Mix 2. The amount of $CaCO_3$ in Mix 2 seems even less as compared to those produced in samples pre-cracked at 7 days of curing. This shows that bacterial activity of calcite production has declined due to decrease in amount of bacteria in Mix 2 with increase in the curing period. Fig. 9 sows that Mix 4 displayed much more crystal formation in 7 days pre-cracked samples as compared to Mix 3. However, as shown in Fig. 10, GNP is no longer able to provide effective cover to bacteria and therefore, calcium carbonate crystal formation in Mix 4 is reduced significantly compare to $CaCO_3$ crystal produced in Mix 3.

These results depict that in samples pre-cracked at 28 days of curing; LWA provides the best cover to bacteria. As described by Sixuan [17], GNP are weak when it comes to multi-axial load application and does not provide better cover to bacteria. Thus, with the increase in completion of hydration reaction and decrease of pore size, healing efficiency of Mix 4 samples decreases. However, LWA provides cover during the mixing phase and provides better protection to spores in the samples as it provides resistance against the pressure developed in samples due to microstructure development. The variation in $CaCO_3$ formation with and without carrier compound conforms the trends seen in the results, in the study carried out by Wiktor and Jonkers [15]. The crystals formation observed in SEM images are also similar to those observed and presented by Wang, Van Tittelboom [11] and Wang, Soens [14] which confirms the formation of calcium carbonate with similar crystalline structure.

5.3. X-ray diffraction analysis

For better understanding of self-healing process and to verify the formation of calcium carbonate in the samples, the healing compounds developed in cracks were subjected to XRD analysis. In order to get the sample, the healing product formed inside the cracks was scratched with great care and was placed in the XRD apparatus. Copper (Cu) was selected as a X-ray target because it can be kept cool easily, due to its high thermal conductivity, and

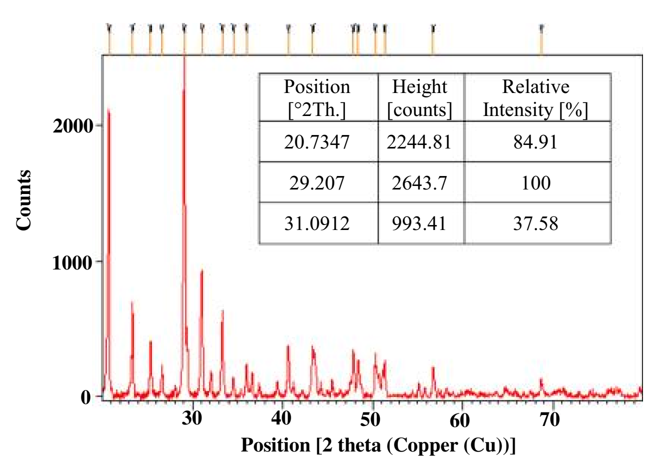

Fig. 11. XRD analysis of healing compound produced in the cracks.

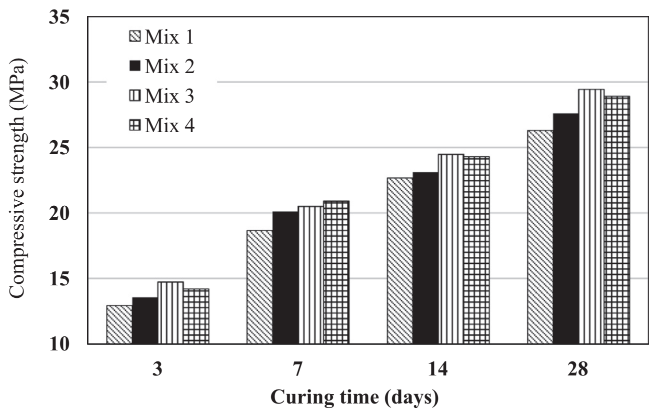

Fig. 12. Compressive strength development with different bacteria incorporation techniques.

which produces strong Kα and Kβ lines. The readings were recorded at a wavelength of 1.54 Å and different representative peaks were obtained as shown in Fig. 11.

It can be seen from the Fig. that no sharp needle like peaks were obtained during XRD. This is due to the reason that sample was scratched from the crack surface and contained a mixture of compounds from concrete surface as well. *B. subtilis* is calcite forming bacteria [19], therefore, the results of XRD were compared with the reference cards of calcite. A highest peak was obtained at the 2 theta (2θ) value of 29.2070° which is quite close to 2θ of 29.455° of pure calcite as observed by Herrington [26]. The slight difference in 2θ value can be due to the impurities in the powder resulted from the scratching off process. This shows that the material produced in the cracks is calcium carbonate in nature and is in harmony with the results obtained from previous studies.

5.4. Compressive strength analysis

Measured compressive strength of self-healing specimens is presented in Fig. 12. It can be seen that all bacterial incorporation techniques result in increased compressive strength of the mix. Samples having LWA as a carrier compound for bacteria incorporation showed maximum strength of 29.43 MPa and improvement of 12% in compressive strength as compared to controlled concrete specimens. The increase in compressive strength are in accordance with the results as recognized in the study in self-healing carried out by Sierra-Beltran and Jonkers [27] and confirms that self-healing is a cause of increase in compressive strength. This increase in compressive strength can be attributed to smaller size of LWA in comparison to regular sized coarse aggregates. This allowed better packing and compaction of concrete matrix around them which gave these specimens much higher strength than controlled specimens.

Specimens containing GNP showed an increase of 9.8% in compressive strength. This improvement in compressive strength can be attributed to the addition of GNP. GNP being a nano sized material acts like a filler material with even and uniform suspension in the mix. Small size of GNP also decreases the formation of weak interfacial transition zone (ITZ) in concrete by allowing filling of porous and crystalline microstructure within ITZ. Decrease in ITZ makes the mortar matrix denser and more compact resulting in higher compressive strength. GNP particles also act as crack arrestors and block and divert crack formation and propagation [17]. GNP therefore, acts in many ways to enhance the compressive strength of concrete.

Direct incorporation of bacteria also showed an increase in compressive strength of concrete. This improvement is because of the presence of calcite producing bacteria in the mix. These calcium carbonate continuously manufactured by the bacteria and calcium lactate provided as organic precursor makes the internal structure of concrete more compact, therefore, results in increase of compressive strength. This improvement seen by the direct introduction of bacteria is in consistence with the results achieved by Ghosh, Mandal [9]. However, after careful comparison of the results achieved by Ramachandran, Ramakrishnan [8] through direct introduction of bacteria, it is evident that there is no difference in strength by introduction of *B. pasteurii* at a rate of 7.2×10^7 cell/cm^3. This shows that as far as compressive strength is related *B. subtilis* is a better choice as compared to *B. pasteurii* as its addition significantly improves the compressive strength of concrete.

6. Conclusions

Based on the results achieved during this study following conclusions are drawn:

- Specimens incorporated with graphite nanoplatelets (GNP) as carrier compound displayed uniform distribution and protection of bacteria at samples pre-cracked at early age of 3 and 7 days, resulting in maximum crack healing efficiency. However, when pre-cracked at later days, such specimens presented a significant decrease in healing of cracks.
- Although specimens incorporated with lightweight aggregate (LWA) as carrier compound, were not as efficient as GNP at early age pre-cracked specimens, they showed consistency in their crack healing efficiency in specimens pre-cracked at later days.
- Specimens incorporated directly with bacteria did not show any effects in crack healing of concrete.
- Compressive strength trends of all mixes suggest that, addition of bacteria "*Bacillus subtilis*" resulted in slight increase in compressive strength, irrespective of the incorporation technique, with significant improvement through LWA technique.

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