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Statistical and experimental investigation on self-healing of microcracks in cement mortar by encapsulation of calcite precipitating bacteria into expanded perlite

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A B S T R A C T

Healing of micro cracks in concrete using calcite precipitating microorganisms has become an interesting research for the past two decades. In this study, an attempt was made to encapsulate strong and efficient calcite precipitating spore-forming bacteria named Bacillus megaterium MTCC 8510. The encapsulation and survival of bacteria in expanded perlite was made successfully by dual coating using sodium silicate and cement. The mechanical properties and healing efficiency of the coated expanded perlite were tested after it was integrated into the cement mortar matrix. The encapsulated bacterial expanded perlite particles were added into cement mortar in 10%, 15%, 20%, and 25% replacement of fine aggregate. The results of 20% replacement of fine aggregate by expanded perlite showed an improvement in healing of microcracks of size varying from 0.154 mm to 0.626 mm. But due to the presence of expanded perlite, there was a decrease in compressive strength. To counteract the reduction in strength 5% of nano-silica was added along with cement, which had given improvement in compressive strength of mortar without compromising in healing. The improvement in healing was observed visually by width repair rate method and the healing in the depth of cracks was determined by Ultrasonic Pulse Velocity (UPV) test method. The crystal shapes and elemental analysis of calcite precipitation were observed using FESEM, and confirmed using XRD analysis. The regression equations were developed for each test procedure by considering the factors which influence the compressive strength and healing efficacy. Statistical model using ANOVA was employed to find the significance of factors and the optimum percentage of expanded perlite replacement. With a p-value of less than 0.05, the factors proved to be significant. A high R^2 value nearing 100 percent and acceptable precision was used to determine the model's predictive efficiency. The model has shown that 20 percentage replacement of fine aggregate by expanded perlite is optimum for healing efficacy without compromising the compressive strength.

1. Introduction

Concrete has been one of the most widely utilized construction materials for decades. The very first Reinforced Concrete Construction was originated in seventeenth-century by incorporating iron tie rods in to masonry work [1]. Most structures' service life is likely to be much shorter than their theoretical maximum lifespans due to various reasons [2]. One major reason is the problems related to durability of materials used. Over a period of time, shrinkage, increase in permeability, environmental pollution and other related factors lead to the deterioration of material's strength and durability [3]. As a result of this there is a possibility of developing micro cracks on the surface of mortar. These micro cracks are of great concern for the damage of the installed reinforcement in the structure. Due to this there arises necessity for repair of the cracks which further increases the cost of construction [4]. To counteract this problem there are many polymeric materials and healing techniques available now a days in construction industry [34]. Among all these available techniques, one of the best approaches is self-healing of micro cracks using calcite precipitating microorganisms [35,37–39]. This attracted the interest of several researchers all over the world.

Lot of research is going on in this particular domain to make autonomous repair of cracks in concrete. The cracks can be healed using microorganisms in two different ways. One way is directly spraying the bacteria on the surface of the cracks and another way is through

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encapsulation technique, where bacteria can be encapsulated into ceramsite [5], diatomaceous earth [6], polyurethane, ceramic pellets, silica gel [7], graphite nano platelets [8], clay pellets [9], light weight aggregate [10], expanded perlite [11], cellulose fibre [3], sugar coated EP [12,13] etc. The advantage of using encapsulation technique is to protect the microorganism from the external pore pressure inside the cement mortar matrix. The life of the encapsulated microorganism should be as long as the designed service life of the building to ensure the healing of microcracks which mitigates the cost of repair [12]. During encapsulation the calcite precipitating microorganism and the corresponding food must be encapsulated together. The previous research work in this field has reported that the calcite precipitating bacteria can live inside the concrete up to 200 years [14].

The survival of bacteria and its specific application for crack healing is possible only by adopting proper encapsulation technique. Several encapsulation techniques have been developed for this particular purpose of healing microcracks in concrete. Factors that influence crack healing are size of crack, age of concrete, temperature, pressure and healing time, pressure of water and air, dosage, size and dispersion of capsules, healing agent and its viscosity, concrete materials composition, expansive agents, calcium ions concentration and mineral admixtures, tolerance to the mechanical forces generated while mixing and compaction of cement mortar [15,16]. The process of encapsulation varies according to the type of encapsulating material used. Encapsulation of bacteria into polymer beads and other chemicals is costly. Recently researchers have shown interest towards low-cost encapsulation techniques and lot of work also has been carried out in this particular area. One amongst such techniques is using expanded perlite coated with sodium silicate and cement [11]. Expanded perlite is having glassy, porous structure, and light weight [17]. It is being used in different applications such as building material, agriculture, chemical and medical industry [18]. The self-healing efficiency of bacteria is mainly depending on its urease activity and amount of calcite precipitation also. The previous research work done in this area by different authors reported that among the various bacteria available for self-healing concrete Bacillus megaterium have shown a good high amount of calcite precipitation as shown in the Table 1.

The present study focuses on immobilization of bacteria into expanded perlite and the effect of its addition in cement mortar. The encapsulation and dual coating were diagrammatically represented in Fig. 1. When the sugar-coated expanded perlite as bacterial carrier [12] mixed in the mortar, the sugar coating being brittle in nature may crack very easily. So, in this research expanded perlite as bacterial carrier by dual coating using sodium silicate and cement coating is adopted. Once the bacteria and nutrients were absorbed completely by expanded perlite particles, coating was carried out with sodium silicate layer and cement layer consequently. The effect of coating materials on the expanded perlite, efficiency of encapsulation, particle size analysis and healing efficiency were checked. The fine aggregate was replaced with bacteria encapsulated coated expanded perlite in 10, 15, 20 and 25 percentages of aggregate weight to find the optimum percentage of replacement through its healing efficiency. Another set of specimens were prepared by replacing the same percentages of aggregate encapsulated with only nutrient broth without bacteria to understand the effect of bacteria clearly. The comparison of results for all test methods were done between only nutrient broth encapsulated specimens (Only N) and specimens prepared with bacterial encapsulation (B-N) to show the effect of bacteria presence in terms of healing. The healing efficiency was determined by means of crack width repair method, UPV test procedures. But consequently, upon replacement of expanded perlite instead of fine aggregate, the compressive strength was found to be significantly reduced. Previous studies have demonstrated that adding nano-silica to concrete or mortar improves its compressive strength [27]. Upon addition of nano silica, the amount C-S-H and C-A-H are increased in the cement paste [28]. The usage of nano silica to complement the strength of mortar shown a significant improvement. In this way the efficient healing can be achieved without compromising in strength of material [29]. A statistical modelling was also performed using ANOVA model to identify optimum percentage of expanded perlite for significant healing without compromising the compressive strength. And also, regression equations for each test were established through regression analysis by considering various factors like, percentage aggregate replacement, nano silica percentage, and n^{th} day of healing. These factors are grouped using the Tukey method with 95 percentage confidence. The ANOVA also showed that healing efficiency due to individual factor model terms are significant with a p- value of less than 0.05 and high adequate precision was ascertained through R-sq value approximately equal to 100 percentages.

Table 1
Various bacterial species and their urease activity and calcite precipitation.

S. No	Bacteria	Urease activity U/ml	Calcite precipitation (mg/100 ml)	References
	B. Pasteurii NCIM 2477	18	–	[19]
	Sporosarcinapasteurii	550	–	[20]
	K. flava CR1	472	–	[21]
	Halomonassp. SR4	374.5	–	[22]
	L. sphaericus CH5	–	980	[23]
	B. thuringiensis	620	167	[24]
	B. megateriumSS3	**690**	**187**	[25]
	Bacillus sp. CR2	432	2.32	[26]

2. Materials and methods

2.1. Expanded perlite

It is an expanded volcanic mineral procured from Casa De Amor, India. The main properties of this material are low density, temperature stability and high porosity. It is generally used for gardening and hydroponics. The material received was clean, odourless, easy to handle and free of any additives or ingredients that were harmful to plant growth. Besides it also allows excess water to drain away quickly while maintaining a small amount of moisture and capturing nutrients that bacteria require to flourish. It holds the right amount of moisture, can hold three to four times its weight in water. The properties of the procured expanded perlite as provided by the supplier are presented in Table 2.

2.2. Encapsulation of bacteria

A gram positive alkaliphilic bacterium was procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. The culture was received in freeze dried condition in an ampoule. The culture medium for the bacteria was containing peptone (5 g), sodium chloride (5 g), beef extract (1.50 g), yeast extract (1.5 g), $CaCl_2$ (20 g), urea (20 g) each was mixed in 1000 ml of distilled water in a laminar chamber to avoid contamination. The culture medium was then sterilized in auto clave for 15 min under pressure of 15 lbs at 121 °C. The culture medium was cooled to normal room temperature and dispensed in conical flasks. Then the bacteria in freeze dried condition was transferred to culture medium in laminar chamber. The solution was kept in orbital shaker for 24 h at 36 ^{0}C to accelerate the growth of the bacteria. The details of the bacteria are tabulated in Table 3. The prepared bacteria were sporulated using heat shock treatment. These spores were centrifuged at 5000 rpm for a duration of 20 min and the spore sludge was transferred to distilled water and diluted to get a concentration of 2.625×10^8 cells/ml. The spores were also tested for calcite precipitation efficiency. To determine the calcite precipitation capacity of the bacteria, a 200 ml solution that containing bacterial spores of above-mentioned concentration, 0.18 m/l of calcium ion concentration and 0.33 m/l of urea concentration was

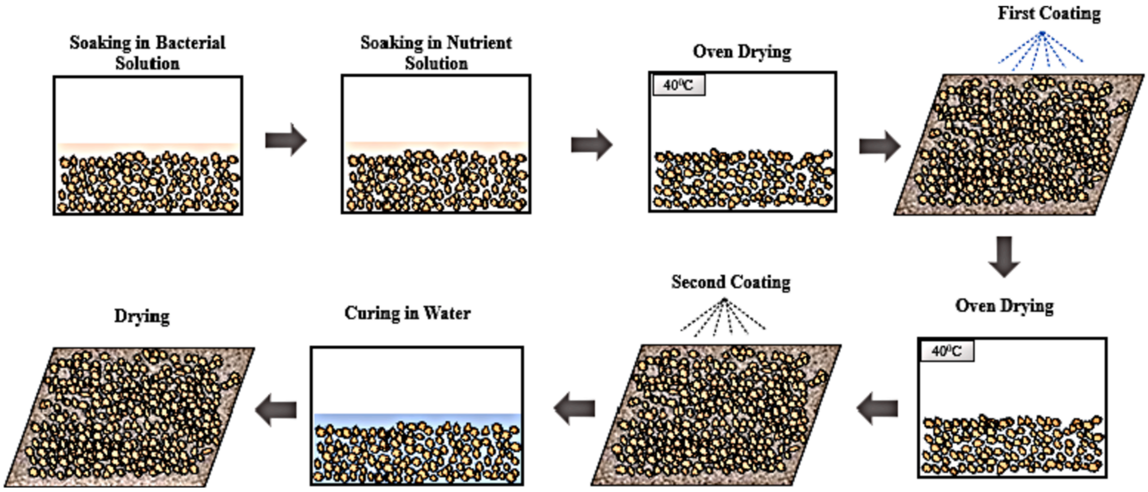

Fig. 1. Schematic representation of bacteria encapsulation.

Table 2
Properties of expanded perlite.

S. No	Property	Value
1.	Bulk density	35 to 60 kg/m^3
2.	Temperature Stability	Up to 1148 ^{0}C
3.	Colour	White
4.	Water Absorption Capacity	154.84% (in 24 h)

Table 3
Details of Bacterial species.

MTCC No.	8510
Genus	Bacillus
Species Name	Megaterium
Isolated or derived from	Ooty, Western Ghats, Nilgiris, Tamil Nadu
Growth Condition	Aerobic
Incubation time and Temperature	24 hours and 37^0 C

kept in an orbital shaker. At every 3rd day the solution was checked for calcite precipitation up to 12 days. The maximum amount of calcite precipitation was found to be 1.19 g on 12th day of incubation in orbital shaker.

The expanded perlite (EP) granules of size varying from 0.15 mm to 4.75 mm were used for encapsulation of bacteria. The encapsulation capacity was determined by using water absorption method on uncoated expanded perlite. The expanded perlite granules were suspended in spore suspension for 24 h at 36 °C in atmospheric pressure to ensure the bacterial cells are absorbed inside the pores of the expanded perlite. It was found that 1 gm of perlite absorbed 1.432 ml of sporulated bacteria in liquid form. For the mentioned sporulated bacterial concentration of 2.625x10^8 cells/ml, it can be mathematically interpolated that 1.432 ml absorption contains 3.73x10^8 cells/g of expanded perlite particles. Then the bacterial encapsulated expanded perlite was sprayed with a solution that contain calcium chloride (20 g/L) and urea (20 g/L) and dried at 40 °C for 24 h [33,38]. Then these granules were coated with sodium silicate and again dried in an oven at 40 ^{0}C for 48 h to ensure the first layer of sodium silicate was formed and hardened on the encapsulated expanded perlite granule. To ensure the safety of perlite granules, since they are too delicate and light in weight, a second coating also applied on the granules using Ordinary Portland cement paste till the granules were fully coated, and dried in oven for 24 h. Then it was cured in water at room temperature for 10 h to hydrate the cement layer sufficiently. Upon formation of hard layer as shown in the Figs. 2 and 3, the granules were ready for further casting in mortar. The recipe for coating is shown in Table 4. The majority of the bacteria in spore form takes time to become a vegetative cell by undergoing activation, germination and outgrowing process [41]. So, in the 24 h of drying process the premature mineralization may not be possible.

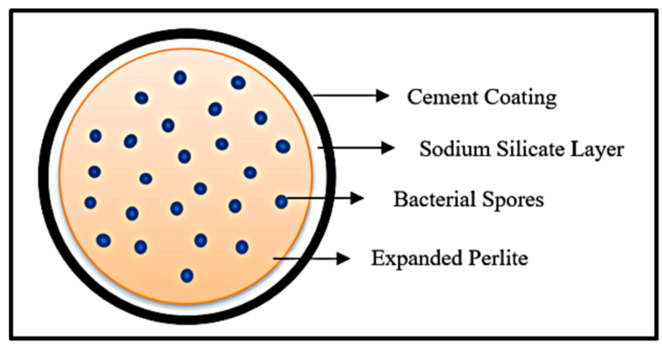

Fig. 2. Coated EP particle.

The particle size distribution of coated and uncoated expanded perlite was studied using sieve analysis procedure conforming to IS: 2720 (Part 4)-1985 [40]. After coating the weight of particle is increased to 5 times of uncoated EP weight and the effect of the coating on the particle size distribution is shown in Fig. 3. As demonstrated in Fig. 4, aggregates with fewer than 11% of particles passing a 2.36 mm sieve, compared to around 48% passing this sieve prior to coating. The resulting particles were added directly into the mortar mixture in different percentages of replacement with fine aggregate.

2.3. Proportioning of mix and specimen preparation

There were three different sorts of mix types made as shown in Table 5. In each mix type, five distinct type of specimens using mortar mixes were made. The different percentages of prepared self-healing granules were replaced instead of fine aggregate in the mixture to find the best percentage of healing for the micro cracks of range from 0.154 mm to 0.626 mm. The materials used for preparing mixes are Ordinary Portland cement 53 grade, three grades of standard sand, water, superplasticizer (PCE based), and nano silica. Among the specimens prepared, the specimens without bacteria were named as "Control". Bacterial cells and nutrients encapsulated EP particles with dual coating are named as "B-N". And finally, the EP particles encapsulated with only nutrients and dual layer coated were named as "Only N". A cube specimen of size 50 mm × 50 mm × 50 mm was also cast with different percentages of EP particles for checking compressive strength and

Fig. 3. Particle size distribution of coated EP.

Table 4
Recipe for dual coating of EP.

Material	Weight in grams	Status of Coating	Oven drying temperature in ^0C	Time of drying (hours)
Encapsulated EP	500	Uncoated EP	40	24
Sodium Silicate solution	500	First coated EP	40	48
Cement paste prepared with 0.4 W/C	1000	Second coated EP	27	10

healing as well. It is obvious that the strength of the specimen would reduce upon increasing the percentage of EP particles, so to improve the strength without compromising the healing, nano silica (NS) was added in 5 percentage of cement weight to the mixture of mortar separately to get the improved strength. The mortar specimens were demoulded after 24 h and maintained in water curing for 28 days. The cylindrical specimens were tightened by using a rubber band during the induction of the crack. Finally, the specimens were subjected to curing in normal water for healing the cracks.

2.4. Crack width observation and measurement of cracked area

The surface of cracks for all the cracked specimens were digitalized using Ultra Wide-angle Macro Lens having phase detection auto focus (PDAF) technology that ensures the clear focus of the object to be digitalized. The images of surface of cracks were taken after healing for 0 days, 3 days, 7 days, 14 days, 21 days, 28 days, 56 days, and 92 days, the specimen surface was observed and the crack healing was measured by using ImageJ software. A specific region along the cracked surface was marked between two black lines of known distance of 1 cm. After setting the scale in the software the area of unhealed crack was measured using a freehand tool as marked in red coloured line shown in Fig. 5. The measured area and corresponding calculation of healed area on n^{th} day is also shown in Fig. 5. All the cracks of the recorded photographs were analysed for healing in the fractured areas after each of the above-mentioned days for measuring the amount of healing by calculating the area of the crack healed as shown in Fig. 13. First the area and length of the cracked specimen were measured. The average width of the crack was measured using equation (1) (Singh et al., 2020).

$$Width\ of\ the\ crack = \frac{C_A}{C_L} \tag{1}$$

where C_A is area of crack at n^{th} day of healing, and C_L is length of crack.

2.5. Quantification of self-healing using UPV method

The specimens with size 50 mm × 50 mm × 50 mm were cast for all three types of mixtures mentioned in Table 5. The specimens were cured for 28 days and cracks were produced by applying controlled loading. Once the minute cracks were started appearing on the surface the loading was stopped and removed the specimen from the loading chamber. The percentage of self-healing can be found by using UPV, a non-destructive testing method [36]. The specimens were tested for UPV for all mortar sample at each 0, 3, 7, 14, 21, and 28th day of healing of cracks. The healing throughout the depth of the cracks is inferred from

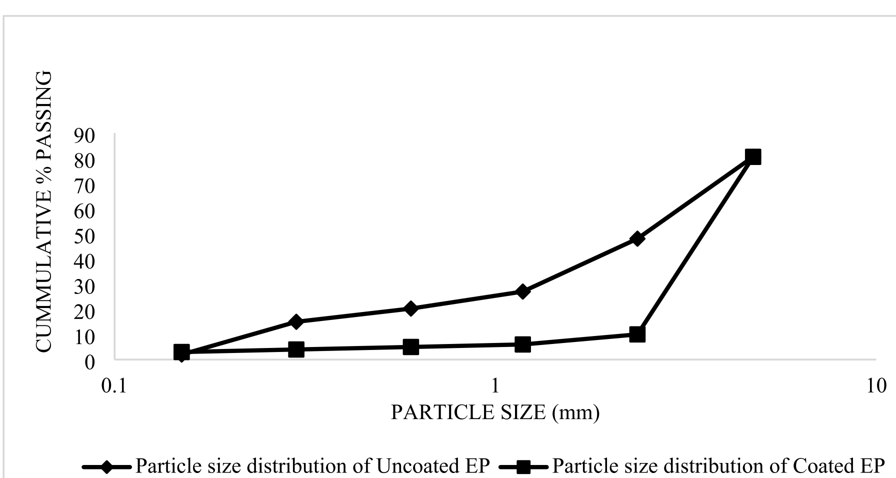

Fig. 4. Sieve analysis of uncoated EP and coated EP.

Table 5
Mixtures used for specimen preparation.

Mix type	% EP replacement	EP weight (grams)	Fine Aggregate	Cement (grams)	Water (ml) Without addition of NS	Addition of nano silica (grams)	Water (ml) With addition of NS	Super plasticizer (ml)
Control	0	0	960	420	131.6	8.4	182.3	1.09
	10	96	864					
	15	144	816					
	20	192	768					
	25	240	720					
B-N	0	0	960	420	131.6	8.4	182.3	1.09
	10	96	864					
	15	144	816					
	20	192	768					
	25	240	720					
Only N	0	0	960	420	131.6	8.4	182.3	1.09
	10	96	864					
	15	144	816					
	20	192	768					
	25	240	720					

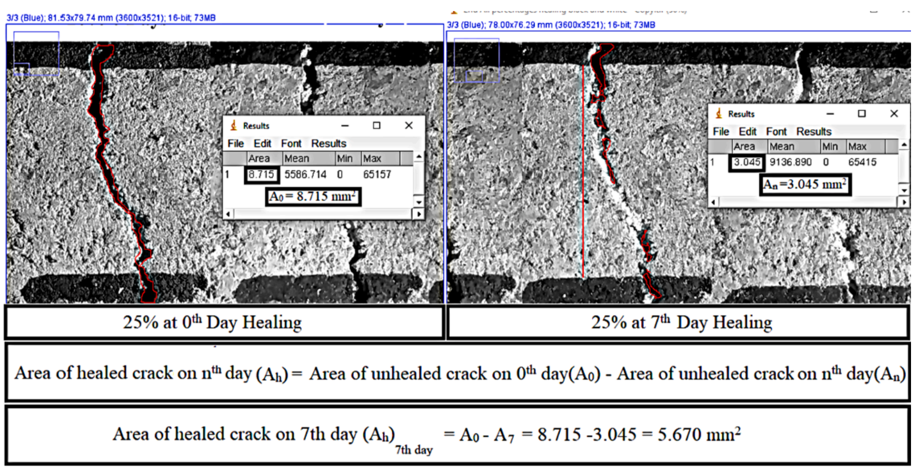

Fig. 5. Measurement and determining area of healed crack.

Fig. 6. UPV test setup.

the results of UPV. The UPV is measured before cracking the specimen in all three types of mortar samples and was compared with all UPV values after cracking and healing after different time intervals also. The percentage of self- healing (S.H) of mortar samples at different ages of healing when compared to unhealed cracked samples can be calculated using equation (4). The test setup and sequence of testing procedures are shown in Fig. 6 and Fig. 7.

$$\text{Percentage Increase in Self Healing (S.H)} = \frac{V_n - V_0}{V_0} \times 100 \quad (4)$$

where V_0 is UPV at 0th day of healing, V_n is the UPV at n^{th} day of healing.

2.6. Compressive strength test

The compressive strength of mortar cubes prepared with and without nano silica/EP were tested according to IS: 4031 (Part 7) – 1988. The fine aggregate was replaced with dual coated expanded perlite granules in 0, 10, 15, 20 and 25 percentages in control mortar cubes to check the change in strength. Since the expanded perlite is more porous and less dense than the fine aggregate there may be a possibility of reduction in strength. For getting accurate test results the testing was conducted using Universal testing machine (UTM) as shown in Fig. 8. With the addition of 5 percentage nano silica into the mix other set of specimens with same percentages of expanded perlite granules replacement for fine aggregate were prepared. The consideration of nano silica addition into the mix is to enhance the strength of mortar specimen without compromising the healing percentage [29].

2.7. Viability of bacteria and sealing performance of encapsulation

The life of bacteria after encapsulation is also a major concern for healing of cracks. The urease enzymatic reactions of bacteria continue to happen even after encapsulation if the bacteria are alive inside the pellet. The bacteria selected for this research work is alkaliphilic ureolytic bacteria named Bacillus Megaterium 8510 that converts the urea into ammonium and carbonate ions. So as a result of these ions formation, the electric conductivity increases [30]. The electric conductivity

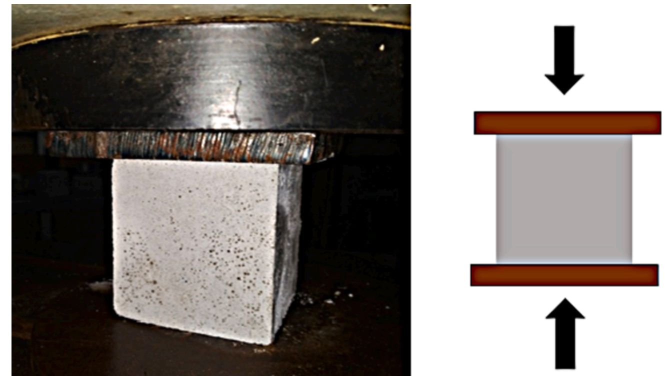

Fig. 8. Loading the specimen in UTM.

was measured using a universal water quality analyser (PE 138 ELICO instruments) at a temperature of 25 °C and the readings were recorded in micro-Siemens. The unbroken B-N pellets that were prepared initially and broken pellet samples were collected from the cracked samples of UPV test at an age of 92 days healing. The fresh unbroken bacteria encapsulated dual coated pellet, broken pellets of only Nutrient broth pellet (Only N) and Bacteria encapsulated broken pellet (B-N) were kept in 1.11 M urea solution. The readings of electric conductivity for three samples were noted at every 5 min interval for a period of one hour. The variation in values of electric conductivity was shown in Fig. 9. The progressive increment in the electric conductivity of B-N pellet is more than the Only N broken pellet and unbroken B-N pellet. From these readings it was clearly evident that the encapsulated bacteria were still alive even at the age of 92 days. Also, from the Fig. 9 it can be observed from the EC values of unbroken encapsulated B-N pellet are less in comparison with other two because of good sealing performance of dual coat and shows relatively no leakage of bacteria.

2.8. Microstructural studies using FESEM

The encapsulated bacteria, dual coating of EP particle and each layer

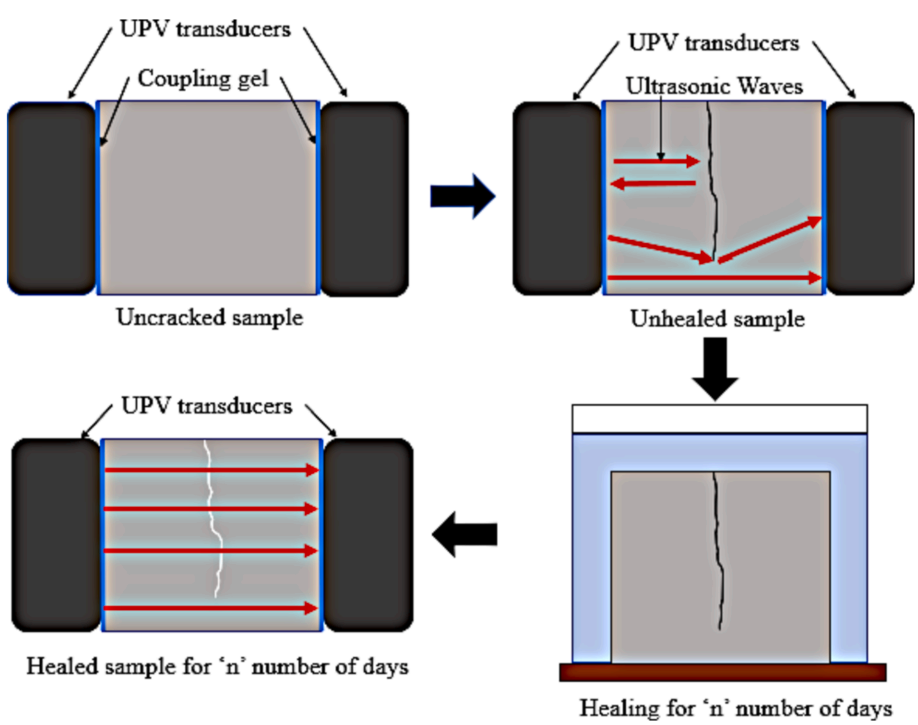

Fig. 7. UPV test sequence.

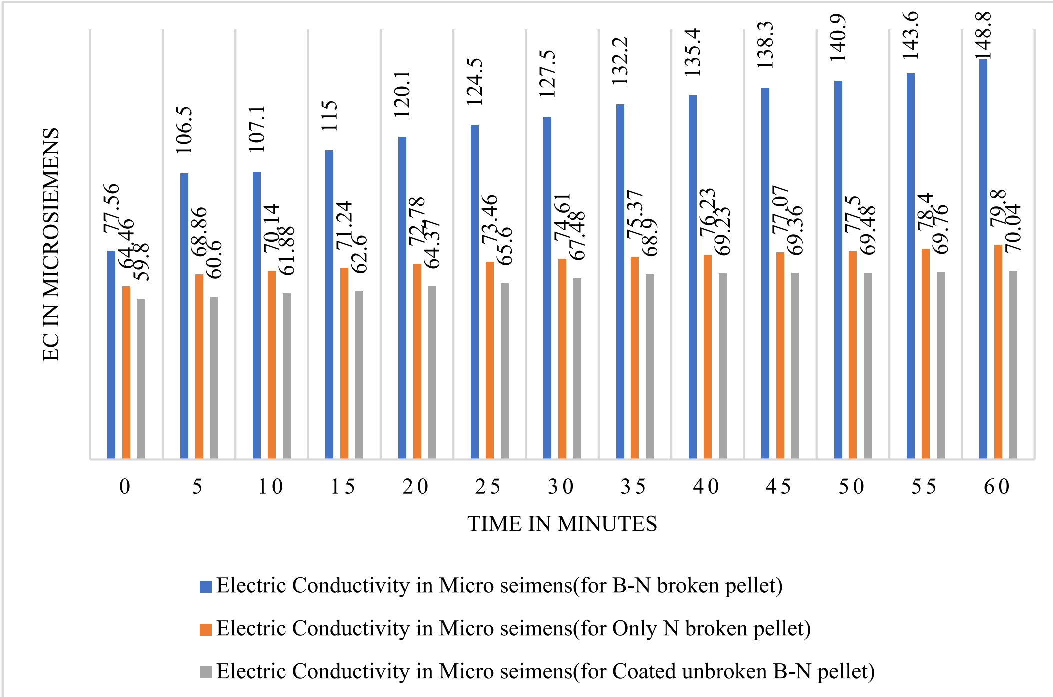

Fig. 9. Electric conductivity values.

thickness over EP particle, inter transition zone (ITZ), and the calcite crystals precipitation inside the cracked capsule was observed in field emission scanning electronic microscope (FESEM). The elemental analysis (EDX) on the precipitated crystal also was conducted for calcite confirmation.

2.9. Analysis of variance (ANOVA)

The ANOVA was performed using statistical analysis software Minitab 7.0 for finding optimum percentage of aggregate replacement to understand the effect of encapsulated perlite for healing cracks. The same was also performed for finding the optimum percentage of aggregate replacement with the addition of nano silica for the enhancement of compressive strength. Usually, large F-values, small p-values (<0.05) and R-sq values nearing 100% are used to validate a regression model [31].

3. Results and discussion

3.1. Compressive strength

The Fig. 10 represents the effect of percentage aggregate replacement on compressive strength of concrete. With an increase in the proportion of aggregate replacement, a continuous decline in

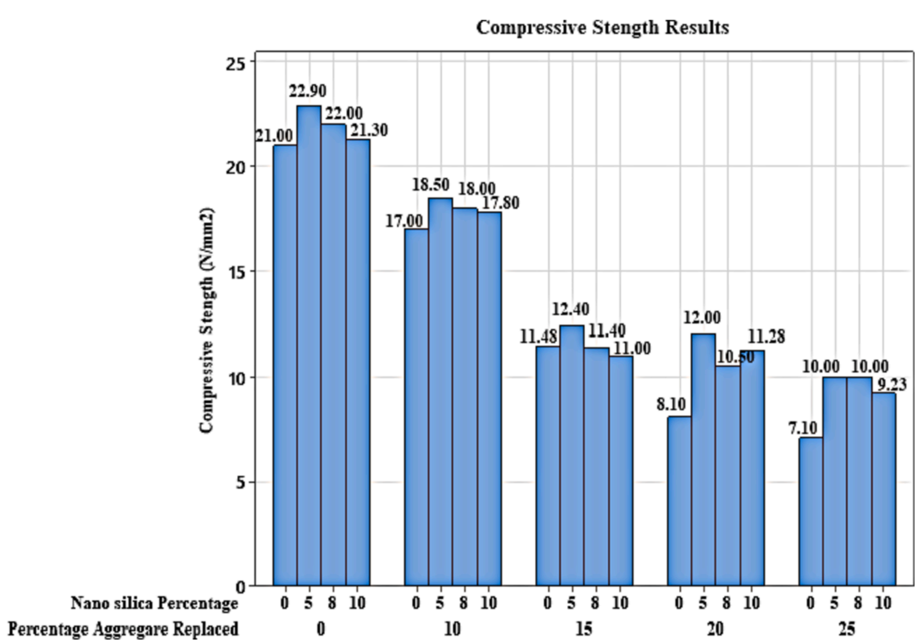

Fig. 10. Compressive strength of specimens With NS and Without NS.

compressive strength was observed. In order to compensate for the loss of compressive strength, 5%, 8% and 10% nano silica is added to the mortar. Fig. 10 also depicts the impact of nano silica content on concrete compressive strength.

The test results revealed that the compressive strength of specimens reduced with the addition of expanded perlite due to its low density, low stiffness and high internal porosity. But the addition of 5 percentage nano silica slightly improved the strength compared to the control. This may be due to the contribution of nano silica in formation of excess amount of C-S-H inside the matrix in addition to already existing C-S-H due to cement hydration. However, as dosage was increased, compressive strength declined. The reduction in compressive strength may be due to the agglomeration of nano silica particles which may have had a detrimental impact on the hydration level and bonding strength of the material. Even though all specimens added with nano silica have shown the increment of strength in comparison with out NS, the specimens prepared with 20 percentage expanded perlite granules with the addition of 5% nano silica have shown 48.15 percentage improvement. Hence the combination of 20 percentage aggregate replacement and 5 percentage NS is considered as optimum percentage for improving compressive strength.

3.2. Visual crack healing observation:

The crack healing was observed for a period of 28 days at every 3rd, 7th, 14th, 21th, 28th day. The area of healed crack at each n^{th} day was measured using ImageJ software. The increment in percentage of self-healing was also calculated for each n^{th} day. Due to presence of different percentage of expanded perlite there was difficulty in producing crack of uniform width. So, the average width of crack was determined by using equation (1). The average width of cracks in each mix type is shown in the Fig. 11. The area of crack healing (A_h) at each corresponding n^{th} day of healing also was measured using ImageJ software as shown in Fig. 5 and is presented in Fig. 11. The area of crack healing was visually observed to be increasing along with the days of healing in cracked B-N samples. And the area of surface crack healing in Only N samples was observed to be zero after 28 days when compared with area of healing in cracked B-N samples. Since there was no healing observed in Only N samples as shown in Fig. 12 the percentage increase was calculated only for B-N samples using equation (5) and is presented in Fig. 13.

$$\textit{Percentage increase in area of self healing} = \frac{A_0 - A_n}{A_0} \times 100 \quad (5)$$

where A_n is the area of unhealed crack on n^{th} day of healing; A_0 is the area of the unhealed crack healing on 0th day of healing.

For 10 and 15 percentages replaced samples the average crack width is 0.30 and 0.36 respectively and the corresponding percentage area of crack healing was determined as 97.98 and 88.32. The average width of crack was observed to be 0.62 mm and 0.60 mm for 20 percentages and 25 percentage replaced specimen respectively. The area of healing percentage is observed to be 96.31 and 96.85 for 20 and 25 percentage replaced specimen. Since almost nearly equal amount of healing was

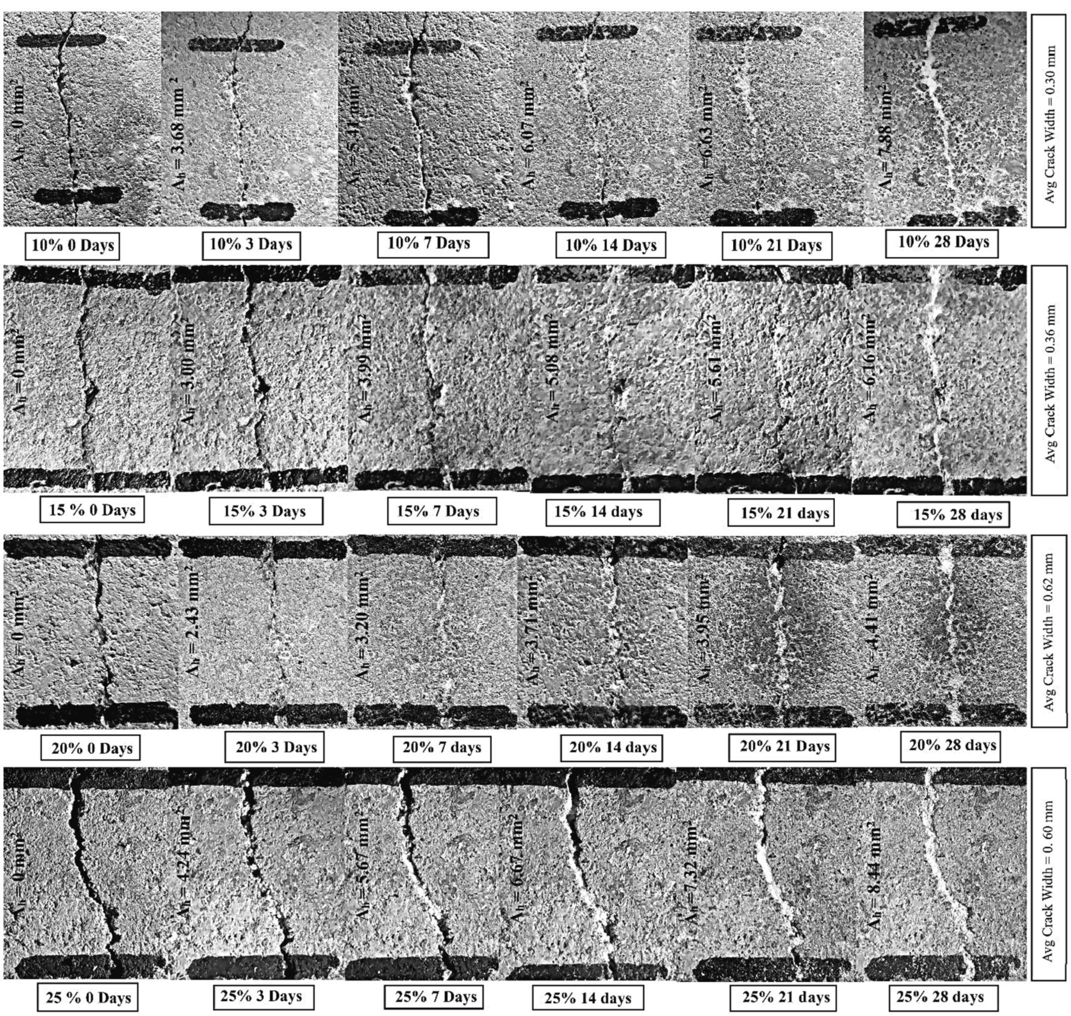

Fig. 11. Visual crack healing (B-N) analysis using ImageJ.

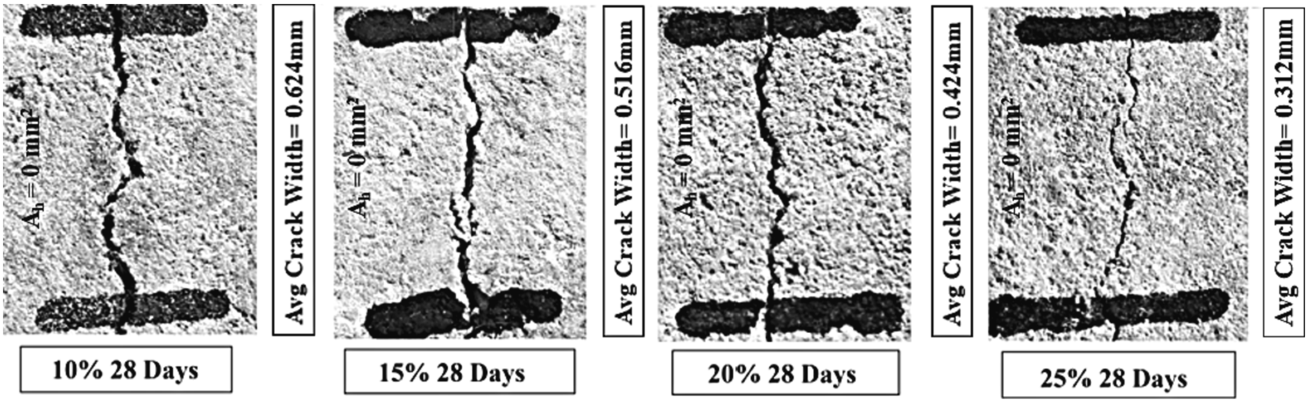

Fig. 12. Visual crack healing (Only N) analysis using imageJ.

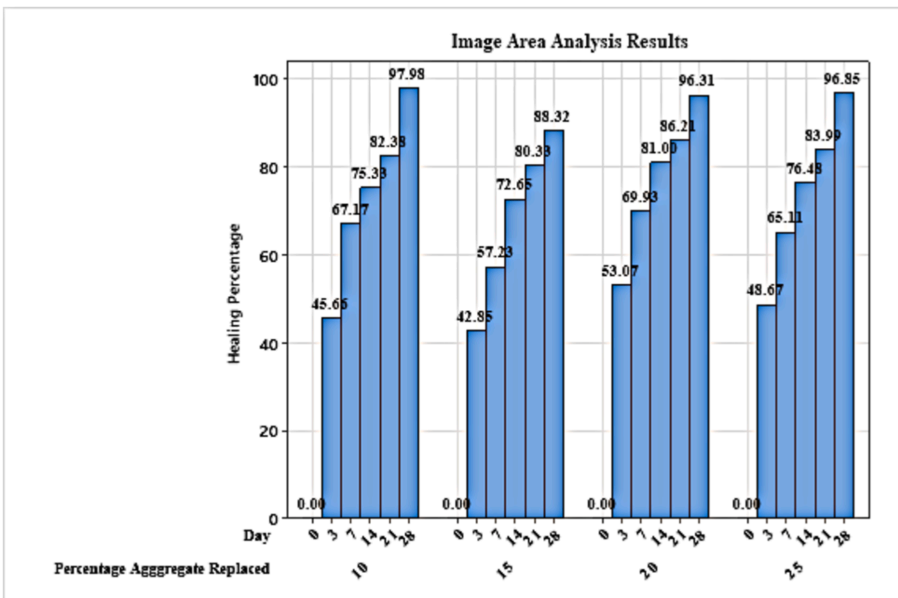

Fig. 13. Effect of percentage aggregate replacement on surface healing.

observed for both 20 and 25 percent aggregate replacement, to find out optimum percentage of aggregate replacement and to find optimum nano silica content that need to be added in order to compensate the lost compressive strength due to the replacement of aggregate, comparison of terms is done by ANOVA general linear model. The corresponding grouping information using turkey method at 95 percentage confidence level is shown in Table 6.

Table 6
Grouping information using the Tukey method and 95% confidence.

Test Name	A (Percentage replacement)	N (No. of observations)	Mean	Grouping	
Compressive Strength	5	5	15.16	A	
	8	5	14.3800	A	
	10	5	14.1220	A	B
	0	5	12.9360		B
UPV	20	5	23.1402	A	
	25	5	21.9840	A	
	10	5	8.0164		B
	15	5	7.7723		B
Visual Crack Healing	20	6	64.4231	A	
	25	6	61.8526	A	
	10	6	61.4275	A	
	15	6	56.9000		B

3.3. UPV test results

Figs. 14 and 15 shows UPV results on different days of healing. From the results it can be observed that percentage healing in ‘B-N’ specimens’ is more than the Only N specimens. This shows that the presence of bacteria in B-N specimens produces more calcite precipitation throughout the region of crack. From Fig. 15 it can be observed that 15% specimens shown prominent healing percentage that may be due to the formation of expansive cementitious products like $Ca(OH)_2$, C-S-H, and $CaCO_3$. Also, sometimes partial healing could also be possible by plugging effect that is due to mixing of water containing dissolved salts of calcium and magnesium silicates that could close the crack partially [32].

The increment in self-healing (S.H) percentages at respective days 3, 7, 14, 21, 28 were calculated by using equation 3. The percentage increment of UPV for ‘B-N’ specimens which were cracked and subjected to healing for 3, 7, 14, 21, 28 days was observed when compared to percentage of UPV values at 0 days of healing. The increase in percentage of UPV values for B-N specimens is shown in Fig. 16. It also represents the effect of percentage aggregate replacement on healing at inner portions of concrete. Maximum healing percentage (39%) at inner portions of concrete was achieved for 20% aggregate replacement.

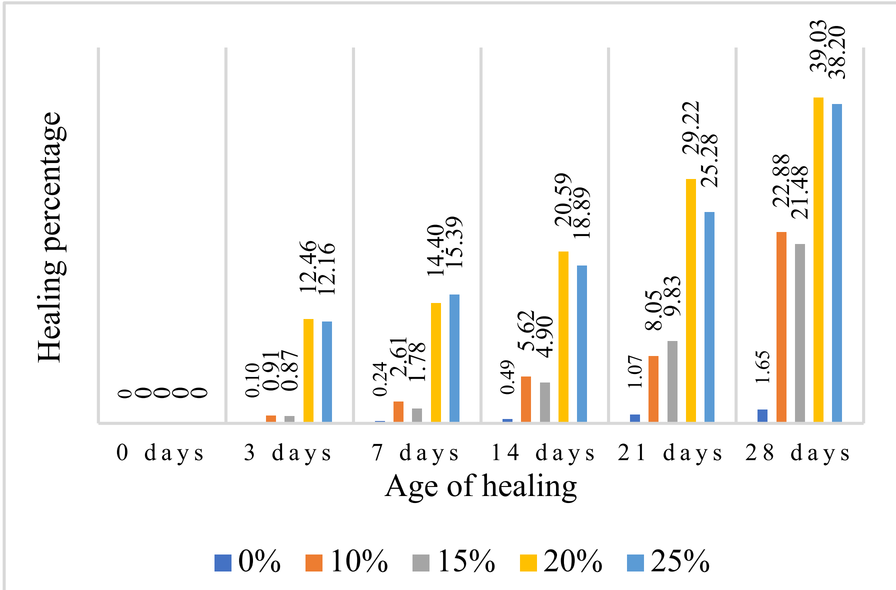

Fig. 14. Percentage increase in UPV values for B-N specimen.

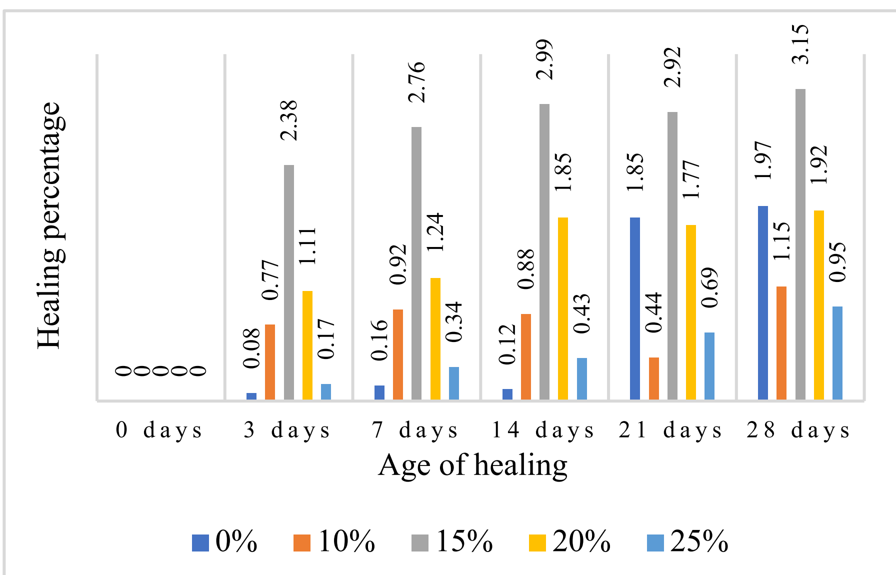

Fig. 15. Percentage increase in UPV values for Only N specimen.

However, similar healing percentage (38%) is also observed for 25% aggregate replacement.

In terms of comparison as listed in Table 6, factors with almost similar mean will share the same letter, indicating that the factors have a similar influence or there is no substantial difference between them. Factors with different letters differ significantly. ANOVA general linear model comparison result showed that 20%, 25% as optimum aggregate replacement percentages and 5%, 8% and 10% as optimum nano silica content, by grouping them under same category 'A'. However, because of their higher mean values, 20% aggregate replacement and 5% nano silica replacement can be considered as optimal percentages.

The regression analysis was also done to establish the equations for all the tests conducted. The corresponding equations for each property are also shown in Table 7. The advantage of establishing regression equations is that for any variation in the values of the variables the result can be estimated without experimentation process.

The findings of ANOVA (analysis of variance) were used to confirm the selected model statistically, and are provided in Table 8. For the validation of regression model, large F-values, small p-values less than 0.05, and R-sq values near to 100 percent suggest the model's suitability for authentication, as seen in ANOVA tables.

3.4. FESEM analysis

The dual coated bacteria encapsulated expanded perlite was taken from broken samples of cement mortar. The sample prepared was coated with gold prior to the testing. The sample was examined at different levels of magnification to visually identify the coating surfaces and pore structure of expanded perlite. The declined strength due to the increase in percentage of EP pellets, weak ITZ between perlite and mortar paste was observed in FESEM images and the increment in strength due to addition of nano silica formed a dense C-S-H, and $Ca(OH)_2$ structure as shown in Fig. 17(a, b and c). The nano silica lumps also can be observed within the hydrated cement matrix as shown in Fig. 17(d). At the 100 µm

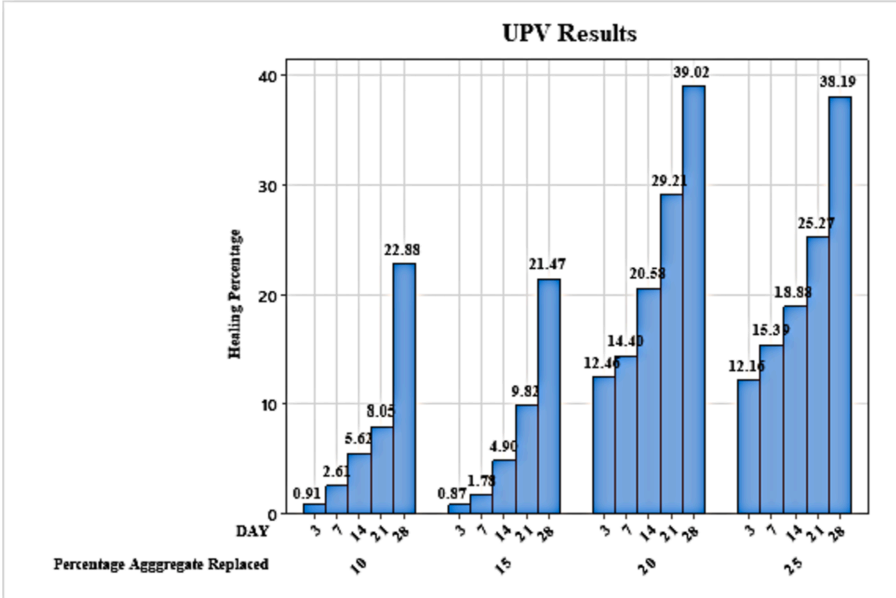

Fig. 16. Effect of percentage aggregate replacement on inner healing using UPV.

Table 7
Regression analysis.

Test Name	Regression Equation	Equation No.
Compressive Strength	C = 20.958–0.5730 X + 0.445 Z where, X = Percentage aggregate replacement, Z = Nano silica percentage, C = Compressive strength	6
UPV	P_{UPV} = -18.03 + 1.145 X + 0.905 Y where, X = Percentage aggregate replacement, Y = n^{th} day P_{UPV} = Healing Percentage	7
Visual Crack Healing	P_{VCH} = 25.0 + 0.176 X + 2.718 Y where, X = Percentage aggregate replacement, Y = n^{th} day P_{VCH} = Healing Percentage	9

magnification level the porous structure inside the perlite was observed very clearly as shown in Fig. 18. These coatings are not uniform over each pellet. The coating shows that it has covered completely on the surface of the perlite particle so that no moisture and air penetrates into the encapsulated capsule to ensure the dormant state of bacteria. The dormant state of bacteria was also confirmed indirectly by measuring EC values as shown in Fig. 11.

The precipitated calcite crystals by the encapsulated bacteria were observed at 1 μm magnification level as shown in Fig. 19. The precipitated calcite from calcite precipitation capacity test (section 2.2) was also observed under the SEM has shown that calcite crystals are in hexagonal shape and bacterial cells are in cylindrical shape as in Fig. 19. Also, the precipitate from the cracked surface, precipitate inside the broken EP particle was also hexagonal shape and hence confirms the calcite precipitation by bacteria. Bacillus megaterium precipitates the similar kind of calcite crystals was observed in previous research work [33]. The EDX analysis of these crystals clearly confirmed the presence of elements calcium, oxygen, and carbon as shown in Fig. 19. These chemical elements are sufficient to form the calcium carbonate crystals.

3.5. XRD analysis

The calcite formed in the cracked area of the specimen was collected separately and tested for ensuring the calcite precipitation. The results from XRD analysis have shown sharp peaks which clearly confirms the presence of crystalline material (Fig. 20). The scanning was done in the range of 5 degree to 90 degree at a scanning rate of 0.020 /sec [12]. The calcite presence was confirmed with highest peak established at an angle of 29.320 and the corresponding peak value is 2148.33 [33].

Table 8
Validation of regression models.

Test Name	Source	DF	Adj SS	Adj MS	F-Value	P-Value	S	R-sq	R-sq(adj)	R-sq (pred)
Compressive strength	A	4	253.604	63.4010	90.65	0.000	0.836325	98.96%	97.66%	93.49%
	B	1	12.365	12.3654	17.68	0.014				
	Error	4	2.798	0.6994	–	–				
	Total	9	268.767	–	–	–				
UPV	A	3	1079.20	359.732	106.32	0.000	1.83941	98.43%	97.51%	95.63%
	B	4	1459.08	364.770	107.81	0.000				
	Error	12	40.60	3.383	–	–				
	Total	19	2578.88	–	–	–				
Visual crack healing	A	3	176.1	58.69	10.46	0.001	2.36855	99.64%	99.45%	99.08%
	B	5	23175.6	4635.12	826.22	0.000				
	Error	15	84.2	5.61	–	–				
	Total	23	23435.8	–	–	–				

DF – Degree of Freedom, Adj SS – Adjusted Sum of Squares Adj MS – Adjusted Mean Squares.

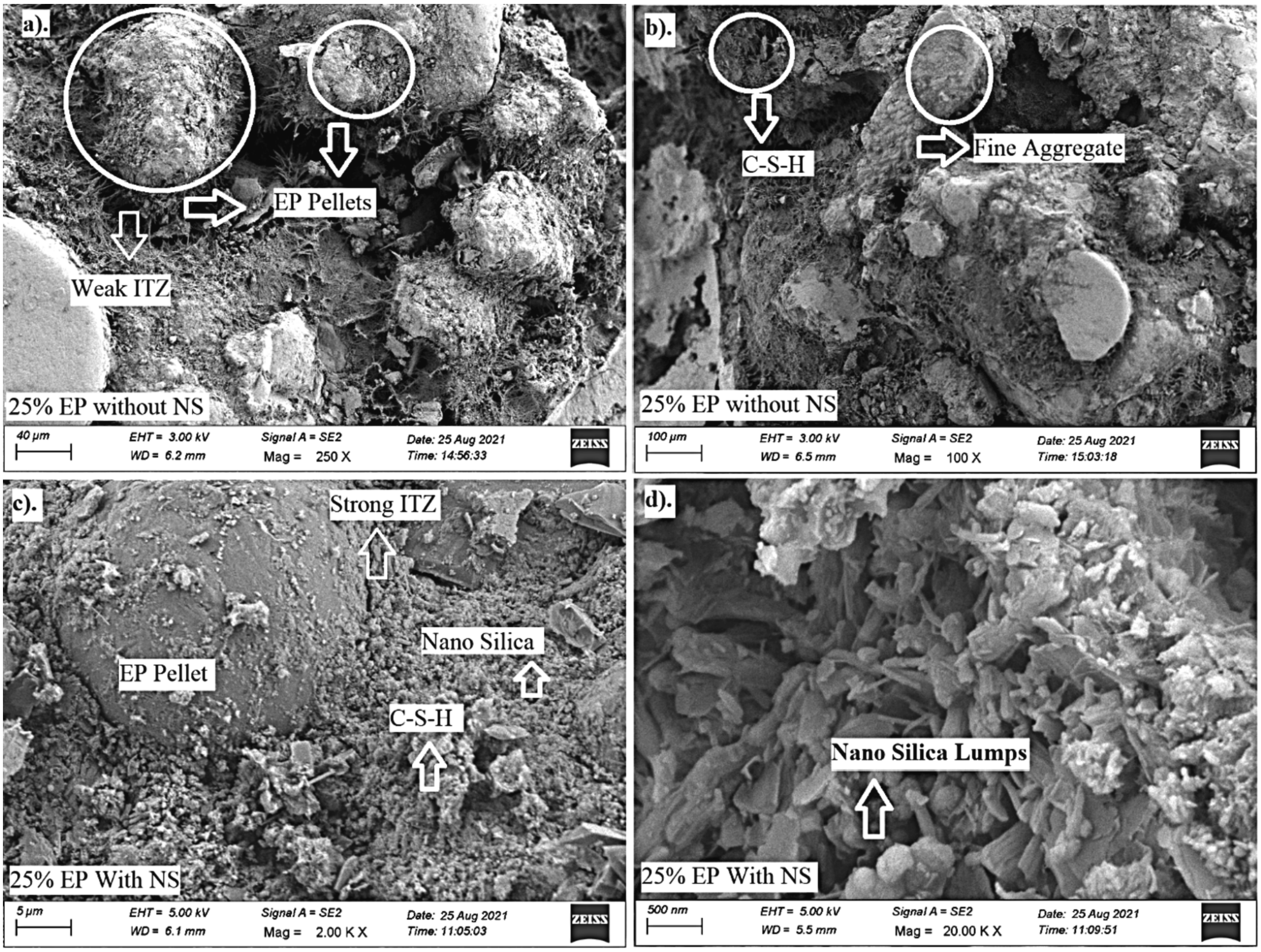

Fig. 17. Microstructure of cement mortar before and after addition of Nano silica.

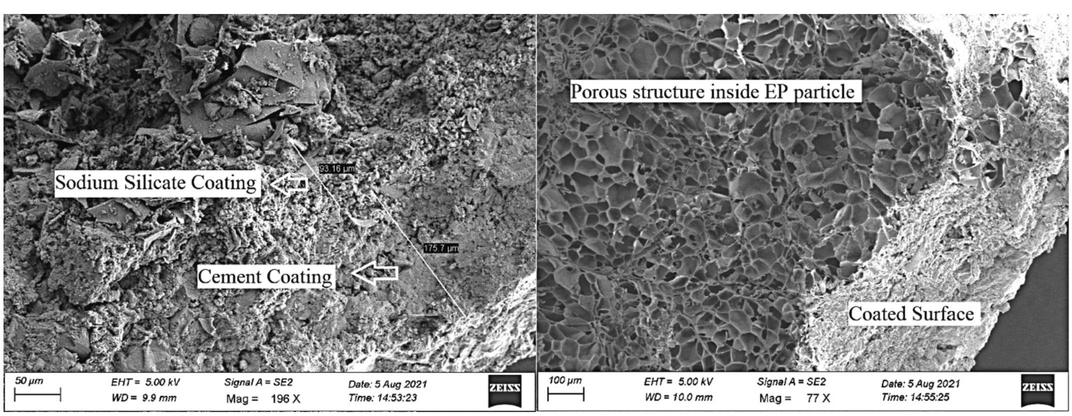

Fig. 18. Dual coating and pore structure in broken coated Expanded Perlite.

4. Conclusions

The capability of dual coated expanded perlite was confirmed based on visualizing and analysing the crack healing at different ages of self-healing and Ultrasonic Pulse Velocity (UPV) method. From the results the following conclusions are made:

- The encapsulated bacteria are viable even after 92 day of encapsulation based on the results from electric conductivity. From the increment in electric conductivity values, it can be concluded that the bacteria were alive inside the expanded perlite particles even after a period of 3 months.
- The calcite precipitation inside the perlite and its crystal shapes were observed in FESEM which was a visual confirmation of biomineralization. The results from XRD also confirmed the precipitated crystals as calcite.
- The encapsulation of bacterial spores into expanded perlite was done successfully with an encapsulation capacity of 3.73×10^8 cells/g which is sufficient enough to heal the range of crack width from 0.154 mm to 0.626 mm.

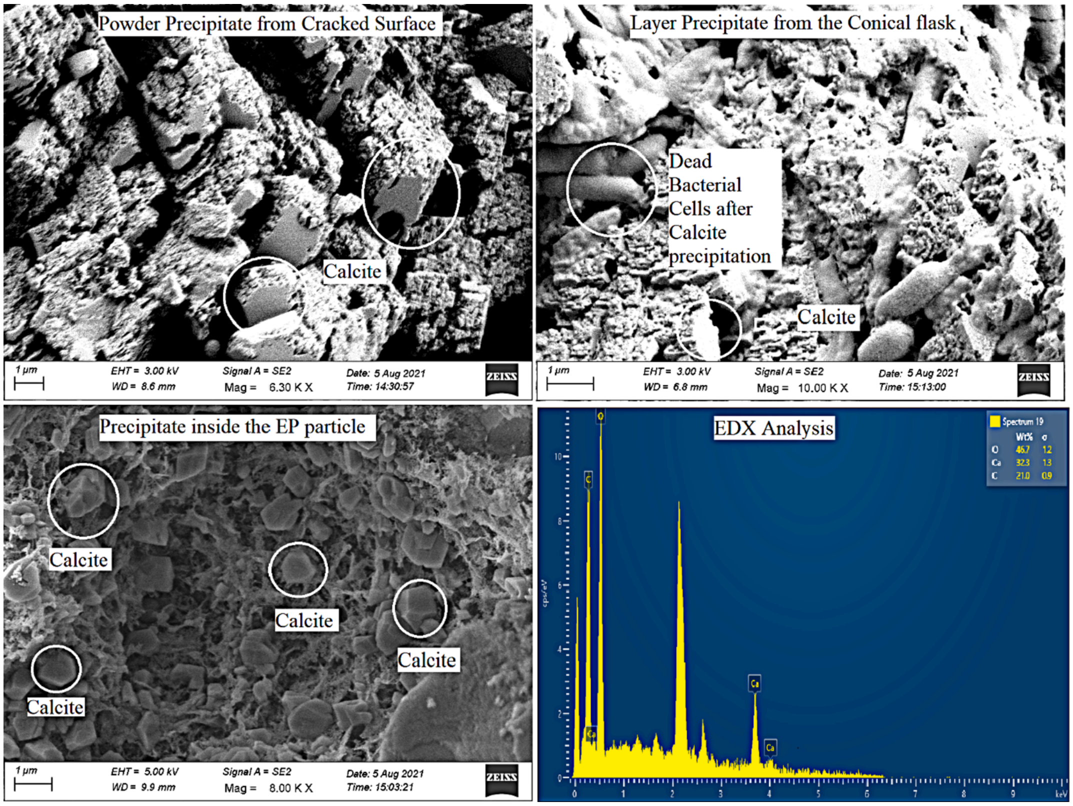

Fig. 19. Calcite precipitation.

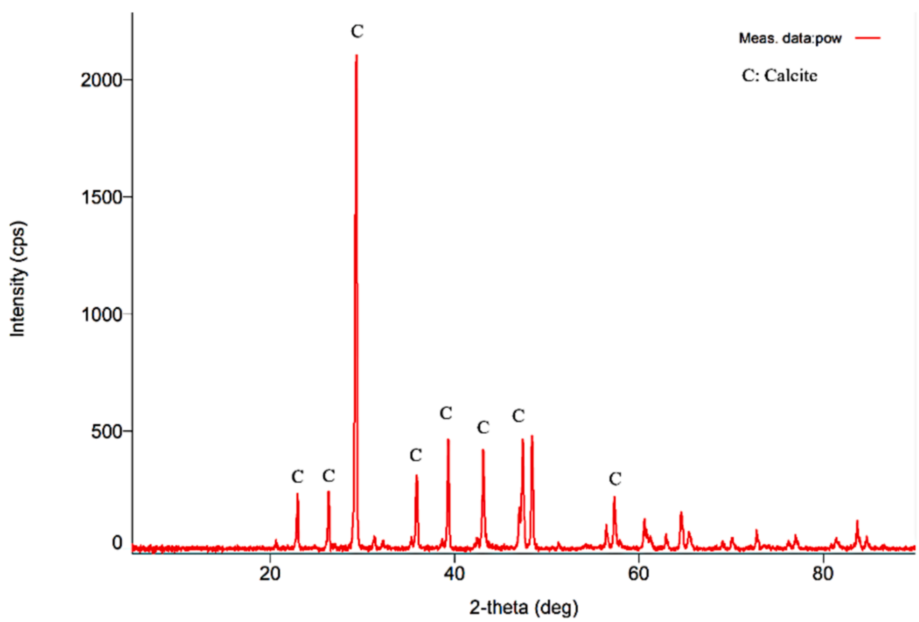

Fig. 20. XRD analysis.

- The maximum amount of healing was observed on the surface of the cracks from visual observation using Image J software compared to the results of UPV method. This is due to the availability of more moisture and oxygen at the surface level. The healing is lesser in deeper parts of the cracks as is evident from the results of UPV method.
- The reduction in compressive strength is observed upon increment in addition of expanded perlite. Even though the addition of perlite enabled the healing of cracks but on the other hand the reduction of strength is observed. So, the addition of 5 percentage nano silica was done and significant improvement in strength was observed without compromising the healing of cracks.
- Concrete properties like compressive strength, UPV and surface healing are successfully modelled using regression equations by considering selected parameters. ANOVA output verified the established model by means of a high R-sq value for all the properties.

Upon performing ANOVA analysis using Minitab statistical analysis software, 20 percentage replacement of expanded perlite was found to be the optimum percentage of replacement of fine aggregate for both the strength and healing parameters.

From the research work done it can be concluded that the bacterial encapsulation techniques are a sustainable way of healing of cracks in unobserved and unidentifiable areas of the cracked structure. The survival of bacteria in the concrete for a longer duration of 100 days is also ensured in the present study. Even though the initial cost of preparation of self-healing concrete is more, the cost associated with the future repair and maintenance works can be minimized. The research work also can be conducted on the effect of different encapsulation capacities of bacteria on different percentages of healing cracks with greater width of cracks.

CRediT authorship contribution statement

Prabhath Ranjan Kumar Soda: Conceptualization, Methodology, Validation, Investigation, Resources, Data curation, Writing – original draft. **Eluri Kalyana Chakravarthi:** Software, Investigation, Formal analysis, Writing – original draft. **Asheer Mogal:** Software, Investigation, Formal analysis, Writing – original draft. **K.M. Mini:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] C.W. Condit, The first reinforced-concrete skyscraper: The Ingalls Building in Cincinnati and its place in structural history, Technol. Culture 9 (1) (1968) 1–33.
[2] J. O'Connor, Survey on actual service lives for North American buildings. InProc. Wood frame Housing Durability and Disaster Issues Conference, Forest Products Society, Las Vegas, USA 2004 Oct 4.
[3] H. Singh, R. Gupta, Cellulose fiber as bacteria-carrier in mortar: Self-healing quantification using UPV, J. Build. Eng. 1 (28) (2020), 101090.
[4] S. Luhar, S. Gourav, A review paper on self healing concrete, J. Civ. Eng. Res. 5 (3) (2015) 53–58.
[5] J. Xu, X. Wang, J. Zuo, X. Liu, Self-healing of concrete cracks by ceramsite-loaded microorganisms, Adv. Mater. Sci. Eng. 2018 (2018).
[6] J.Y. Wang, N. De Belie, W. Verstraete, Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete, J. Ind. Microbiol. Biotechnol. 39 (4) (4 2012) 567–577.
[7] J. Wang, K. Van Tittelboom, N. De Belie, W. Verstraete, Use of silica gel or polyurethane immobilized bacteria for self-healing concrete, Constr. Build. Mater. 26 (1) (2012) 532–540.
[8] W. Khaliq, M.B. Ehsan, Crack healing in concrete using various bio influenced self-healing techniques, Constr. Build. Mater. 102 (2016) 349–357.
[9] V. Wiktor, H.M. Jonkers, Quantification of crack-healing in novel bacteria-based self-healing concrete, Cement Concr. Compos. 33 (7) (8 2011) 763–770.
[10] J. Zhang, Y. Liu, T. Feng, M. Zhou, L. Zhao, A. Zhou, Z. Li, Immobilizing bacteria in expanded perlite for the crack self-healing in concrete, Constr. Build. Mater. 148 (2017) 610–617.
[11] M. Alazhari, T. Sharma, A. Heath, R. Cooper, K. Paine, Application of expanded perlite encapsulated bacteria and growth media for self-healing concrete, Constr. Build. Mater. 30 (160) (2018) 610–619.
[12] L. Jiang, G. Jia, C. Jiang, Z. Li, Sugar-coated expanded perlite as a bacterial carrier for crack-healing concrete applications, Constr. Build. Mater. 30 (232) (2020), 117222.
[13] G. Souradeep, H.W. Kua, Encapsulation technology and techniques in self-healing concrete, J. Mater. Civ. Eng. 28 (12) (2016) 04016165.
[14] H. M. Jonkers, "Bio-concrete" set to revolutionise the building industry: Dutch inventor of self-healing concrete named finalist for European Inventor Award", 21 April 2015, EPO, https://www.epo.org/newsevents/press/releases/archive/2015/20150421i.html.
[15] S.S. Lucas, C. Moxham, E. Tziviloglou, H. Jonkers, Study of self-healing properties in concrete with bacteria encapsulated in expanded clay, Sci. Technol. Mater. 1 (30) (2018) 93–98.
[16] M.G. Meharie, J.W. Kaluli, Z. Abiero-Gariy, N.D. Kumar, Factors affecting the self-healing efficiency of cracked concrete structures, Am. J. Appl. Scientific Res. 3 (6) (2017) 80–86.
[17] A.G. Celik, A.M. Kilic, G.O. Cakal, Expanded perlite aggregate characterization for use as a lightweight construction raw material, Physicochemical Problems of Mineral Processing 49 (2013).
[18] O. Sengul, S. Azizi, F. Karaosmanoglu, M.A. Tasdemir, Effect of expanded perlite on the mechanical properties and thermal conductivity of lightweight concrete, Energy Build. 43 (2–3) (2011) 671–676.
[19] D. Sarada, H.S. Choonia, D.D. Sarode, S.S. Lele, Biocalcification by Bacillus pasteurii urease: a novel application, J. Ind. Microbiol. Biotechnol. 36 (8) (2009) 1111–1115.
[20] V. Achal, A. Mukherjee, P.C. Basu, M.S. Reddy, Strain improvement of Sporosarcina pasteurii for enhanced urease and calcite production, J. Ind. Microbiol. Biotechnol. 36 (7) (2009) 981–988.
[21] V. Achal, X. Pan, D. Zhang, Remediation of copper-contaminated soil by Kocuria flava CR1, based on microbially induced calcite precipitation, Ecol. Eng. 37 (10) (2011) 1601–1605.
[22] V. Achal, X. Pan, D. Zhang, Q. Fu, Bioremediation of Pb-contaminated soil based on microbially induced calcite precipitation, J. Microbiol. Biotechnol. 22 (2) (2012) 244–247.
[23] V. Achal, X. Pan, Q. Fu, D. Zhang, Biomineralization based remediation of As (III) contaminated soil by Sporosarcina ginsengisoli, J. Hazard. Mater. 30 (201) (2012 Jan) 178–184.
[24] N.K. Dhami, M.S. Reddy, A. Mukherjee, Biomineralization of calcium carbonate polymorphs by the bacterial strains isolated from calcareous sites, J. Microbiol. Biotechnol. 23 (5) (2013) 707–714.
[25] N.K. Dhami, M.S. Reddy, A. Mukherjee, Synergistic role of bacterial urease and carbonic anhydrase in carbonate mineralization, Appl. Biochem. Biotechnol. 172 (5) (2014) 2552–2561.
[26] V. Achal, X. Pan, Influence of calcium sources on microbially induced calcium carbonate precipitation by Bacillus sp. CR2, Appl. Biochem. Biotechnol. 173 (1) (2014) 307–317.
[27] P. Aggarwal, R.P. Singh, Y. Aggarwal, Use of nano-silica in cement based materials—a review, Cogent Eng. 2 (1) (2015) 1078018.
[28] J.I. Tobón, J.J. Payá, M.V. Borrachero, O.J. Restrepo, Mineralogical evolution of Portland cement blended with silica nanoparticles and its effect on mechanical strength, Constr. Build. Mater. 1 (36) (2012) 736–742.
[29] A.M. Mohamed, Influence of nano materials on flexural behavior and compressive strength of concrete, HBRC J. 12 (2) (2016) 212–225.
[30] S. Krishnapriya, D.V. Babu, Isolation and identification of bacteria to improve the strength of concrete, Microbiol. Res. 1 (174) (2015) 48–55.
[31] D. Patnaik, A.K. Verma, S. Panda, N.K. Sahoo, Bio Cementation Process in the Concrete using Bacillus Subtilis: A Statistical Modeling Study. InIOP Conference Series: Materials Science and Engineering 2020 Nov 1 (Vol. 970, No. 1, p. 012027). IOP Publishing.
[32] J. Parks, M. Edwards, P. Vikesland, A. Dudi, Effects of bulk water chemistry on autogenous healing of concrete, J. Mater. Civ. Eng. 22 (5) (2010) 515–524, https://doi.org/10.1061/(ASCE)MT.1943-5533.0000082.
[33] Prabhath Ranjan Kumar Soda, K.M. Mini, Performance Enhancement and Remediation of Micro cracks in Cement Mortar by Doping Calcite Precipitating Microorganism". Journal of Materials in Civil Engineering, ASCE, Volume 34-Issue 4- April 2022. doi: 10.1061/(ASCE)MT.1943-5533.0004176.
[34] Rahul Goushis, K.M. Mini, Effectiveness of polymeric and cementitious materials to secure cracks in concrete under diverse circumstances. Int. J. Adhesion Adhesives. 114 (2022) 103099.
[35] J. Zhang, C. Zhao, A. Zhou, et al., Aragonite formation induced by open cultures of microbial consortia to heal cracks in concrete: insights into healing mechanisms and crystal polymorphs, Constr. Build. Mater. 224 (2019) 815–822.
[36] N.F. Ariffin, M.W. Hussin, A.R. Sam, H.S. Lee, N.H. Khalid, N.H. Lim, M. Samadi, Mechanical properties and self-healing mechanism of epoxy mortar, Jurnal Teknologi 77 (12) (2015).
[37] V. Poornima, R. Venkatasubramani, V. Sreevidya, S. Subash, J. Karingamanna, Environmental influence on bacterial efficiency in ensuring self-healing mortars. European journal of environmental and civil engineering. 2021 Jun 21.
[38] P.R.K. Soda, V. Poornima, V. Rameshkumar, R. Venkatasubramani, Influence of ureolytic bacteria in improving performance characteristics of concrete, Eclgy. Env. Cons 23 (2017) 57–63.
[39] M.G. Giriselvam, V. Poornima, R. Venkatasubramani, V. Sreevidya, Enhancement of crack healing efficiency and performance of SAP in biocrete. In IOP Conference Series: Materials Science and Engineering (Vol. 310, No. 1,2018, p. 012061). IOP Publishing.
[40] Standard IS. 2720 (Part 4). 1985. Methods of Test for Soils: Grain Size Analysis.
[41] L. Sinai, A. Rosenberg, Y. Smith, E. Segev, S. Ben-Yehuda, The molecular timeline of a reviving bacterial spore, Mol. Cell 57 (4) (2015) 695–707.