STUDIES ON COMPRESSIVE STRENGTH MICROSTRUCTURAL ANALYSIS OF SELF-COMPACTING MORTAR WITH BACTERIA

Middela Sravanthi [1], S. Venkateswara Rao [1], Kanthala Krishnaveni [2,*], Venkata Kamal Lal Meenuga [2], Sujana Kariveda [3]

[1]Department of Civil Engineering, National Institute of Technology, Warangal, Telangana, India
[2]Department of Civil Engineering, Anurag University, Telangana, India
[3]Department of Biotechnology, Osmania University, Telangana, India

*E-mail of corresponding author: krishnavenikanthala@gmail.com

Resume

This research proposed bacteria-treated concrete, which is highly desirable because the mineral precipitation induced from microbial activities is pollution free and natural. This study investigates the effects of Bacillus Licheniformis, Bacillus Cohnii and mixed bacteria on compressive strength, ultrasonic pulse velocity test, rapid chloride penetration test, microstructural analysis and bacteria cell concentration of 10^{5} cell/ml of normal and self-compacting mortars made with and without fly ash for 7 and 28 days. With self-compacting mortar, the compressive strength of Bacillus Licheniformis improved by 30.3% and by adding Bacillus Cohnii in 28 days, the ion penetration value decreased. Finally, it was observed that the deposition of calcium carbonate in mortar improved compressive strength using SEM-EDS and XRD with compound polymorphisms of calcite, vaterite, quartz, and Hautirite.

Available online: https://doi.org/10.26552/com.C.2022.4.D183-D200

Article info

Received 29 April 2022
Accepted 14 September 2022
Online 17 October 2022

Keywords:

self-compacting mortar
compressive strength
bacillus licheninformis
bacteria cohnii
class-c fly ash
durability test

ISSN 1335-4205 (print version)
ISSN 2585-7878 (online version)

1 Introduction

In the field of civil engineering for the construction of modern buildings, concrete has the highest compressive strength compared to steel [1]. It is also the most affordable. Due to its many properties, such as high compressive strength, durability and rigidity, concrete is the most commonly used building material [2]. Workable concrete is defined as concrete that has been laid and compacted uniformly without bleeding or segregation. Microbiologically Induced Calcite Precipitation (MICP) is a natural bio-mineralization process in which live microorganisms (such as bacteria) generate layers of Calcium Carbonate ($CaCO_3$) that fill cracks and improve concrete's strength and durability [3].

Numerous kinds of research have been conducted and more are being conducted to determine the use of bacteria in self-repairing cracked concrete. A new generation of bio-additives for Self-Compacting Concrete (SCC) was recently created using this ground-breaking technology [4-6]. Self-Compacting Concrete is a flowable self-consolidating concrete mixture. The concrete's durability is reduced due to cracking and is more prone to corrosion. Cracks in concrete surfaces are often repaired with cement paste, penetrating cement grout, or epoxy grout [7-8].

Self-healing concrete self-heals by forming a lime layer with the help of moisture and air from the environment. The reaction between cement and water is exothermic and the heat release is called heat of hydration. The heat of hydration is one of the well-known methods to measure reactivity. Concerning the heat of hydration study, 50% of the total heat is released between 1 and 3 days, 75% in 7 days and 83-91% in 28 days for Ordinary Portland Cement (OPC). The age at which 20-30% hydration will be completed depends on the water-to-cement ratio, relative humidity, curing method and temperature and cement composition. The non-hydrated cement will hydrate when cracks emerge on the concrete surface [9-10]. The lime particles in cement will then react with the moisture in the air, filling up the fissures. When the crack width is between 0.05 and 0.1 mm, the crack may automatically fill. Alternative repair procedures, based on chemical healing agents, can be employed for crack widths less than 0.3 mm when the crack width reaches 0.1mm. The use of biological healing agents can also be incorporated into this approach [11]. Bacterial activity produces calcium carbonate or other calcium-based chemicals, which are then utilized to fill fissures. Microbiologically Induced Calcium Precipitation (MICP) is the name for this concept [12-13]. The MICP generates CO_2 in the presence of water by microbial metabolism, leading to formation of carbonate ions. Calcium carbonate is formed when the carbonate ion interacts with calcium ions under the right conditions [14-17].

The MICP is influenced by the bacterial concentration, ionic strength, availability of water, air space and nutrients, the matrix's pH and the presence of nucleation sites. Some of these essential criteria are built into concrete, while others can be easily achieved. *Bacillus subtilis (B. subtilis*), a common soil Gram-positive bacteria, is extremely effective in MICP when utilized in concrete. *B. subtilis* attaches to cement particles' surfaces and produces MICP nucleation sites [18-19]. Existing research provides information on the role of bacteria in successful crack restoration and improving concrete's mechanical and durability properties, which are explained as follows:

Sushree et al. [20] used an alkali-activated Ground Granulated Blast-furnace Slag (GGBS) and fly ash to replace cement in self-compacting concrete mixes. The authors look at the effects on the fresh and hardened properties of Self-Compacting Geopolymer Concrete (SCGC) mixtures due to *Bacillus Licheniformis*. Moreover, Mohd Nasim et al. [21] have investigated the effect of crystalline admixture (CA), fly ash (FA) and PVA fibre on the ability of early-age cracks in concrete to self-heal. M1 is the control mix, M2 is the 0.1 percent Polyvinyl Acetate (PVA) fibre by volume mix, M3 is the 20% partial cement replacement with fly ash mix and M4 is the 2% CA by cement mass mix; these are the four mixes used. Then, Tsampali et al. [22] investigated the qualities of the self-healing cement paste compositions comprising nano-CaO and nano-SiO_2 at 1.5% w/w, as well as a combination of the two, with 0.5 percent NL and 1% NS.

Glass Fibre Reinforced Concrete (GFRC) is used by Guzlena et al. [23] to investigate concrete that has been assisted to heal by the addition of crystals. The GRC was chosen due to the fibre reinforcement, which allowed the breadth of the crack to be controlled during the pre-cracking of the sample. Moreover, Dongsheng et al. [24] enhanced the self-healing efficiency of cemented coral fine aggregate by using Urea Formaldehyde (UF) microcapsules. Further, Manvith et al. [25] looked into various methods for employing mineralizing bacteria to improve the structural strength of concrete. Then, Pavan et al. [26] provided a quick overview of the many features of bacterial concrete. Microcracks are essentially present in concrete.

Luhar et al. [27stated that autonomous crack sealing using microorganisms in the concrete without human intervention is a possible solution to the problem of long-term concrete improvement. Algaifi et al. [28] used Gene Expression Programming (GEP) modelling a new predicted mathematical formula for compressive strength of bacterial concrete was established using 69 experimental tests with various amounts of calcium nitrate tetra-hydrate, yeast extract, urea, bacterial cells and time. Almohammed et al. [29] utilized M5P, Random Tree (RT), Reduced Error Pruning Tree (REPT), Random Forest (RF) and Support Vector Regression (SVR) techniques were evaluated and compared to Multiple Linear Regression (MLR) -based models for predicting the compressive strength of concrete (with bacteria). Ramagiri et al. [30] disclosed that since the primary goal of creating these blends is to reduce environmental impact, a full Life Cycle Analysis (LCA) is required.

Moreover, *Bacillus Licheniformis* has been studied in self-compacting geopolymer concrete, but no research on *Bacillus Licheniformis, bacillus cohnii*, or mixed bacteria in mortar, has been done. Very little research has focused on impact of the self-compacting concrete on *bacillus cohnii's* self-healing mechanism under various curing conditions. Concrete that self-compacts, when *Bacillus Licheniformis* bacteria are added, is not the subject of much research. The main contribution of this research is as follows:

Bacillus Licheniformis and Bacillus cohnii, a laboratory cultured bacteria, are used in this research. Thus, this research investigated the new properties, Ultrasonic Pulse Velocity (UPV) test and compressive strength of self-compacting and normal mortars with bacteria incorporation using single and mixed bacteria. Microstructural and durability evaluations of self-compacting and normal mortars with bacterial integration utilized single and mixed bacteria.

The following sections are laid out: The materials and their properties are covered in Section 2, along with a thorough account of the experimental work. The outcome and a discussion of the suggested strategy are presented in Section 3. The paper is concluded in Section 4.

2 Materials and methods

The following materials and methods were utilized in this research.

2.1 Materials

Materials such as cement, fine aggregate, water, admixture and bacteria (nutrient broth, nutrient agar) were gathered from local sources. The chemical and physical properties of collected materials are examined in the laboratory and tabulated as follows.

2.1.1 Cement

Cement is the most essential and crucial material that regulates mortar performance since it is required

Table 1 Physical properties of cement

Properties	Result	Permissible limits
Fineness	8%	It should not be more than 10
Specific gravity	3.12	3.1-3.16
Normal consistency	34%	25-35%
Initial setting time	35min	It should not be less than 30 min

Table 2 Chemical Composition of Cement

Oxide	Concentration (%)
CaO	63.3
SiO_2	21.8
Al_2O_3	4.8
Fe_2O_3	3.8
MgO	0.9
SO_3	2.2
K_2O	0.46
TiO_2	0.13
Na_2O	0.17

Table 3 Fineness of Sand

IS sieve size (mm)	Weight of retained (g)	% of retained	Cumulative % of retained	% of passing	As per IS383:1970 Specification limit			
					I	II	III	IV
10	0	-		100	100	100	100	100
4.75	10	1	1	99	90-100	90-100	90-100	95-100
2.36	18	1.8	2.8	97.2	60-95	75-100	85-100	95-100
1.18	106	10.9	13.7	86.6	30-70	55-90	75-90	90-100
0.6	358	35.7	49.4	50.8	15-34	35-59	60-79	80-100
0.3	498	49.8	98.2	1	5-20	8-30	12-40	15-50
0.15	10	1	100.2	0	0-10	0-10	0-10	0-15
			265.3					

Table 4 Physical Properties of Fine Aggregate

Properties	Result
Fineness modulus	2.65
Zone	II
Specific gravity	2.62
Bulk density (loose)	1550 kg/m^3
Bulk density (compacted)	1692 kg/m^3

to bond sand and aggregate. According to IS 12269-2013, this research utilized Ordinary Portland cement 53 grade (OPC-53). Table 1 shows the physical properties of cement, such as fineness, specific gravity, normal consistency and the first setting time. The OPC 53 grade cement results are compared to IS 4031-1988.

Table 2 shows the chemical characteristics of cement, such as Calcium Oxide (CaO), Aluminum Oxide (Al_2O_3), Silicon Dioxide (SiO_2), Ferric Oxide (Fe_2O_3), MgO (Magnesium Oxide), Sulphur Trioxide (SO_3), Titanium Oxide (TiO_2), Potassium Oxide (K_2O) and Sodium oxide (Na_2O), examined in this research.

2.1.2 Fine aggregate

Aggregate material helps in the compacting of cementitious materials. It minimizes cement and water usage while contributing to the mechanical strength of mortar and it is an essential component in the construction process. In this research, river sand easily available nearby was collected and processed. According to IS.2386:1963, specific gravity and fineness are evaluated based on equation (1), which is stated in Table 3.

$$\text{Fineness modulus} = (\text{Cumulative percentage of retained})/100 \quad (1)$$

The fine aggregate chosen has a fineness modulus of 2.65 and confirms Zone-II, according to the results. The sand was rinsed and screened on-site to remove harmful elements before being tested according to IS: 2386-1963. The physical properties of fine aggregate results are illustrated in Table 4.

2.1.3 Water

The mortar unit bond is reduced when water in the mortar mix evaporates or is absorbed by the masonry units and wet mixes that are simple to deal with, yielding the highest bond strengths. According to IS.456: 2000, in this research the fresh potable water was used for curing and mixing. For both vibrated and self-compacting mortar, the water-cement (w/c) ratio utilized in this research is 0.4.

2.1.4 Bacteria

This research utilizes laboratory-cultured bacterium such as *Bacillus Lechiniformis* and *Bacilluscohnii*, where the class and order of the bacteria are illustrated in Tables 5 and 6

Cultivation of Bacteria: The features of pure bacteria from the microbial culture collection are listed below. *Bacillus Licheniformis* and *Bacillus cohnii* are sorts of bacteria found in soil. They are commonly observed on ground-dwelling birds (like sparrows) and aquatic species' feathers, specifically the plumage on the chest and back (like ducks). It is a mesophilic gram-positive bacterium. It thrives at temperatures around 50 °C but can withstand much higher temperatures. The ideal temperature for enzyme secretion is 37 °C.

Nutrient Broth: Nutrient broth is a liquid medium, which is depicted in Figure 1. It is essential for the faster growth of bacteria, in which an autoclave, oven, conical flask, test tubes, glass rod and cotton are employed to prepare and sterilize nutrient broth. Moreover, for 500 ml, peptone 2.5 g, beef extract 1.50 g, NaCl 2.5 g/l and distilled water are all the ingredients of nutrient broth. Combine the ingredients in a mixing bowl, heat and shake properly to dissolve them. Then, add distilled water to reach the desired volume and partially dilute each test tube or conical flask with the medium. After dilution, cotton plugs should be placed in all flasks. After that, sterilize the prepared solutions for 15 minutes in an autoclave at 121 °C. Finally, Remove the flasks and preserve them at room temperature until suitable to use.

Nutrient Agar: Nutrient agar is the nutrient broth shown in Figure 2; the only difference is that it is hardened with agar. The ingredients are Peptone 5 g/l, NaCl 5 g/l, Meat extract 1.50 g/l, Agar 15 g/l and Yeast extract 1.20 g. Except for the agar, all ingredients are dissolved in distilled water in the correct ratio. The agar powder is added to the medium and heated to dissolve the agar, obtaining a clear liquid. Then, the media is

Table 5 *Class and Order of Bacillus Licheniformis*

Class of Bacillus Licheniformis	Order of Bacillus Licheniformis
MTCC number	2588
Genes/species	*Bacillus Licheniformis*
Growth condition	Aerobic
Subculturing period	30 days
Temperature	37°C
Incubation	36 h
Growth media number	3

Table 6 *Class and Order of Bacillus cohnii*

Class of Bacillus cohnii	Order of Bacillus cohnii
MTCC number	3616
Genes/species	*Bacillus cohnii*
Growth condition	Aerobic
Subculturing period	5days
Temperature	37°C
Incubation	24 h
Growth media number	239

Figure 1 *Nutrient Broth*

Figure 2 *Nutrient Agar*

uniformly distributed in the tubes or slants covered with cotton. After that, sterilize the prepared solutions for 15 minutes in an autoclave at 121 °C. Finally, cool the tube in a slanting position.

2.1.5 Admixtures

Ingredients added to the concrete batch right before or during the mixing are admixtures. In this research, fly ash and superplasticizer are used as admixtures, of which the chemical and physical properties are explained as follows:

Fly ash: Fly ash is a fine grey powder primarily made up of spherical, glassy particles and produced as a by-product of coal-fired power plants. This research discovers the quality standards for fly ash according to IS 3812-2003. Fineness, low carbon content and strong reactivity are the characteristics of good fly ash. Sub-bituminous coal usually produces fly ash with a CaO value of more than 10%. Moreover, fly ash from class C possesses both pozzolanic and cementitious characteristics. Thus, this research utilized class C fly ash. Class C fly ash's chemical and physical properties are illustrated in Tables 7 and 8.

Super Plasticizer: Superplasticizers (SPs) are a type of additive used in the production of high-strength concrete. They are also known as high-range water reducers. Plasticizers are chemicals that allow concrete to be made with up to 15% less water. Water content can be reduced by as much as 30%. Chemical admixtures are concrete additives that improve the concrete's strength and longevity by increasing penetration, lowering the amount of water in the concrete and improving the concrete's strength and longevity. They decrease the amount of water in the product while maintaining its workability. Table 9 shows the physical characteristics of the superplasticizer.

Table 7 *Physical Properties of Class C Fly ash*

Properties	Results Obtained
Normal consistency	28%
Initial setting time	50min
Specific gravity	2.10

Table 8 *Chemical Properties of Class C Fly ash*

Oxide	Percent content
Calcium Oxide (CaO)	16
Silicon Dioxide (SiO_2)	40
Aluminum Oxide (Al_2O_3)	17
Ferric Oxide (Fe_2O_3)	6
Magnesium Oxide (MgO)	1.81
Sulphur Trioxide (SO_3)	3
Potassium Oxide (K_2O)	2.19
Titanium Oxide (TiO_2)	0.94
Sodium Oxide (Na_2O)	0.62

Table 9 *Physical Properties of Super Plasticizer*

Base	Sulphonated Naphthalene formaldehyde
Specific gravity	1.23 - 1.25
Sight appearance	Dark brown
Solid content	40%
pH	> 6
Dosage	1to2 to litresper100kg of Cement

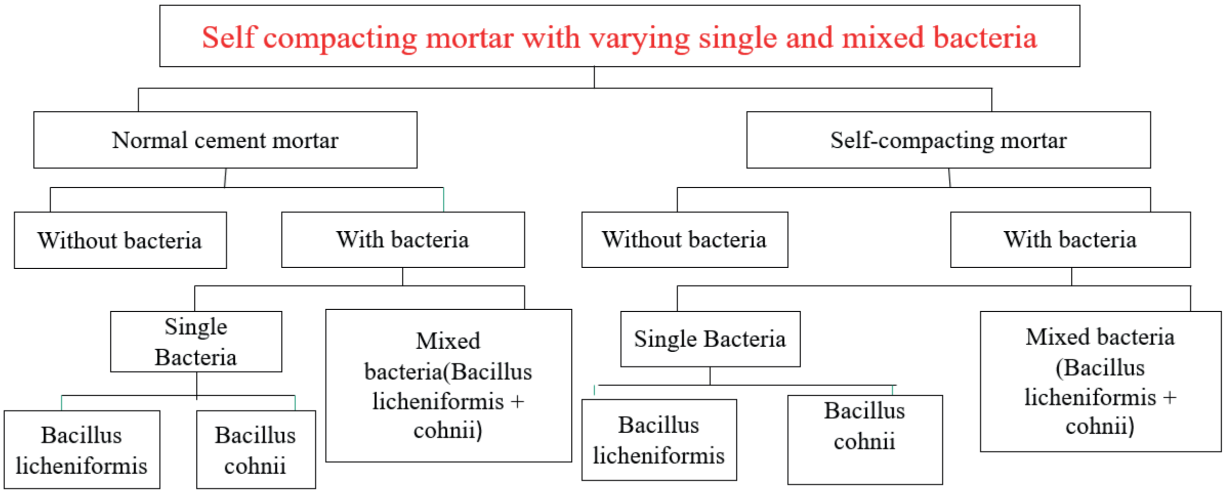

Figure 3 *Architecture of Self-compacting mortar with single and mixed bacteria*

2.2 Methods

The testing was performed using the following mix compositions - cement, fine aggregate, water and admixtures for the preparation of self-compacting mortar. Mix proportions for various combinations of mineral admixtures were evaluated using the cement replacement materials. The Mini v-funnel and Mini slump are all used to determine the workability of concrete. Mechanical Properties such as the non-

Table 10 *Mix Proportion of Normal Mortar*

Mix proportions		CEMI	Fly ash	Sand	Water	Bacteria (cells/ml)
1:1	Weight (kg/m^3)	760	190	970	0.30	10^5
	Volume (m^3)	0.243	0.09	0.37	0.230	

Table 11 *Mix Proportion of Self-Compacting Mortar*

Mix proportions		CEMI	Fly ash	Sand	Water	W/C	Sp	Bacteria (cells/ml)
1:1	Weight (kg/m^3)	760	190	970	300	0.4	3.0	10^5
	Volume (m^3)	0.243	0.09	0.37	0.3		0.00244	

(a) Casting Concrete (b) Curing Concrete

Figure 4 *Preparation of Test Specimen*

destructive test, Compressive strength test and durability test of SCM mixes were tested using various degrees of cement replacement material with a constant aggregate and water-binder ratio. The following Figure 3 shows the architecture of the proposed approach.

2.2.1 Mix proportions

The mixing process greatly impacts the fresh-state properties of self-compacting mortars when it comes to efficiently utilizing superplasticizers. They indicated that the ratio of time spent mixing before adding the superplasticizer has no effect on its effectiveness and, as a result, modifies the self-compatibility of these mortars. The overall mixing process takes 10 minutes and begins with addition of fine aggregates (sands) and fine materials, followed by one minute of mixing. The initial water fraction, equivalent to 80% of the total mixing water, is gradually added to optimize homogenization without pausing the mixing process for another minute. The second water component (the remaining 20 %), combined with the superplasticizer, is gradually put into the mix without interfering. The mixing continues at its constant speed for another 5 minutes. After that, the mixer is turned off, giving the mixture two minutes to settle. If necessary, take this step to clean the mixer's paddle. Self-compacting mortar is selected 1:1 ratio for this research. The water-cement ratio in this mix is 0.4, with fly ash replacing 20% of the cement. The following Tables 10 and 11 describe the mixed proportion of normal and self-compacting mortar. This research evaluated the mix design, which is discussed in Appendix-I.

2.2.2 Preparation of test specimen

The specimens were made in cast-iron moulds measuring 70.6 mm x 70.6 mm x 70.6 mm, in which oil was sprayed within the moulds to facilitate easier removal. The specimens are cast with and without bacteria and then demoulded and cured in fresh water in the curing tank after 24 hours. Finally, the specimens were removed and stored in the shade after completing the curing period. Figure 4 shows the casting concrete and curing concrete.

2.3 Test performed on SCM

The test was performed on self-compacting mortar in both fresh and hardened states. In fresh SCM, Mini-cone slump-flow test for SCM, Mini V-funnel test for SCM are conducted as well as in hardened SCM Compression Test, Ultrasonic Pulse Velocity Test; Rapid Chloride Penetration Test is performed. Scanning

Electron Microscopy and X-Ray Diffraction Analysis are conducted for the microstructure analysis.

2.3.1 Ultrasonic pulse velocity test

The ultrasonic pulse velocity test operates on the principle that an electro-acoustical transducer induces an ultrasonic pulse into the concrete, which then generates multiple reflections at the different material phase boundaries of the concrete specimen. The PUNDIT (Portable Ultrasonic Non-destructive Digital Indicating Tester) is used. The PUNDIT instrument is made in Switzerland by Proceq. The ultrasonic pulse velocity approach is used to evaluate the mortar for homogeneity, fractures, voids and other flaws. The ultrasonic waves' passage through the hardened mortar specimens was timed in microseconds. The pulse velocity was calculated by dividing the time required by the distance travelled.

2.3.2 Rapid chloride penetration test

After 28 days of curing, a rapid chloride penetration test was performed on mortar specimens 100 mm in diameter and 50 mm thick for proportions of standard mortar and self-compacting mortar with and without fly ash. After effectively attaching the tank and preparing the samples, this test was performed by ASTM C 1202-19 without any leaks. The diffuser cell contains two terminals, one of which is positive and the other negative. A 3.0% sodium chloride (NaCl) solution was added to the tank connected to the positive terminal. In contrast, a 0.3 N solution of sodium hydroxide (NaOH) was added to the tank attached to the negative terminal. A 60 V current was then supplied for six hours to both reservoirs. The initial reading is obtained and further readings were taken every 30 minutes up to 360 minutes.

2.3.3 Scanning electron microscopy

A specimen of fractured concrete is examined using the scanning electron microscopy. This investigation was carried out to examine the morphology of mortar. The SEM images of the fillers detected in sample cracks. The fragmented specimen is initially fixed to either metal or a graphite stub using a rapid fading adhesive. The stub was covered with glue or double-sided tape before the mortar powder was sprinkled. The sample was then compressed with aluminium sheets to remove the loose granules. Cement paste and concrete need to be coated in a thin layer of electrically conductive substance, such as gold plated, because they are typically non-conductive. The specimens were prepared for the SEM analysis once the coating was finished and the thickness on the specimen surface had been applied.

2.3.4 X-ray diffraction analysis

This research uses X'Pert High Score Plus software to determine the crystalline material phase and their chemical compositions to analyze X-ray diffraction. Amorphous to Crystalline nature is indicated by XRD patterns with scan ranges of 3° to 80° (2θ). For this examination, a powder sample was taken from mortar specimens that had been crushed while being tested for mechanical qualities. Calcite, Vaterite, Quartz and Hematite are some of the hydration products of Crystalline that develop in mortar.

3 Result and discussions

This section describes different test results from the proposed approach, such as workability, compressive strength, ultrasonic pulse velocity test and microstructural analysis.

3.1 Performance evaluation

The workability of self-compacting mortar is evaluated using the Mini-cone slump-flow test and the Mini V-funnel test for SCM. Furthermore, Compression strength with and without bacteria, mixed bacteria, ultrasonic pulse velocity and rapid chloride penetration tests are conducted.

3.1.1 Workability of self- compacting mortar

The workability of the self-compacting mortar is evaluated by mixing different ratios of cement, fine aggregate and water, in which the water-cement ratio is constant. The new properties of mortar are evaluated by Mini slump cone and V funnel slump.

Table 12 illustrates values for the Mini Slump and Mini V-Funnel test on self-compacting mortar. According to the research findings, incorporating bacteria into the mortar has no impact on its workability. Table 12

***Table 12** Workability Test Results*

S. No	Mix (1:1)				Workability	
	Water	Cement	Fine aggregate	W/C	Mini slump (mm)	Mini V-Funnel (s)
1.	120 ml	300	300	0.4	260mm	8 s

Figure 5 *Ultrasonic pulse velocity test setup*

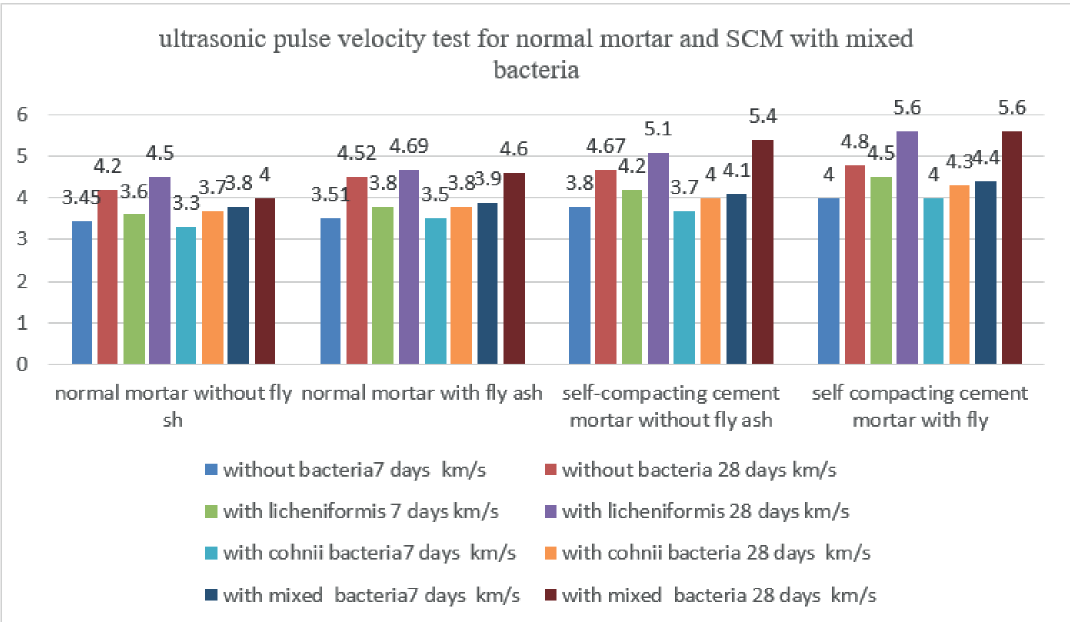

Figure 6 *Ultrasonic pulse velocity test for normal mortar and SCM with mixed bacteria*

shows that the workability of bacterial mortar mixes is determined by the slump; Mini V-Funnel is 260 mm and 8 s when the dose of cell concentration of bacteria is 10^5 cells/ml.

3.1.2 Ultrasonic pulse velocity test

Ultrasonic pulse velocity tests were conducted on mortar specimens measuring 70.6 mm x 70.6 mm x 70.6 mm that had been cured in water for 7 days and 28 days. The test configuration for UPV is shown in Figure 5.

The mortar can be examined for homogeneity, cracks, voids and other faults using the ultrasonic pulse velocity method.

Figure 6 compares the ultrasonic pulse velocity test without fly ash for 7 days and 28 days. Compared to the normal mortar, the self-compacting mortar has a high pulse velocity of 5.4 km/s for *bacteria Licheniformis* in the 4th week. The ultrasonic pulse velocity results were obtained using fly ash in normal and self-compacting mortar for 7 days and 28 days. Compared to the normal mortar with fly ash, the self-compacting mortar with fly ash has a high pulse velocity of 5.6 km/s *bacteria Licheniformis* and mixed bacteria at 28 days. Furthermore, all the bacterial mortar specimens had ultrasonic pulse velocities greater than 3.0 km/s. Therefore, the bacterial mortar can efficiently enhance mortar strength.

***Figure 7** Compression strength test setup*

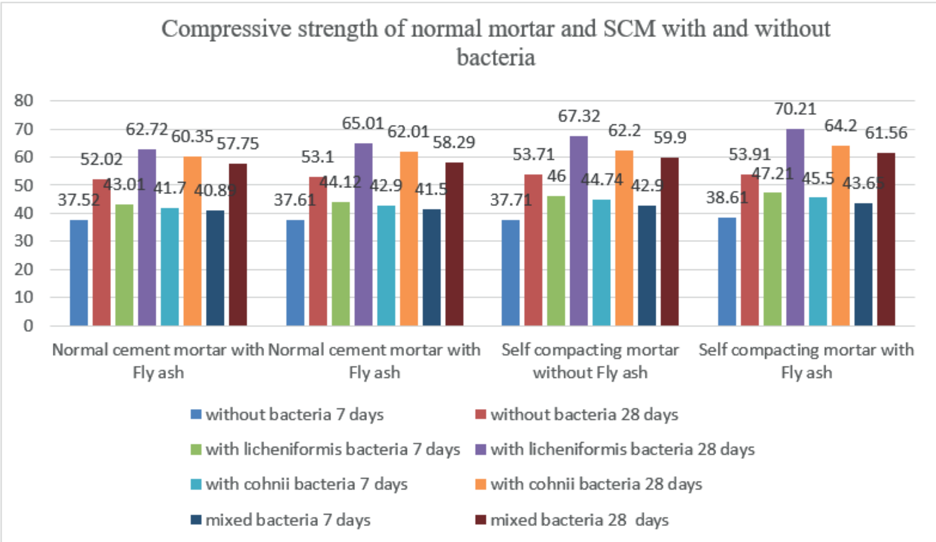

***Figure 8** Compressive strength of normal mortar and SCM with and without bacteria*

3.1.3 Compression strength

Compressive testing equipment with a 2000 kN capability was used to measure the compression strength of normal and bacterial mortar. The tests were performed once the specimen was centered in the testing apparatus. Once the dial gauge needle had just begun to change direction, the loading process was continued. The direction of the needle's motion has changed, indicating that the specimen has failed. The current dial gauge value was used to calculate the maximum load. The ultimate load, divided by the specimen's cross-sectional area, yields the ultimate cube compressive strength. The test configuration for compressive strength on a bacterial concrete cube specimen is shown in Figure 7.

The compressive strength test was performed on the normal and self-compacting mortar after curing for 7 and 28 days. The test setup for the compressive strength on a bacterial concrete cube specimen is shown in Figure 7.

In Figure 8, Compressive strength on self-compacting mortar without bacteria is 38.61 N/mm^2 and 53.91 N/mm^2 for 7 days and 28 days, respectively. Compared to the normal mortar, the compressive strength with and without fly ash increases in self-compacting mortar. Compared the seven days of curing and 28 days of curing enhances the strength of the self-compacting mortar. The Compressive strength on normal mortar with *Bacillus* Licheninformis bacteria, with and without fly ash for 7 days and 28 days are 43.01 N/mm^2, 44.12 N/mm^2, 67.32 N/mm^2, 65.01 N/mm^2. Compressive strength on self-compacting mortar with *Bacillus Licheniformis bacteria*, with and without fly ash for 7 days and 28 days are 46 N/mm^2, 47.21 N/mm^2, 67.32 N/mm^2, 70.21 N/mm^2. As a result, self-compacting mortar with fly

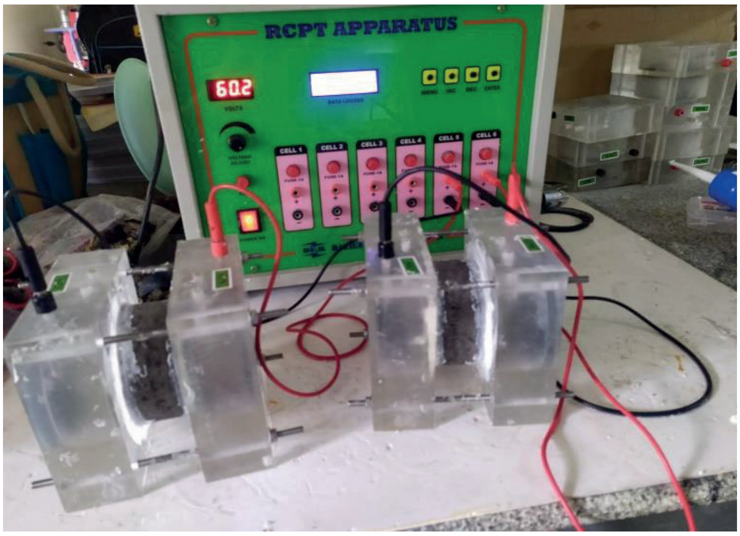

Figure 9 *Rapid chloride penetration test setup*

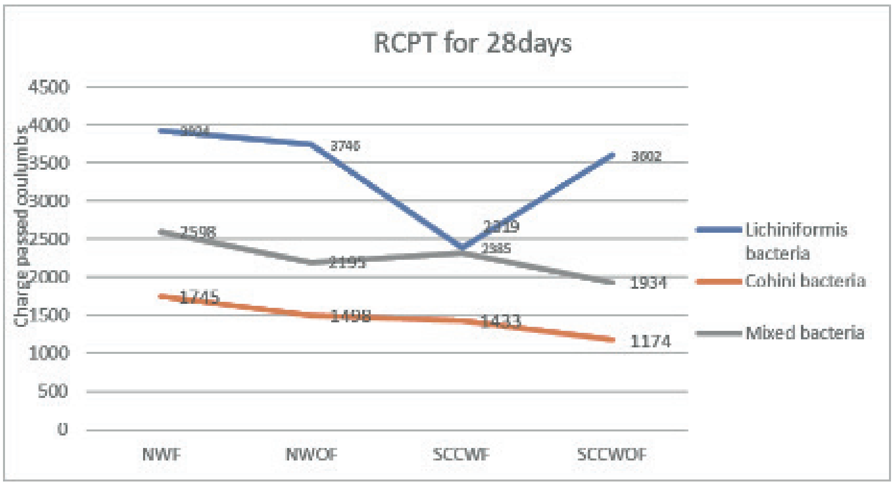

Figure 10 *Rapid chloride penetration test results*

ash and *Bacillus Lichneniformis bacteria* has a higher compressive strength of 70.21 N/mm^2 at 28 days.

The Compressive strength on normal mortar with *bacteria Cohnii*, with and without fly ash for 7 days and 28 days, is 41.7 N/mm^2, 42.9 N/mm^2, 60.35 N/mm^2 and 62.01 N/mm^2. Compressive strength on self-compacting mortar with *bacteria Cohnii*, with and without fly ash, for 7 days and 28 days, is 44.74 N/mm^2, 45.5 N/mm^2, 62.2 N/mm^2 and 64.2 N/mm^2. As a result, self-compacting mortar with fly ash and *bacteria Cohnii* has a higher compressive strength of 64.2 N/mm^2 at 28 days.

A compressive strength test was conducted on normal mortar and self-compacting mortar with mixed bacteria such as *Bacillus Licheniformis* and *Bacteria Cohnii*. Compressive strength on normal mortar with mixed bacteria, with and without fly ash for 7 days and 28 days, is 40.89 N/mm^2, 41.5 N/mm^2, 57.75 N/mm^2 and 58.29 N/mm^2. Compressive strength on self-compacting mortar with mixed bacteria, with and without fly ash, for 7 days and 28 days, is 42.9 N/mm^2, 43.65 N/mm^2, 59.9 N/mm^2 and 61.56 N/mm^2. As a result, self-compacting mortar with fly ash and mixed bacteria attains higher compressive strength of 61.56 N/mm^2 at 28 days.

The result of the compression strength test shows that *Bacillus Licheniformis bacteria* has the highest compressive strength in 7 and 28 days when compared to all other control mortars and bacteria.

3.1.4 Rapid chloride penetration test

After 28 days of curing, a rapid chloride penetration test was conducted on mortar specimens with a diameter of 100 mm and a thickness of 50 mm for proportions of normal mortar and self-compacting mortar, with and without fly ash. The test configuration for RCP is shown in Figure 9.

Figure 10 shows the rapid chloride penetration test of *Bacillus Licheninformis*, *Bacteria Cohnii* and *Mixed Bacteria*. The *Bacillus Licheninformis* has a higher penetration value than the *bacteria cohnii*, mixed bacteria and other mortar mixes. According to the test results, bacillus Cohnii has much lower chloride permeability than other bacteria.

3.1.5 Micro-structural analysis

The microstructure of bacterial mortar specimens can be examined using a scanning electron microscope (SEM) and an X-ray diffractometer. The SEM images, X-ray diffraction patterns and energy dispersive X-ray spectra of mortar samples, acquired from testing specimens, are described as follows.

SEM-EDS: Scanning electron microscopy can be used to examine a cracked concrete specimen. The goal of this study was to look into the morphology of mortar. A scanning electron microscope (SEM) was used to examine the microstructure of the concrete mixes, allowing the microstructure of the hydrated cement paste to be observed.

The formation of calcium carbonate crystals, the precipitation of calcite crystals within the mortar and the filling of pores are all visible. In this research, SEM analysis of bacteria mortar revealed unique calcite crystals included in the mortar. The presence of crystalline calcite in the form of CaCO3 in bacterial mortar specimens with fly ash was confirmed by high calcium levels. The presence of calcium (Ca) and the weight fraction of calcium are indicated by the Energy Dispersive X-Ray (EDX) spectrum of bacteria mortar. Moreover, the SEM-EDS test results include *Bacillus Licheniformis bacteria* with and without fly ash, *Bacteria Cohnii* with and without fly ash and mixed bacteria with and without fly ash of self-compacting mortar, are shown in Figures 11-16.

X-Ray Diffraction Analysis: The microstructure of various mixes was examined using an X-Ray Diffraction test and the peaks of graphs were utilized to identify minerals such as Quartz, Gypsum and C-S-H gel. In mortar mixtures, quartz aids in the development of bonding qualities. Gypsum is primarily important for controlling the time required for mortar to set. Calcite is a crucial component in the development of mortar strength qualities. Calcium silica hydrates gel improves mortar's strength while making it more durable and workable. Moreover, the X-ray diffraction test results include *Bacillus Licheniformis* bacteria with and without fly ash, *Bacteria Cohnii* with and without fly ash and Mixed bacteria with and without fly ash of self-compacting mortar, as shown in Figures 17-19

Microbes considerably impact the characteristics of bacteria in mortar, as demonstrated by SEM and XRD. According to the test results, self-compacting mortar with fly ash produces more calcium carbonate or calcite. XRD measurements revealed a higher percentage of calcite in Bacillus Licheniformis fly ash specimens with a cell concentration of 10^5 cells/ml. Bacteria mortar contains a lot of calcite, CH and C-S-H, as well as fly ash.

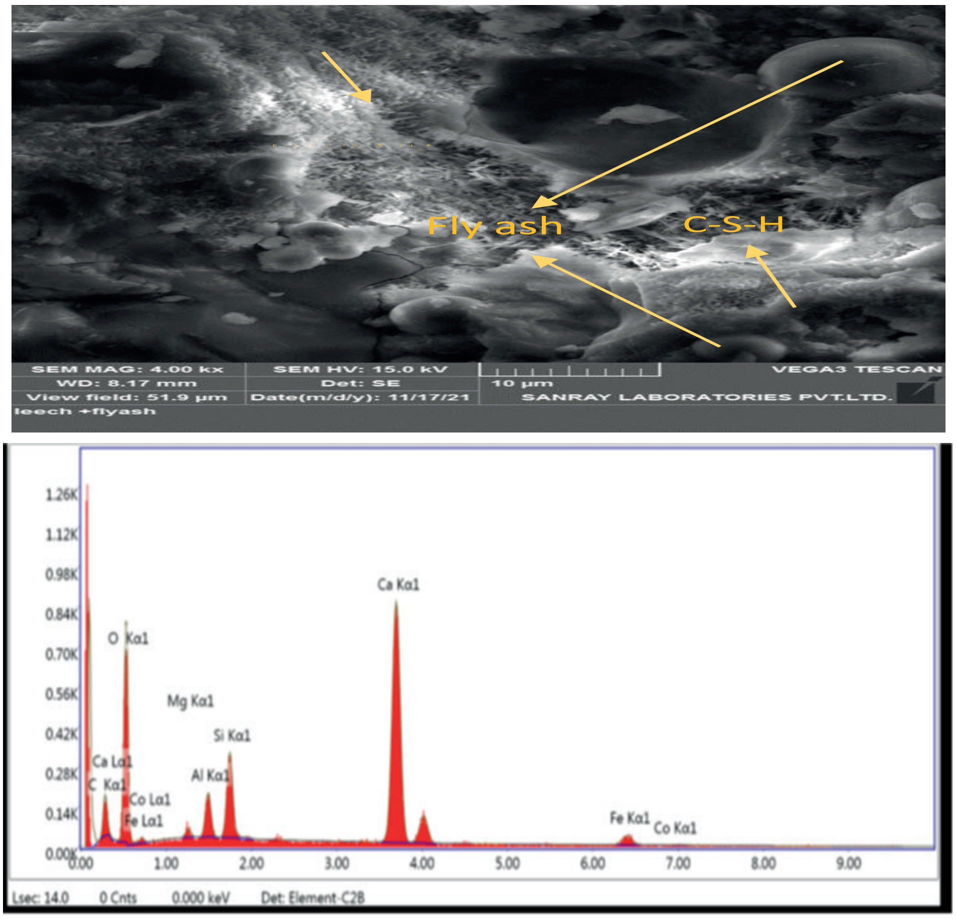

Figure 11 *Bacillus Licheniformis bacteria with fly ash SEM and EDS after 28 days of curing*

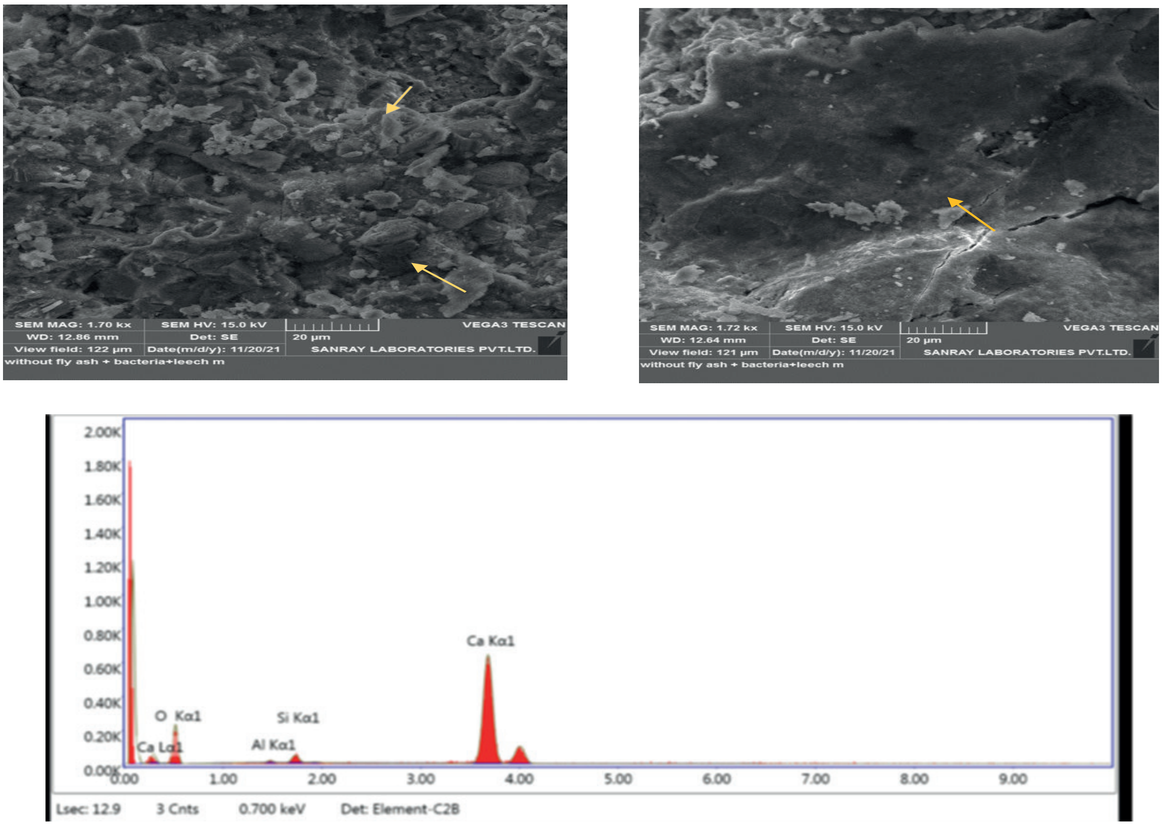

Figure 12 *Bacillus Licheniformis bacteria without fly ash SEM and EDS after 28 days of curing*

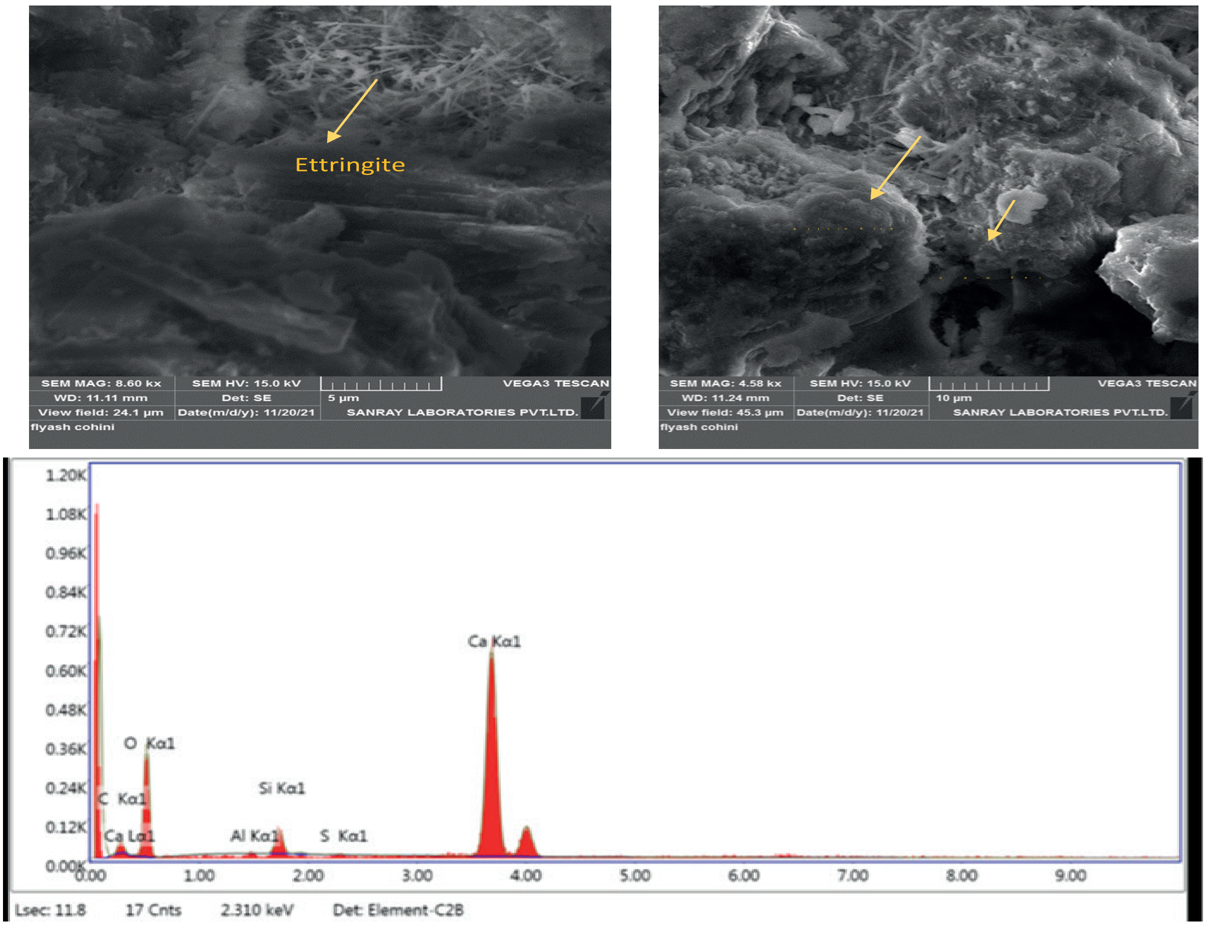

Figure 13 *Bacteria Cohnii with fly ash SEM and EDS after 28 days of curing*

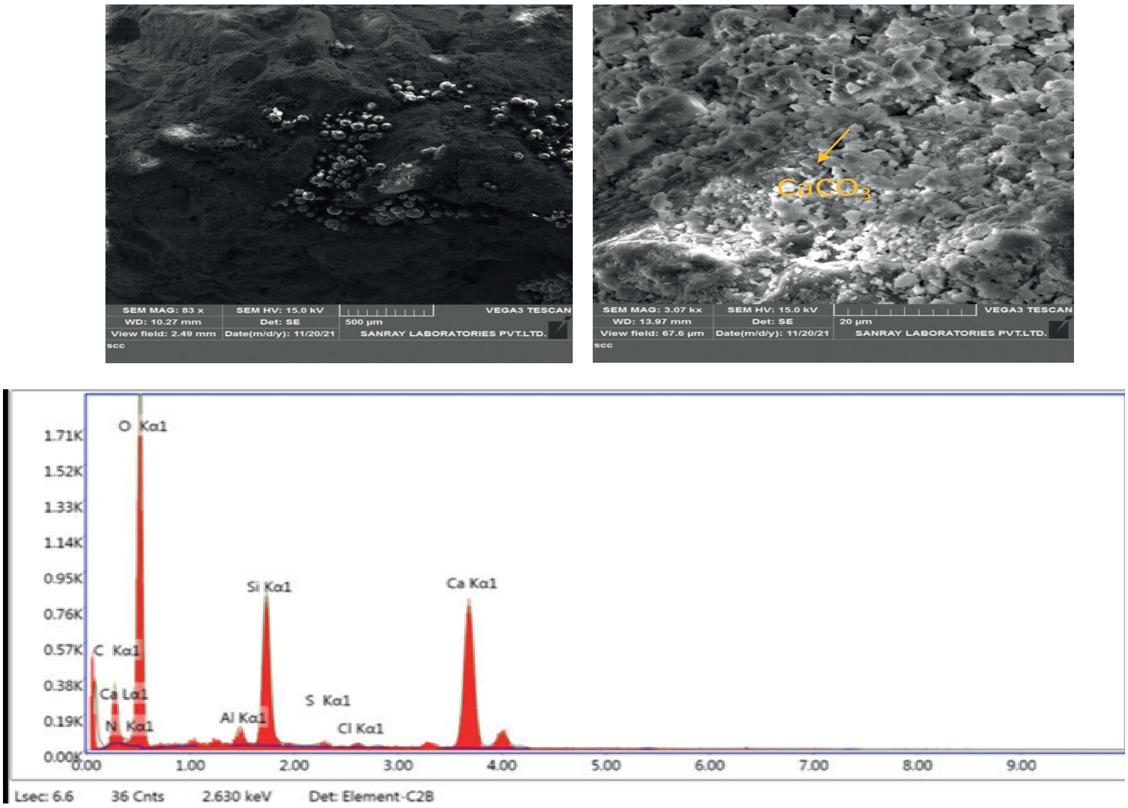

Figure 14 *Bacteria Cohnii without fly ash SEM and EDS after 28 days of curing*

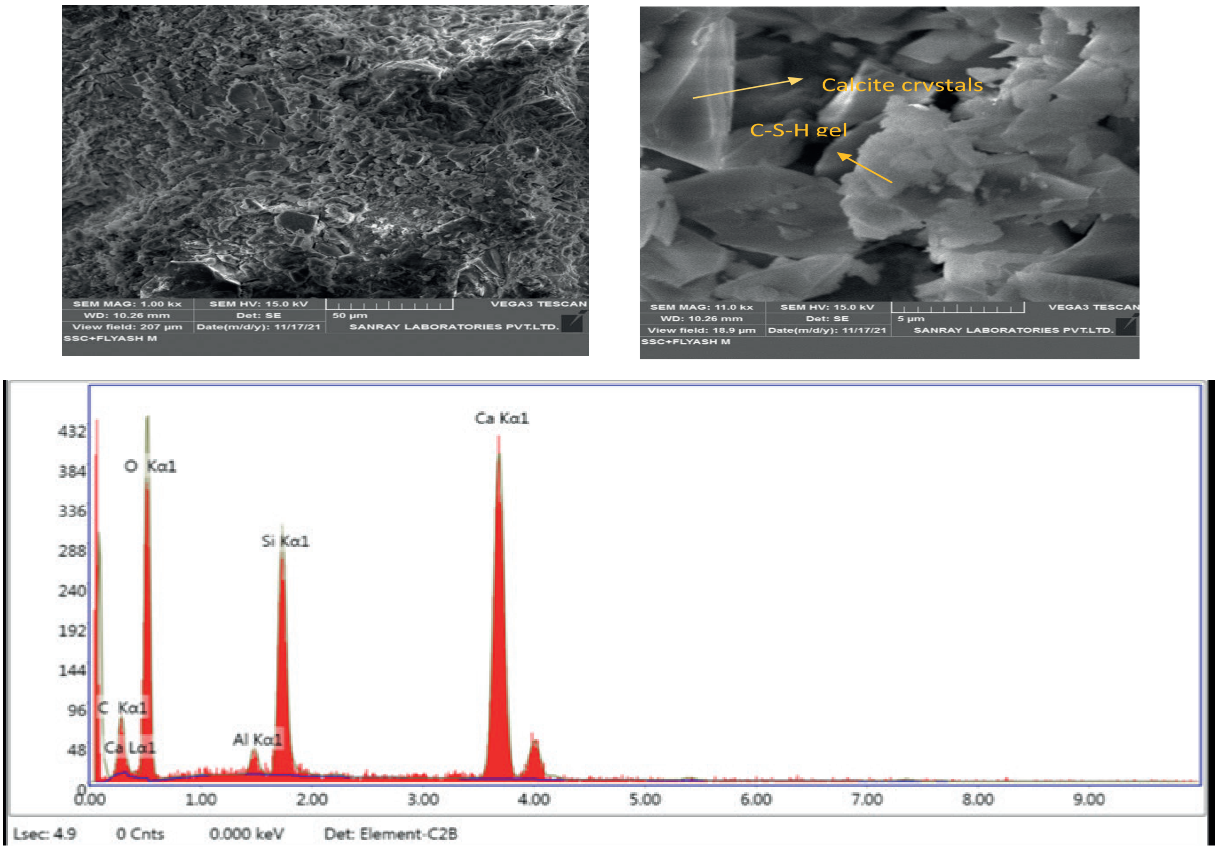

Figure 15 *Mixed Bacteria with fly ash SEM and EDS after 28 days of curing*

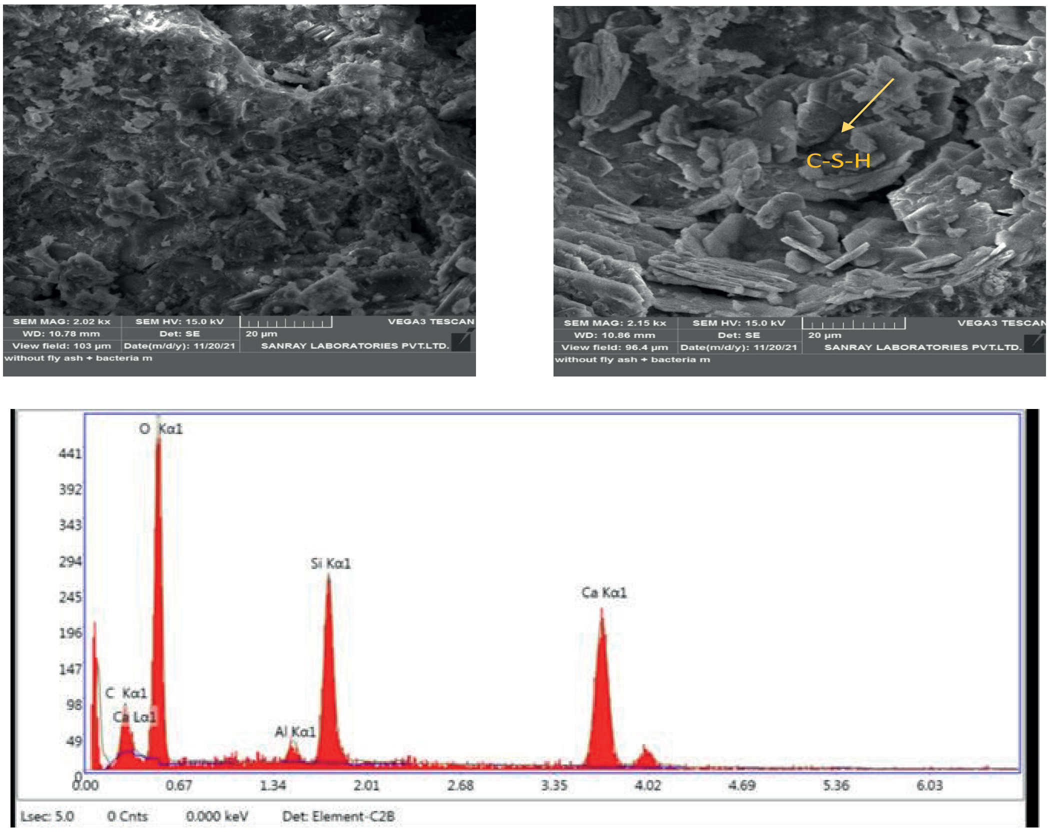

***Figure 16** Mixed Bacteria without fly ash SEM and EDS after 28 days of curing*

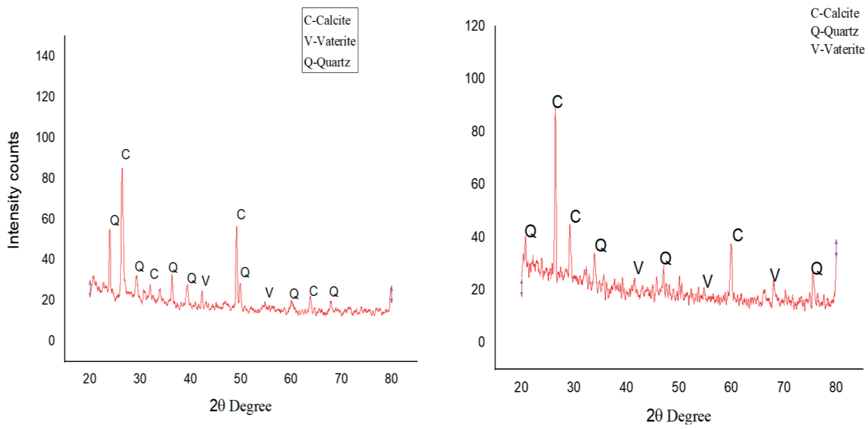

***Figure 17** X-ray diffraction for Bacillus Licheniformis with and without fly ash*

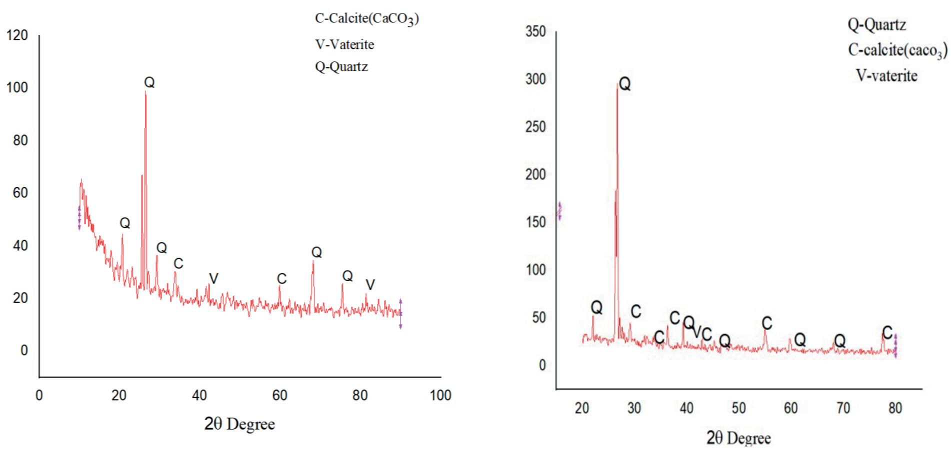

***Figure 18** X-ray diffraction for Bacteria Cohnii with and without fly ash*

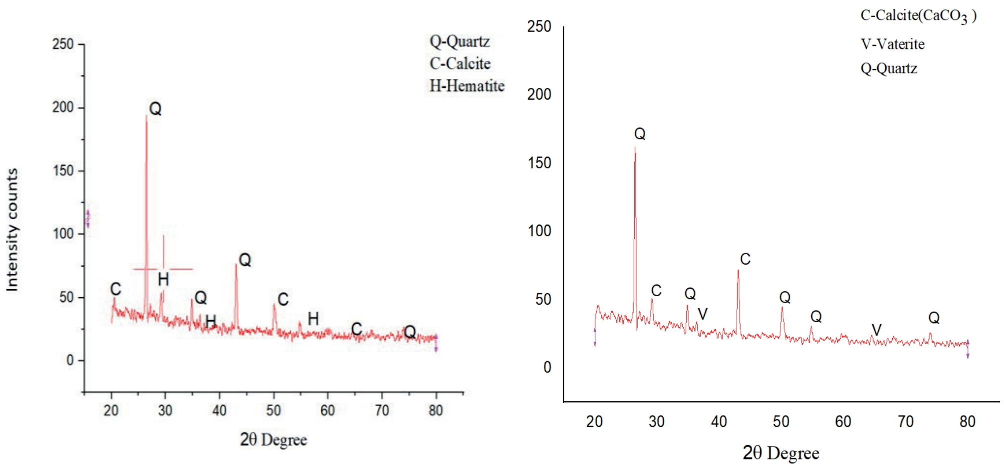

***Figure 19** X-ray diffraction for Mixed Bacteria with and without fly ash*

4 Conclusion

- *Bacillus Licheniformis* significantly increases the compressive strength up to 30% for SCM by adding fly ash compared to normal mortar at 28 days. *Bacillus Licheniformis*, *Bacillus cohnii and mixed bacteria (Licheniformis + cohnii)* increased compressive strength by 30 %,19.08% and 14.19%, respectively. There is an increase in compressive strength due to the settlement of calcite as a filling material in the inner portion of cracks on the concrete surface.
- The non-destructive testing studies, i.e. the Ultrasonic pulse velocity test, could identify microbes' crack healing nature by understanding the variation in pulse velocity. It is observed that the quality of the normal mortar and SCM specimens with bacteria is excellent compared to normal mortar and SCM without bacteria. There is an increase in the velocity of waves by the increased $CaCo_3$ formation of nucleation effect of bacterium cell walls.
- SEM-EDX analysis showed the crystals of calcium carbonate morphology and C-S-H gel. It was confirmed through the elemental composition of mortar samples, which showed the presence of C, O and Ca elements in EDX, indicating the presence of $CaCO_3$.
- The XRD analysis observed the presence of calcite, quartz and vaterite mineral phases. The presence of calcite ($CaCO_3$) improves the strength. Previous studies also revealed the formation of vaterite and calcite due to self-healing, confirming the presented results. In the future, concrete mixes could incorporate mineral admixtures such as silica fume, GGBS and their combinations, chemical admixtures and microorganisms.

Self-Compacting Mortar has many benefits over traditional abiotic reinforced concrete. It can heal cracks caused by water with no human intervention and lasts much longer. Moreover, traditional concrete is expensive to manufacture and difficult to recycle effectively, whereas bacterial concrete is environmentally friendly nor economically feasible. In the long term, the self-healing concrete saves money on construction costs and minimizes the waste generated during the demolition of traditional concrete structures because it may regenerate itself and increase the lifespan of the buildings it comprises.

References

[1] DEGLOORKAR, N. K., PANCHARATHI, R. K. Investigating microstructure characterization of mortars from 800 years old heritage structures in the Southern part of India. *Journal of Archaeological Science: Reports* [online]. 2020, **34**(A), 02634. ISSN 2352-409X. Available from: https://doi.org/10.1016/j.jasrep.2020.102634

[2] DEGLOORKAR, N. K., PANCHARATHI, R. K. Characterization of ancient mortar for sustainability of an 800-year-old heritage site in India. *Materials Today: Proceedings* [online]. 2020, 32(4), p. 734-739. ISSN 2214-7853. Available from: https://doi.org/10.1016/j.matpr.2020.03.472

[3] RAO, S., SILVA, P., DE BRITO, J. Experimental study of the mechanical properties and durability of self-compacting mortars with nanomaterials (SiO2 and TiO2). *Construction and Building Materials* [online]. 2015, **96**, p. 508-517. ISSN 0950-0618. Available from: https://doi.org/10.1016/j.conbuildmat.2015.08.049

[4] GHOSH, P., MANDAL, S., CHATTOPADHYAY, B. D., PAL, S. Use of microorganism to improve the strength of cement mortar. *Cement and Concrete Research* [online]. 2005, **35**(10), p. 1980-1983. ISSN 0008-8846. Available from: https://doi.org/10.1016/j.cemconres.2005.03.005

[5] SHASHANK, B. S., NAGARAJA, P. S. Durability studies on low-strength bacterial concrete [online]. In: *Advances in Sustainable Construction Materials*. BISWAS, S., METYA, S., KUMAR, S., SAMUI, P. (eds.). Lecture Notes in Civil Engineering. Vol. 124. Singapore: Springer, 2021. ISBN 978-981-33-4589-8, eISBN 978-981-33-4590-4, p. 639-650. Available from: https://doi.org/10.1007/978-981-33-4590-4_60

[6] SHASHANK, B. S., NAGARAJA, P. S. Fracture behaviour study of self-healing bacterial concrete. *Materials Today: Proceedings* [online]. 2022, **60**(1), p. 267-274. ISSN 2214-7853. Available from: https://doi.org/10.1016/j.matpr.2021.12.520

[7] CHAHAL, N., SIDDIQUE, R., RAJOR, A. Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of fly ash concrete. *Construction and Building Materials* [online]. 2012, **28**(1), p. 351-356. ISSN 0950-0618. Available from: https://doi.org/10.1016/j.conbuildmat.2011.07.042

[8] TAYEBANI, B., MOSTOFINEJAD, D. Penetrability, corrosion potential and electrical resistivity of bacterial concrete. *Journal of Materials in Civil Engineering* [online]. 2019, **31**(3), 04019002. ISSN 0899-1561, eISSN 1943-5533. Available from: https://doi.org/10.1061/(ASCE)MT.1943-5533.0002618

[9] REDDY, P. Y., RAMESH, B., KUMAR, L. P. Influence of bacteria in self-healing of concrete-a review. *Materials Today: Proceedings* [online]. 2020, **33**, p. 4212-4218. ISSN 2214-7853. Available from: https://doi.org/10.1016/j.matpr.2020.07.233

[10] ZHENG, T., QIAN, C. Self-healing of later-age cracks in cement-based materials by encapsulation-based bacteria. *Journal of Materials in Civil Engineering* [online]. 2020, **32**(11), 04020341. ISSN 0899-1561, eISSN 1943-5533. Available from: https://doi.org/10.1061/(ASCE)MT.1943-5533.0003437

[11] STANASZEK-TOMAL, E. Bacterial concrete as a sustainable building material? *Sustainability* [online]. 2020, **12**(2), 696. eISSN 2071-1050. Available from: https://doi.org/10.3390/su12020696

[12] REDDY, B. M., REVATHI, D. An experimental study on effect of Bacillus sphaericus bacteria in crack filling and strength enhancement of concrete. *Materials Today: Proceedings* [online]. 2019, **19**, p. 803-809. ISSN 2214-7853. Available from: https://doi.org/10.1016/j.matpr.2019.08.135

[13] NGUYEN, T. H., GHORBEL, E., FARES, H., COUSTURE, A. Bacterial self-healing of concrete and durability assessment. *Cement and Concrete Composites* [online]. 2019, **104**, 103340. ISSN 0958-9465. Available from: https://doi.org/10.1016/j.cemconcomp.2019.103340

[14] SEIFAN, M., BERENJIAN, A. Application of microbially induced calcium carbonate precipitation in designing bio self-healing concrete. *World Journal of Microbiology and Biotechnology* [online]. 2018, **34**(11), 168. ISSN 0959-3993, eISSN 1573-0972. Available from: https://doi.org/10.1007/s11274-018-2552-2

[15] AMERI, F., SHOAEI, P., BAHRAMI, N., VAEZI, M., OZBAKKALOGLU, T. Optimum rice husk ash content and bacterial concentration in self-compacting concrete. *Construction and Building Materials* [online]. 2019, **222**, p. 796-813. ISSN 0950-0618. Available from: https://doi.org/10.1016/j.conbuildmat.2019.06.190

[16] LORS, C., DUCASSE-LAPEYRUSSE, J., GAGNE, R., DAMIDOT, D. Microbiologically induced calcium carbonate precipitation to repair microcracks remaining after autogenous healing of mortars. *Construction and Building Materials* [online]. 2017, **141**, p. 461-469. ISSN 0950-0618. Available from: https://doi.org/10.1016/j.conbuildmat.2017.03.026

[17] DUCASSE-LAPEYRUSSE, J., GAGNE, R., LORS, C., DAMIDOT, D. Effect of calcium gluconate, calcium lactate and urea on the kinetics of self-healing in mortars. *Construction and Building Materials* [online]. 2017, **157**, p. 489-497. ISSN 0950-0618. Available from: https://doi.org/10.1016/j.conbuildmat.2017.09.115

[18] ZHANG, L. V., SULEIMAN, A. R., ALLAF, M. M., MARANI, A., TUYAN, M., NEHDI, M. L. Crack self-healing in alkali-activated slag composites incorporating immobilized bacteria. *Construction and Building Materials* [online]. 2022, **326**,126842. ISSN 0950-0618. Available from: https://doi.org/10.1016/j.conbuildmat.2022.126842

[19] BAYATI, M., SAADABADI, L. A. Efficiency of bacteria-based self-healing method in alkali-activated slag (AAS) mortars. *Journal of Building Engineering* [online]. 2021, **42**, 102492. ISSN 2352-7102. Available from: https://doi.org/10.1016/j.jobe.2021.102492

[20] RAUTRAY, S. S., MOHANTY, B. N., DAS, M. R. Performance of self-compacting geopolymer concrete using Bacillus Licheniformis. *Materials Today: Proceedings* [online]. 2020, **26**, p. 2817-2824. ISSN 2214-7853. Available from: https://doi.org/10.1016/j.matpr.2020.02.587

[21] NASIM, M., DEWANGAN, U. K., DEO, S. V. Effect of crystalline admixture, fly ash and PVA fibre on self-healing capacity of concrete. *Materials Today: Proceedings* [online]. 2020, **32**, p. 844-849. ISSN 2214-7853. Available from: https://doi.org/10.1016/j.matpr.2020.04.062

[22] TSAMPALI, E, STEFANIDOU, M. Effect of nano-SiO2 and nano-CaO in autogenous self-healing efficiency. *Materials Today: Proceedings* [online]. 2021, **37**, p. 4071-4077. ISSN 2214-7853. Available from: https://doi.org/10.1016/j.matpr.2020.09.253

[23] GUZLENA, S., SAKALE, G. Self-healing of glass fibre reinforced concrete (GRC) and polymer glass fibre reinforced concrete (PGRC) using crystalline admixtures. *Construction and Building Materials* [online]. 2021, **267**, 120963. ISSN 0950-0618. Available from: https://doi.org/10.1016/j.conbuildmat.2020.120963
[24] XU, D., CHEN, W., FAN, X. Experimental investigation of particle size effect on the self-healing performance of microcapsule for cemented coral sand. *Construction and Building Materials* [online]. 2020, **256**, 119343. ISSN 0950-0618. Available from: https://doi.org/10.1016/j.conbuildmat.2020.119343
[25] REDDY, C. M., RAMESH, B., MACRIN, D. Influence of bacteria Bacillus subtilis and its effects on flexural strength of concrete. *Materials Today: Proceedings* [online]. 2020, **33**, p. 4206-4211. ISSN 2214-7853. Available from: https://doi.org/10.1016/j.matpr.2020.07.225
[26] JOGI, P. K., LAKSHMI, T. V. Self-healing concrete based on different bacteria: a review. *Materials Today: Proceedings* [online]. 2021, **43**, p. 1246-1252. ISSN 2214-7853. Available from: https://doi.org/10.1016/j.matpr.2020.08.765
[27] LUHAR, S., LUHAR, I., SHAIKH, F. U. A Review on the performance evaluation of autonomous self-healing bacterial concrete: mechanisms, strength, durability and microstructural properties. *Journal of Composites Science* [online]. 2022, **6**(1), 23. eISSN 2504-477X. Available from: https://doi.org/10.3390/jcs6010023
[28] ALGAIFI, H. A., ALQARNI, A. S., ALYOUSEF, R., BAKAR, S. A., IBRAHIM, M. W., SHAHIDAN, S., IBRAHIM, M., SALAMI, B. A. Mathematical prediction of the compressive strength of bacterial concrete using gene expression programming. *Ain Shams Engineering Journal* [online]. 2021, **12**(4), p. 3629-3639. ISSN 2090-4479. Available from: https://doi.org/10.1016/j.asej.2021.04.008
[29] ALMOHAMMED, F., SIHAG, P., SAMMEN, S. S., OSTROWSKI, K. A., SINGH, K., PRASAD, C. V., ZAJDEL, P. Assessment of soft computing techniques for the prediction of compressive strength of bacterial concrete. *Materials* [online]. 2022, **15**(2), 489. eISSN 1996-1944. Available from: https://doi.org/10.3390/ma15020489
[30] RAMAGIRI, K. K., CHINTHA, R., BANDLAMUDI, R. K., DE MAEIJER, K. P., KAR, A. Cradle-to-gate life cycle and economic assessment of sustainable concrete mixes-alkali-activated concrete (AAC) and bacterial concrete (BC). *Infrastructures* [online]. 2021, **6**(7), 104. eISSN 2412-3811. Available from: https://doi.org/10.3390/infrastructures6070104