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A study on the mechanical and durability properties of bacterial culture with Ground Granulated Blast Furnace Slag (GGBS) as partial replacement for cement

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ABSTRACT

In concrete structures, the formation of cracks leads to reduction in the strength of the structures. Bioconcrete is an environmentally friendly material used for healing of cracks. In this study, the indigenous bacteria *Bacillus cereus* KOV15 obtained from the soil is used in bioconcrete with Ground Granulated Blast Furnace Slag (GGBS) as partial replacement for cement. Five different mixes of concrete such as conventional concrete and various mixes of concrete with bacterial broth culture (30% GGBS + 5% bacterial broth culture), (30% GGBS +10% bacterial broth culture), (40% GGBS + 5% bacterial broth culture) and (40% GGBS + 10% bacterial broth culture) were used to find the mechanical and durability properties and to study the microstructure of bioconcrete. The maximum percentage increase in the cube compressive strength, the split tensile strength and the flexural strength of bioconcrete was 26.79%, 11.69% and 21.3% respectively for concrete with 30% cement replaced with GGBS and 10% bacterial broth culture in comparison with the control concrete at the 28th day. The XRD, SEM and EDX analyses were performed to identify the calcium carbonate formation in bioconcrete. The SEM images of the bioconcrete with GGBS as replacement for cement have better hydrated form and have lesser pores than the conventional concrete. The EDX results exhibited a significant increase in the amount of calcium in the bioconcretewith 30% GGBS and 10% bacterial broth culture by 103.82% than that of the conventional concrete.The permeability of chloride ion was very low (903.2 Coulombs) in concrete with 10% bacterial broth culture and 30% GGBS as partial replacement for cement. The water absorption was maximum (3.03%) in the conventional concrete specimens when compared to other bioconcrete specimens with bacterial broth culture and GGBS as partial replacement for cement. Bioconcrete showed very low permeability and higher acid resistance than the conventional concrete. The Load deflection studies exhibited higher ultimate load and ductility factor for bioconcrete and the failure pattern of bioconcrete indicated lesser number of cracks, minimum crack width and no shear failure pattern. The indigenous Bacillicus Cereus KOV15 strains can be used for the synthesis of green construction materials like calcite-based biocement.

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1. Introduction

Concrete is a readily available building material which is used widely in construction. Its durability is the main criterion which decides the life span of a structure. Crack formation in concrete structures allows chlorides, carbon dioxide, oxygen and water to enter into it which leads to corrosion and ultimately reduces the strength of concrete [1]. Healing of the cracks found in concrete structures is necessary to strengthen the structures, increase the durability and enhance the service life of the structure [2].

The development of concrete in construction leads to heat production, CO_2 emission and consumption of a large number of natural resources. Sustainable materials are preferred to minimize pollution. Construction with sustainable concrete is to be adopted by the construction industry. Bacterial concrete is a concrete intended for crack healing in an environmentally friendly manner. This can be achieved by introducing biocement which is recently developed for improving the engineering properties of soils through microbial activity [3,4]. Microbiologically induced calcium carbonate precipitation (MICP) is a bio-geochemical process that induces precipitation of calcium carbonate in the soil matrix [5]. In order to achieve soil biocementation, MICP is used which forms calcium carbonate ($CaCO_3$) that helps in binding the soil particles resulting in increased soil strength and stiffness [6].

The formation of $CaCO_3$ by bacteria via urea hydrolysis is a mechanism widely used for biocement production [7]. The $CaCO_3$ precipitation is easily controllable in the MICP process which produces higher concentration of $CaCO_3$ in a specific period of time [8]. Currently, the study of soil microbes has been widely used for greener construction materials including biocement [2–4]. Biocement production due to hydrolysis of urea from various microbes such as *Synechococcus*, *Bacillus pasteurii*, *Chlorella, Bacillus sphaericus*, *Halomonas*sp. SR4, and *B. thuringiensis* have been investigated [9,10]. The bacteria like *Lysinibacillus* sp. WH, *Bacillus Sphaericus*, *Bacillus pasteurii*, and *Bacillus flexus* have the potential to precipitate calcite [11].

An experimental investigation has been conducted on bacterial concrete using *Bacillus pasteurii* for the production of $CaCO_3$[12]. The repair and high maintenance costs of concrete structures are reduced due to self-healing [13]. Biological mortar is produced by the mechanism of oxidative deamination of amino acids using the bacteria *Bacillus cereus* [14]. Higher compressive strength and durability in cement mortar are achieved through different calcite-forming bacteria [15]. Bacteria can survive in lower to higher ranges of salinity (or) acidity, extreme temperatures and high pressure and the pH varies from 5 to 7 [16]. Calcium carbonate precipitation on the cement mortar specimen has been evaluated by [17] in some of the early studies.

The morphological effect on concrete by the calcium carbonate precipitation has been analyzed previously [18]. The Crystal morphology obtained with different bacterial culture is due to the level of the actual urease activity [18]. The morphology of the crystals is influenced by the calcium source [10]. The SEM and XRD images reveal the presence of $CaCO_3$ produced microbially due to the addition of bacterial strains [12].

Concrete has very little resistance to cracking and low tensile strength. It is susceptible to cracking because of external loads and temperature changes that cause shrinkage [19]. Concrete that uses biological principles for self-healing cracks is intended to improve durability. Through a bioprocess called microbial-induced-calcite-precipitation (MICP), specific microorganisms can precipitate calcite in various ways. The life of concrete structures can be extended by microbial self-healing without the need for expensive or time-consuming interventions. These microbes produce minerals to fill up the pores and cracks in concrete, it also increases the pH and promotes the durability of the structures [20,21]. The general aim of this research study is to screen the bacterial strains, investigate their activity in self-healing application of crack-filling through biocementation, detect the mechanical, microstructural and durability property of bioconcrete, apply the optimised value in beams and study their structural behaviour.

According to [22], the experiment's concrete with the bacterial cells added had higher compressive strength and a higher maximum load than control concrete and was suitable for use in building construction. It could therefore be used to create building materials for structural purposes.

The compressive strength increases in concrete (22.62%) and mortar (19%) by adding *Escherichia coli* in concrete at 10^5 concentration per mL. by the precipitation of inert filler matter [23]. Calcium carbonate ($CaCO_3$) precipitated by *Bacillus Sphaericus* in the concrete gives greater resistance to corrosion and improved durability [7]. The bacteria are used for making concrete with enhanced durability and also biomineralization is used for surface treatments in concrete [24]. By incorporating *Bacillus Subtilis* bacteria into concrete, the density, durability, strength, and microstructure of concrete can be improved ([25]; Bathena and Gadkar,2014). *Sporosarcina pasteurii* bacteria in the concrete can increase the durability of concrete due to $CaCO_3$ precipitation [10].

However, limited study has been reported so far on the usage of ureolytic bacteria screened from soil and hence a study was conducted on the mechanical and durability properties of bioconcrete using *Bacillus cereus* KOV15 and micro structural analysis of bioconcrete. Therefore, the present study is focused on use of urease producing bacterium *Bacillus cereus* KOV15 obtained from natural limestone deposited fields soil samples for the production of bioconcrete, evaluating their application in biocement formation finding the mechanical and durability properties and microstructure of the bioconcrete with GGBS as replacement for cement.

2. Experimental investigation

2.1. Materials used

Ordinary Portland Cement 53 grade was used as the binding material for concrete. The fine aggregate used was manufactured sand (M-sand) obtained locally from Chennai. The fine aggregate conforms to grading zone II. Blue granite crushed stone aggregates of nominal size 20 mm were used as coarse aggregate.

GGBS used in the present investigation was obtained from Chennai. It is highly pozzolanic in nature. Torbopol CEM50 was used as

the super plasticizer with a specific gravity of 1.135. The bacterium *Bacillus cereus* KOV15 was used for biocement production as well as for promoting self-healing in construction. All the tests on materials were performed as per Bureau of Indian Standards (IS) and are represented in Table 1. Chemical composition of cement and GGBS are shown in Supplementary Information Table S1.

2.2. Screening and Identification of the Bacteria

Screening of ureolytic bacteria from 524 soil samples obtained from various places of Tamil Nadu, India (shown in Supplementary Information Fig. S1) and their efficiency in the formation of calcium carbonate ($CaCO_3$) to be used for the production of biocement was described in our previous publication [26].

Briefly, all the soil samples were cultured by agar medium using Zobell Marine Agar. Thirteen out of 524 samples were ureolytic positive which was evident when the color of agar medium changes from yellow to magenta in the presence of bacteria producing urease enzyme as shown in Fig. S2 (Supplementary Information, Fig. S2).

From the thirteen ureolytic positive bacteria investigated, four strains - KLK13, KOV15, KNT2, and NKP14 were selected as potential bacteria for biocement production test (Supplementary Information, Fig. S3).

The highest urease activity was found at urea concentrations 0.3% and the bacterial strain values were 2.02, 4.15, 4.64, 3.95 and 4.02 U/mL for control, KLK13, KOV15, KNT2, and NKP14, respectively as shown in Fig. S4 (Supplementary Information, Fig. S4).

Among all investigated strains, the KOV15strain was selected for the production of biocement based on the maximum $CaCO_3$ (18.31 g/L after 48 h incubation) and substrate hydrolysis.The KOV15 strain was identified as a member of *Bacillus cereus* and given the name *B. Cereus* KOV15 as a result of the phylogenetic analysis (GenBank accession number MW865710) by NCBI.[26].

2.3. Details of the Specimens

Concrete specimens were prepared for mechanical strength studies on different mixes, which are shown in Table 2 below.

The workability tests were carried out as per Bureau of Indian Standards (IS) IS7320:1974 code. The grade of concrete was M25 and the mix design was performed according of IS 10262:2009. The mix proportion for various mixes used in the study was shown in Supplementary Information Table S2. The mix proportioning was 1:2.21:2.96 for cement: fine aggregate: coarse aggregates, 0.36 water cement ratio, 1% by weight of superplasticizer, 385 Kg/m^3 of cement in the control mix, 7 kg/m^3 of bacterial broth culture for mixes with 5% bacterial culture and 14 kg/m^3 for 10% bacterial culture.

Specimens for strength determination was prepared in triplicates for all the 5 different mixes of concrete and tested after 7 days and 28 days of curing as per IS standards. The average value obtained for three specimens was used as the strength for each mix. The microstructure of concrete mixes was later studied.

2.4. Tests for finding the mechanical and durability properties of bioconcrete

2.4.1. Compressive, split tensile and flexural strengths

The AIMIL Compression Testing Machine (CTM Digital-Electrically operated - AIM 317E-DG - 081433) capacity- 200 T was used to perform the test for finding the compressive strength after 7 and 28 days of curing. The average value of the compressive strengths obtained for three specimens was used as the compressive strength for each mix. The size of the specimen was 150 mm × 150 mm × 150 mm. The test was conducted according to IS 3495 (Part I): 2002.

Cylinder-shaped concrete specimens (150 mm diameter and 300 mm height) of the various mixes of concrete were tested after 7 days and 28 days of curing on a universal testing machine of 100 MT capacity (Model: TUC-CN-1000, Make: Fine spavy Associates &Engg, Sl no: 2008/88) to determine thesplit tensile strength. The procedure based on the IS 5816 – 1999 was used.

Flexural strength test was conducted on prism specimens (100 mm × 100 mm c/s dimensions and 500 mm length) cast for every mix, subjected to two-point loading, in the Universal Testing (STEK brand) and a capacity of 400 kN on the 7th and 28th days of curing. The IS code followed for flexural strength was IS: 516–1959.

2.4.2. Rapid chloride permeability test

The quality of concrete was assessed by conducting the Rapid Chloride Permeability Test (RCPT) as per ASTM C 1202–97. Accordingly, cylindrical specimens of size 100 mm diameters and 50 mm thickness was prepared for all the mixes and cured for 28 days. After curing, all the samples were air-dried for 12–16 h. Thus, prepared specimens are used for RCPT (Caltech Engineering Services, Mumbai, with a vacuum pump and 8 Channels). During the test, one surface of the sample was in contact with sodium

Table 1
Physical properties of raw materials.

Property	Cement	GGBS	Coarse aggregates	M-sand
Colour	Grey	Off white	-	-
Specific gravity (Kg/m^3)	3.15	2.9	2.77	2.67
Specific surface (m^2/kg)	-	350	501	468
Fineness in number	7.00	-	6.97	2.92
Water absorption (%)	-	-	0.34	1.0

Table 2
Tests were investigated in description of Mixes.

Mix designation	Description of Mixes
Mix I	Conventional concrete (without bacterial broth culture and GGBS)
Mix II	Concrete with 5% bacterial broth culture *(Bacillus cereus)* and 40% GGBS
Mix III	Concrete with 5% bacterial broth culture and 30% GGBS
Mix IV	Concrete with 10% bacterial broth culture and 40% GGBS
Mix V	Concrete with 10% bacterial broth culture and 30% GGBS
Mix VI	Concrete with 5% bacterial broth culture without GGBS
Mix VII	Concrete with 10% bacterial broth culture without GGBS

chloride solution while the other one was in contact with sodium hydroxide solution. A potential difference of 60 V, DC was maintained across the specimen. The charge passed through the specimen for 6 h was measured which defines the degree of resistance to chloride ion penetration.

2.4.3. Water absorption test

Water absorption test was conducted as per IS 1881–122: 2011. Briefly, cube specimens of size 150 mm were prepared and submerged in water throughout the test period. Before immersion, dry weight of the concrete specimen was taken. Later, the specimens were submerged in water for 24 h and again the concrete cubes were weighed. Increase in the weight of the specimens expressed as a percentage was taken as water absorption.

2.4.4. Acid attack

Cubes of size 150 mm was prepared for all the mixes and cured for 28 days. Later, they were immersed in 10% sulphuric acid and hydrochloric acid solutions for 28 days. The acid solutions were periodically replaced with a freshly prepared batch. The acid exposed specimens were inspected for percentage loss in compressive strength, loss in weight and also visual inspection.

2.5. Microstructural analysis of bioconcrete

2.5.1. XRD analysis

XRD analysis on different mixes was performed to analyse phase composition and crystalline structure of the conventional concrete and bioconcrete using XRD SHIMADZU XRD-6100 instrument. The analyzed material was finely ground, homogenized, and the average bulk composition was determined. X-ray diffraction was based on constructive interference of monochromatic X-rays and a crystalline sample. These X-rays were generated by a cathode ray tube, filtered to produce monochromatic radiation, collimated to concentrate, and directed toward the sample. The angle of incidence of the X-ray is θ. The angle at which the X-rays are diffracted is equal to the angle of incidence, θ. The angle of diffraction is the sum of these two angles, 2θ. The interaction of the incident rays with the sample produces constructive interference when conditions satisfy Bragg's Law ($n\lambda=2d \sin \theta$). To ascertain the nature of the materials using XRD patterns, the nature of Bragg's peaks appearing in the XRD pattern are observed. If a very broad humped peak is obtained, then the material is amorphous with short range ordering. If sharp peaks are obtained in the XRD pattern, then the material is crystalline. The peak intensity shows the extent of crystallinity of the particular plane. The compounds in the concrete were identified using X-Pert High Score Plus.

2.5.2. SEM analysis

In the present study, the change in the morphology due to the incorporation of GGBS and bacterial broth culture to concrete was examined with the help of Scanning Electron Microscope (SEM). The SEM analysis was carried out on different mixes of concrete using JEOL JSM-7600 F instrument.

The type of electron microscope which uses a focused beam of electrons to scan the sample and produce an image of it is known as SEM. The atoms present in the sample produce numerous signals on interaction with electrons. Based on these signals, the composition of the samples and the topographic characteristics are determined. A special detector is used to collect the secondary electrons which create the image and show the topography of the surface. SEM analysis is normally used to analyse cracks and identify the defects in structures. The microstructure of the samples is described in three objectives, i.e., the hydrated cement particles, other hydrated particles and unhydrated particles or pores. The hydrated cement particles consist of high-density C-S-H gel, they are distinctively visible in the image and are seen as individual solid particles. Other hydrated particles are the spaces which bind the cement together, grow within the capillary pore space and appear in grey, which are referred to as groundmass. Unhydrated particles or pores are entrapped air voids and pores with cement particles that are left unhydrated in the concrete and appear as discrete black patches in the SEM analysis.

2.5.3. EDX analysis

The chemical microanalysis method used in conjunction with SEM is called as EDX system. Totally, 10 specimens were tested using a JEOL JSM-7600 F machine, which includes 5 different mixes of concrete with 2 specimens in each mix. EDX images of the samples of bacterial concrete and conventional concrete were obtained at 28 days curing.

2.6. Application of bacterial concrete in structural elements

The reinforced concrete beams of five mixes were used to find out the flexural behavior. The reinforcement details of the reinforced concrete beams are shown in Supplementary Information Fig. S3. The length of the beam was 1200 mm and c/s size was 100 mm × 150 mm. Two numbers of 10 mm bars at the top, three numbers of 12 mm bars at the bottom and two legged 6 mm diameter stirrups at 150 mm c/c was provided as the reinforcement to the beams. The beams were tested under two-point loading applied at one-third of the supporting span. The specimens were tested in a loading frame of 400 kN capacity at a constant load increment of 5 kN up to failure. Linear variable differential transformer (LVDT) was used to measure the deflection of the specimen. It was placed in such a manner that it measured deflection at the centre of the beam at the bottom. The loads and deflections were measured up to failure of the specimen. A strain gauge was used to monitor the strain continuously. The load at first crack, cracking behavior, cracking pattern, pattern of deflections and load at failure were observed.

3. Results and discussion

3.1. The cube compressive strength of bioconcrete

The cube compressive strength test results of the different mixes of concrete with GGBS and bacterial broth culture on the 7thday and 28th day are represented in Fig. 1.

Apparently, from Fig. 1, the cube compressive strength of concrete with GGBS and bacterial broth culture is higher than the conventional concrete. The cube compressive strengths of the mixes Mix II to Mix VII at 28 days increased by 10.65%, 14.37%, 19.59%,26.79%,4.92% and 5.34% respectively. The maximum cube compressive strengths of concrete at 7 and 28 days of curing was 25.136 N/mm^{2}and 39.288 N/mm^{2}for concrete with 10% bacterial broth culture and 30% GGBS as replacement for cement (Mix V). Since the percentage increase in the compressive strength of concrete bioconcrete without GGBS (Mix VI, VII) at 7 days and 28 days period was marginal, the strength of the bacterial concrete shown to increase using GGBS. Addition of 30% and 40% GGBS was decided based on the preliminary tests conducted in the laboratory and well substantiated in the literature [27,28]. The increase in the compressive strength is due to the pozzolanic material GGBS which fills the voids and due to $CaCO_3$ deposition in the pores. Analysing deeper, when bacterial broth culture is kept constant, an increase in GGBS percent (30–40%) decreased the compressive strength by 1.15 (5%) and 2.23 (10%). On contrast, increase in broth culture increased the compressive strength for the same percentage of GGBS. For example, for 30% GGBS, the compressive strength increased by 3.85% for increase in bacterial broth culture from 5% to 10%. An increase in production of calcium carbonate leading to greater biocementation enhanced the compressive strength (Mahawish et al., 2019; [29,20,30]).

3.2. The split tensile strength of bioconcrete

The split tensile strength test was conducted on cylindrical specimens of concrete with GGBS (30% and 40%) replacement for cement and bacterial broth culture (5% and 10%) after 7 days and 28 days of curing (Fig. 2).

Higher values of split tensile strength were observed for all the mixes with GGBS and bacterial broth culture (Bacillus cereus) at 7 days and 28 days compared to the conventional concrete. The maximum values of 2.02 N/mm^2 and 3.32 N/mm^{2}were obtained on the 7th day and the 28th day for concrete with 10% Bacterial broth culture and 30% GGBS as replacement for cement (Mix V). The increase in the split tensile strengths of the mixes Mix II to Mix VII at 28 days was 5.57%, 6.66%, 8.38%,11.69%,2.97% and 3.63% respectively compared to conventional concrete. Even in the case of split tensile strength, specimens with constant GGBS and increasing bacterial

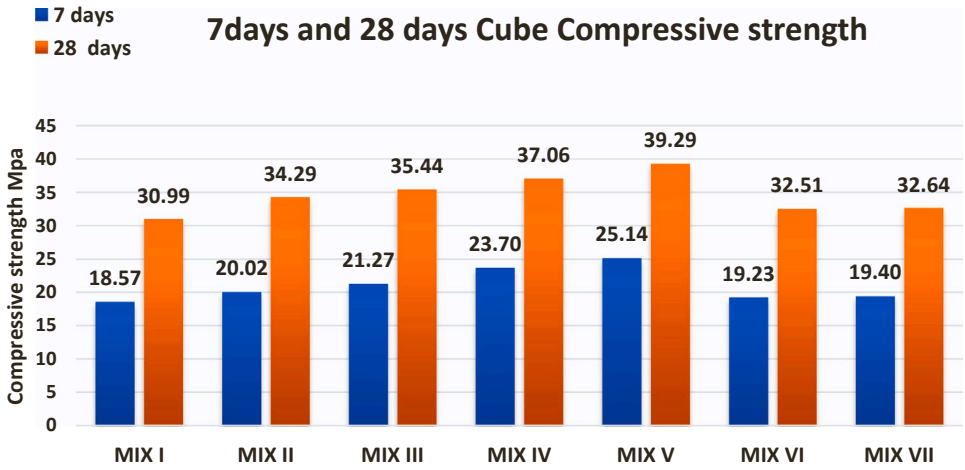

Fig. 1. The Cube Compressive Strength of Different Mixes of Concrete with GGBS and Bacterial Broth Culture on the 7thday and 28th day.

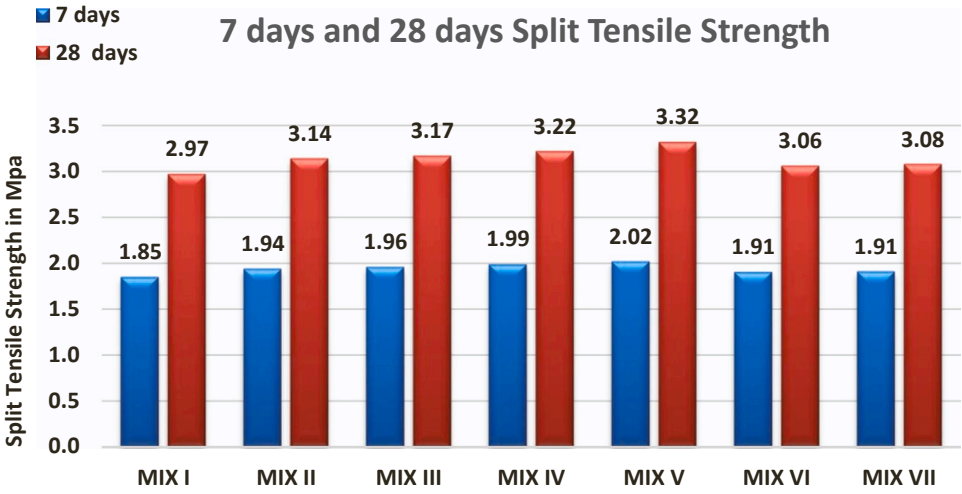

Fig. 2. The Split Tensile Strength of Different Mixes of Concrete with GGBS and Bacterial Broth Culture on the 7thday and 28th day.

broth culture resulted in increased split tensile strength. Using GGBS, it was demonstrated that the strength of the bacterial concrete was at its maximum because the percentage increase in the split tensile strength of concrete at 7 days and 28 days was negligible (roughly 2.97–3.67%). Though there is a decrease in the tensile strength for a constant bacterial broth culture and increasing GGBS percentage, the difference is not very significant. The presence of $CaCO_3$ in the pores causes a reduction in the porosity and enhances the bonding strength of the mix by filling the interfaces between the constituents of the bioconcrete which offers high resistance to splitting of the concrete. The increase in split tensile strength is mainly due to the bacteria induced precipitation of calcium carbonate of filling of the pores inside the concrete [20].

3.3. The flexural strength of bioconcrete

The flexural strength test was conducted on prism specimens of different mixes of concrete with GGBS and bacterial broth culture on the 7th day and 28th day and the results of the flexural strength test are represented in Fig. 3. The flexural strengths for all the mixes of bioconcrete Mix II to Mix VII after 7and 28 days curing were higher than the flexural strength of conventional concrete. The maximum values of flexural strength of concrete 3.49 N/mm^2 and 4.8 N/mm^2 were obtained at 7 days and 28 days for Mix V. The increase in the flexural strengths of the mixes Mix II to Mix V at 28 days was in the range of 5.57–21.3%. After 28 days, it was discovered that concrete with 5% and 10% bacterial broth cultures without GGBS (Mix VI & VII) had flexural strengths of 2.27% and 3.03%, respectively. The percentage increase in the flexural strength of bacterial concrete without GGBS was negligible. The maximum increase in the flexural strength was 21.3% at 28 days for Mix V. The increase in the flexural strength is due to GGBS which modifies the compactness of the paste, enhances the adherence at the aggregate paste interface, alters the pore structure and reduces the

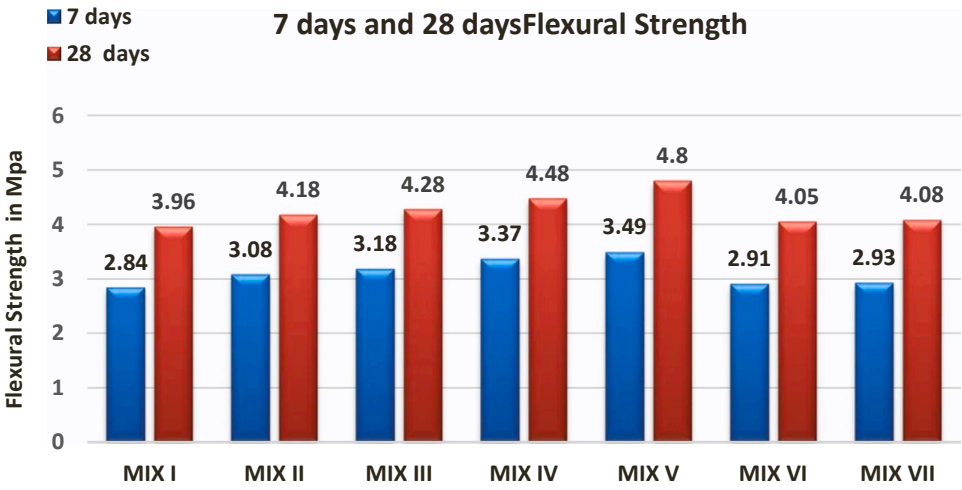

Fig. 3. The Flexural Strength of Different Mixes of Concrete with GGBS and Bacterial Broth Culture on the 7thday and 28th day.

permeability which results in higher strength [31]. The filling of the pores due to calcite formation by bacteria enhances the flexural strength of bioconcrete. ([19,32,33]; Venkata SRPC et al., 2018; [20,34,21]).

3.4. Rapid chloride permeability test results

RCPT is a popular test to check the durability of concrete. The penetrability of chloride is classified on the basis of the current passed through the specimen in the test setup. The RCPTresults are shown in Fig. 4. Chloride ion penetrability in proportion to the charge passed in the specimen for mixes I to IV comes under low as per ASTM 1997 scale (1000–2000) whereas for mix V it is very low (100–1000). The chloride penetration reduced in the presence of bacterial broth which is in conjunction with its percentage. GGBS content affects the chloride penetrability [27], in the present study for a common percentage of bacterial culture the variation in chloride penetrability with respect to the percentage of GGBS is low. In another case, constant GGBS percentage and variation in bacterial culture percentages has shown a noticeable result. Hence, we can say that in the present study, influence of GGBS on chloride penetrability is less than that of bacterial culture. This shows that the mix V has excellent quality of concrete [35].

3.5. Water Absorption Results

Table 3 shows the results of water absorption in terms of % water absorption and compressive strength. The % water absorption values are compared to that of assessment criteria for water absorption (CEB, 1989). According to CEB 1989, if % water absorption is less than 3 then the absorption rating is considered low and concrete quality is good. If the absorption lies within 3–5, then absorption rating and concrete quality are average. If the value is greater than 5, then the rate of absorption is high while the quality of concrete is poor (Nadir and Sujatha, 2017).

The concrete cubes with *Bacillus cereus* bacterial cells absorbed lesser amount of water than the control concrete cubes over a period of 28 days. Water absorption in conventional concrete was maximum perhaps due to the occurrence of micro-voids around the surface of the specimen enabling more water absorption. Quality of bioconcrete fell in to good category in terms of water absorption. Likewise compressive strength was also minimum for conventional concrete after submersion of cubes in water for 28 days. Higher bacterial content contributed to lesser loss of compressive strength when compared to 28 days compressive strength as shown in Fig. 1. The possible reason is that there was a reduction in the permeation properties after the bacterial deposition of a layer of calcium carbonate crystals on the surface of the concrete specimens. As a consequence, the ingress of substances is reduced. A similar result was observed by (Nemati and Voordouw 2003), wherein there was a decrease in permeability of sandstone cores after injection of $CaCO_3$ forming reactants. Formation of carbonate crystals due to the selected bacterial isolate enhanced resistance of cementitious materials to various degradation processes (Nemati and Voordouw, 2003).

3.6. Acid attack results

The durability of the concrete cube specimens with conventional concrete (Mix I), Mix II, Mix III, Mix IV, Mix V was assessed by conducting the acid test on concrete cube specimens. The acid resistance of concrete was determined in terms of weight loss and residual compressive strength. For conducting the acid test, concrete cubes of size 150 mm × 150 mm x 150 mm were cast and stored in a place at a temperature of 27 °C for 24 h and then the specimens were water cured for 28 days. After 28 days curing, the specimens were taken out and allowed to dry for one day as shown in Fig. 5. The initial weights of the concrete cube specimens were recorded. For acid attack test, 10% concentration of hydrochloric acid (HCl) and 10% concentration of sulphuric acid (H_2SO_4) by volume of water were used. After taking the initial weights, the cubes of all the five mixes were immersed in the above said acid solutions for a period of 28 days. The specimens were visually inspected after immersion in the above said acid water and the weight loss and residual compressive strength of the cubes were determined. The percentage loss in compressive strength and weight was calculated by taking

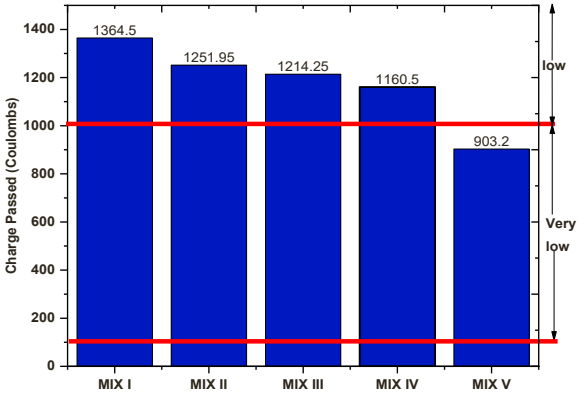

Fig. 4. RCPT Results of various mixes.

Table 3
Percentage water absorption and compressive strength of the concrete cubes for studies mixes with and without bacterial broth culture.

Mix designation	% Water absorption	Quality of concrete as per CEB 1989	Compressive strength
Mix I	3.03	Average	32.20
Mix II	2.44	Good	34.69
Mix III	1.83	Good	35.65
Mix IV	1.84	Good	36.99
Mix V	1.84	Good	37.90

Fig. 5. Cubes immersed in hydrochloric acid and sulphuric acid for 28 days.

into account the difference in compressive strength and weight between the conventional concrete and different mixes of bacterial concrete.

The concrete specimens subjected to 10% hydrochloric and 10% sulphuric acid solutions for 28 days were tested for % loss of weight and the compressive strength as represented in Table 4.

The comparison of the percentage loss in compressive strength and weight loss values of the hydrochloric acid and sulphuric acid attacked specimens at the end of 28 days are shown in Fig. 6 and Fig. 7 respectively.

It can be noticed that, the percentage loss in weight of the cubes immersed in Hydrochloric acid and sulphuric acid for 28 days was maximum in conventional concrete specimens and minimum for bacterial concrete with 10% bacterial broth culture and 30% GGBS as partial replacement for cement. The decrease in loss of compressive strength both in acid as well as hydrochloric and sulphate attack was due to the presence of more amount of internal voids that has been filled up by the use of GGBS and bacterial broth culture. Likewise, the compressive strength of conventional concrete was minimum when subjected to acid attack. The presence of *Bacillus cereus* bacteria has indeed improved the resistance of concrete towards acid attack. More resistance was exhibited by mix V compared to other mixes. The damage to the bacterial concrete was more in the sulphate environment than the chloride environment. Same kind of results were reported by other researchers also [35].

Based on the HCL and H_2SO_4 acid test, the strength loss is highly observed for conventional concrete than bacterial concrete. Similarly, after 24 h the surface damage was observed for conventional concrete, whereas the bacterial concrete can withstand the acid attach are shown in Fig. 8.

The crack healing capacity of the bacterial concrete specimens was studied. In order to identify the formation of $CaCO_3$ in the cracks, artificial cracks were formed in the cube specimens and the bacteria's healing activity with calcite precipitation was monitored continuously. The cracks were observed for 3,7,14 and 28 days. The deposition of a layer of calcium carbonate crystals on the surface resulted in a decrease of the permeation properties. Consequently, the ingress of harmful substances may be limited. Carbonate crystal deposition on the surface by bacterial isolate augments the resistance potential of cementitious materials towards deleterious substances and attacks (Nemati and Voordouw, 2003). Fig. 9 depicts the healing of cracks in bacterial concrete cubes Mix V at the age of 3rd, 7th, 14th and 28th day. Similar kind of self-healing was observed by several researchers [32,21,33,36–39].

Table 4
Percentage loss in weight and compressive strength of concrete cubes with and without bacterial broth culture exposed to acid attack.

Mix designation	10% Hydrochloric acid			10% Sulphuric acid		
	% Weight loss	Compressive strength N/mm^2	% Loss in compressive strength	% Weight loss	Compressive strength N/mm^2	% Loss in compressive strength
Mix I	2.38	27.81	13.61	2.98	19.50	39.43
Mix II	1.81	30.19	12.98	3.01	21.26	38.71
Mix III	1.81	31.36	12.03	2.41	21.92	38.52
Mix IV	1.20	32.82	11.26	2.41	22.84	38.26
Mix V	1.20	33.83	10.74	2.41	23.52	37.93

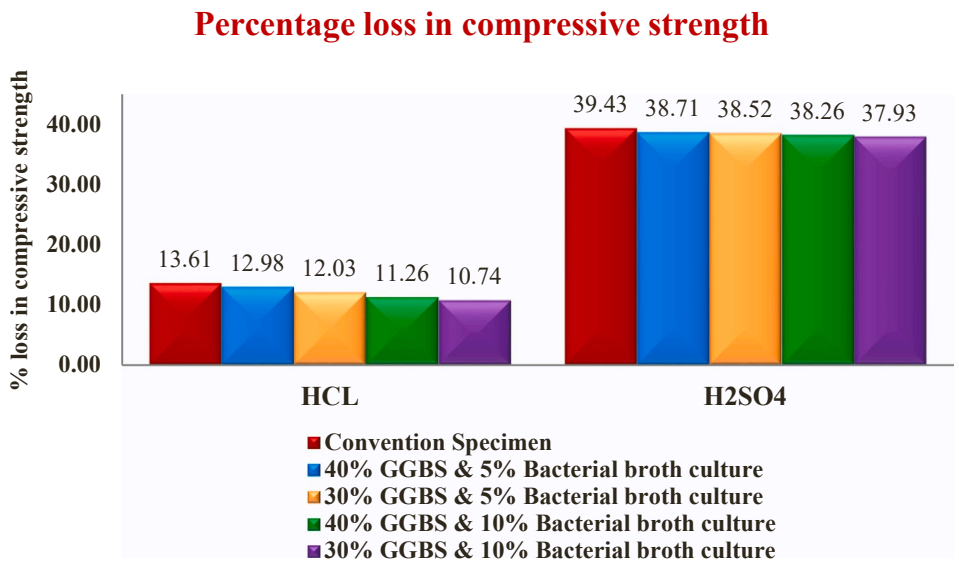

Fig. 6. Comparison of the percentage loss in compressive strength of the cubes immersed in hydrochloric acid and sulphuric acid at the end of 28 days.

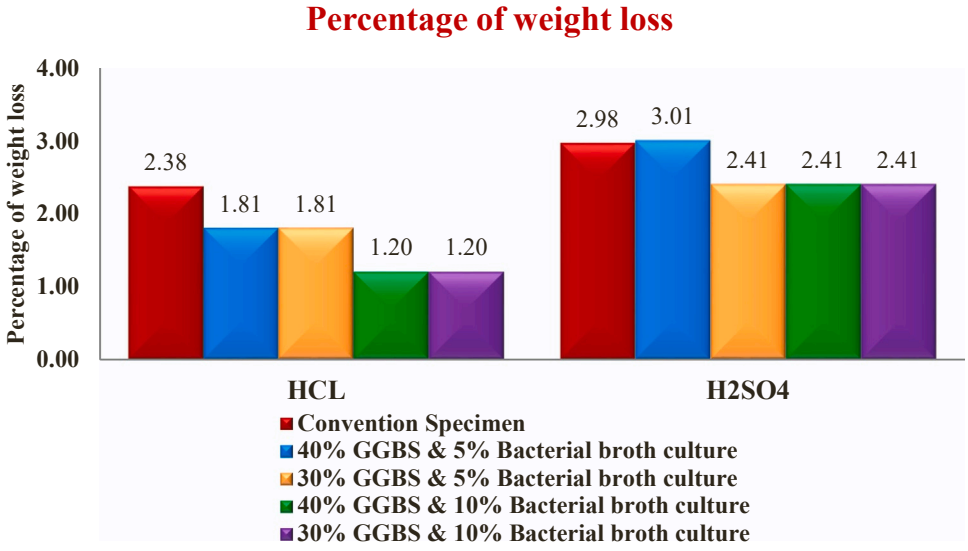

Fig. 7. Comparison of the weight loss values of hydrochloric acid and sulphuric acid attacked specimens at the end of 28 days.

3.7. XRD analysis

XRD peaks of five mixes are shown in Fig. 10. It can be observed that the conventional concrete exhibited broad humped peaks while that of *Bacillus cereus* bacterial broth cultured bioconcrete mixes had sharp peaks.

The broad humped peaks of conventional concrete indicated that the material was amorphous with short range ordering. Sharp peaks indicated crystalline nature of the bacterial concrete mixes. Further, calcite appeared at 30° in the bacterial culture medium of KOV 15 [26]. Accordingly, the Mixes II to V with bacterial broth culture had given peaks at 2θ value of 30°and no calcite content in the case of control concrete (Mix I). Similar peaks corresponding to calcite were reported by earlier researches incorporating bacterial culture for the biocement (Zhao et al., 2022). Peaks in XRD at 22◦ indicate the presence of silicates in the bioconcrete samples [14]. Other diffraction peaks at 28˚, 36˚, 39◦,42˚, 48˚, and 56˚ were reported to belong to various types of calcite type biocement crystals particularly in the presence of GGBS (Zhao et al., 2022; [22]). Mixes IV and V with higher bacterial content has higher intensity peaks corresponding to calcite and other hydration products of biocement.

Fig. 8. Failure mode and surface damage in cubes subjected to chemical attack.

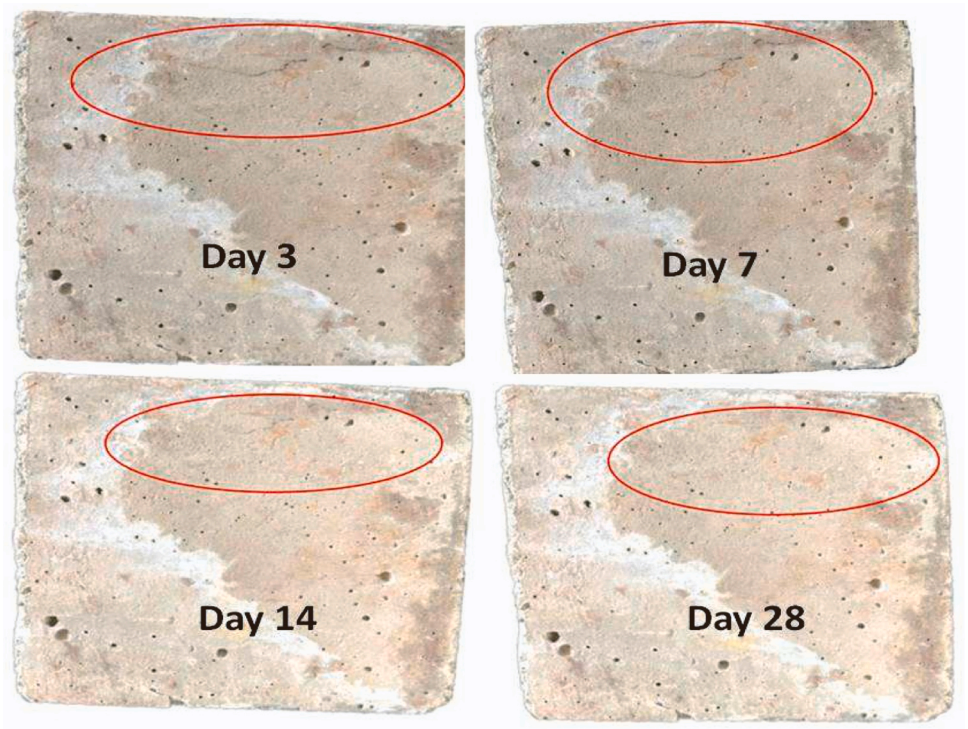

Fig. 9. Crack Healing of Bioconcrete Cubes.

3.8. SEM analysis

The SEM images of conventional concrete and various mixes of bioconcrete with GGBS as replacement for cement and bacterial broth culture at 28 days are shown in Fig. 11. In comparison with conventional concrete images, the bacterial concrete images with GGBS as replacement for cement had better hydrated form, possessed good homogeneity and had lesser pores. The SEM image of conventional concrete showed more of white areas that represent unreacted cement and lesser amount of grey areas that represent C-S-H gels which are products of hydration [40]. The SEM analysis results showed that the samples with 30% GGBS as replacement for cement and with 10% Bacterial broth culture exhibited more of C-S-H gels, less pores, densely packed structure and less unreacted

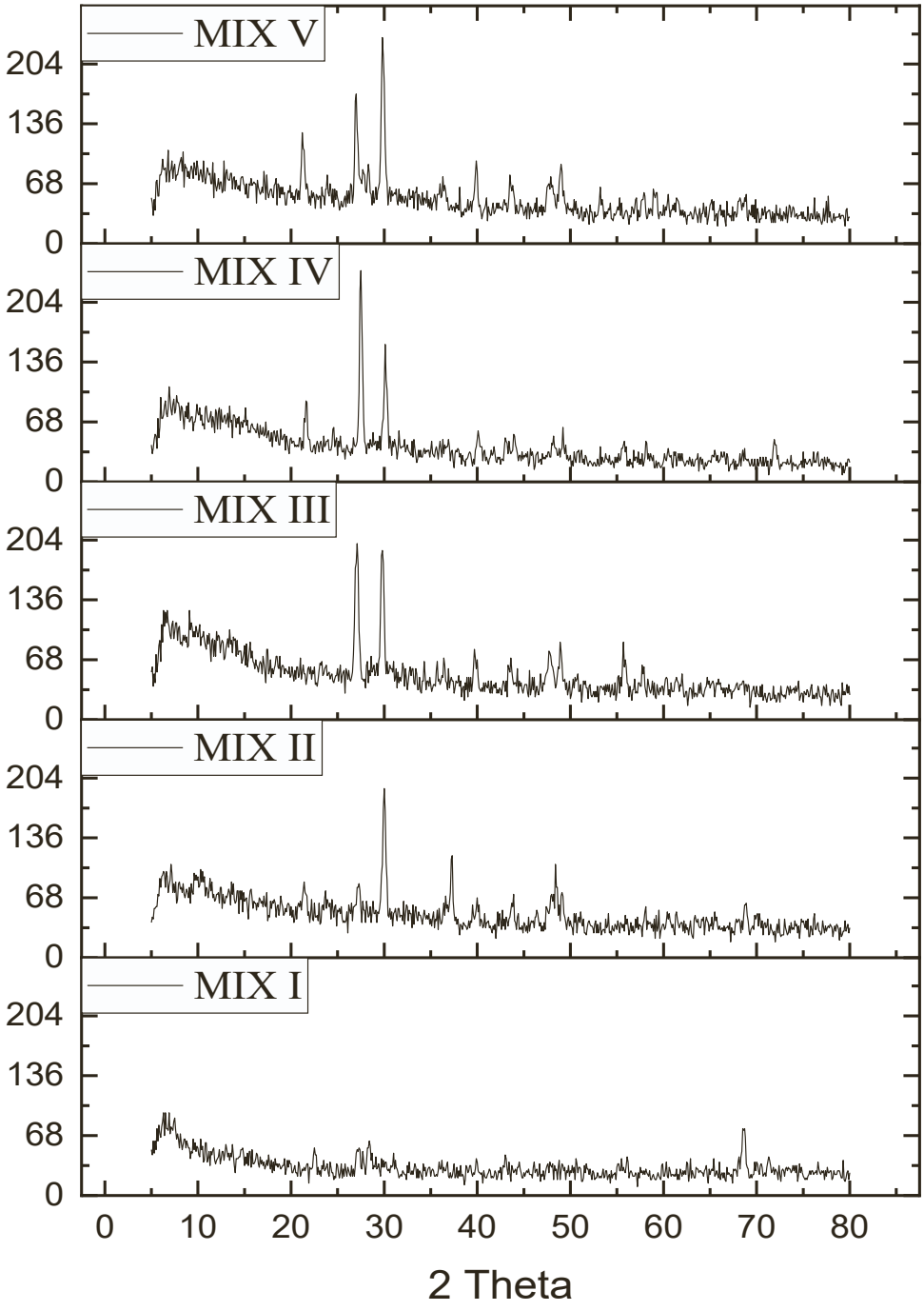

Fig. 10. XRD pattern of Conventional Concrete (Mix I) and Bacterial Concrete (Mix II - V).

cement particles. [41].

3.9. EDX analysis

The EDX data generated by the analysis consist of a spectrum that displays the peaks correlated to the elemental composition of the sample being investigated [42]. The EDX analysis for various samples of bioconcrete and conventional concrete was carried out and the EDX images of the samples of conventional concrete (Mix I) and bioconcrete with 30% GGBS and 10% Bacterial broth culture at 28 days (Mix V) are shown in Fig. 12. The elemental composition of different mixes of concrete with GGBS and bacterial broth culture at

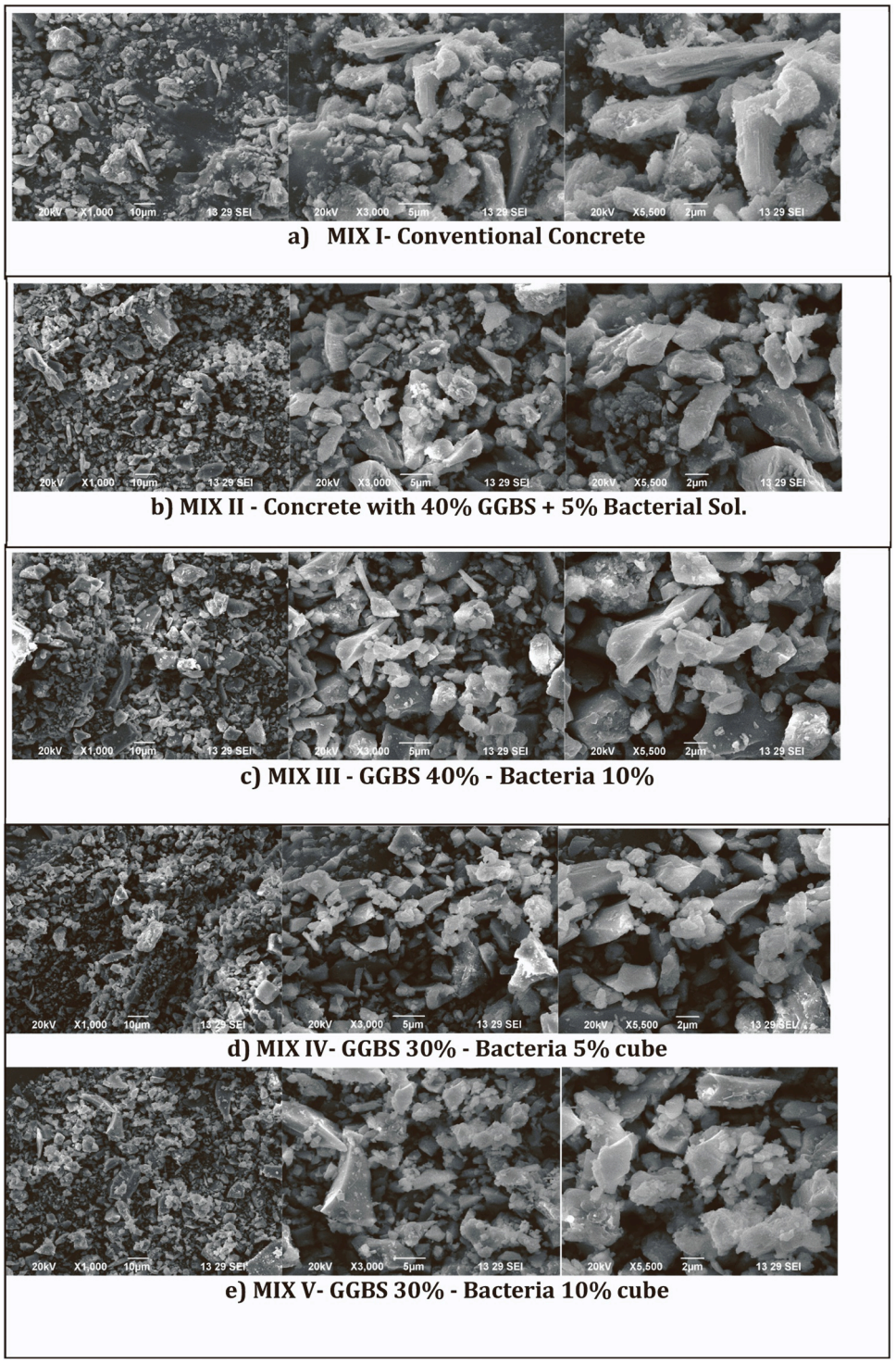

Fig. 11. The SEM Images of Conventional Concrete (Mix I) and Bacterial Concrete (Mix II to Mix V) at 28 days.

28 days are shown in Table 5.

Calcium and Silica are responsible for the higher strength of concrete. The amount of calcium increased by 64.82%, 78.52%, 79.84% and 103.82% for Mixes II to V respectively when compared to the conventional concrete.

In this study, the effect of bacterial healing agents on the crystal polymorphs of $CaCO_3$ was confirmed. It can be concluded from the above discussions that strain KOV15 could produce higher $CaCO_3$ content, greater amino acid acidity and more suitable ions than those

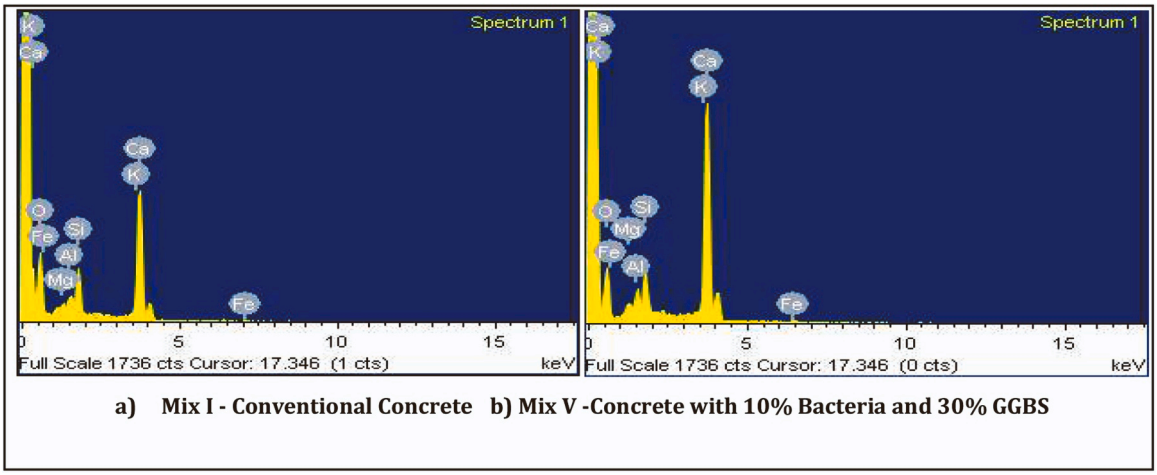

Fig. 12. EDX Images of Conventional Concrete (Mix I) and Concrete with 30% GGBS and 10% Bacterial Broth Culture at 28 days (Mix V).

Table 5
Elemental composition of different mixes of concrete with GGBS and Bacterial broth culture at 28 days.

Element	Atomic %				
	Mix I	Mix II	Mix III	Mix IV	Mix V
O K	84.35	79.05	76.92	78.83	78.87
Mg K	0.96	1.52	1.30	1.11	0.49
Al K	1.61	2.10	2.53	1.66	1.66
Si K	4.40	4.47	3.93	4.39	3.03
K K	0.68	0.09	0.20	0.18	0.30

presented in the MICP process, thereby leading to the formation and stabilization of aragonite in the final biocement [20].

3.10. Behavior of bioconcrete when applied as structural element

Five beams with five different mixes were studied for load deflection behavior as shown in Fig. 13 and Table 6 represents the load at the initial crack formation, the ultimate load, the deflection in beams and the ductility factor of RC beams.

From the load deflection studies of RC beams, it can be inferred that the ultimate load was minimum and the deflection was maximum for control beam without bacterial broth culture. Bioconcrete exhibited good ultimate load and lesser deflection compared to the control beam. The increase in the ultimate load when compared to the control beam was 6.29%, 12.25%, 17.55% and 20.53% for mixes II to V respectively. Within the various mixes, Mix V has shown superior properties than the others. Once again, the same phenomenon of increased quality of concrete with increased bacterial content can be observed for load deflection behavior of RC beams which is in consistence with the bioconcrete cubes. The structural response of bacterial concrete with varying percentages of

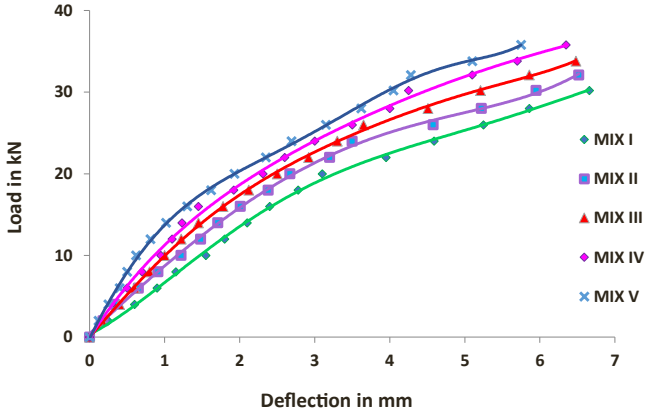

Fig. 13. Load deflection behavior of concrete beams according to five different mixes.

Table 6
Initial crack load, ultimate load, deflection and ductility factor of RC beams with and without bacterial broth culture.

Mix designation	Initial crack load kN	Ultimate load kN	Deflection in mm	Ductility Factor du = Δu/Δy
Mix I	14	30.2	6.66	1.75
Mix II	20	32.1	6.52	1.98
Mix III	18	33.9	6.48	2.03
Mix IV	20	35.5	6.35	2.27
Mix V	22	36.4	5.75	2.88

bacterial culture indicates that the increase in bacterial culture percentage has improved the structural properties through the self-healing mechanism.

Ductility means the ability of a structure to undergo large amplitude of cyclic deformation in the inelastic range without a substantial reduction in strength. The displacement ductility factor defined as the ratio of failure displacements to yield displacements, according to the Committee Euro-International, was calculated using the following formula.

$du = \frac{\Delta u}{\Delta y}$ where Δu is the failure displacement which is lateral displacement at 80% of the ultimate load at the descending part of the curve and Δy is yield displacement which is lateral displacement at 80% of the ultimate load at the ascending part of the curve and du is the ductility factor.

The ductility factors were calculated for all the beams as shown in Table 6. As an example of determination of the values in the equation, the control beam and the beam with mix V concrete are shown in Fig. S4. The ductility factor was maximum for the beam with 30% GGBS and 10% bacterial broth culture and minimum for the control beam. The ductility factor value increased for the concrete specimens with bacterial culture and in proportion to the percentage increase of bacterial culture. These results show that *Bacillus cereus* bacteria derived from soil has improved the requisite qualities of concrete.

Further, failure pattern of the reinforced concrete beams of control mix and 30% GGBS containing concrete with both 5% and 10% *Bacillus cereus* (Mix III and Mix V) was observed. In the initial stages of loading, flexural cracks appeared only in the flexural zone and not in the shear zone. In the later stages of loading, shear cracks occurred in the shear span between the loading point and the support. Crushing of concrete occurred in the compression zone. Many flexural cracks appeared in the tension zone and propagated towards the compression zone in the control beam. Fig. 14 depicts the crack pattern in control beam, Mix III and Mix V.

The dominant failure mode for reinforced bioconcrete beams was formation of flexural crack during the initial crack load, followed by many flexural cracks and shear cracks near collapse. The number of cracks were very less in the case of bacterial concrete beam with 10% bacterial culture and 30% GGBS.

4. Conclusion

The current research work was done on the bacterial broth culture concrete samples prepared using cultured bacteria obtained from autoclaved soil along with GGBS as fractional substitution for cement. The performance of the concrete was evaluated based on the study of the mechanical properties, such as the compressive strength, the split tensile strength and the flexural strength. Water absorption and durability studies were carried out in this research. Based on the study, the following conclusions are drawn: (i) KOV-15 bacteria (*Bacillus cereus*) is an efficient bacteria for biocement production as well as for promoting self-healing in construction, (ii) The values of the cube compressive strength (39.288 N/mm^2), split tensile strength (3.32 N/mm^2) and flexural strength (4.8 N/mm^2) were maximum for concrete with 30% GGBS as replacement for cement and 10% bacterial broth culture (Mix V) at 28 days, (iii) The maximum percentage increase in the cube compressive strength, split tensile strength and flexural strength of bioconcrete was 26.79%,

Fig. 14. Crack pattern in reinforced concrete beams with and without bacterial broth culture.

11.69% and 21.3% respectively for Mix V compared to the conventional concrete, (iv) The SEM images of the bioconcrete with GGBS as replacement for cement have better hydrated form, possess good homogeneity and have less pores when compared to the images of conventional concrete, (v) From the EDX images and the elemental constituents of the various mixes of bioconcrete, the amount of calcium increased by 64.82%, 78.52%, 79.84% and 103.82% for Mixes II to V respectively when compared to the conventional concrete (Mix I), (iv) Bioconcrete has shown good resistance towards acid attack, has lower water absorption, and higher ductility factor, and (v) The application of bioconcrete in structural member exhibiting significant strength properties and lesser crack formation compared to the conventional concrete also suggested that the indigenous *Bacillus cereus* KOV15 can be used for synthesis of greener construction materials such as bioconcrete with the optimum usage of 30% GGBS as replacement for cement.

5. Future directions

The research work can be extended further to the following areas in future:

i. The study can be extended to select various bacterial broth culture such as B.megaterium, B.subtilis, B. thuringiensis, D.halophila, Pseudomonas, Aerobacteraerogenes, Sporosarcina, Sporosarcinapasteurii, etc.,
ii. Higher grades of concrete such as M30, M35, and M40, etc., with different broth cultures can be studied.
iii. The study can be extended to the use of selected bacterial broth culture in different proportions such as 15%, 20%, 25%, 30%, etc. weight of cement for concrete.
iv. The present study which was done for 7 days and 28 days could be extended for 56 days and beyond.
v. The incorporation of different proportions of GGBS such as 45%, 50%, 55%, 60%, etc., as fractional substitution for cement along with bacterial broth culture can be investigated.
vi. The study can be extended to fly ash, silica fume, micro silica, etc., as fractional substitution for cement along with bacterial broth culture.
vii. The behaviour of other structural members such as columns and slabs made of bioconcrete using Bacillus cereus (KOV 15) can be investigated.

CRediT authorship contribution statement

Shanmugam Kirupakaran: Methodology, Investigation, Formal analysis. **Preethi:** Conceptualization, Methodology, Formal analysis, Supervision. **Angeline Prabhavathy:** Investigation, Writing, Formal analysis. **Preyadarshi S:** Experimental Investigation. **Sri Chandana:** Data curing, Writing – review & editing, Supervision.

Declaration of Competing interest

With reference to the present manuscript, on behalf of all the authors, as a correspond author, hereby I declare that there is no competing interest / conflict of interest either financial or personal relationships in concern with the research work reported in this paper.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.cscm.2023.e02325.

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