EXPERIMENTAL INVESTIGATIONS ON BEHAVIOUR OF BASCILLUS BACTERIA IN GEOPOLYMER CONCRETE

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Abstract - *Using cement as an aggregate binder has become standard practise on all construction sites since it is simple to use and has good strength. It is generally known that considerable amounts of carbon dioxide are emitted during the production of cement. Geopolymer Concrete (GPC), environmentally friendly concrete that reduces CO2 emissions by generating aluminosilicates-based inorganic polymers by activating source material rich in alumina and silica with alkaline liquids. GPC can address the problems of unsustainable use of limestone and pollution associated with cement production by employing supplementary cementitious materials (SCMs) including fly ash (FA), ground granulated blast furnace slags (GGBS), metakaolin, and other materials instead of cement. GPCs provide considerable ecological and environmental benefits. GPC requires addition and modification. GPC must be changed by the inclusion of specific components, such as bacteria that affects the substance's strength and curing environment. In compliance with applicable standards and protocol, a series of tests were carried out to verify the essential characteristics of BGPC in the fresh and solid states. Extraction, isolation, and identification of Bacillus bacteria; mix design of bacterial-based geopolymer concrete (BBGPC); and evaluation of mechanical properties for various BBGPC mixes are among the goals that the current project is expected to achieve. In order to characterize BBGPC and forecast its compressive strength, researchers used the Plate Test, the Urease Test, Field Emission Scanning Electron Microscopy with EDAX (SEM-EDAX), and Field Emission Scanning Electron Microscopy (FE-SEM).*

***Key Words*: Polymerization, bacteria, supplementary cementitious materials, alkaline solution.**

1. INTRODUCTION

A polymeric reaction between an alkaline liquid and a source material made of natural minerals or a byproduct, such as supplemental cementitious material without cement, produces geopolymer, which may hold the key to lowering emissions from the manufacture of cement. In the field of concrete, geopolymer is a recent innovation in which pozzolanic elements such as fly ash, GGBS, metakaolin, silica fume, etc. completely replace cement as a binder in the concrete mix. In an effort to create a sustainable building material, this study aims to demonstrate how bacteria can be used in geopolymer concrete. A unique study area called geopolymer bacterial concrete can be employed for cementitious materials that can alter the behaviour of geopolymer concrete and cure on their own through a bio-mineralization mechanism. It is intended that bacteria be added to concrete, which aids in the precipitation of calcite in pores and microscopic hollow spaces.

A ureolytic bacterium called Bacillus species can create calcite, which reduces the pores in concrete and increases its strength and endurance. Bacillus pasteurii, Bacillus sphaericus, Bacillus cohnii, Bacillus pseudofirmus, Bacillus subtilis, Bacillus Megaterium, and Bacillus alkalinitrilicus are spore-forming bacteria that have all been used by researchers in their studies. Additionally, Sporosarcina pasteurii has replaced Bacillus pasteurii in the classification. This study truly looks into how the calcification and geopolymerization processes used by certain Bacillus bacteria species might enhance the toughness and longevity of geopolymer concrete.

Strength properties and durability studies on modified geopolymer concrete composites incorporating GGBS and metakaolin was studied by Srikanth Divvala and M. Swaroopa Rani (2021) In this investigation GPC (100:0) mix specimens attained the maximum compressive strength split tensile strength and flexural strength values of 63.22, 2.63 and 10.6 for 28 days and 67.55, 3.1 and 12.8 for 90 days. The 16 M specimens exhibit much better strength properties in all aspects compared to 20 M specimens. Ramin Andalib et al. (2015) did research on bacterial concrete made using geopolymer. In that study, microorganisms are used to examine whether certain Bacillus bacterial species can strengthen geo-polymer concrete based on bio-mineralization. In this study, it was discovered that at 90 days, Geo-polymer bacterial concrete experienced the highest growth in compressive strength compared to regular bacterial concrete.

3. EXPERIMENTAL PROGRAM

3.1 Materials

Supplementary cementitious materials like ground granulated blast furnace slag (GGBS), Fly Ash, Metakaolin, Silica fume, were obtained as shown in fig.1. The chemical composition of the GGBS, Fly Ash, Metakaolin, Silica fume are shown in Table 1. A combination of Sodium Hydroxide and

Sodium Silicate solutions were used to react with the Aluminium and the Silica in the SCMs. Fine sand and 20mm and 12 mm aggregates were also used in saturated surface dry condition. The mixtures were designed for the grade of 30 MPa. The Geo-polymer concrete mix was based on the different ratio of materials to optimize the best mixture. The appropriate components of ordinary and Geopolymer concrete with optimum percentage of bacteria mixed with geopolymer concrete are shown at Table 2 respectively.

3.2 Activating solution (AS)

Alkaline activating solution made using distilled water, a mixture of NaOH and Na2SiO3, and prepared prior to the 24 hour mix. For concentrations of 8, 10,12, 16, and 20 NaOH, alkaline solutions are made. The utilized sodium hydroxide is with a purity of 98%. For the creation of alkaline solution, some of the sodium silicate is purchased in powder form, while the remainder is purchased in liquid form. The difference between NaOH solids and distilled water depends on the solution's concentration. Because the solution releases a significant amount of heat during preparation, it is safest to prepare it with the utmost care at least 24 hours before to mixing.

Fig 1 Materials used

Table -1: The chemical composition of the GGBS, Fly Ash, Metakaolin and silica fume (mass %)

Cementitious Materials	SiO2 %	Al2O3 %	CaO %	Fe2O3 %	LOI %	Sp. Gr
GGBS	48.74	10.78	43.34	0.42	0.6	2.25
Fly Ash	57.79	20.18	2.97	7.04	4.22	2.05
Metakaolin	19.43	5.64	61.60	4	1.85	3.2
Silica Fume	92	0.32	0.34	0.07	0.001	2.64

4. Mix proportion

BGPC combinations were classified based on the amount of bacteria they contained. The ratios of these combinations per cubic meter of concrete are shown in Tables 2. The water-to-geopolymer solids ratio parameter was taken into account by mass with regard to mixture design. The parameters selected for the mixture's constituents for the alkaline activators include a sodium silicate solution to sodium hydroxide solution mass ratio of 2.5, a sodium hydroxide solution molarity in the range of 12 M, and a mass ratio of 0.35 for the activator solution to fly ash. Concrete made using the control mixture's additional cementitious ingredients.

Table2: Bacterial Geo-polymer concrete mix proportions with different ratio of ingredients to achieve 30 MPa strength

MIX ID	M12MC	M12MB 25	M12MB 50	M12MB 75	M12MB 100	M12MB 125
BACTERIA ml/litre	0	22.5	45	67.5	90	112.5
GGBS (kg/m3)	246	246	246	246	246	246
MK (kg/m3)	51.25	51.25	51.25	51.25	51.25	51.25
FA (kg/m3)	82	82	82	82	82	82
SF (kg/m3)	30.75	30.75	30.75	30.75	30.75	30.75
FREE WATER	100	100	100	100	100	100
ADMIXTURES	10.25	10.25	10.25	10.25	10.25	10.25
AAS	143	143	143	143	143	143
NAOH SOLID (kg/m3)	13.25	13.25	13.25	13.25	13.25	13.25
NAOH WATER	27.61	27.61	27.61	27.61	27.61	27.61
SODIUM SILICATE	102.14	102.14	102.14	102.14	102.14	102.14
FINE AGGREGATE (kg/m3)	560	560	560	560	560	560
COARSE AGGREGATE (kg/m3)	1265	1265	1265	1265	1265	1265

5. SPECIMEN PREPARATION

The specimens are prepared by mixing the supplementary cementitious material like GGBS, fly ash, Metakaolin, and Silica fume with alkaline solution with incorporation of bacteria in different percentages and the BGPC concrete is prepared in concrete mixer and the bacterial concrete is allowed for ambient curing as shown in fig 2.

Fig 2. Prepared Specimens - Marking of Specimen ID and GPC Specimens under ambient curing

6. TESTING OF SPECIMENS AND RESULTS

Fig 3. Testing of specimen samples

7. HARDENED CONCRETE PROPERTIES

7.1 Mechanical Strength

The compressive strength of all the concrete mixes (GPC and BGPC) at 3, 7, and 28 days were determined using a standard 100 × 100 × 100 mm cube specimen per IS 516. The modulus of rupture and split tensile strengths were also determined using the concrete bar of size 100 breadth × 100 depth × 500 length mm and cylinders with 100-mm diameter and 150-mm height, respectively, at 7 and 28 days.

7.2 Durability properties

7.2.1 Water Absorption Test

The cube specimens of GPC and BGPC (measuring 100 x 100 x 100 mm) underwent the water absorption test. The mass of these concrete cubes was maintained constant by oven drying them at 110 °C for 24 hours before weighing them as W1. After being submerged in water for 24 hours, the specimens were weighed as W2. The water absorption percentages were computed using the formula = W2 - W1/W1*100

7.2.2 Sorptivity Test

By measuring the capillary rise absorption rate on sufficiently homogeneous material, the sorptivity can be ascertained. As the test fluid, water was employed. The cylinders following casting and a 28-day cure period. After being dried in an oven at a temperature of 100 + 10 °C, the specimen, measuring 100 mm in diameter and 150 mm in height, was drowned as shown in with water level not more than 5 mm above the base of the specimen. The flow from the peripheral surface was then properly stopped by sealing it with non-absorbent coating. Weighing the specimen allowed us to calculate how much water was absorbed over the course of 30 minutes.

7.3 MICROSTRUCTURAL ANALYSIS

The concrete samples are collected for microstructural study after mechanical and durability assessments. The FESEM analysis is undergone with EDS to study the shape of structure and composition of materials in the bacterial concrete. To determine the presence of bacteria in concrete several microscopic studies have been in consideration like plate test, Urease test, FTIR test for $CaCO_3$ precipitation.

8. RESULTS AND DISSCUSSION

8.1 Fresh concrete properties - Workability – Slump Cone Test

The slump cone value provides the concretes workability condition by slump cone apparatus and the chart 1 shows the slump value and workability condition for different mixes. The test shows that the mix BGPC 125 shows the condition of highly workable than other mixes.

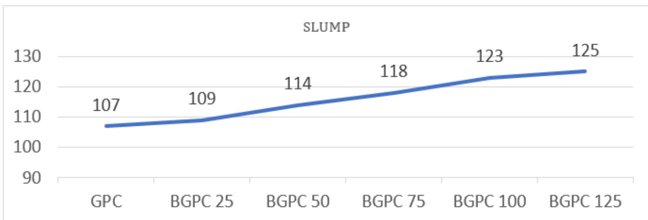


Chart 1 – Slump Cone Values for Different Mixes

8.2 Mechanical properties

The test results shows that the mechanical properties like compression strength, split tensile strength, flexural strength have optimal mix at BGPC100 compared to other mixes and conventional GPC as well. The test results are given below in the chart 2, 3 and 4.

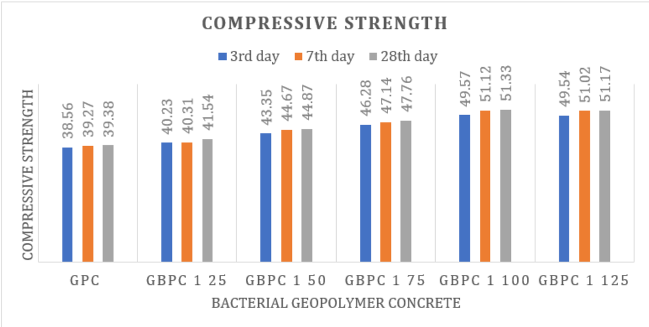


Chart 2. Compressive strength results

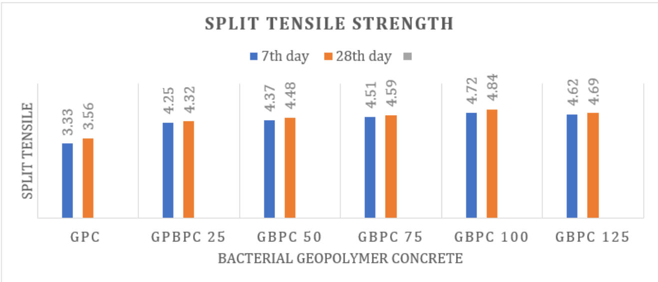


Chart 3. Split tensile strength results

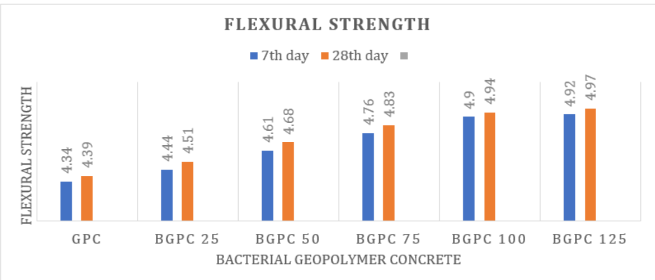


Chart 3. Flexural strength results

8.3 Durability results

8.3.1 Water absorption

The water absorption results are shown below in table 3 which represents the increase of bacterial percentage results in decrease in the water absorption percentage.

Table 3. water absorption percentages

S.NO	SPECIMEN	WATER ABSORPTION (%)
1	GPC	2.83
2	BGPC 25	2.61
3	BGPC 50	2.38
4	BGPC 75	2.17
5	BGPC 100	2.02
6	BGPC 125	2.01

8.3.2 Sorptivity Test results

The measurement of the capillary rise absorption rate which involves the bacterial geopolymer concrete mixes which absorbs how much mm is the results of the sorptivity which is given in the table 4 below.

Table 4. sorptivity results

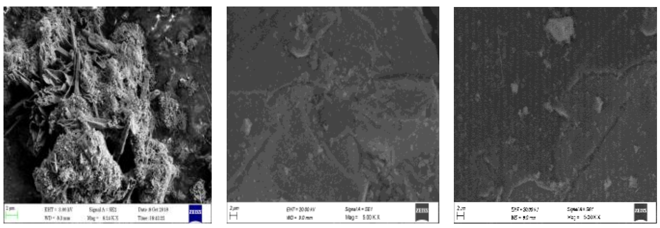
S.NO	SPECIMEN	SORPTIVITY (mm)/min
1	GPC	0.45
2	BGPC 25	0.42
3	BGPC 50	0.37
4	BGPC 75	0.34
5	BGPC 100	0.28
6	BGPC 125	0.21

8.4 MICRO STRUCTURAL ANALYSIS

8.4.1 FESEM ANALYSIS

The existence of calcite crystals precipitated by bacteria inside concrete sample is verified via FE-SEM analysis. Five BBGC samples were analyzed using FE-SEM after 28 days. In general, Fig. 4 and 5 shows that $CaCO_3$ precipitates occur in all samples excluding the control sample. These photographs demonstrated how the bacteria in the bacterial-based geopolymer mortars functioned and precipitated calcium carbonate. To demonstrate that bacterial activity in the geopolymer samples caused calcium carbonate to precipitate, EDS analysis was carried out. To ascertain the condition of the level of calcium precipitation, the Si, Al, Na, Ca, C, and O peaks in the EDS were analyzed. The Si and Al peaks in the EDS spectroscopies revealed the primary products of geopolymerization.

Formation of few individual crystals in the control concrete mix BMC, (ii) little precipitation of $CaCO_3$, (iii) more crystals and more precipitation of $CaCO_3$ for the mixes incorporating various bacterial dosages, (iv) and the development of rod-shaped bacteria in the mixes incorporating various bacterial dosages (v) An increase in the number of bacteria and more $CaCO_3$ precipitation until the bacterial cultures are dosed properly.



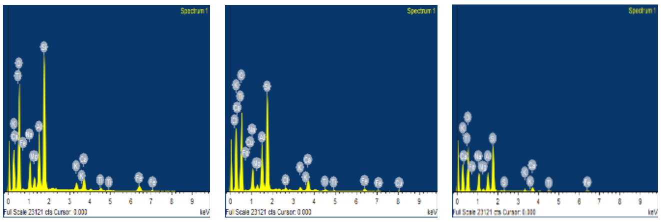


Fig 4. SEM and EDS results

8.4.2 PLATE TEST OF BACTERIA BASED GEOPOLYMER CONCRETE

At the Centre for Bioscience and Nanoscience Research (CBNR), Coimbatore, the bacteria is evaluated and cultured. A Petri dish called a nutrient agar plate is used to cultivate microorganisms. It comprises agar as a solid growth medium and nutrients. Colonies of individual microbes will form as they are placed on the plate. It is essentially a dilution technique that includes covering the surface of an agar plate with a loop full of cultures. Eubacteria, a gram-positive, spore-producing bacteria, is present in soil. It is one of the largest Eubacteria and has a rod-like form. Due to the cell wall's sticky polysaccharides, colonies grow in chains. By using procedures for enrichment culture, Bacillus bacteria are isolated. Different dilutions of the soil suspension were drawn. It was added to the Hicrome Bacillus Agar medium, which is a selective medium for isolating Bacillus bacteria and produces colonies that are yellow in hue. Consequently, it may be inferred from the test that the Bascillus bacteria is Bascillus Megaterium.

8.4.3 UREASE TEST RESULTS

It is a carbonic acid diamide Ammonia and carbon dioxide are released as it hydrolyzes. The urease enzyme, which is found in many organisms, is notably common in those that affect the urinary system. It can split urea in the presence of water to release ammonia and carbon dioxide. The indicator phenol red changes from its initial orange-yellow colour to a brilliant pink hue when the indicator phenol red reacts with the ammonia, carbon dioxide, and water to generate ammonium carbonate, which makes the medium alkaline.

Out of all the mixes, a urease test was done for a few (BGPC3, control) at 28 days. It has been noted that the control mixtures' colour remains the same yellow and is not altered. All five of the mixes have had their colours changed from yellow to pink, save from the control mixtures. Even after 28 days, the BBGPC still exhibits the positive active Bacillus bacteria bascillus megaterium as seen by the colour change from yellow to pink.

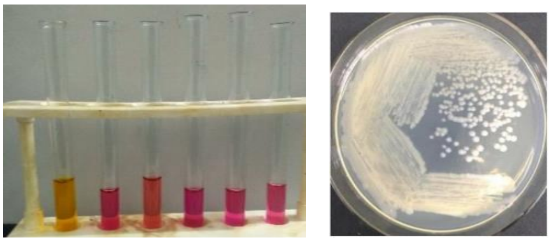


Fig 5. Colonies and urease enzyme

9. CONCLUSIONS

- Compressive strength has been evaluated for various BBGC mixes in comparison with conventional geopolymer concrete. In all mixes, the variation includes the % bacterial proportions in geopolymer concrete.
- In each mix, Bacteria has been added in different dosage. Early Compressive strength is attained in day 3 to day 7. According to the results of SEM and EDS analysis, as the pH increased, the bond strength of the mortar increased and more stable $CaCO3$ structures were formed
- With the addition of bacterial dosage in Various proportions, there is about 15 – 20% strength enhancement is observed. The reason could be due to its ability to form calcite which is observed in FESEM analysis which block the pores of concrete structure in addition to other SCMs (FA, SF and Mk) in different proportions.
- The durability properties like water absorptions and sorptivity also shows decremental progress in the bacterial geopolymer concrete compared to conventional concrete.

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