

## EXPERIMENTAL STUDY ON INFLUENCE OF VARIOUS BACTERIA ON WORKABILITY, STRENGTH AND DURABILITY CHARACTERISTICS OF GEOPOLYMER CONCRETE

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**Abstract:** Cement is widely used as a crucial raw material in construction across the globe. To meet the demands of the construction industry, significant quantities of cement are produced annually, leading to the release of substantial amounts of CO<sub>2</sub> into the atmosphere. With growing awareness of global warming, geopolymer concrete (GPC), an inorganic polymer binder, was developed as an eco-friendly alternative to ordinary Portland cement (OPC) concrete. Recent research has focused on improving the viscosity and workability of GPC, leading to the development of self-consolidating geopolymer concrete (SCGC). This experimental study examines the effects of three microorganisms—*E. coli*, *Bacillus subtilis*, and *Bacillus pseudofirmus*—on the workability, strength, durability, and microstructural characteristics of SCGC. Four different SCGC mixes were prepared and subjected to various workability, strength, and durability tests, alongside SEM and XRD analyses to study their microstructural development. The results indicated that the SCGC mix containing *E. coli* bacteria exhibited superior microstructural development, the highest workability with a slump value of 706 mm, and the maximum compressive strength of 44.30 MPa after 28 days of ambient curing. Additionally, the hardened SCGC mix with *E. coli* showed enhanced resistance to acid and alkali attacks and minimal water absorption.

**Keywords:** Bacterial concrete; E-Coli, Bacillus Subtilis, and Bacillus Pseudofirnis; Self-consolidating; Geopolymer concrete.

### 1. Introduction

The construction industry has experienced significant growth in recent times, driven by the development of infrastructure such as bridges, buildings, and roads. Concrete and steel are extensively utilized in this sector. However, the cement industry, a major component of construction, is the second-largest source of carbon dioxide emissions. Concrete, the most commonly used construction material globally, contributes nearly 8% of global CO<sub>2</sub> emissions due to the production and use of Portland cement. The CO<sub>2</sub> emissions from concrete production are directly proportional to the cement content in the mix, with 900 kg of CO<sub>2</sub> released for every ton of cement produced. This accounts for 88% of the emissions in an average concrete mix. The main issue lies in the calcination process, where each molecule of calcium oxide, also known as "quicklime" or "burnt lime," releases one molecule of CO<sub>2</sub>. With an annual global cement production of approximately 4.5 billion tons, this translates to 2.7 billion tons of CO<sub>2</sub> emissions.

To mitigate these environmental impacts, the development of alternative binding materials is critical. One such innovation is Geopolymer Concrete (GPC), introduced by Prof. Joseph Davidovits in 1978. GPC is an environmentally friendly material that utilizes industrial by-products like fly ash (from thermal power plants) and ground granulated blast furnace slag (from iron production) as a complete replacement for cement in concrete. This sustainable approach not only reduces cement consumption but also offers superior strength and durability compared to Portland Cement Concrete (PCC). Consequently, the adoption of GPC in the construction industry can significantly reduce environmental pollution and reliance on PCC.

Self-Consolidating Geopolymer Concrete (SCGC), a variation of GPC, possesses the ability to flow under its own weight, eliminating the need for manual compaction. It can seamlessly fill gaps in formwork and densely reinforced areas, enhancing construction quality while significantly reducing long-term maintenance costs.

Concrete structures often suffer from deterioration caused by naturally occurring cracks. These cracks can be effectively sealed by incorporating *Bacillus* species bacteria into the concrete, eliminating the need for external chemical treatments. These bacteria are capable of surviving in harsh conditions and sealing cracks by producing calcite. This study investigates the effects of *Bacillus* species on the workability, strength, and durability of SCGC. While research on bacteria-enhanced SCGC is still limited, studies have shown that incorporating bacteria improves concrete by filling cracks with calcite and enhancing the mechanical properties of geopolymer concrete.

## 1. Materials and Methodology

### 1.1. Materials Used

The binder constituents (GGBFS, Alccofine and Flyash) utilized for preparing self-consolidating geopolymer concrete (SCGC) were procured from Ultratech ready mix concrete plant located at peenya, Bangalore. Table 2 and Table 8 represent physical properties of binder constituents. Table 6 and Table 7 represent chemical properties of fly ash, GGBFS and alccofine respectively. Three different bacteria namely *E. coli*, *Bacillus subtilis*, and *Bacillus pseudofirmus* procured from National Centre for Microbial Resource (NCMR), Pune in solution form were added to SCGC mixes. Table 1 represents characteristics of bacteria. Alkaline activator liquids utilized in preparation of SCGC mixes were commercially available Sodium hydroxide (NaOH) flakes with 94% purity and Sodium silicate (Na<sub>2</sub>SiO<sub>3</sub>) solution with 55% water content. Table 3 exhibits properties of Na<sub>2</sub>SiO<sub>3</sub> solution. Locally available M-sand was utilized as fine aggregate. Coarse aggregate of size below 20mm procured from local vendor was utilized in preparation of SCGC. Table 4 represent physical properties of both aggregates. BASF Master Glenium 8233 procured from local vendor was utilized as superplasticizer for preparing the mix. Table 5 represent properties of superplasticizer.

**Table 1.** Bacterium properties (Data source: NCMR, Pune)

NCMR Accession Number	MCC 2819	MCC 2412	MCC 2183
Taxonomic Designation	<i>Bacillus Pseudofirmus</i>	<i>Escherichia coli</i>	<i>Bacillus Licheniformis</i> (Ehrenberg 1835) Cohn 1872
Strain Designation	LAP217	Seattle 1946	LS-1
Source of Isolation	Lonar Lake water sample	Clinical isolate	Lonar lake soil sample

Medium Name and Number	34C (Alkaline Nutrient Agar)	34b (Nutrient Agar)	72a (Horikoshi and Akiba Agar (HAA)/Broth (HAB))
pH of Medium	10	7	12
Temperature of Growth in 0C	28-30	37	30
Incubation Period	2 Days	1 Day	1 Day
Risk Group	Do not Know	2	--
Oxygen Requirement	Aerobic	Aerobic	Aerobic

**Table 2:** Physical Properties of GGBFS and Alccofine

Physical Requirements	Ph Specific Gravity	Slag Activity Index in %		Fineness (m <sup>2</sup> /kg)
		7 Days	14 Days	
GGBFS	2.9	72.4	382	110.4
Alccofine	2.91	73.3	383	114
Requirements as per IS 16715: 2018	--	60 (Min)	320 (Min)	75 (Min)

**Table 3:** Properties of Na<sub>2</sub>SiO<sub>3</sub> solution

Properties	Specifications
Solids to Liquid Ratio	48:52
Color	Colorless
Density (g/cc)	2.7

**Table 4:** Physical properties of Aggregates

Test Data	Fineness Modulus	Water Absorption (%)	Specific Gravity
Fine Aggregate	3.61	0.4	2.7
Coarse Aggregate	20 mm: 6.4 12.5 mm: 2.82	0.4 1.5	2.8 2.7

**Table 5:** Properties of Superplasticizer

Test Data	pH	Relative Density	Aspect	Chloride ions
Superplasticizer	>6 at 250 C	1.1 to 0.01 at 250C	Dark Brown Liquid	<0.18 %

**Table 6:** Chemical Properties of Fly ash

Chemical Requirements	SiO2	Na2O	MgO	SO3	SiO2+Al2O3 + Fe2O3	Total Chlorides	Loss of Ignition	
Content in %	52.72	0.80	1.94	0.23	87.56	0.004	3.2	
Requirements as per IS 3812:2013								
Part 1	Siliceous Pulverized Fuel Ash in % Calcareous	35.0	1.5	5.0	3.0	70.0	0.05	5.0
	Pulverized Fuel Ash in % Siliceous	25.0	1.5	5.0	3.0	50.0	0.05	5.0
Part 2	Pulverized Fuel Ash in % Calcareous	35.0	1.5	5.0	5.0	70.0	0.05	7.0
	Pulverized Fuel A	25.0	1.5	5.0	5.0	50.0	0.05	7.0

**Table 7:** Chemical Properties of GGBFS and Alccofine

Sl No	Chemical Requirements	Requirements as per IS 16715:2018	Alccofine	GGBFS
1.	Al2O3	-	18.13	17.68

2.	MgO	(Max) 17.0%	7.1	8.3
3.	CaO	-	36.59	38
4.	MnO	(Max) 5.5%	0.42	0.3
5.	SiO <sub>2</sub>	-	32.44	32.65
6.	SO <sub>3</sub>	(Max) 3.0%	0.4	0.1
7.	Loss of Ignition	(Max) 3.0%	0.3	0.2
8.	S (Sulphide Sulphur)	(Max) 2.0%	0.5	0.56
9.	Insoluble Residue	(Max) 3.0%		0.2
10.	Glass Content	(Min) 85%	88	98
11.	Moisture Content	(Max) 1.0%	0.2	0.2
12.	(CaO+MgO+Al <sub>2</sub> O <sub>3</sub> )/SiO <sub>2</sub>	(Min) 1.0%	1.93	1.9
13.	Cl (Chloride)	(Max) 0.1%	0.02	0.007

**Table 8:** Physical Properties of Fly ash

Physical Requirements	Soundness	Lime Reactivity (N/mm <sup>2</sup> )	Fineness (m <sup>2</sup> /kg)	Specific Gravity	Residue on 45 Micron Sieve in %
Results	0.032	0.1	5/1	2.4	1.1
Requirements as per IS 3812:2013					
Part 1	0.8 (Min)	4.5 (Min)	320 (Min)	--	34.9 (Max)
Part 2	0.8 (Max)	--	200 (Min)	--	50 (Max)

### 2.2 Methodology

The bacteria *E. coli*, *Bacillus subtilis*, and *Bacillus pseudofirmus* used in this study were cultured in the laboratory at M S Ramaiah University of Applied Sciences, Bangalore, using Luria Bertani (LB) broth as the growth medium. The LB broth consisted of Tryptone (10 g/L), Yeast Extract (5 g/L), and NaCl (5 g/L). The bacteria were cultured at a temperature of 30°C with continuous shaking for 24 hours. The bacterial cell concentration in each milliliter of the medium was manually determined after 24 hours using the Quadrant Plate technique.

Four different Self-Consolidating Geopolymer Concrete (SCGC) mixes were prepared for this study. One mix served as the control mix and did not contain any bacteria, while the other three mixes included *E. coli*, *Bacillus subtilis*, and *Bacillus pseudofirmus* at a bacterial cell concentration of 3% of the total binder content. The binder content for all SCGC mixes was kept constant at 450 kg/m<sup>3</sup>. The proportions of the binder components—GGBFS, Class F Fly Ash, and Alccofine—were fixed at 65%, 30%, and 5%, respectively, across all mixes.

All SCGC mixes were prepared using a 13M Sodium Hydroxide (NaOH) solution, with the ratio of Sodium Silicate (Na<sub>2</sub>SiO<sub>3</sub>) to NaOH maintained at 1. The quantities of free water and superplasticizer were also held constant at 12% and 1% of the total binder content, respectively. Ambient curing was applied to all SCGC mixes. Mix identification details are provided in Table 9.

**Table 9:** SCGC mix identification

Mix Description	Mix ID			
	M1	M2	M3	M4
GGBFS (%)	65	65	65	65
Fly Ash (%)	30	30	30	30
Alccofine (%)	5	5	5	5
Type of Bacteria	No Bacteria	E-Coli	Bacillus Subtilis	Bacillus Pseudofirmus
Bacteria Cell Concentration (%)	3	3	3	3

**2.3 Mix Design:**

The Self-Consolidating Geopolymer Concrete (SCGC) mixes used in this study were developed based on prior research, as no standardized guidelines for SCGC mix design currently exist. The workability of the SCGC mixes was enhanced by incorporating superplasticizer and free water. The optimal percentages of free water and superplasticizer required to achieve higher workability were determined through slump flow tests and marsh cone tests, respectively. The slump flow test results are provided in Table 10, while Figure 1 illustrates the results of the marsh cone test.

The proportions of binder constituents and the molarity of the Sodium Hydroxide (NaOH) solution used in this study were initially established by performing compressive strength tests on 7-day-old hardened SCGC cubes. Based on the initial test results, the mix design for this study was adapted from the research conducted by Nishanth L and Dr. Nayana N. Patil (2022). Details of the mix design for all SCGC mixes are presented in Table 11.

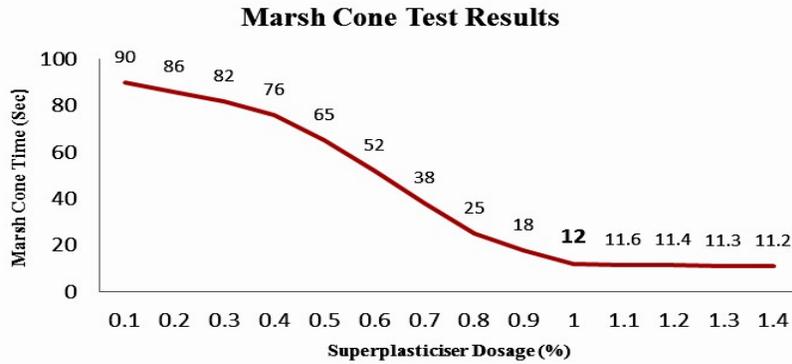


Fig 1: Marsh Cone Test Results

Table 10: Slump Flow Test Results

Mix ID	S0	S1	S2	S3	S4	S5	S6	S7
% of Free Water	0	2	4	6	8	10	12	14
Slump Flow (mm)	39	156	262	393	480	551	675	811

Table 11: Mix Design Details

Mix ID	Molarity	NaOH solid (kg/m <sup>3</sup> )	Sodium silicate (kg/m <sup>3</sup> )	GGBF S (kg/m <sup>3</sup> )	Fly ash (kg/m <sup>3</sup> )	Alccofine (kg/m <sup>3</sup> )	Coarse Aggregate (kg/m <sup>3</sup> )	Fine Aggregate (kg/m <sup>3</sup> )	SP(%)	Extra Water (kg/m <sup>3</sup> )
M1										
M2	13M	135	135	292.5	292.5	292.5	925	925	1	54
M3										
M4										

### 3. Test Results and Discussion

#### 3.1 Test results of fresh concrete

According to EFNARC guidelines, the self-compacting ability of Self-Consolidating Geopolymer Concrete (SCGC) are defined by its filling ability, segregation resistance, and passing ability. SCGC must demonstrate high workability while maintaining its strength characteristics. The workability test results are summarized in Table 12.

Based on the test results, the SCGC mix without bacteria showed the lowest slump flow, which can be attributed to an enhanced polymeric reaction caused by the high molarity of the NaOH solution and a lower water content, leading to accelerated hardening of the concrete. In contrast, SCGC mixes containing bacteria displayed improved filling and passing ability. This improvement

is likely due to the increased water content introduced by the addition of the Luria Bertani (LB) broth medium containing the bacteria. The presence of the LB broth medium delayed the polymeric reaction by reducing the effective molarity of the NaOH solution, thereby extending the concrete’s hardening time. Among all the mixes, SCGC mix M2, which incorporated *E. coli* bacteria, exhibited the best workability characteristics. It is important to note that all SCGC mixes met the EFNARC guidelines for self-compacting concrete.

**Table 12:** Workability test results

TEST RESULTS						
Sl. No	MIX ID	Slump Test (mm)	T-50 Slump (Sec)	L- Box Test (mm)	V- Funnel Test(sec)	J – Ring Test(mm)
1	M1	692	4.8	0.86	9.74	8.1
2	M2	735	1.5	0.83	5.61	4.5
3	M3	706	2.3	0.95	6.25	5.2
4	M4	703	2.6	0.96	7.28	6.1
Acceptable Values AS PER EFNARC						
Minimum Values		650	0	0.80	6	0
Maximum Values		800	6	1.0	12	10

**3.2**Hardened concrete test results

Compression, split-tensile, and flexural strength tests were performed on hardened SCGC mixes after 7, 14, and 28 days of ambient curing, following the procedures outlined in IS 516-1959. The test results are presented in Table 13, with Figures 2, 3, and 4 illustrating the results graphically. The SCGC mix M2, which contained *E. coli* bacteria at a cell concentration of 3% of the total binder content, achieved the highest strength values after 28 days. It recorded a compressive strength of 44.30 MPa, a split-tensile strength of 8.35 MPa, and a flexural strength of 20.5 MPa. The significant strength gain in this mix is attributed to the development of a tetrahedral aluminosilicate structure, which forms due to the creation of geopolymeric gels such as C-S-H, C-A-S-H, and N-A-S-H gels. These gels are generated through an exothermic reaction facilitated by the high calcium oxide (CaO) content in Alccofine and GGBFS.

In comparison, the SCGC mix M1, which did not incorporate bacteria, exhibited the lowest strength values after 28 days of ambient curing. It achieved a compressive strength of 30.72 MPa, a split-tensile strength of 6.18 MPa, and a flexural strength of 13 MPa.

**Table 13:** 7, 14 and 28 days strength tests results

Mix ID	Compression strength (MPa)			Split-tensile strength (MPa)			Flexural Strength (MPa)		
	7 Days	14 days	28 Days	7 days	14 days	28 Days	7 days	14 days	28 Days
M1	25.62	29.98	30.72	2.61	4.75	6.18	6	11	13
M2	34.50	36.32	44.30	3.78	8.09	8.35	13	16	20.5
M3	32.51	35.84	38.47	3.41	7.34	8.16	10	12.5	15
M4	30.72	33.80	37.05	3.27	4.77	7.61	7	12	13.5

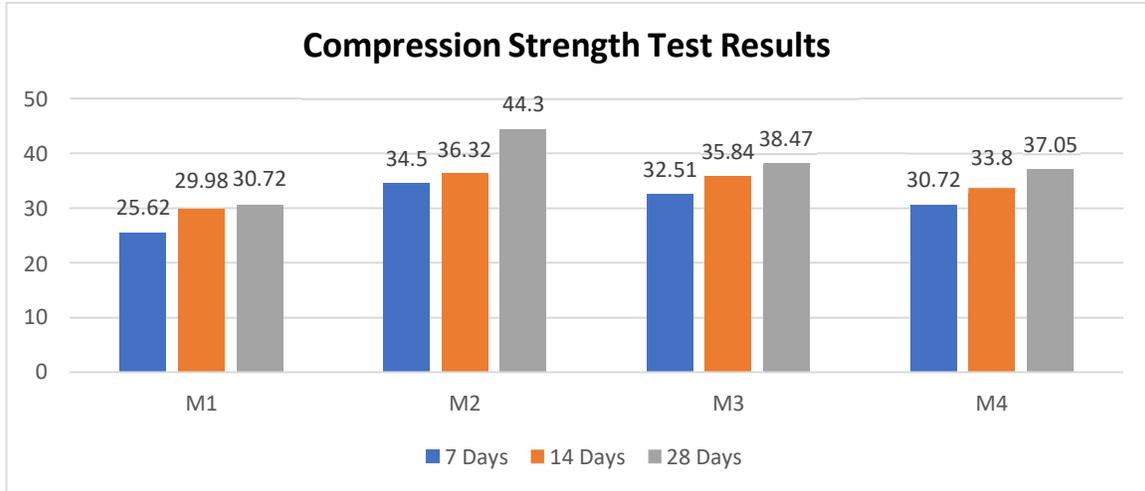


Fig 2: Compression strength test results

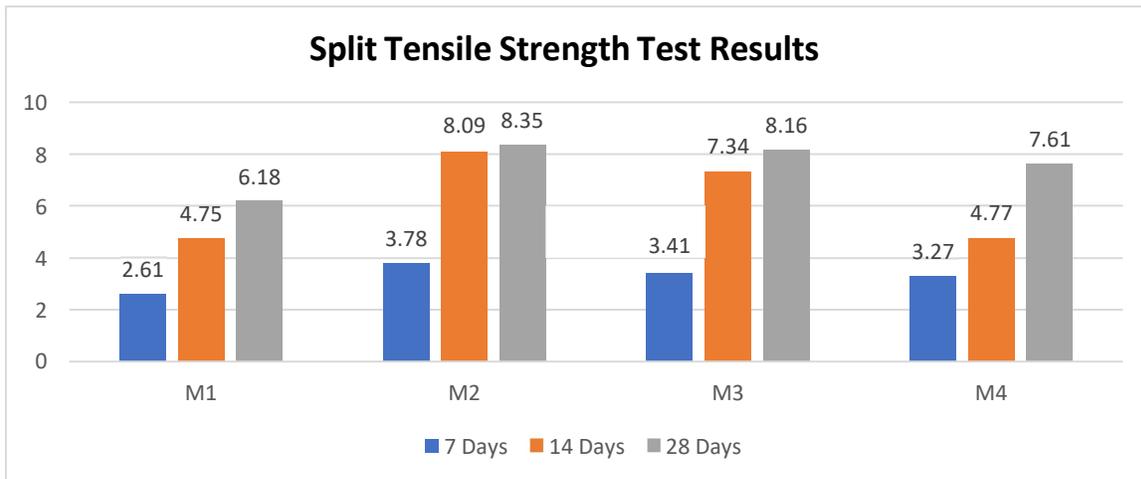


Fig 3: Split-tensile strength test results

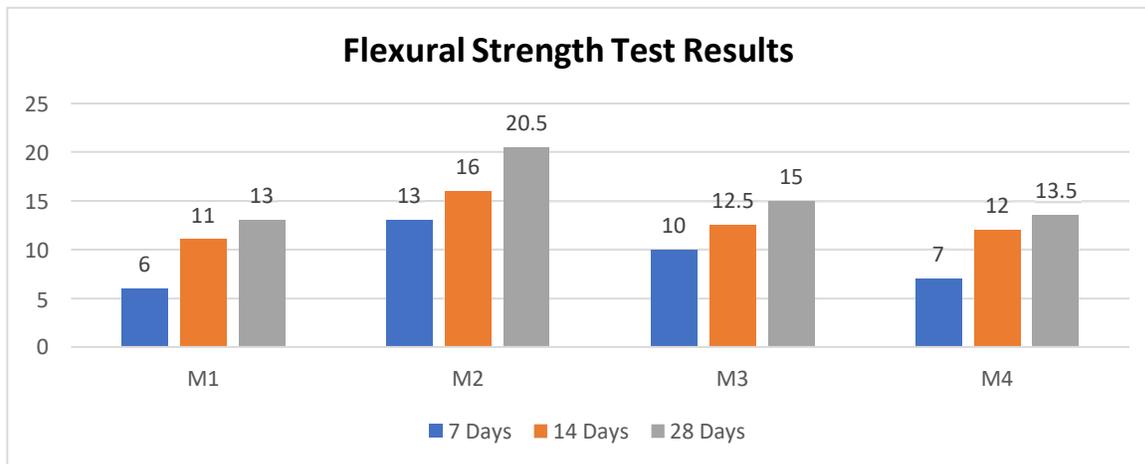


Fig 4: Flexural strength test results

**3.3 durability tests**

**3.3.1 Acid attack test**

The acid attack test was conducted by immersing cylindrical specimens measuring 100 mm in diameter and 150 mm in height into a 3% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) solution for 28 days to evaluate changes in weight and strength. Figure 5 illustrates the weight loss, while Figure 6 shows the strength reduction after 28 days of acid exposure. The SCGC mix M1, which did not contain bacteria, exhibited the highest weight loss of 4.63% and the greatest strength reduction of 5.1% after the 28-day immersion period. The detailed test results are presented in Table 14.

**Table 14: Acid Attack Test Results**

Mix ID	Change in W			Change in Strength		
	Before Immersion	After Immersion	% Loss in Weight	Before Immersion	After Immersion	% Loss in Strength
M1	3.683	3.520	4.63%	3.15	2.92	5.1%
M2	3.708	3.626	2.13%	5.11	4.93	3.1%
M3	3.695	3.568	3.5%	4.73	4.57	4.6%
M4	3.697	3.528	3.2%	4.63	4.48	4.1%

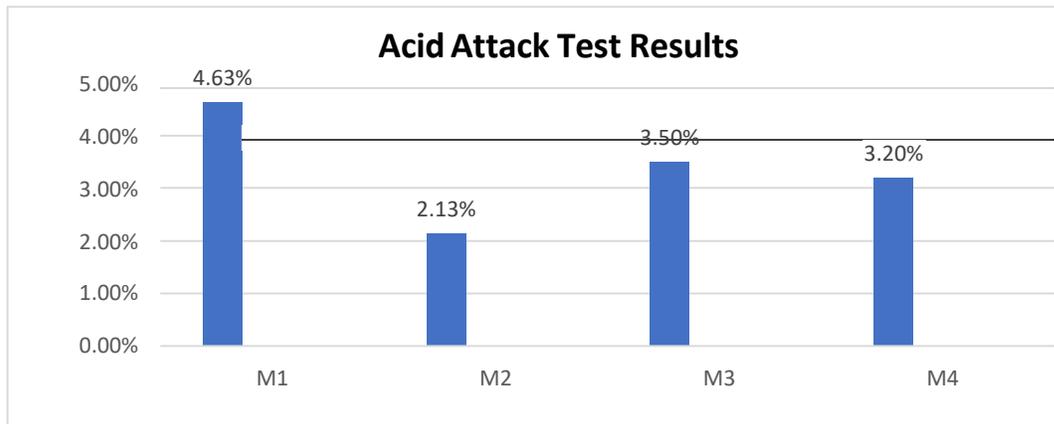


Fig 5: Change in Weight

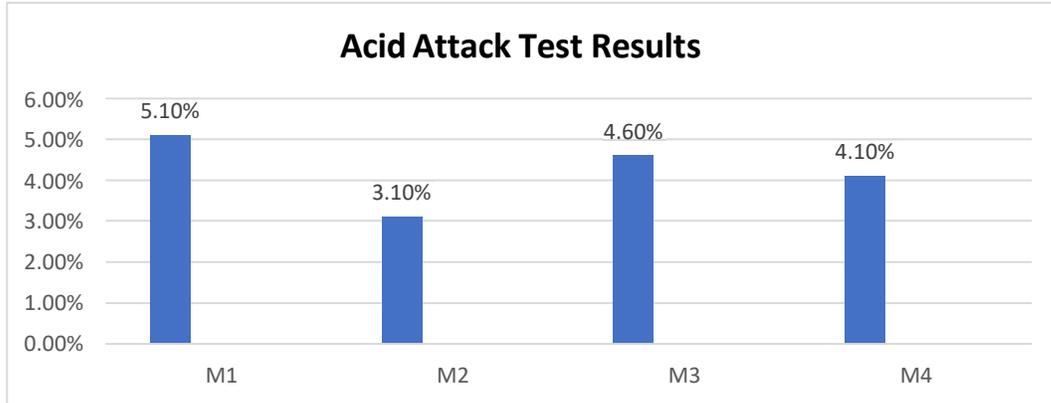


Fig 6: Change in Strength

3.3.2 Alkali Resistance Test

The alkali resistance test was performed by immersing cylindrical specimens with dimensions of 100 mm in diameter and 150 mm in height into a 5% magnesium sulfate (MgSO<sub>4</sub>) solution for 28 days to assess changes in weight and strength. Figure 7 shows the weight loss, while Figure 8 depicts the reduction in strength after 28 days of alkali exposure. The SCGC mix M1, which did not contain bacteria, demonstrated the highest weight loss of 2.6% and the greatest strength reduction of 2.2% after the 28-day immersion period. The detailed results are provided in Table 15.

Table 15: Alkali Attack Test Results

Mix	Change in Weight			Change in Strength		
	Before Immersion	After Immersion	% Loss in Weight	Before Immersion	After Immersion	% Loss in Strength
M1	3.683	3.598	2.6%	3.15	3.05	2.2%
M2	3.708	3.682	0.6%	5.11	5.04	1.6%
M3	3.695	3.622	1.9%	4.78	4.69	1.8%
M4	3.697	3.625	2.0%	4.63	4.59	1.9%

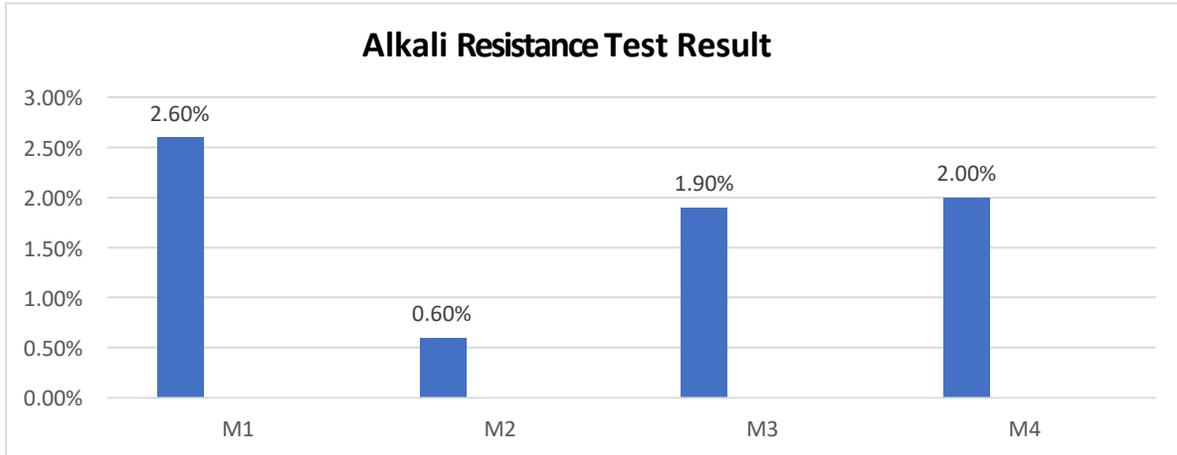


Fig 7: Change in Weight

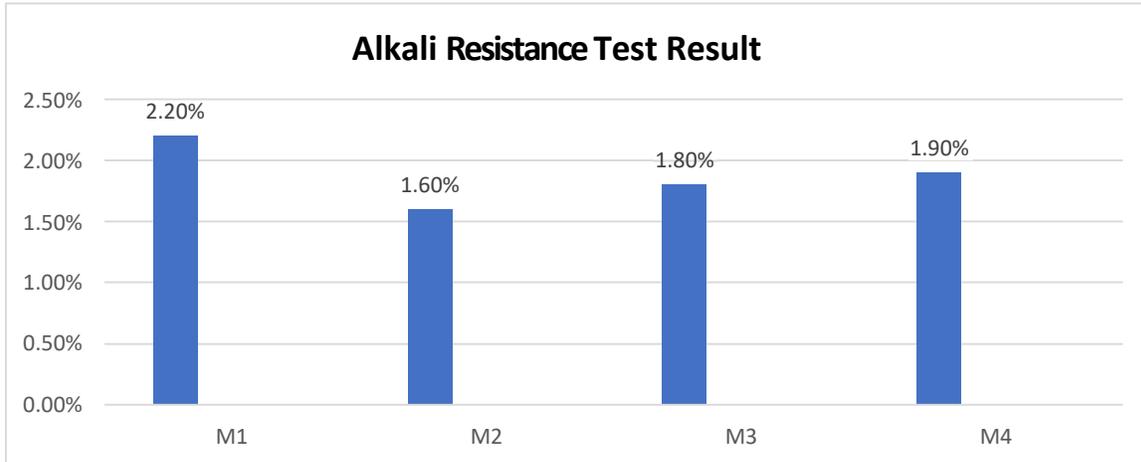


Fig 8: Change in Strength

### 3.3.3 Water absorption test

Water absorption test was conducted by immersing oven dry 100mm dia and 150mm height cylindrical samples in water for 24 hours to determine the change in weight. After 24 hours the specimens are removed from water, patted dried and weighed. Fig 9 represent test result. SCGC mix M1 indicated highest water absorption of 2.8% after immersion in water for 24 hours. This may be due to porous nature of concrete. Table 16 represent test result.

**Table 16: Water Absorption Test Result**

Mix ID	Change in Weight		
	Before Immersion	After Immersion	% Gain in Weight
M1	3.693	3.798	2.8%
M2	3.703	3.769	1.8%

M3	3.693	3.784	2.4%
M4	3.688	3.772	2.3%

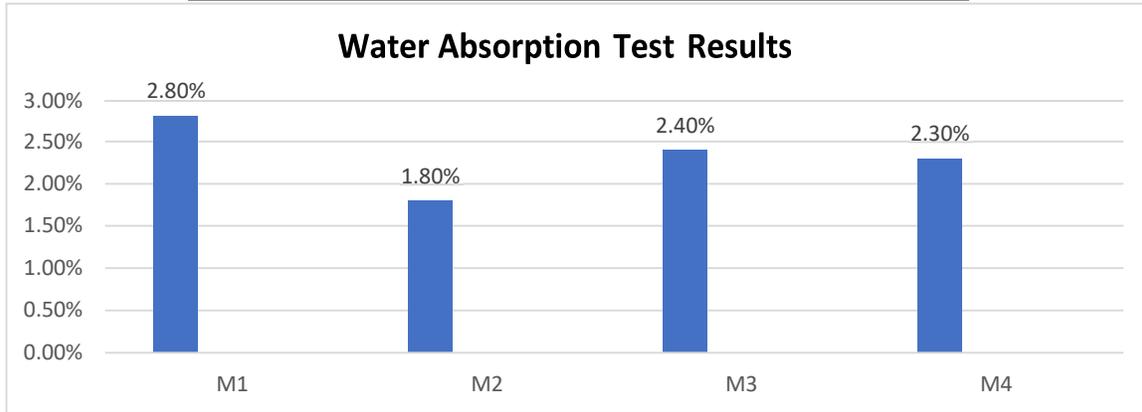


Fig 9: Change in Weight

### 3.3.4: Sorptivity Test

The sorptivity test was conducted to assess the rate of absorption of different SCGC mixes, and the results are presented as the rate of absorption (mm/sec<sup>2</sup>) for the initial and secondary absorption stages. For the initial absorption, the control mix showed a rate of  $4.25 \times 10^{-7}$  mm/sec<sup>2</sup>, while the mixes containing *E. coli*, *Bacillus subtilis*, and *Bacillus pseudofirmus* exhibited slightly lower rates of  $3.57 \times 10^{-7}$  mm/sec<sup>2</sup>,  $3.82 \times 10^{-7}$  mm/sec<sup>2</sup>, and  $4.00 \times 10^{-7}$  mm/sec<sup>2</sup>, respectively. In the secondary absorption phase, the control mix had a rate of  $8.36 \times 10^{-6}$  mm/sec<sup>2</sup>, with the bacterial mixes showing slightly reduced rates: *E. coli* at  $7.37 \times 10^{-6}$  mm/sec<sup>2</sup>, *Bacillus subtilis* at  $7.82 \times 10^{-6}$  mm/sec<sup>2</sup>, and *Bacillus pseudofirmus* at  $8.12 \times 10^{-6}$  mm/sec<sup>2</sup>. These results indicate that the inclusion of bacteria led to a slight decrease in the rate of absorption in both initial and secondary stages compared to the control mix. Table 17 represent test result.

Table 17: Sorptivity Test Results

SL NO	Rate of Absorption(mm/s ec <sup>2</sup> )	M1	M2	M3	M4
1.	Initial Absorption	$4.25 \times 10^{-7}$	$3.57 \times 10^{-7}$	$3.82 \times 10^{-7}$	$4.00 \times 10^{-7}$
2.	Secondary Absorption	$8.36 \times 10^{-6}$	$7.37 \times 10^{-6}$	$7.82 \times 10^{-6}$	$8.12 \times 10^{-6}$

### 3.4: Microstructure Analysis

After 28 days of ambient curing, Scanning Electron Microscope (SEM) and X-ray Diffraction (XRD) analyses were performed on Self-Consolidating Geopolymer Concrete (SCGC) mix M2, as it met both workability and strength criteria, to examine the impact of *E. coli* bacteria on the microstructure of the hardened SCGC. Figure 5 displays the SEM micrograph of SCGC mix M2. The SEM analysis reveals a dense microstructure with a uniform distribution of geopolymeric gels. Minor cracks and pores, which resulted from the evaporation of excess water due to the addition of the LB broth medium, were filled by the initial precipitation of calcite from *E. coli* bacteria. The high SiO<sub>2</sub> content from the partial replacement of fly ash with GGBFS and Alccofine promoted the formation of C-A-S-H gels, which also contributed to the filling of microcracks and pores. This dense microstructure, with a consistent distribution of C-S-H and C-A-S-H gels, enhanced both the workability and strength of SCGC mix M2. The XRD analysis helps identify the chemical composition and crystalline phases that contribute to the development of the microstructure. Figure

6 shows the intensity vs. position (in degrees) curve for SCGC mix M2. The X-ray pattern indicates the typical amorphous structure of geopolymer concrete. Between  $2\theta$  values of 20 to 70, minor peaks can be observed, representing crystalline phases of minerals such as quartz, illite, and mullite, along with C-A-S-H, C-S-H, and N-A-S-H gels. The formation of these geopolymeric gels, combined with quartz, contributes to the strength characteristics of SCGC mix M2.

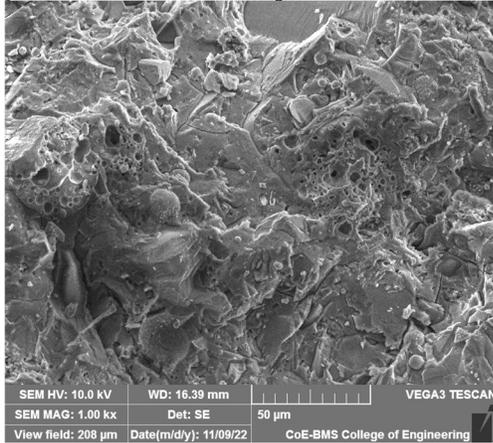


Fig 10: SEM Analysis

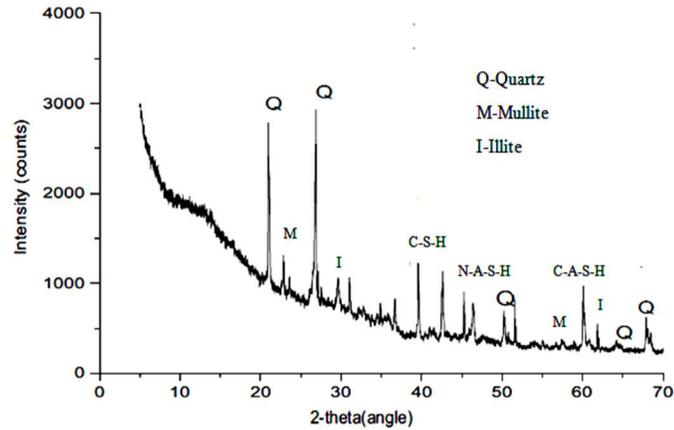


Fig 11: XRD Analysis

## Conclusion

In conclusion, this experimental investigation demonstrated the significant influence of bacteria *E. coli*, *Bacillus subtilis*, and *Bacillus pseudofirmus* on the workability, strength, and durability characteristics of Self-Consolidating Geopolymer Concrete (SCGC). Among the bacterial strains tested, the incorporation of *E. coli* bacteria at a cell concentration of 3% of the total binder content showed remarkable improvements in the performance of SCGC. The addition of *E. coli* enhanced the workability, as indicated by a 6.1% increase in slump flow. Furthermore, it resulted in substantial gains in the mechanical properties of SCGC, with compressive strength improving by 45.83%, split-tensile strength increasing by 35%, and flexural strength rising by 57.69%.

The bacteria also played a crucial role in reducing the pore structure within the concrete matrix, as observed in the durability tests, which in turn contributed to improved durability. This reduction in pores is directly linked to the enhanced strength characteristics of the concrete, making it more resilient to environmental stresses. Given the benefits observed, bacteria-incorporated SCGC, especially with *E. coli*, can be considered a promising and eco-friendly alternative to Ordinary Portland Cement (OPC) concrete. This innovative approach not only improves the properties of the concrete but also aligns with sustainability goals by reducing the need for traditional cement, which is a major contributor to carbon emissions in the construction industry.

## References

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