

# Comparative Analysis of Bacterial Concrete (PPC M40 Grade with *Bacillus subtilis*) and Conventional OPC Concrete

Himanshu Kumar Singh<sup>1</sup>, Anjali Rai<sup>2</sup>

<sup>1</sup>M-Tech Research Scholar, Civil Engineering Department, IET Lucknow, Pin Code- 226014

<sup>2</sup>Assistant Professor, CED, IET Lucknow, Pin code-226021

\*\*\*

**Abstract** - Concrete's lifespan decreases when cracks form, which is a common issue regardless of mix design. Self-healing concrete, utilizing microbiologically induced calcium carbonate precipitation, aims to prevent structural damage caused by cracks. This process allows controlled material passage while maintaining structural integrity. This concrete type can autonomously initiate biological activity and perform self-repair. In our study, we explored the impact of *Bacillus Subtilis* bacteria on PPC M40-grade concrete. We compared Ordinary Portland Cement (OPC) with Bacteria-Stimulated PPC concrete of the same grade. *Bacillus subtilis* bacteria were introduced to the PPC concrete in varying volumes (10, 20, and 30 ml) with concentrations of  $10^8$  cells per ml to find the optimal dosage for maximum strength. We conducted compressive strength, split tensile strength, and flexural strength tests on the concrete samples at 7 days, 14 days, and 28 days. The results indicated that PPC-based bacteria (30 ml) significantly activated the concrete, leading to a remarkable 29.7% increase in compressive strength, a 28.2% rise in flexural strength, and a 12.7% boost in split tensile strength compared to conventional concrete. Scanning Electron Microscopy (SEM) analysis confirmed the presence of nanoparticles i.e. calcite precipitates, contributing to densification and strength enhancement.

**Key Words:** bacterial concrete, *bacillus subtilis*, M40 grade concrete, PPC, SEM.

## 1. INTRODUCTION

The paper delves into the significance of concrete as a widely used construction material but underscores its susceptibility to cracks, especially in tension. Left untreated, these cracks can lead to structural issues and costly repairs. To address this concern, the concept of self-healing concrete is introduced.

Self-healing concrete involves the use of microorganisms, specifically bacteria, to trigger a self-repair mechanism within the concrete. One of the key challenges in this field is finding bacteria that are readily available, harmless to living organisms, and capable of facilitating biochemical reactions for long-lasting repairs. Research has shown that the bacterium "*Bacillus subtilis*" meets these criteria and can be naturally sourced from soil.

The bacteria, in combination with a nutrient broth (food for the bacteria), can be mixed directly into the concrete during the casting process. These bacteria

remain dormant within the concrete until a crack appears. When a crack forms, water and other substances enter, initiating a reaction with the bacteria. The bacteria react with water and precipitate calcite, filling the crack. Simultaneously, they consume oxygen, converting soluble calcium lactate into insoluble limestone. This not only repairs the crack but also densifies the concrete, making it more impermeable. The formation of limestone prevents corrosion and ensures the structural integrity of the concrete.

The experimental investigation presented in the manuscript focuses on comparing M40 grade concrete made with fly ash-based cement, Portland pozzolana cement (PPC), and conventional concrete. PPC is preferred for its environmental benefits, as it uses less cement and reduces carbon emissions. The concentration of bacteria is varied to determine the optimal concentration that results in maximum strength. Scanning Electron Microscopy (SEM) analysis is also conducted to visualize the growth of nanoparticles, specifically calcite precipitates, which contribute to concrete densification and enhanced strength.

## 2. MATERIALS AND TESTING METHOD

### 2.1 Cement

PPC conforming to IS 1489 (part-1) with 32% fly ash in it was used. OPC conforming to IS 269 The physical properties of Pozzolanic Portland cement and Ordinary Portland Cement were determined such as specific gravity to be 2.88 and 3.14 respectively.

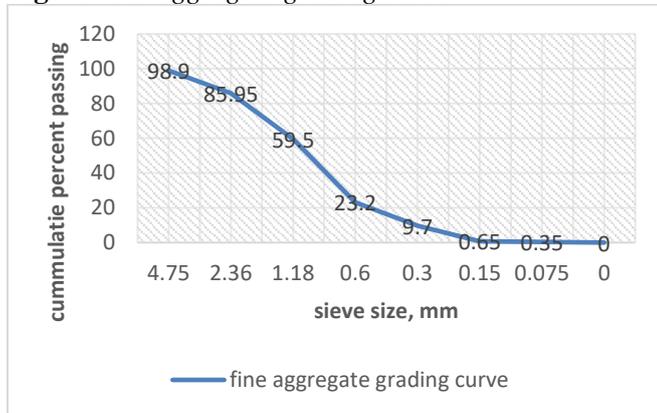
**Table -1:** Physical properties of cement

PROPERTIES	OPC	PPC
Fineness of cement	0.34 m <sup>2</sup> /g	0.24 m <sup>2</sup> /g
Initial setting time	40	38 min
Standard Consistency	31%	32%
Specific Gravity	3.14	2.88
Final setting time	122 min	525 min

### Fine aggregate

For fine aggregate, local river sand was used in this test and its particle size distribution curve is shown in fig:1. Specific gravity of fine aggregate was found to be 2.66 and using the particle size distribution curve it was graded to zone 1.

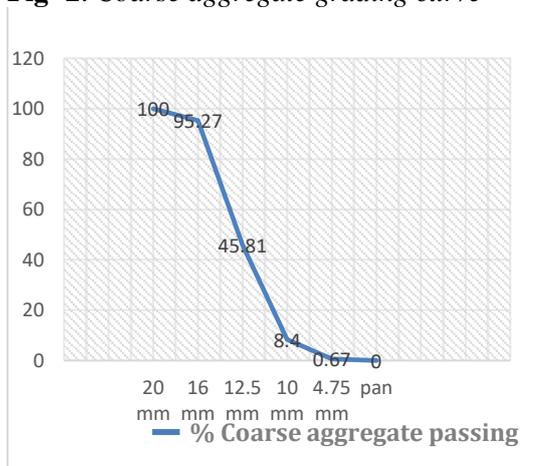
**Fig -1:** Fine aggregate grading curve



### 2.2 Coarse aggregate

For coarse aggregate, crushed angular stone of 20mm size was used. Its specific gravity was found to be 2.73 and water absorption was found to be 0.59 percent.

**Fig -2:** Coarse aggregate grading curve



### 2.3 Water

For this test, local drinking water was used for all types of casting.

### 2.4 Microorganism

The microorganism which was used is *Bacillus Subtilis* which was cultured at the Bio-Tech laboratory of the Institute of Engineering & Technology, Lucknow, India.

### 2.5 Culture of bacteria

The bacteria were initially stored in a dried freeze form within a test tube. To culture them, they were removed from this state and mixed in two 50ml nutrient broth

flasks. These flasks were then placed in a shaker cum incubator for 24 hours at room temperature, with the shaker operating at a speed of 100-120 rpm. The nutrient broth served as the bacteria's food source and was prepared using peptone, NaCl, and yeast extract at concentrations of 5 g/lit, 5 g/lit, and 3 g/lit, respectively. The concentration of bacteria was adjusted to 10<sup>8</sup> ppm as needed for the experiments.

The cell concentration is obtained by the equation given below:

$$Y = 8.59 \times 10^7 \times X^{1.3627}$$

Where,  
X = Reading at OD 600

Y = Concentration of bacterial cells per ml

$$X = OD = 1.13$$

$$Y = 8.59 \times 10^7 \times 1.13^{1.3627}$$

Y = 1.01466 × 10<sup>8</sup> Concentration of bacteria cell per ml

### 2.6 Mixture design

The mix design for M40 grade concrete was conducted following the guidelines of IS 10262:2019. In this research, concrete was mixed and designed for M40 grade, with varying proportions of 10ml, 20ml, and 30ml of a bacterial solution containing *Bacillus Subtilis*. This was done to assess the impact of bacteria on the workability, strength, and durability of the concrete specimens.

Materials that were required for this design per one cube of concrete are described in Table 2.

**Table 2:** Mix design proportion

Mix notation	NC	BC10	BC20	BC30
Cement (kg/m <sup>3</sup> )	392	392	392	392
Fine aggregate (kg/m <sup>3</sup> )	699	699	699	699
Coarse aggregate (kg/m <sup>3</sup> )	1199	1199	1199	1199
Admixture (kg/m <sup>3</sup> )	3.92	3.92	3.92	3.92
Bacteria concentration (ppm)	-	10 <sup>8</sup>	10 <sup>8</sup>	10 <sup>8</sup>
Volume of bacteria (ml)	-	10	20	30

W/C ratio	0.38	0.38	0.38	0.38
-----------	------	------	------	------

NC: Normal Concrete, BC: Bacterial Concrete.

### 2.7 Compressive strength test

A compression test was conducted on both bacterial and conventional concrete specimens, each measuring 15×15×15 cm, following the specifications outlined in IS 516-1959. The testing procedure was carried out using a UTM (Universal Testing Machine). The concrete specimens were cast and allowed to cure for 7, 14, and 28 days in accordance with the standards defined in IS 456-2000. The test results are provided in the table. The compressive strength of the cubes was calculated using the formula provided below:

$$\text{Compressive Strength} = \frac{\text{load}}{\text{area}} \text{ N/mm}^2$$

### 2.8 Flexural strength test

For the flexure test, a specimen of size 50 × 10 × 10 cm was cast for 7, 14 and 28 days and tested as per IS 516-1959.

### 2.9 Split tensile test

The tensile test of the cylinder is also performed by the Universal Testing Machine (UTM). By this test, we can determine the ultimate tensile strength, breaking strength, maximum elongation and reduction in area. The split tensile strength with and without bacteria is performed at 7 days and 28 days. The tensile strength of the cylinder is calculated as per the formula given below:

$$\text{Tensile Strength. } f_t = \frac{2P}{\pi DL}$$

P = Compressive load at failure.

L = 0.3m, Length of cylinder.

D = 0.15m, Diameter of cylinder.

### 2.10 SEM test

A Scanning Electron Microscope (SEM) operates by scanning a focused electron beam across a surface to generate an image. The electrons in the beam interact with the sample, producing various signals that are used to gather information about the surface's topography and composition. In this test, a beam of electrons is generated, typically through a tungsten filament or a field emission gun, and then accelerated using a high voltage of approximately 20,000 V. The electrons pass through a series of apertures and electromagnetic lenses, resulting in a thin electron beam directed onto the

specimen's surface. Recoiled electrons are collected by a detector suitably positioned for this purpose. These signals are then used to determine surface topography, composition, and other properties.

In this research, we will collect data to identify the presence of calcium deposits and surface density. This information will allow us to confirm the deposition of calcite and analyse the efficiency of self-healing concrete.

## 3. Discussion of the test result.

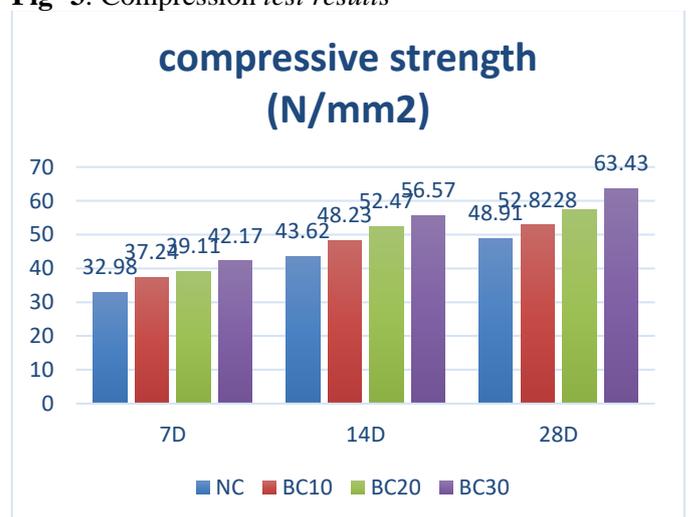
### 3.1 Compressive strength test

At 28 days, the bacterial concrete mixes maintain their superior performance in terms of compressive strength when compared to normal concrete. The strength gain over time is noticeable, and the highest concentration of the bacterial agent (BC30) results in the highest compressive strength with an increase of 29.7% at 28 days as compared to normal concrete. Overall, the results suggest that the addition of a bacterial agent has a positive impact on the compressive strength of concrete at various curing ages. As the concentration of the bacterial agent increases, so does the improvement in strength. This information is valuable for assessing the effectiveness of bacterial concrete additives in enhancing concrete performance.

**Table -3:** compression test results

Mix notation	f <sub>c</sub> , MPa 7d	14d	28d	% increase in strength at 28d
NC	32.98	43.62	48.91	-
BC10	37.24	48.23	52.82	7.99
BC20	39.11	52.47	57.22	16.99
BC30	42.17	56.57	63.43	29.7

**Fig -3:** Compression test results



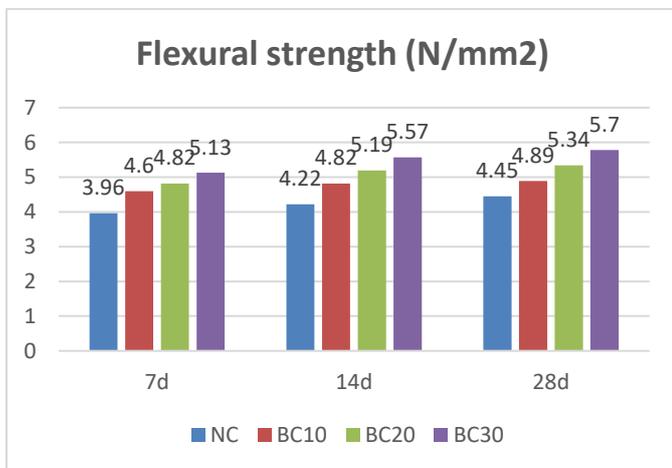
### 3.2 Flexural strength test

The results presented in Table 4 demonstrate that the inclusion of a bacterial agent consistently enhances the flexural strength of concrete across various curing periods. Notably, the concrete mix with the highest bacterial agent concentration (BC30) exhibits the most substantial percentage increase in flexural strength, recording a notable improvement of 28.2%. These findings strongly support the notion that bacterial concrete additives play a beneficial role in improving flexural strength.

**Table -4:** flexural test results

Mix notation	$f_c$ , MPa 7d	14d	28d	% increase in strength at 28d
NC	3.96	4.22	4.45	-
BC10	4.60	4.82	4.89	9.88
BC20	4.82	5.19	5.34	20
BC30	5.13	5.57	5.70	28.2

**Fig-4:** Flexure strength test results



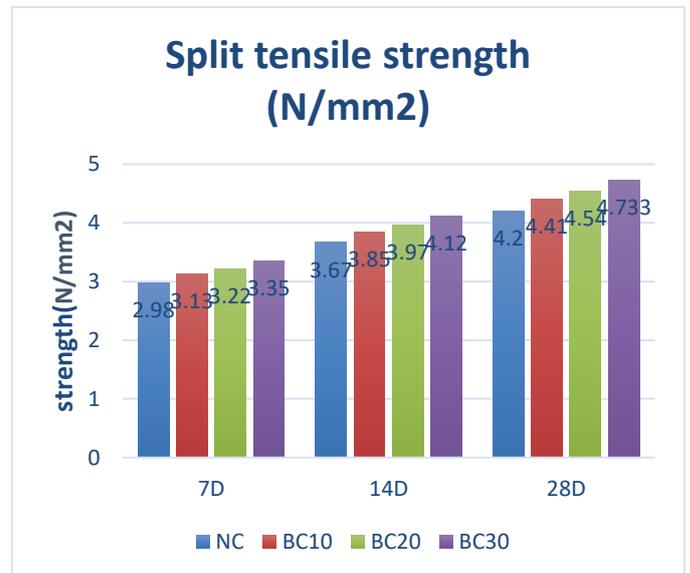
### 3.3 Split tensile strength test

The concrete mix with a 30% concentration of the bacterial agent (BC30) consistently exhibited a significant and sustained improvement of 12.7% in split tensile strength compared to the normal concrete (NC) at all tested curing ages. This outcome underscores the effectiveness of the bacterial concrete additive in enhancing split tensile strength, making BC30 a promising choice for applications where improved tensile performance is critical.

**Table -5:** slit tensile test results

Mix notation	$f_c$ , MPa 7d	14d	28d	% increase in strength at 28d
NC	2.98	3.67	4.20	-
BC10	3.13	3.85	4.41	5
BC20	3.22	3.97	4.54	8.09
BC30	3.35	4.12	4.733	12.7

**Fig -5:** Split tensile strength test results



### 3.4 Scanning electronic microscopy (SEM)

The detection of calcite deposition within micro-cracks in concrete, attributed to bacterial activity, was carried out using scanning electron microscopy (SEM). Analysis of the graphical data confirms the presence of calcite precipitation in concrete specimens that incorporate bacteria. Clearly visible calcite layers were observed within the pores of each bacterial concrete sample, contributing to increased concrete strength. Precipitated calcite was identified within the concrete pores, enhancing structural robustness. A comparative examination of standard and bacterial concrete specimens after a 28-day curing period reveals that concrete containing bacteria exhibits greater compaction and density. This study also underscores the role of calcite formation in enhancing concrete strength, emphasizing the superior mechanical performance of bacterial-infused concrete.

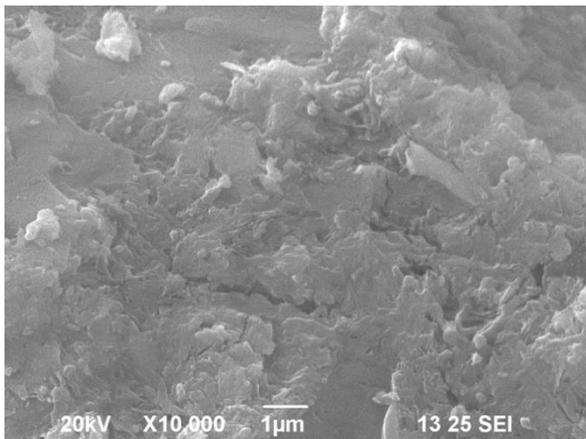


Fig -6.4 (a): SEM image

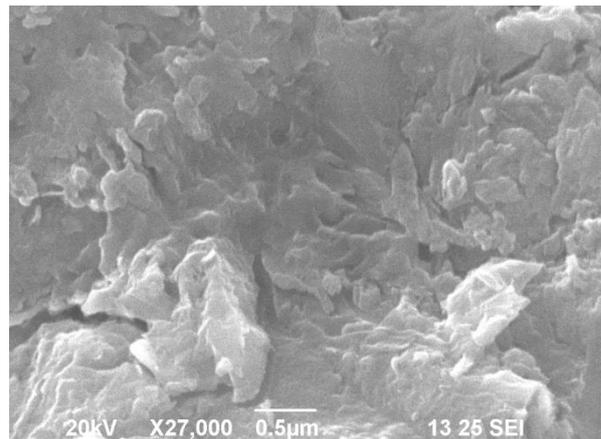


Fig -6.4 (d): SEM image

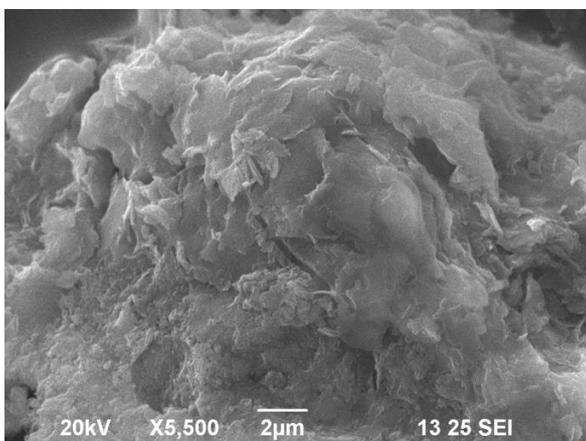


Fig -6.4 (b): SEM image

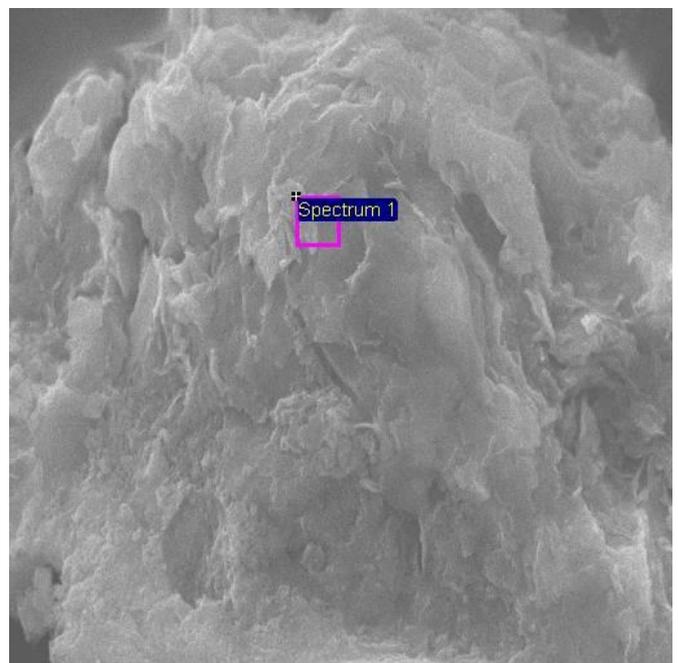


Fig -7: EDS image

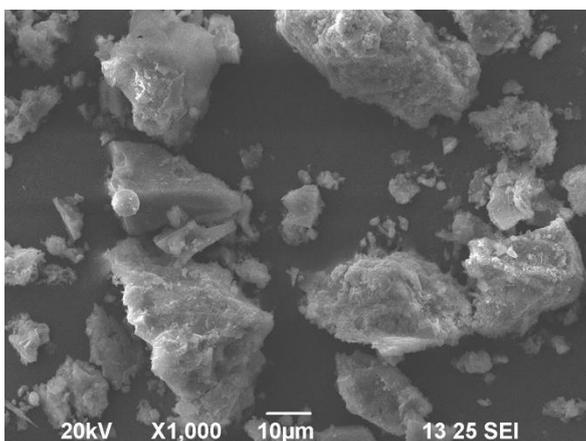


Fig -6.4 (c): SEM image

Table -6: Concrete composition

ELEMENT	WEIGHT%	ATOMIC%
O K	51.67	74.23
Mg K	0.53	0.50
Al K	3.04	2.59
Si K	6.17	5.05
K K	0.80	0.47
Ca K	24.41	14.0
Mn K	0.51	0.21
Fe K	2.4	0.99
Sb L	7.79	1.47
I L	2.68	0.49
Total	100.00	

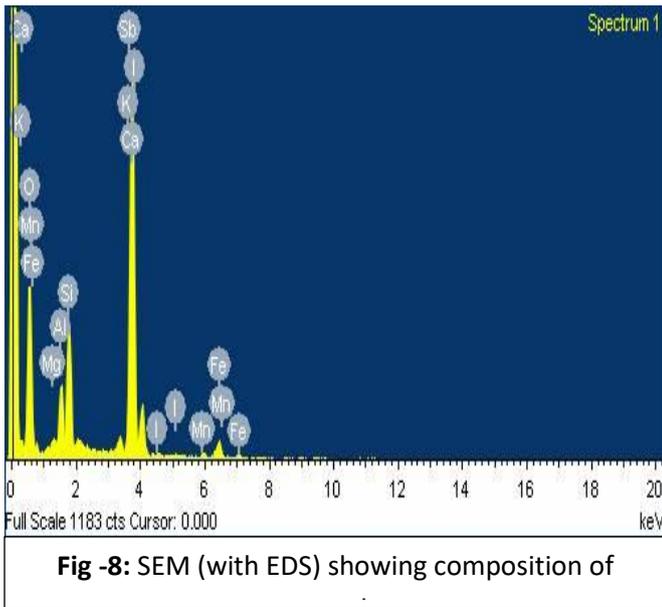


Fig -8: SEM (with EDS) showing composition of

#### 4 CONCLUSION

The passage provides a summary of the key findings from the experimental investigation of bacterial concrete in comparison to conventional Portland Pozzolana Cement (PPC) concrete. It highlights several important outcomes:

1. **Strength Enhancement:** Bacterial concrete demonstrates significant improvements in its mechanical properties. It exhibits a 29.7% increase in compressive strength, a 28.2% increase in flexural strength, and a 12.7% increase in split tensile strength compared to conventional PPC concrete. These improvements indicate that the addition of *Bacillus subtilis* bacteria has a positive impact on the strength characteristics of concrete.
2. **Environmental Benefits:** The use of PPC cement is emphasized due to its environmental advantages. PPC cement is known for being cost-effective, reducing carbon emissions, and saving energy during the cement production process. A key feature is that PPC cement typically replaces 30% of cement with fly ash, which is an eco-friendly practice. When combined with bacterial concrete, these benefits are further enhanced.
3. **Increased Impermeability:** The Scanning Electron Microscopy (SEM) test results reveal a higher concentration of calcium deposits, signifying the presence of calcite. Calcite plays a crucial role in the concrete by filling voids and densifying the material. Additionally, it enhances the concrete's impermeability, making it less susceptible to the ingress of water and potentially harmful substances.

In summary, the findings suggest that the combination of PPC cement and *Bacillus subtilis* bacteria in concrete offers a sustainable and durable solution. This approach not only improves the concrete's mechanical properties but also contributes to environmental sustainability by reducing maintenance costs and enhancing impermeability.

#### 5 REFERENCE

1. C. Sonali Sri Durga, N. Ruben, M. Sri Rama Chand et al., Performance studies on the rate of self-healing in bio concrete, *Materials Today: Proceedings*, <https://doi.org/10.1016/j.matpr.2019.09.151>
2. C. Venkata Siva Rama Prasad and T. V. S. Vara Lakshmi et al., Experimental investigation on bacterial concrete strength with *Bacillus subtilis* and crushed stone dust aggregate based on ultrasonic pulse velocity, *Materials Today: Proceedings*, <https://doi.org/10.1016/j.matpr.2020.01.478>
3. Olatokunbo M. Ofuyatan et al., Development of high-performance self-compacting concrete using eggshell powder and blast furnace slag as partial cement replacement, *Construction and Building Materials* 256 (2020) 119403 *Materials Today: Proceedings*, <https://doi.org/10.1016/j.conbuildmat.2020.119403>
4. Rafat Siddique, Karambir Singh et al., Properties of bacterial rice husk ash concrete, *Construction and Building Materials* 121 (2016) 112–119, *Materials Today: Proceedings*, <http://dx.doi.org/10.1016/j.conbuildmat.2016.05.146>
5. Rafat Siddique, Abir Jameel et al., Effect of bacteria on strength, permeation characteristics and micro-structure of silica fume concrete, *Construction and Building Materials* 142 (2017) 92–100, *Materials Today: Proceedings*, <http://dx.doi.org/10.1016/j.conbuildmat.2017.03.057>
6. Jun Feng, Bingcheng Chen, Weiwei Sun, Yang Wang et al., Microbial induced calcium carbonate precipitation study using *Bacillus subtilis* with application to self-healing concrete preparation and characterization, *Construction and Building Materials* 280 (2021) 122460, *Materials Today: Proceedings*, <https://doi.org/10.1016/j.conbuildmat.2021.122460>
7. S. Jena, B. Basa, K. C. Panda et al., Impact of *Bacillus subtilis* bacterium on the properties of concrete, *Materials Today: Proceedings*, <https://doi.org/10.1016/j.matpr.2020.03.129>
8. Kunamineni Vijay et al., Bacteria based self-healing concrete – A review, *Construction and Building Materials* 152 (2017) 1008–1014, *Materials Today: Proceedings*, <http://dx.doi.org/10.1016/j.conbuildmat.2017.07.040>
9. S. Krishnapriya et al., Isolation and identification of bacteria to improve the strength of concrete, *Microbiological Research* 174 (2015) 48–55 *Materials Today: Proceedings*, <http://dx.doi.org/10.1016/j.micres.2015.03.009>
10. J. Wang, K. Van Tittelboom, N. De Belie, W. Verstraete, Use of silica gel or polyurethane immobilized bacteria for self-healing concrete, *Constr. Build. Mater.* 26 (1) (2012) 532–540, <https://doi.org/10.1016/j.conbuildmat.2011.06.054>.

11. V.C. Li, E. Herbert, Robust self-healing concrete for sustainable infrastructure, *J. Adv. Concr. Technol.* 10 (6) (2012) 207–218, <https://doi.org/10.3151/jact.10.207>.
12. H.M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, E. Schlangen, Application of bacteria as self-healing agent for the development of sustainable concrete, *Ecol. Eng.* 36 (2) (2010) 230–235.
13. S. Reddy, K.A. Satya, M.V. Rao, M. Azmatunnisa, A biological approach to enhance strength and durability in concrete structures, *Int. J. Adv. Eng. Technol.* (2012), ISSN 2231-1963.
14. S. Zwaag (Ed.), *Self-healing materials: an alternative approach to 20 centuries of materials science*, Vol. 30, Springer Science + Business Media BV, 2008, doi: 10.1007/978-1-4020-6250-6.
15. H.M. Jonkers, E. Schlangen, Crack repair by concrete-immobilized bacteria, in *Proceedings of the first international conference on self-healing materials*, April 2007, pp. 18–20.
16. C. Joseph, A.D. Jefferson, M.B. Cantoni, Issues relating to the autonomic healing of cementitious materials, in: *First International Conference on Self-Healing Materials*, April 2007.
17. M.R. Kessler, N.R. Sottos, S.R. White, Self-healing structural composite materials, *Compos. An Appl. Sci. Manuf.* 34 (8) (2003) 743–753, [https://doi.org/10.1016/S1359-835X\(03\)00138-6](https://doi.org/10.1016/S1359-835X(03)00138-6).
18. K.R. Santhosh, V. Ramakrishnan, E.F. Duke, S.S. Bang, SEM Investigation of Microbial Calcite Precipitation in Cement, in: *Proceedings of the International Conference on Cement Microscopy*, International cement microscopy association, vol. 22, 2000, pp. 293–305.
19. V. Ramakrishnan, K.P. Ramesh, S.S. Bang, Bacterial concrete, in: *Smart Materials*, Vol. 4234, International Society for Optics and Photonics, April 2001, pp. 168–177.
20. H.M. Jonkers, Bacteria-based self-healing concrete, *Heron* 56 (1/2) (2011). [12] H.E.J.G. Schlangen, H.M. Jonkers, S. Qian, A. Garcia, Recent advances on self-healing of concrete, in: *FraMCoS-7: Proceedings of the 7th International Conference on Fracture Mechanics of Concrete and Concrete Structures*, 23–28 May 2010, Jeju Island, Korea, 2010.
21. IS:4031, *Methods of Physical Test for Hydraulic Cement – Determination of Consistency of Standard Cement Paste*, Bureau of Indian Standard, New Delhi, 1980.
22. IS: 516, *Methods of Tests for Strength of Concrete*, Bureau of Indian Standard, New Delhi, 1959.
23. J. Feng, W. Sun, L. Wang, L. Chen, S. Xue, W. Li, Terminal ballistic and static impactive loading on thick concrete target, *Construction and Building Materials* 251 (2020) 118899.
24. Y. Su, J. Feng, Q. Zhan, Y. Zhang, C. Qian, Non-ureolytic microbial self-repairing concrete for low-temperature environment, *Smart Material Structures* 28 (2019) 12 pp
25. D. Yoo, J. Kim, B. Chun, Dynamic pull out behaviour of half-hooked and twisted steel fibres in ultra-high-performance concrete containing expansive agents, *Composites Part B Engineering* 167 (2019) 517–532.
26. R. Ma, L. Guo, S. Ye, W. Sun, J. Liu, Influence of hybrid fibre reinforcement on mechanical properties and autogenous shrinkage of an ecological uhpfrcc, *Journal of Materials in Civil Engineering* 31 (5) (2019) 04019032.
27. D. Zhang, J. Yu, H. Wu, B. Jaworska, B.R. Ellis, V.C. Li, Discontinuous micro- fibres as intrinsic reinforcement for ductile engineered cementitious composites (ECC), *Composites Part B: Engineering* 184 (2020) 107741.
28. J. Feng, X. Gao, J. Li, H. Dong, W. Yao, X. Wang, W. Sun, Influence of fibre mixture on impact response of ultra-high-performance hybrid fibre reinforced cementitious composite, *Composites Part B: Engineering* 163 (2019) 487–496.
29. A. Al-Tabbaa, C. Litina, P. Giannaros, A. Kanellopoulos, L. Souza, First UK field application and performance of microcapsule-based self-healing concrete, *Construction and Building Materials* 208 (30) (2019) 669–685.
30. S. Abdallah, M. Fan, D.W.A. Rees, Predicting pull-out behaviour of 4d/5d hooked end fibres embedded in normal-high strength concrete, *Engineering Structures* 172 (2018) 967–980.
31. X. Wang, Y. Huang, Y. Huang, J. Zhang, C. Fang, K. Yu, Q. Chen, T. Li, R. Han, Z.a. Yang, Laboratory and field study on the performance of microcapsule-based self-healing concrete in tunnel engineering, *Construction and Building Materials* 220 (30) (2019) 90–101.
32. S. Mondal, A.D. Ghosh, Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete, *Construction and Building Materials* 183 (20) (2018) 202–214.
33. C. Manvith Kumar Reddy, B. Ramesh, Macrin D. et al., Influence of bacteria *Bacillus subtilis* and its effects on flexural strength of concrete, *Materials Today: Proceedings*, <https://doi.org/10.1016/j.matpr.2020.07.225>
34. N Iswaryaa et al., Experimental investigation on strength and durability of lightweight bacterial concrete, *Materials Today: Proceedings* 22 (2020) 2808–2813.
35. P. Kumar Jogi and T.V.S. Vara Lakshmi, Self-healing concrete based on different bacteria: A review, *Materials Today: Proceedings*, <https://doi.org/10.1016/j.matpr.2020.08.765>.