

Research Article

Performance Evaluation of Bio Concrete by Cluster and Regression Analysis for Environment Protection

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The focus of this research is to isolating and identifying bacteria that produce calcite precipitate, as well as determining whether or not these bacteria are suitable for incorporation into concrete in order to enhance the material's strength and make the environment protection better. In order to survive the high "potential of hydrogen" of concrete, microbes that are going to be added to concrete need to be able to withstand alkali, and they also need to be able to develop endospores so that they can survive the mechanical forces that are going to be put on the concrete while it is being mixed. In order to precipitate CaCO_3 in the form of calcite, they need to have a strong urease activity. Both *Bacillus sphaericus* and the *Streptococcus aureus* bacterial strains were evaluated for their ability to precipitate calcium carbonate (CaCO_3). These strains were obtained from the Department of Biotechnology at GLA University in Mathura. This research aims to solve the issue of augmenting the tension and compression strengths of concrete by investigating possible solutions for environmentally friendly concrete. The sterile cultures of the microorganisms were mixed with water, which was one of the components of the concrete mixture, along with the nutrients in the appropriate proportions. After that, the blocks were molded, and then pond-cured for 7, 28, 56, 90, 120, 180, 270, and 365 days, respectively, before being evaluated for compressibility and tensile strength. An investigation into the effect that bacteria have on compression strength was carried out, and the outcomes of the tests showed that bacterial concrete specimens exhibited an increase in mechanical strength. When compared to regular concrete, the results showed a maximum increase of 16 percent in compressive strength and a maximum increase of 12 percent in split tensile strength. This study also found that both bacterial concrete containing 106, 107, and 108 cfu/ml concentrations made from *Bacillus sphaericus* and *Streptococcus aureus* bacteria gave better results than normal concrete. Both cluster analysis (CA) and regression analysis (RA) were utilized in this research project in order to measure and analyze mechanical strength.

1. Introduction

Concrete is thought of as a homogeneous substance since it is created by combining cement, coarse and fine aggregate, and water in a certain ratio. Concrete is a porous material and is sensitive to various assaults such as chloride, CO_2 , sulfate, freeze and thaw cycles, and others because it is made up of voids that are referred to as pores [1, 2]. Since these

pores are typically associated with one other, concrete is a porous substance. Concrete has a design life of fifty years, but owing to these assaults, it deteriorates considerably more quickly than expected. The infrastructure is built of concrete [3–5]. Cementitious concrete is the most often used building material and is also one of the most essential substances used in construction business. Its annual production is around 10 km^3 /per year, and it is one of the very important materials

in the construction industry. Cement is the sole component that is made, whereas the rest are naturally occurring and sourced from the area [6]. The manufacturing of 1 ton of cement results in the release of around 1 ton of carbon dioxide, and the building industry is responsible for approximately fifty percent of the world's total CO₂ emissions. Because of its adaptability, concrete is employed in the construction of a wide variety of structures, including bridges, large buildings, off-shore constructions, airports, sidewalks, railroad beds, and deep foundations, despite the fact that it is fragile and has a low resistance to stress [7–9]. A great number of concrete buildings are plagued by early deterioration issues such as carbonation and chloride attack, both of which ultimately result in the buildings needing to be repaired and retrofitted. Over the last several years, research into microbial (CaCO₃) calcium carbonate has been more popular in the field of building engineering. It is seen as a potentially fruitful novel method for extending the useful life of cement-based buildings [10–12]. The CaCO₃ calcium carbonate precipitation that results from metabolic activity of various microbe species, such as sulfate-reducing microbes, ureolytic microbes, nitrate reducing microbes, and oxidation of organic microbes, is what allows this method to self-heal the gaps in the concrete and the inevitable microcracks that will form in the concrete. For instance, ureolytic microbes are responsible for the production of an enzyme known as urease, which decomposes urea into carbonate ions [13, 14]. According to the findings of our earlier studies, the process of carbonate precipitation by microbes was carried out by utilizing ureolytic microbes. Since they produce an enzyme called urease, these microbes are able to affect the precipitation of CaCO₃ in a given environment. This enzyme catalyzes the hydrolysis of urea into carbon dioxide and ammonia, which ultimately results in a rise in both the pH and the amount of carbonate in the surrounding/environment in which the microbes are found. The incorporation of microbial species into concrete has increased its strength and endurance, providing additional benefits in the form of environmentally friendly and cost-effective alternatives. Since bacteria thrive at alkaline pH, the concrete's resistance to alkali assault, chemical attacks, freeze-thaw strike, and drying shrinkage is significantly improved [15–17]. In a similar vein, the compression strength of Bio-Concrete was the subject of a great deal of research in order to evaluate the efficacy of bacteria-based self-healing in the existing body of scholarly work. This is due to the fact that the strength of concrete is regarded as an essential metric that represents the uniformity of a concrete mix as well as the components of the material being used [18–20]. The compressive strength test has a direct bearing on the general performance of the concrete as well as its attributes. As a result, the compression strength technique has been widely utilized to analyze the process of microbes-based self-healing in cementitious concrete, combining bacteria and associated chemical compounds in the research that has been published [21, 22]. An examination of the relevant literature reveals that virtually all bacteria are capable of producing calcium carbonate (CaCO₃) as a precipitate; however, for the selected bacteria to provide the

greatest advantages, they must be alkaliphilic and thermophilic. *Bacillus sphaericus*, which is an alkaliphile and precipitates (CaCO₃) calcium carbonate with a higher density, and *Streptococcus aureus*, which is also an alkaliphile and increases the compressive strength of concrete, are both beneficial microorganisms [23, 24]. For instance, the research group “Chattopadhyay et al.” found that the compression strength of bacterial mortar increased with time, specifically at seven, twenty-eight, and fifty-six days, in comparison to the normal mix. The precipitation of CaCO₃, which filled the gaps and improved the texture of the concrete as a result, was thought to be responsible for the increase in compression strength that was observed. Additionally, “Ryu et al.” came to the conclusion that the highest increase in compression strength was observed at a microbial cell concentration of 30 × 10⁵ cfu/mL, and also that the compressive strength declined once this value was exceeded [25]. In a similar vein, the “Manikandan et al.” study indicated that a rise in compressive strength occurred even at a high concentration level of 10⁸ cells/mL. In the same vein, the greatest notable improvement in compression strength was seen when there were 10⁶ cells per milliliter of solution [26]. Predictions of compressive strength are extremely valuable because of the significant savings in both money and time that they provide. Because of this fact, researchers have been motivated to construct a mathematical model that correctly forecasts the strength of various forms of concrete. Despite this, there still is not a prediction formula or code requirements for estimating the strength properties of microorganism concrete [27, 28].

In order to find a solution to the problem of increasing the tensile and compression strengths of concrete, the purpose of this study is to investigate potential options. Microorganisms grown in sterilized cultures were combined with water, which was one of the components of the concrete mixture, along with the nutrients in the right amounts. After that, the blocks were cast, and then they were pond-cured for 7, 28, 56, 90, 120, 180, 270, and 365 days, respectively, before being assessed for compressibility and tensile strength. In order to assess and investigate the nature of mechanical strength, this research study made use of both cluster analysis (CA) and regression analysis (RA). After a thorough study of old papers, the researcher found that not much research has been done with *Bacillus sphaericus* and *Streptococcus aureus* bacteria and also found that till date no researcher has done a thorough study of the results through cluster analysis for *Bacillus sphaericus* and *Streptococcus aureus* bacterial concrete. The researcher used three different concentrations, namely 10⁶, 10⁷, and 10⁸ cfu/ml, of *Bacillus sphaericus* and *Streptococcus aureus* bacteria to determine the relationship between bacterial concrete and normal concrete.

This paper shows the introduction of the study in Section 1 and the materials used for the research in Section 2, bacteria (*Bacillus sphaericus* and *Streptococcus aureus*) used in Section 2.1, and research methodology in Section 3.1; Section 3.2 shows bacterial culture techniques, Section 3.3 shows concrete mix design, Section 4 shows results and discussion, and Section 5 shows conclusions.

2. Materials Used

OPC (ordinary Portland cement) of the grade 43 was utilized in this experimental endeavor. In accordance with IS 4031-1996 [29], this OPC was evaluated for both its physical qualities and its optical properties. The specific gravity, Blaine's fineness [30], soundness, and compressive strength of Portland cement were all measured and analyzed to identify their respective qualities [31, 32]. In this particular project, the fine aggregates consisted of river and crushed stone sand, both of which were sourced locally and were readily accessible. Sand from rivers typically has a specific gravity of about 2.68. As a coarse aggregate for this project, crushed granite-shattered stone with a nominal size of 20 millimeters is being employed [33–35]. In the experimental study, safe and potable water that was readily available in the local area was utilized for all combinations. Table 1 presents the variables studied and the procedure of bacterial concrete.

2.1. Bacillus sphaericus Bacteria and Streptococcus aureus Bacteria. Both *Bacillus sphaericus* and *Streptococcus aureus* were obtained from the Department of Biotechnology at GLA University in Mathura. The fact that the microorganisms *Bacillus sphaericus* and *Streptococcus aureus* are able to survive in the highly alkaline surroundings of concrete during the formation of CaCO_3 crystals on concrete suggests that the presence of the microbes had no negative implication on the hydration of reaction, which forms a dense CaCO_3 crystal in liquid medium [39, 61]. Ureolytic, Gram +ve, anaerobic, and round-spore-forming *Bacillus sphaericus* and *Streptococcus aureus* at a temperature of four degrees Celsius were grown on nutrient agar slants, and they were subcultured once every fifteen days on a medium that had been filtered and sterilized [59, 62].

3. Methodology

3.1. Research Methodology. The gathering of particular experimental data that contributes to a better understanding of microorganism concrete and the features it possesses is the primary aim of the current experimental research (durability and strength). In the current experimental inquiry, tests on the behavior of hardened and fresh characteristics of ordinary concrete grade and normal grade concrete without and with the inclusion of microbes have been carried out. These studies were carried out as part of the experimental study. Concrete in its hardened state is subjected to the appropriate laboratory tests, which enable the concrete strength, such as compression strength and tension strength. Research process methodology is shown in Figure 1.

The primary purpose of this experimental inquiry is to investigate the strength of normal concrete and bacterial concrete. The current work may be broken down into three distinct stages:

Phase 1: growth of bacteria and culturing technique of bacterial concrete

Phase 2: to study the compression strength and split tensile strength of bacterial concrete

Phase 3: regression analysis and cluster analysis

3.2. Culturing Technique of Bacteria. Both *Bacillus sphaericus* and *Streptococcus aureus* were cultivated in a medium that was designed for the improved generation of CaCO_3 . This medium includes baking soda, ammonium chloride, urea, and CaCl_2 , and it was dissolved in distilled water [36]. In order to determine the growth curve, the colonies were cultivated in batch culture aerobic endurance at 37 degrees Celsius and 100 revolutions per minute for a period of time [37, 41]. During this process, aliquots of the cells were taken out for optical density measurements and standard plate enumeration. We used a UV-vis 3000plus dbl spectrometer [63] to determine the initial concentration at 600 nm, also known as OD 600 (Department of Biotechnology, GLA University, Mathura).

3.3. Mix Design of Concrete. Concrete of the grade M30 was mixed in accordance with the standards set out in IS 10262-1982 [64]. For an exposure level of moderate and a water to cement ratio of 0.46, the amount of cement necessary to produce 1 m^3 of concrete is 400 kg. Casting concrete into cubes of size 100 millimeters by 100 millimeters by 100 millimeters is carried out to determine the concrete's compressive strength, and casting concrete into cylinders of size 100 millimeters in diameter and 200 millimeters in length is carried out to determine the concrete's split tensile strength. Mix design is shown in Table 2.

4. Result and Discussion

4.1. Compression Strength. The evaluation of the effect that the incorporation of microorganisms into the mixture has on the compression strength and stiffness of cement concrete blocks is another factor that is taken into consideration [65–67]. The compression strength of the concrete was enhanced as a result of the influence of the bacterial isolates, as can be seen in Figures 2, 3, and Table 3. It is clear that the compression strength of microorganism concrete is significantly higher than that of the conventional concrete. The increase in compressive strength that is brought about by bacteria is presumably brought about by the deposition of calcium carbonate [67] on the surfaces of the microbe cell and the gaps inside the concrete, which plugs the holes that are present in the binder matrix [68, 69]. The filling of the gaps inside the concrete with microbiologically generated concrete mixes is the primary cause of the increase in compression strength [70]. The findings of the study indicated that the addition of bacteria to concrete led to an increase in compressive strength, which, in turn, would lead to an improvement in the concrete's overall performance.

4.2. Split Tensile Strength. In order to evaluate the tension strength of each mixture, three cylinders measuring 100 mm by 200 mm were casted for every combination. In order to create these cylinders, a mold made of cast iron and steel was

TABLE 1: Variables studied and procedures of bacterial concrete.

Materials used	Durability and mechanical test	Existing structure
Pseudomonas bacteria [36–39]	Compression strength [40–42]	Self-healing [16, 43–45]
Bacillus subtilis [2, 4, 34]	Tension strength [5, 46–48]	Resettlement of original structure [1, 16, 49, 50]
Megaterium bacteria [5, 51, 52]	Flexural strength [3], [28, 53, 54]	
Cereus and sphaericus bacteria [55–57]	Durability characteristics [12, 19, 58–60]	

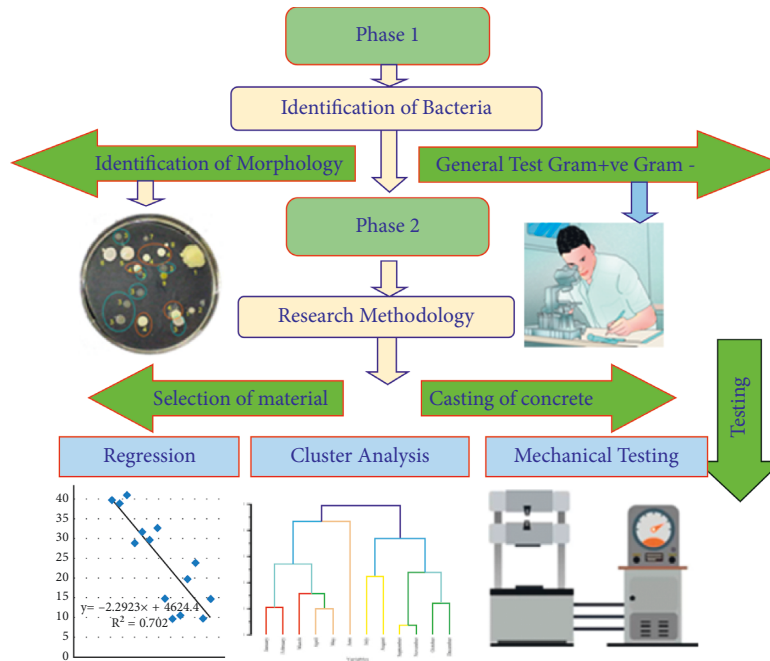


FIGURE 1: Research process methodology.

TABLE 2: Materials utilized in concrete mix design in detail.

Concrete	Mix Id	Sample	Bacteria cell concentration (ml)	OPC	Coarse aggregate	Fine aggregate	Water
Normal concrete	Conv.	A	—	400	1457	940	168
		B	—	400	1457	940	168
		C	—	400	1457	940	168
Bacillus sphaericus bacterial concrete	SP1	A	SP10 ⁶	400	1457	940	168
		B	SP10 ⁷	400	1457	940	168
		C	SP10 ⁸	400	1457	940	168
	SP2	A	SP10 ⁶	400	1457	940	168
		B	SP10 ⁷	400	1457	940	168
		C	SP10 ⁸	400	1457	940	168
	SP3	A	SP10 ⁶	400	1457	940	168
		B	SP10 ⁷	400	1457	940	168
		C	SP10 ⁸	400	1457	940	168
Streptococcus aureus bacterial concrete	SA1	A	SA10 ⁶	400	1457	940	168
		B	SA10 ⁷	400	1457	940	168
		C	SA10 ⁸	400	1457	940	168
	SA2	A	SA10 ⁶	400	1457	940	168
		B	SA10 ⁷	400	1457	940	168
		C	SA10 ⁸	400	1457	940	168
	SA3	A	SA10 ⁶	400	1457	940	168
		B	SA10 ⁷	400	1457	940	168
		C	SA10 ⁸	400	1457	940	168

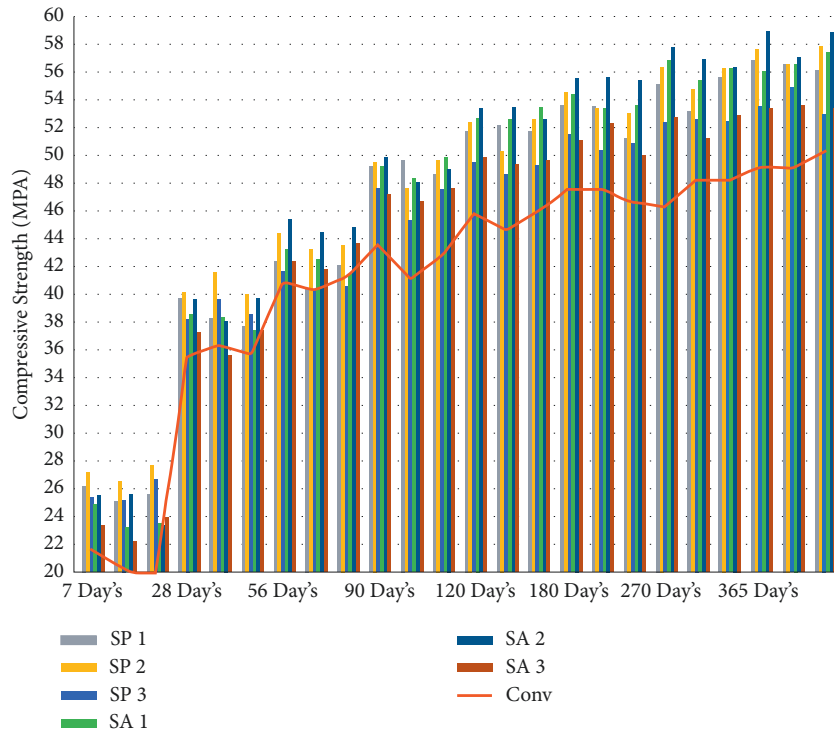


FIGURE 2: Graphical representation of compression strength result at different days' intervals.

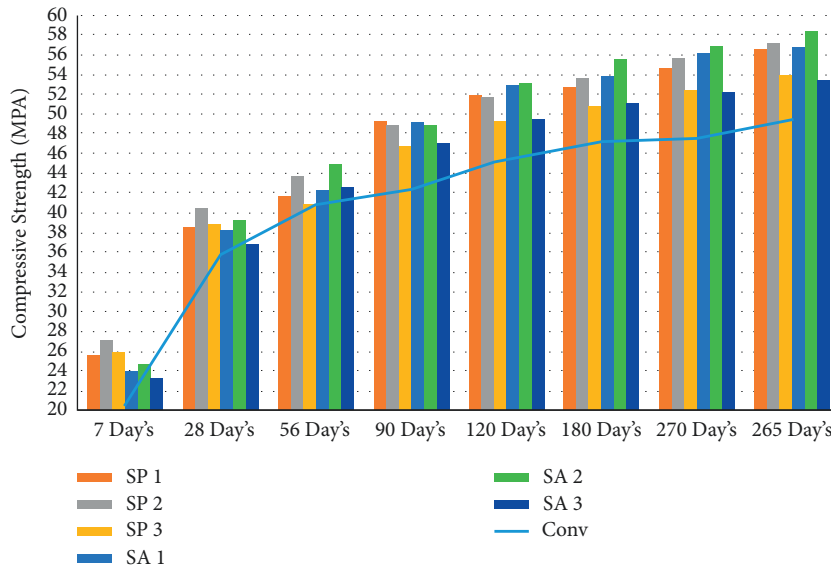


FIGURE 3: Graphical representation of average compression strength result at different days' intervals.

utilized. Casting and curing times for each mix percentage were as follows: seven days, twenty-eight days, fifty-six days, ninety days, one hundred eighty days, two hundred seventy days, and three hundred sixty-five days. In accordance with the Indian Standard code IS 516–1959 [71], a split tensile strength test is performed at the ages of seven days, twenty-eight days, fifty-six days, ninety days, one hundred twenty days, one hundred eighty days, two hundred seventy days, and three hundred sixty-five days using a compressive testing machine with a capacity of two thousand

kilonewtons. The loading part and the surface of the cylinder specimen are separated by a wooden strip so that there is no direct impact from the loading on the cylinder [55, 72, 73]. As shown in Figures 4, 5, and Table 4, the effect of the bacterial isolates resulted in an increase in the split tension strength of the concrete. This improvement can be attributed to the fact that the concrete was allowed to cure at a higher temperature [58]. It would suggest that the addition of *Bacillus sphaericus* and *Streptococcus aureus* was responsible for the rise in the splitting tension strength.

TABLE 3: Compression strength result at different days' intervals.

Day	Sample	Normal concrete	Bacillus sphaericus bacterial concrete			Streptococcus aureus bacterial concrete		
			10 ⁶ cfu/ml	10 ⁷ cfu/ml	10 ⁸ cfu/ml	10 ⁶ cfu/ml	10 ⁷ cfu/ml	10 ⁸ cfu/ml
7 days	A	21.71	26.32	27.31	25.53	24.91	25.61	23.36
	B	20.39	25.16	26.63	25.32	23.31	25.74	22.34
	C	19.86	25.72	27.81	26.72	23.67	23.31	23.91
28 days	A	35.61	39.72	40.19	38.32	38.61	39.65	37.31
	B	36.32	38.31	41.62	39.61	38.39	38.23	35.64
	C	35.68	37.69	40.03	38.59	37.42	39.69	37.65
56 days	A	40.88	42.39	44.39	41.67	43.23	45.37	42.37
	B	40.32	40.61	43.27	40.31	42.64	44.47	41.86
	C	41.23	42.13	43.53	40.63	41.35	44.83	43.73
90 days	A	43.61	49.24	49.57	47.67	49.23	49.91	47.21
	B	41.08	49.67	47.62	45.34	48.27	48.07	46.63
	C	42.71	48.63	49.67	47.57	49.86	49.03	47.67
120 days	A	45.83	51.71	52.37	49.54	52.71	53.43	49.87
	B	44.65	52.16	50.33	48.62	52.65	53.41	49.31
	C	45.79	51.75	52.64	49.37	53.53	52.62	49.64
180 days	A	47.64	53.62	54.45	51.47	54.43	55.53	51.07
	B	47.64	53.49	53.43	50.39	53.41	55.61	52.31
	C	46.59	51.35	53.06	50.87	53.63	55.39	50.03
270 days	A	46.23	55.12	56.39	52.39	56.86	57.74	52.75
	B	48.23	53.19	54.71	52.61	55.37	56.81	51.21
	C	48.17	55.64	56.23	52.39	56.26	56.37	52.86
365 days	A	49.13	56.87	57.61	53.67	56.07	58.96	53.39
	B	49.08	56.61	56.63	54.89	56.61	57.07	53.61
	C	50.23	56.12	57.81	52.93	57.37	58.83	53.37

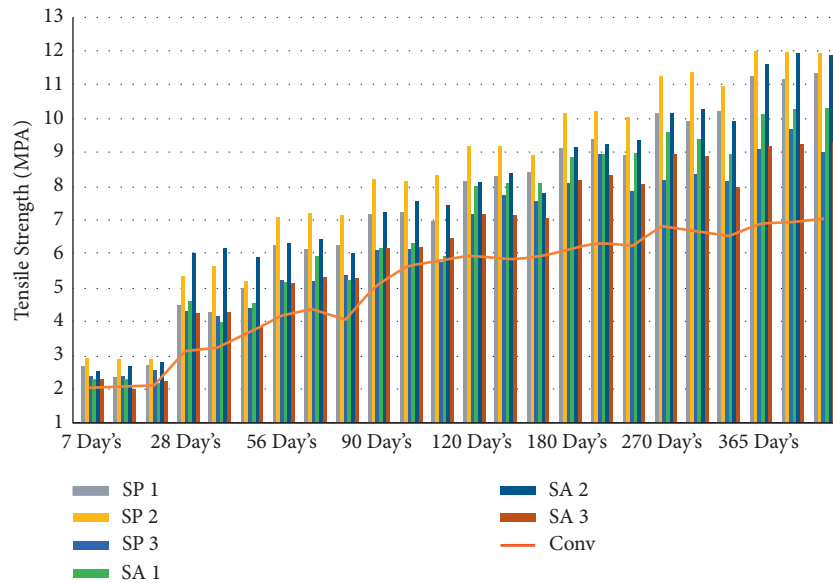


FIGURE 4: Graphical representation of split tension strength result at different days' intervals.

4.3. *Predicted Split Tension Strength.* The findings of the experiments were evaluated using regression analysis [43], which led to the discovery of the link between the compression strength of microbiological and normal concrete as well as tension strength of microbiological and normal concrete. This relationship may be expressed as equation (1), [74]. The predicted tensile strength's results at different day intervals are shown in Table 5 and Figures 6 and 7.

$$F_{\text{tensile}} = 0.23 f_{\text{compression}}^{0.73}. \quad (1)$$

4.4. *Regression Analysis.* The results of an experiment are depicted in Figure 8, which shows the link between the tension strength and the compression strength of bacterial concrete that was made with the bacteria *Bacillus*

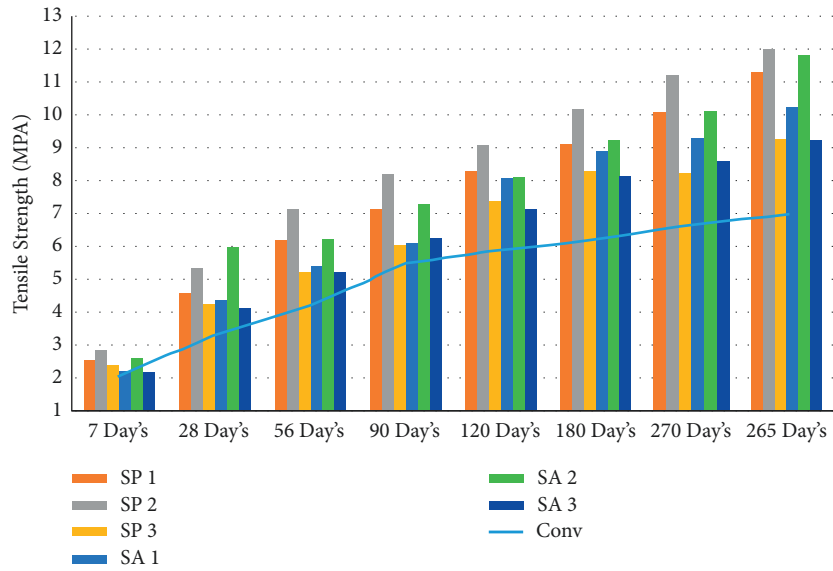


FIGURE 5: Graphical representation of average split tension strength result at different days' intervals.

TABLE 4: Tension strength result at different days' intervals.

Day	Sample	Normal concrete	Bacillus sphaericus bacterial concrete			Streptococcus aureus bacterial concrete		
			10 ⁶ cfu/ml	10 ⁷ cfu/ml	10 ⁸ cfu/ml	10 ⁶ cfu/ml	10 ⁷ cfu/ml	10 ⁸ cfu/ml
7 days	A	2.03	2.68	2.91	2.39	2.31	2.56	2.3
	B	2.11	2.39	2.89	2.41	2.29	2.63	2.16
	C	2.13	2.7	2.93	2.52	2.17	2.81	2.21
28 days	A	3.16	4.51	5.31	4.31	4.62	6.02	4.27
	B	3.23	4.31	5.62	4.16	3.98	6.13	4.31
	C	3.68	5.03	5.17	4.39	4.57	5.87	3.92
56 days	A	4.16	6.23	7.08	5.23	5.17	6.31	5.13
	B	4.39	6.13	7.16	5.16	5.93	6.42	5.31
	C	4.08	6.27	7.11	5.39	5.23	5.98	5.27
90 days	A	5.09	7.18	8.21	6.08	6.14	7.21	6.17
	B	5.67	7.23	8.18	6.17	6.31	7.52	6.19
	C	5.83	6.98	8.31	5.87	5.93	7.43	6.45
120 days	A	5.98	8.17	9.17	7.17	8.03	8.13	7.17
	B	5.87	8.31	9.19	7.77	8.12	8.39	7.13
	C	5.92	8.42	8.92	7.52	8.11	7.81	7.06
180 day's	A	6.13	9.16	10.16	8.13	8.87	9.16	8.16
	B	6.34	9.38	10.23	8.91	8.92	9.23	8.32
	C	6.24	8.93	10.08	7.86	8.99	9.33	8.05
270 days	A	6.87	10.19	11.26	8.17	9.62	10.19	8.91
	B	6.67	9.89	11.38	8.39	9.39	10.27	8.89
	C	6.54	10.21	10.97	8.17	8.91	9.89	7.98
365 days	A	6.91	11.31	12.03	9.16	10.11	11.61	9.17
	B	6.97	11.22	11.98	9.71	10.27	11.91	9.23
	C	7.08	11.34	11.91	8.98	10.33	11.89	9.31

sphaericus and Streptococcus aureus. The linear equation displays not only the percentage correlations between compression strength and split tensile strength (σ) [75] but also the regression coefficients (R^2) [76] that were derived from the equation that was shown below.

$$y = 0.249_x - 4.4595. \tag{2}$$

The following value for the regression coefficient, R^2 (0.8813), indicates that the regression line as well as the statistics of compression strength and split tensile strength

TABLE 5: Predicted tensile strength result at different days' intervals.

Day	Sample	Normal concrete	Bacillus sphaericus bacterial concrete			Streptococcus aureus bacterial concrete		
			10 ⁶ cfu/ml	10 ⁷ cfu/ml	10 ⁸ cfu/ml	10 ⁶ cfu/ml	10 ⁷ cfu/ml	10 ⁸ cfu/ml
7 days	A	2.038227629	2.503436224	2.571832482	2.448358056	2.404809379	2.453956321	2.294631801
	B	2.077793632	2.422404145	2.524926774	2.433640017	2.291045402	2.463043444	2.221051093
	C	2.038227629	2.461646233	2.606120893	2.531153289	2.316821444	2.291045402	2.333946661
28 days	A	3.121586521	3.380684022	3.409839839	3.293278443	3.31145373	3.376333718	3.229685754
	B	3.166899629	3.292651047	3.497988291	3.373846899	3.297668971	3.287630294	3.123506065
	C	3.126064779	3.253665605	3.399924843	3.310201448	3.236634043	3.37881986	3.251144495
56 days	A	3.452476917	3.545113386	3.6664519	3.501055467	3.596259797	3.725366843	3.543892297
	B	3.417887928	3.435816163	3.598688612	3.417269093	3.560363902	3.671274354	3.512701689
	C	3.474030048	3.529227066	3.614461166	3.437051316	3.481408306	3.692946494	3.626576596
90 days	A	3.619309138	3.954758866	3.97408953	3.862307051	3.954172543	3.993969665	3.835064357
	B	3.464799065	3.979940439	3.859349338	3.723568456	3.897734599	3.885938698	3.800612473
	C	3.564629716	3.91893403	3.979940439	3.856390786	3.991048415	3.942439324	3.862307051
120 days	A	3.752902069	4.098616165	4.136738945	3.972333635	4.156327281	4.197696287	3.991632728
	B	3.682116322	4.124623116	4.018477076	3.918345731	4.152873001	4.196549189	3.958862225
	C	3.750510674	4.100930361	4.152297184	3.962378139	4.203430041	4.151145462	3.978185501
180 days	A	3.860532523	4.208587947	4.256045786	4.084720819	4.254904531	4.317506874	4.061522956
	B	3.860532523	4.201136887	4.197696287	4.021973619	4.196549189	4.322046643	4.13327862
	C	3.79823223	4.077766587	4.176456144	4.049905639	4.209160903	4.309558025	4.000977445
270 days	A	3.776785136	4.294212764	4.366217491	4.137892149	4.392753571	4.442279551	4.158629545
	B	3.895376476	4.18392344	4.270871825	4.150569557	4.308422033	4.389933406	4.069647769
	C	3.891838301	4.323748602	4.357170323	4.137892149	4.358867196	4.365086974	4.164958342
365 days	A	3.948307546	4.393317523	4.434976112	4.21145244	4.348116202	4.510604955	4.195401974
	B	2.503436224	4.378646039	4.379775259	4.281124862	4.378646039	4.404590959	4.208014962
	C	4.012647003	4.350946363	4.446210333	4.168983907	4.421481136	4.503342676	4.194254644

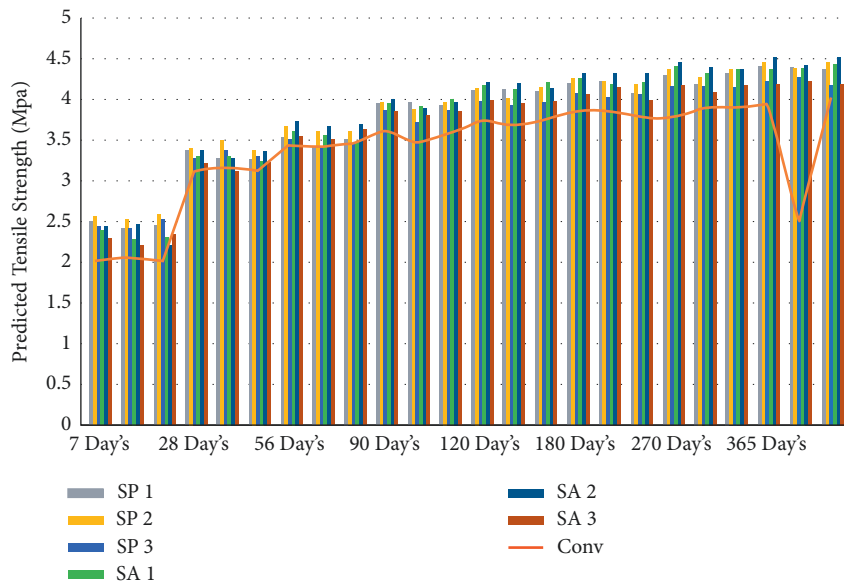


FIGURE 6: Graphical representation of predicted split tension strength result at different days' intervals.

values has a solid connection with one another. As can be seen from equation (2), the tensile strength improves as the compressive strength does.

4.5. Cluster Analysis. Multivariate statistical techniques are typically utilized for the categorization, analysis, and interpreting of big data sets. These approaches are also employed for the decrease of a dimension of complicated datasets with a minimal loss of original data [77]. Cluster

analysis is a method of unsupervised pattern classification that organizes the objects into the categories (clusters) on the basis of their commonalities within a category and their differences from other categories. The findings of CA lend a hand in data interpretation and point to patterns in the data. The square root of the average squared of the distinctions among corresponding values is computed in order to derive the distance from site in the Euclidean distance, which is one of the measurements that is utilized most frequently in order

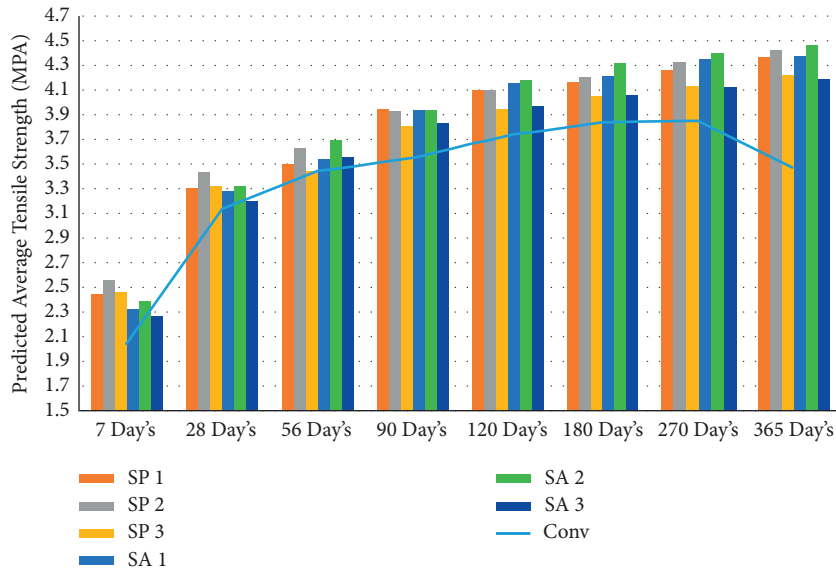


FIGURE 7: Graphical representation of average predicted split tensile strength result at different days' intervals.

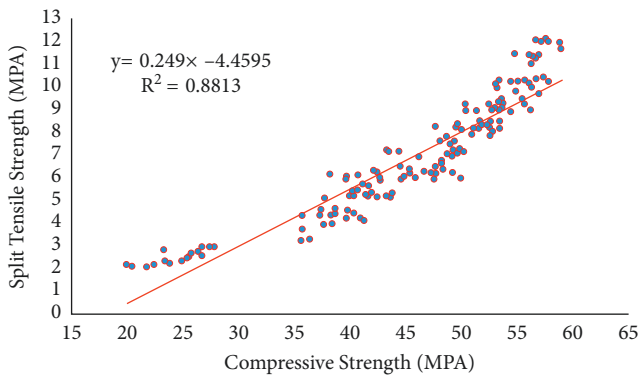


FIGURE 8: Linear regression analysis between compressive and tensile strength.

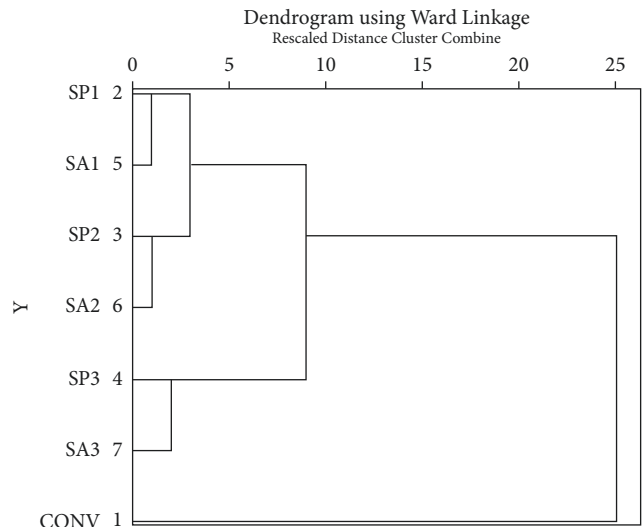


FIGURE 10: Cluster analysis result of split tensile strength.

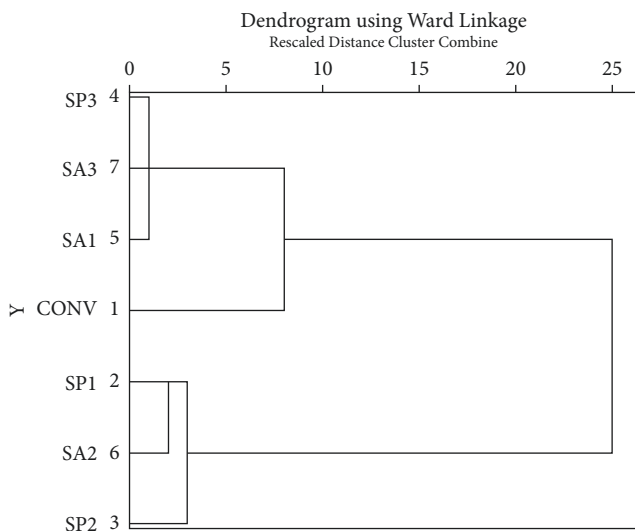


FIGURE 9: Cluster analysis result of compressive strength.

to determine the degree to which two cases are comparable to one another.

Through cluster analysis, it was found that the compression strength of bacterial concrete SP1, SA 1, SP 2, and SA 2 increases in the same way, and similarly, compression strength of SP3 and SA3 increases in the same group. Also, the compression strength of normal concrete sets itself apart from bacterial concrete. Cluster analysis results for compression strength are displayed in Figure 9.

Through cluster analysis, it was found that the tension strength of bacterial concrete SP 3, SA3, and SA1 increases in the same way, and tensile strength of SP 1, SA2, and SP 2 increases in the same group. Also, the split tensile strength of normal concrete sets itself apart from bacterial concrete. Cluster analysis results for split tensile strength are displayed in Figure 10.

5. Conclusion

- (i) The study of this research work has shown that the mechanical strength of concrete can be increased by using *Bacillus sphaericus* and *Streptococcus aureus* bacteria for bio-concrete.
- (ii) Using both bacteria in concrete with different concentrations showed that microbial concrete with a concentration of 10^7 cfu/ml produced better results for mechanical strength.
- (iii) This study also found that both bacterial concrete containing 10^6 , 10^7 , and 10^8 cfu/ml concentrations made from *Bacillus sphaericus* and *Streptococcus aureus* bacteria gave better results than normal concrete.
- (iv) Value of the regression coefficient indicates that the regression line as well as the statistics of compression strength and split tensile strength values has a solid connection with one another.
- (v) Through cluster analysis, it was found that the compression strength of bacterial concrete SP1 and SA1 increases almost equally, similarly, the compression strength of bacterial concrete SP2 and SA2 increases almost equally, and both the compressive strength of both SP3 and SA increases almost equally in a similar group. And in the cluster analysis for split tensile strength, three groups were formed, in which in the first group the tensile strength of SP3, SA3, and SA1 increases almost equally, and in the second group the tensile strength of SP1, SA2, and SP2 is mutual, which grows almost evenly, and in the third group comes the normal concrete.
- (vi) Research organizations from all over the world-wide have been interested in the employment of microbes for the purpose of increasing the longevity of construction materials as a consequence of the encouraging findings obtained so far. Our grasp of the opportunities and constraints presented by biotechnological processes on construction materials may unquestionably benefit from the work that has been carried out by a number of research groups that have concentrated their attention on a variety of materials. Studies are still being conducted on the preservation of nutrients and microbial products since these factors have an effect on the survival, development, and creation of biofilms. “*Bacillus sphaericus* and *Streptococcus aureus* bacterial concrete” appears to become the most viable technique for generating crack-resistant concrete in the coming days, according to the findings of this research as well as the prior research that has been conducted.

Data Availability

Data are available on request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

References

- [1] P. N. Reddy and B. V. Kavyateja, “Experimental study on strength parameters of self repairing concrete,” *Annales de Chimie Science des Matériaux*, vol. 43, no. 5, pp. 305–310, 2019.
- [2] M. V. S. Rao, V. S. Reddy, and C. Sasikala, “Performance of microbial concrete developed using *Bacillus subtilis* JC3,” *Journal of the Institution of Engineers*, vol. 98, no. 4, pp. 501–510, 2017.
- [3] N. N. T. Huynh, N. M. Phuong, N. P. A. Toan, and N. K. Son, “*Bacillus subtilis* HU58 immobilized in micropores of diatomite for using in self-healing concrete,” *Procedia Engineering*, vol. 171, pp. 598–605, 2017.
- [4] B. Tayebani and D. Mostofinejad, “Penetrability, corrosion potential, and electrical resistivity of bacterial concrete,” *Journal of Materials in Civil Engineering*, vol. 31, no. 3, Article ID 04019002, 2019.
- [5] C. Venkata Siva Rama Prasad and T. Vara Lakshmi, “Experimental investigation on bacterial concrete strength with *Bacillus subtilis* and crushed stone dust aggregate based on ultrasonic pulse velocity,” *Materials Today Proceedings*, vol. 27, no. xxxx, pp. 1111–1117, 2020.
- [6] K. Vijay and M. Murmu, “Effect of calcium lactate on compressive strength and self-healing of cracks in microbial concrete,” *Frontiers of Structural and Civil Engineering*, vol. 13, no. 3, pp. 515–525, 2019.
- [7] A. Shukla and N. Gupta, “Study on the efficacy of natural pozzolans in Cement Mortar,” in *Calcined Clays for Sustainable Concrete*, Rilem Publication, pp. 469–480, Springer, Berlin, Germany, 2020.
- [8] A. Talaiekhazan, A. Keyvanfar, A. Shafaghat, R. Andalib, M. Z. A. Majid, and M. A. Fulazzaky, “A review of self-healing concrete research development,” *J. Environ. Treat. Tech*, vol. 2, no. 1, pp. 1–11, 2014.
- [9] A. Shukla, N. Gupta, A. Gupta, R. Goel, and S. Kumar, “Study on the behaviour of green concrete by the use of industrial waste material: a review,” *IOP Conference Series: Materials Science and Engineering*, vol. 804, no. 1, Article ID 012036, 2020.
- [10] V. Achal, A. Mukherjee, and M. S. Reddy, “Microbial concrete: way to enhance the durability of building structures,” *Journal of Materials in Civil Engineering*, vol. 23, no. 6, pp. 730–734, 2011.
- [11] A. Gupta, N. Gupta, A. Shukla, R. Goyal, and S. Kumar, “Utilization of recycled aggregate, plastic, glass waste and coconut shells in concrete - a review,” *IOP Conference Series: Materials Science and Engineering*, vol. 804, Article ID 012034, 2020.
- [12] V. Achal, A. Mukherjee, and M. Sudhakara Reddy, “Biogenic treatment improves the durability and remediates the cracks of concrete structures,” *Construction and Building Materials*, vol. 48, pp. 1–5, 2013.
- [13] V. Achal, A. Mukherjee, and M. S. Reddy, “Effect of calcifying bacteria on permeation properties of concrete structures,” *Journal of Industrial Microbiology & Biotechnology*, vol. 38, no. 9, pp. 1229–1234, 2011.
- [14] F. B. Silva, N. Boon, N. De Belie, and W. Verstraete, “Industrial application of biological self-healing concrete:

- challenges and economical feasibility,” *Journal of Commercial Biotechnology*, vol. 21, no. 1, pp. 31–38, 2015.
- [15] N. Gupta, A. Shukla, A. Gupta, R. Goel, and V. Singh, “A review on the selection of the variant water in concreting,” *IOP Conference Series: Materials Science and Engineering*, vol. 804, no. 1, p. 012037, 2020.
- [16] Y. Ç. Erşan, E. Gruyaert, G. Louis, C. Lors, N. De Belie, and N. Boon, “Self-protected nitrate reducing culture for intrinsic repair of concrete cracks,” *Frontiers in Microbiology*, vol. 6, p. 1228, 2015.
- [17] H. J. Kim, H. J. Eom, C. Park et al., “Calcium carbonate precipitation by *Bacillus* and *Sporosarcina* strains isolated from concrete and analysis of the bacterial community of concrete,” *Journal of Microbiology and Biotechnology*, vol. 26, no. 3, pp. 540–548, 2016.
- [18] J. Xu, Y. Tang, X. Wang, Z. Wang, and W. Yao, “Application of ureolysis-based microbial CaCO_3 precipitation in self-healing of concrete and inhibition of reinforcement corrosion,” *Construction and Building Materials*, vol. 265, Article ID 120364, 2020.
- [19] M. J. C. Alonso, C. E. L. Ortiz, S. O. G. Perez et al., “Improved strength and durability of concrete through metabolic activity of ureolytic bacteria,” *Environmental Science and Pollution Research*, vol. 25, no. 22, Article ID 21451, 2017.
- [20] A. Shukla, N. Gupta, A. Gupta, R. Goel, and S. Kumar, “Natural pozzolans a comparative study: a review,” *IOP Conference Series: Materials Science and Engineering*, vol. 804, no. 1, Article ID 012040, 2020.
- [21] R. K. Verma, L. Chaurasia, V. Bisht, and M. Thakur, “Bio-mineralization and bacterial carbonate precipitation in mortar and concrete,” *Biosci. Bioeng.*, vol. 1, no. 1, pp. 5–11, 2015.
- [22] N. Chahal and R. Siddique, “Permeation properties of concrete made with fly ash and silica fume : influence of ureolytic bacteria,” *Construction and Building Materials*, vol. 49, pp. 161–174, 2013.
- [23] S. Jena and K. C. Panda, “Effect of bacteria on the properties of concrete - a review,” *UKIERI Concr. Congr. Concr. Glob. Build.*, 2019.
- [24] O. Öztürk, G. Yıldırım, Ü. S. Keskin, H. Siad, and M. Şahmaran, “Nano-tailored multi-functional cementitious composites,” *Composites Part B: Engineering*, vol. 182, Article ID 107670, 2020.
- [25] Y. Ryu, K. E. Lee, I. T. Cha, and W. Park, “Optimization of bacterial sporulation using economic nutrient for self-healing concrete,” *Journal of Microbiology*, vol. 58, no. 4, pp. 288–296, 2020.
- [26] A. T. Manikandan and A. Padmavathi, “An experimental investigation on improvement of concrete serviceability by using bacterial mineral precipitation,” *Int. J. Res. Sci. Innov.* 3, vol. 2, pp. 46–49, 2015.
- [27] A. Shukla, N. Gupta, and K. Kishore, “Experimental investigation on the effect of steel fiber embedded in marble dust based concrete,” *Materials Today Proceedings*, vol. 26, pp. 2938–2945, 2020.
- [28] V. Nagarajan, T. K. Prabhu, M. G. Shankar, and P. Jagadesh, “A study on the strength of the bacterial concrete embedded with *Bacillus megaterium*,” *Int. Res. J. Eng. Technol.*, vol. 4, no. 12, pp. 1784–1788, 2017.
- [29] IS 4031- Part I, *Method of Physical Tests for Hydraulic Cement: Determination of Fineness by Dry Sieving*, Bur. Indian Stand, New Delhi, 2005.
- [30] P. Sharma, N. Sharma, P. Singh, M. Verma, and H. S. Parihar, “Examine the effect of setting time and compressive strength of cement mortar paste using iminodiacetic acid,” *Materials Today Proceedings*, vol. 32, no. 4, pp. 878–881, 2020.
- [31] S. Krishnapriya and D. L. Venkatesh Babu, “Isolation and identification of bacteria to improve the strength of concrete,” *Elsevier GmbH.*, vol. 174, pp. 48–55, 2015.
- [32] N. De Belie, “Application of bacteria in concrete: a critical evaluation of the current status,” *RILEM Tech. Lett.*, vol. 1, p. 56, 2016.
- [33] V. Nežerka, Z. Slížková, P. Tesárek, T. Plachý, D. Frankeová, and V. Petráňová, “Comprehensive study on mechanical properties of lime-based pastes with additions of metakaolin and brick dust,” *Cement and Concrete Research*, vol. 64, pp. 17–29, 2014.
- [34] S. Joshi, S. Goyal, A. Mukherjee, and M. S. Reddy, “Microbial healing of cracks in concrete: a review,” *Journal of Industrial Microbiology and Biotechnology*, vol. 44, no. 11, pp. 1511–1525, 2017.
- [35] R. Bansal, N. K. Dhami, A. Mukherjee, and M. S. Reddy, “Biocalcification by halophilic bacteria for remediation of concrete structures in marine environment,” *Journal of Industrial Microbiology and Biotechnology*, vol. 43, no. 11, pp. 1497–1505, 2016.
- [36] B. Radha Kiranmaye, J. Ray Dutta, A. Kar, C. Parimi, and S. Raju, “Optimization of culture parameters of *Pseudomonas alcaligenes* for crack healing in concrete,” *Materials Today Proceedings*, vol. 28, pp. 713–716, 2020.
- [37] S. Heidari Nonakaran, M. Pazhouhandeh, A. Keyvani, F. Z. Abdollahipour, and A. Shirzad, “Isolation and identification of *Pseudomonas* azotoformans for induced calcite precipitation,” *World Journal of Microbiology and Biotechnology*, vol. 31, no. 12, pp. 1993–2001, 2015.
- [38] Y. Ç. Erşan, N. Boon, and N. De Belie, “Microbial Self-Healing concrete: Denitrification as an Enhanced and Environment-Friendly Approach,” in *Proceeding of the 5th Int. Conf. Self-Healing Mater*, Durham, NC, USA, September 2019.
- [39] Y. S. Lee, H. J. Kim, and W. Park, “Non-ureolytic calcium carbonate precipitation by *Lysinibacillus* sp. YS11 isolated from the rhizosphere of *Miscanthus sacchariflorus*,” *Journal of Microbiology*, vol. 55, no. 6, pp. 440–447, 2017.
- [40] P. Pachavannan, C. Hariharasudhan, M. Mohanasundram, and M. Anitha Bhavani, “Experimental analysis of self healing properties of bacterial concrete,” *Materials Today Proceedings*, vol. 33, pp. 3148–3154, 2020.
- [41] P. Kumar Jogi and T. V. S. Vara Lakshmi, “Self healing concrete based on different bacteria: a review,” *Materials Today Proceedings*, vol. 43, no. xxxx, pp. 1246–1252, 2021.
- [42] N. Karimi and D. Mostofinejad, “*Bacillus subtilis* bacteria used in fiber reinforced concrete and their effects on concrete penetrability,” *Construction and Building Materials*, vol. 230, Article ID 117051, 2020.
- [43] S. Farhadi and S. Ziadloo, “Self-healing microbial concrete-a review,” *Materials Science Forum*, vol. 990, pp. 8–12, 2020.
- [44] S. Bifathima, T. V. S. Vara Lakshmi, and B. N. Matcha, “Self healing concrete by adding *Bacillus megaterium* MTCC with glass & steel fibers,” *Civil and Environmental Engineering*, vol. 16, no. 1, pp. 184–197, 2020.
- [45] Y. S. Lee and W. Park, “Current challenges and future directions for bacterial self-healing concrete,” *Applied Microbiology and Biotechnology*, vol. 102, no. 7, pp. 3059–3070, 2018.
- [46] N. Iswarya, R. Adalarasan, V. Subathra Devi, and M. Madhan Kumar, “Experimental investigation on strength and durability of light weight bacterial concrete,” *Materials Today Proceedings*, vol. 22, pp. 2808–2813, 2020.

- [47] P. Jagannathan, K. S. Satya Narayanan, K. devi arunachalam, and S. kumar annamalai, "Studies on the mechanical properties of bacterial concrete with two bacterial species," *Materials Today Proceedings*, vol. 5, no. 2, pp. 8875–8879, 2018.
- [48] R. Pei, J. Liu, S. Wang, and M. Yang, "Use of bacterial cell walls to improve the mechanical performance of concrete," *Cement and Concrete Composites*, vol. 39, pp. 122–130, 2013.
- [49] M. S. Jafarnia, M. Khodadad Saryazdi, and S. M. Moshtaghioun, "Use of bacteria for repairing cracks and improving properties of concrete containing limestone powder and natural zeolite," *Construction and Building Materials*, vol. 242, Article ID 118059, 2020.
- [50] N. De Belie and J. Wang, "Bacteria-based repair and self-healing of concrete," *Journal of Sustainable Cement-Based Materials*, vol. 5, no. 1, pp. 35–56, 2015.
- [51] R. Andalib, M. Z. Abd Majid, M. W. Hussin et al., "Optimum concentration of Bacillus megaterium for strengthening structural concrete," *Construction and Building Materials*, vol. 118, pp. 180–193, 2016.
- [52] X. Sun, L. Miao, L. Wu, and R. Chen, "Improvement of biocementation at low temperature based on Bacillus megaterium," *Applied Microbiology and Biotechnology*, vol. 103, no. 17, pp. 7191–7202, 2019.
- [53] A. Toghroli, P. Mehrabi, M. Shariati, N. T. Trung, S. Jahandari, and H. Rasekh, "Evaluating the use of recycled concrete aggregate and pozzolanic additives in fiber-reinforced pervious concrete with industrial and recycled fibers," *Construction and Building Materials*, vol. 252, Article ID 118997, 2020.
- [54] A. K. Parande, B. R. Babu, K. Pandi, M. S. Karthikeyan, and N. Palaniswamy, "Environmental effects on concrete using Ordinary and Pozzolana Portland cement," *Construction and Building Materials*, vol. 25, no. 1, pp. 288–297, 2011.
- [55] J. Y. Wang, N. De Belie, and W. Verstraete, "Diatomaceous earth as a protective vehicle for bacteria applied for self-healing concrete," *Journal of Industrial Microbiology and Biotechnology*, vol. 39, no. 4, pp. 567–577, 2012.
- [56] S. Luhar, "A review paper on self healing concrete," *Journal of Civil Engineering Research*, vol. 5, no. 3, pp. 53–58, 2015.
- [57] W. De Muynck, K. Cox, N. D. Belie, and W. Verstraete, "Bacterial carbonate precipitation as an alternative surface treatment for concrete," *Construction and Building Materials*, vol. 22, no. 5, pp. 875–885, 2008.
- [58] R. Jakubovskis, A. Jankutė, J. Urbonavičius, and V. Grišniak, "Analysis of mechanical performance and durability of self-healing biological concrete," *Construction and Building Materials*, vol. 260, Article ID 119822, 2020.
- [59] E. Schlangen and S. Sangadji, "Addressing infrastructure durability and sustainability by self healing mechanisms - recent advances in self healing concrete and asphalt," *Procedia Engineering*, vol. 54, pp. 39–57, 2013.
- [60] F. Nosouhian, D. Mostofinejad, and H. Hasheminejad, "Concrete durability improvement in a sulfate environment using bacteria," *Journal of Materials in Civil Engineering*, vol. 28, no. 1, pp. 1–12, 2016.
- [61] A. K. Parashar and A. Gupta, "Investigation of the effect of bagasse ash, hooked steel fibers and glass fibers on the mechanical properties of concrete," *Materials Today Proceedings*, vol. 44, pp. 801–807, 2021.
- [62] J. Jayaprakash, "Advances in construction materials and systems," *Proc. Int. Conf. Chennai*, vol. 2, 2017.
- [63] N. Chahal, R. Siddique, and A. Rajor, "Influence of bacteria on the compressive strength, water absorption and rapid chloride permeability of concrete incorporating silica fume," *Construction and Building Materials*, vol. 37, pp. 645–651, 2012.
- [64] I. S. Is 10262, *Indian Concrete mix design guide lines*, vol. 1982, New Delhi, Bur. Indian Stand, Article ID 10262.
- [65] V. Karthekeyan R, D. R. Manju, and S. T, "Study on strength and durability properties of bacterial concrete," *International Journal of Advanced Research in Science, Communication and Technology*, pp. 13–20, 2022.
- [66] A. Paknahad, M. Goudarzi, N. W. Kucko, S. C. G. Leeuwenburgh, and L. J. Sluys, "Calcium phosphate cement reinforced with poly (vinyl alcohol) fibers: an experimental and numerical failure analysis," *Acta Biomaterialia*, vol. 119, pp. 458–471, 2021.
- [67] A. K. Parashar and A. Gupta, "Experimental study of the effect of bacillus megaterium bacteria on cement concrete," *IOP Conference Series: Materials Science and Engineering*, vol. 1116, no. 1, Article ID 012168, 2021.
- [68] H. Rong, G. Wei, G. Ma et al., "Influence of bacterial concentration on crack self-healing of cement-based materials," *Construction and Building Materials*, vol. 244, Article ID 118372, 2020.
- [69] Y. Ç. Erşan, E. Hernandez-Sanabria, N. Boon, and N. De Belie, "Enhanced crack closure performance of microbial mortar through nitrate reduction," *Cement and Concrete Composites*, vol. 70, pp. 159–170, 2016.
- [70] M. N. H. Khan, G. G. N. N. Amarakoon, S. Shimazaki, and S. Kawasaki, "Coral sand solidification test based on microbially induced carbonate precipitation using ureolytic bacteria," *Materials Transactions*, vol. 56, no. 10, pp. 1725–1732, 2015.
- [71] IS 516, *Method of Tests for Strength of Concrete*, 2018.
- [72] R. P. Santos, T. M. Ramos, B. M. Borges et al., "A selected bacterial strain for the self-healing process in cementitious specimens without cell immobilization steps," *Bioprocess and Biosystems Engineering*, vol. 44, no. 1, pp. 195–208, 2021.
- [73] A. R. Suleiman and M. L. Nehdi, "Effect of environmental exposure on autogenous self-healing of cracked cement-based materials," *Cement and Concrete Research*, vol. 111, no. May, pp. 197–208, 2018.
- [74] M. Sarkar, D. Adak, A. Tamang, B. Chattopadhyay, and S. Mandal, "Genetically-enriched microbe-facilitated self-healing concrete-a sustainable material for a new generation of construction technology," *RSC Advances*, vol. 5, no. 127, Article ID 105363, 2015.
- [75] N. Sharma and P. Sharma, "Effect of hydrophobic agent in cement and concrete: a Review," *IOP Conference Series: Materials Science and Engineering*, vol. 1116, no. 1, Article ID 012175, 2021.
- [76] H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen, "Application of bacteria as self-healing agent for the development of sustainable concrete," *Ecological Engineering*, vol. 36, pp. 230–235, 2010.
- [77] K. R. Singh, R. Dutta, A. S. Kalamdhad, and B. Kumar, "Risk characterization and surface water quality assessment of Manas River, Assam (India) with an emphasis on the TOPSIS method of multi-objective decision making," *Environmental Earth Sciences*, vol. 77, no. 23, p. 780, 2018.