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Optimization of Bacterial Self-healing Concrete Using *Bacillus licheniformis* with Micro Silica and Fly Ash Aggregates

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ABSTRACT

This research investigates the application of bacterial self-healing materials for resilient concrete using a cement matrix. It focuses on incorporating *Bacillus licheniformis* (*B. licheniformis*), a gram-positive microorganism, to repair structural and non-structural cracks at the nano-scale. The study addresses the challenge of microbial sustainability in concrete mixtures by employing an efficient immobilization technique using iron oxide nanoparticles. These nanoparticles were characterized using XRD and FTIR techniques. The effects of immobilized *B. licheniformis* on the concrete's strength and healing mechanisms were analyzed by measuring compressive and split tensile strengths per ASTM standards. Fly ash aggregates partially replaced coarse aggregates in varying proportions, with the mix containing 25% fly ash substitution demonstrating a 50% increase in compressive strength and complete healing of wider cracks. Furthermore, the optimization of fly ash, micro silica, and bacteria content in cement mortar was conducted using Response Surface Methodology (RSM). The RSM-based models were validated through ANOVA and found significant for all parameters ($p < 0.05$), with no significant lack of fit. The optimal mix achieved a 30% substitution of fly ash and micro silica at normal temperature, yielding compressive strengths of 23.22 MPa at 7 days and 28.72 MPa at 28 days and split tensile strengths of 1.68 MPa and 2.53 MPa, respectively. The findings highlight the potential of bacterial concrete in achieving self-healing and strength recovery, with a desirability factor of 1 confirming the precision of the optimization results. This study underscores the synergy between bacterial activity, pozzolanic materials, and statistical optimization techniques in enhancing the performance of concrete.

Keywords: *Bacillus licheniformis*; Characterization; Fly ash; Micro organisms; Micro silica; Self-healing.

1. INTRODUCTION

The increasing demand for concrete has led to a daily rise in the use of component materials. The behavior and characteristics of concrete are influenced by the materials employed in its composition. Notably, concrete's vulnerability to tension makes it prone to developing cracks in the tension zone, which can compromise its durability by allowing the ingress of moisture and harsh chemicals from the environment (Bogas *et al.* 2021). To address this issue, self-healing concrete has been introduced as a solution, incorporating both autonomous and autogenous processes in its production. The autogenous process involves mineral additives, nanofillers, and healing agents, while the autonomous process employs techniques such as electrode position, capsules, and microorganisms. In the realm of self-healing, the autonomous process is considered superior to the autogenous one. The autonomous self-healing process, which involves bacteria-induced calcium carbonate precipitation has shown significant potential in improving the durability of concrete structures. Among various bacterial strains studied *Bacillus licheniformis* has demonstrated superior self-healing capabilities due to its high survivability in harsh concrete environments rapid calcite precipitation, and ability to form a dense crystalline layer that effectively seals cracks. Compared to other bacterial strains such as *Bacillus pasteurii* and *Sporosarcina pasteurii*, *B. licheniformis* exhibits better resistance to the alkaline conditions within the concrete matrix and can remain viable for extended periods without significant loss of activity. This characteristic makes it a promising candidate for enhancing concrete's self-repairing efficiency. Another critical challenge in bacterial concrete technology is the long-term performance of self-healing concrete under varying environmental conditions, including exposure to moisture, temperature fluctuations, and chemical attacks. While this study optimizes the mix using Response Surface Methodology (RSM) for strength and healing efficiency, further research is needed to evaluate bacterial survivability and sustained healing efficiency over extended periods in different climates. Future investigations should focus on the effects of environmental variations such as freeze-thaw cycles, sulfate attack and carbonation resistance on the optimized mix to ensure its practical applicability in real-world infrastructure projects. The accumulation of fly ash, an industrial waste product from thermal power plants, is a growing concern as it poses environmental pollution and respiratory health risks due to its daily increase. Addressing the challenges posed by fly ash depositions is imperative. Simultaneously, excessive mining is depleting natural aggregate resources. To counter this, the production of lightweight fly ash aggregates has commenced, aiming to reduce reliance on both natural aggregates and mitigate the issues associated with fly ash deposits. Fly ash, recognized as a pozzolanic material, plays a crucial role by filling voids in composites and forming ettringite phase materials (Golewski, 2022; Mohamed, 2011; Chu *et al.*, 2022). Incorporating fly ash in concrete manufacturing not only addresses environmental concerns but also contributes to a reduction in CO_2 emissions during the production process (Golewski, 2021).

Initially, fly ash aggregates exhibit a higher porosity, making them ideal to serve as containers for bacteria. These aggregates prove excellent in the production of lightweight concrete. The pores within these aggregates act as capsules, facilitating the entry of bacteria. Once within the voids, these bacteria induce calcium precipitation, a process that enhances the concrete's strength by creating voids within the material. The resulting phenomenon is referred to as microbially induced calcium carbonate precipitation (MICP). Various strains of bacteria, primarily belonging to the Bacillus family, such as *Bacillus pasteurii*, *Bacillus licheniformis*, *Bacillus scohnii*, and *Bacillus lintus*, have been extensively studied in research.

Additionally, strains from other bacterial families like Sporosarcina, Pseudomonas, Escherichia, Shewanella, and Coli have also been subjects of investigation in numerous research studies (Kiran and Ratnam, 2014). Due to their negatively charged cell walls, bacteria exhibit an affinity for pulling cations from their surroundings. This process leads to the eventual coating of the bacteria's surface with Ca2+ ions as a result of Ca2+ demineralization. Subsequently, these Ca2+ ions combine with CO_3 2– ions, giving rise to $CaCO_3$ precipitation (Soundharya and Nirmalkumar, 2014; Reddy and Safiuddin, 2016). The carbonate is produced extracellularly by both autotrophic and mixed metabolic pathways. In the autotrophic process, bacteria employ various mechanisms such as oxygenic photosynthesis, non-methylotrophic methanogenesis, and oxygenic photosynthesis to convert carbon dioxide into carbonate (Soundharya and Nirmalkumar, 2014). On the other hand, calcium carbonate crystals precipitate through the heterotrophic route, growing in diverse natural environments. Notably, Bacillus species undergo heterotrophic reproduction.

Bacterial concrete, or self-healing concrete, is precisely engineered to prolong the durability of concrete buildings. Its self-recovering property is attributed to the production of calcite crystals by bacteria. Cracks that develop on the concrete's surface can be healed through this process. Various bacterial strains, including *Bacillus pasteurii*, *Bacillus cohnii*, *Bacillus pseudofirmus*, *Sporosarcina pasteurii*, and *Arthobacter crystallopoietes*, contribute to the increased compressive strength of the concrete through their biomass. Additionally, *Bacillus sp*. CT-5 can reduce corrosion in reinforced concrete, while *Sporosarcina pasteurii* bacteria demonstrate the ability to decrease permeability to water and chloride.

In the ongoing research, different proportions of fly ash and micro silica were introduced into bacterial concrete within an experimental framework. The strength of both regular concrete and bacterial concrete was assessed through various tests. The principal objective of the study is to leverage the bacterium *Bacillus licheniformis* for the mending of cracks in structural elements. Synthetic polymers can also be employed to address environmental cracks that demand intricate analytical techniques such as XRD, and FTIR. A critical aspect of the research involves the imperative need to diminish cement production, as it emits CO_2, contributing to worsened air pollution. The substitution of cement with fly ash and micro silica is employed in the utilization of thermal power plant waste, which possesses cementitious qualities. This strategic substitution serves the dual purpose of maintaining structural integrity while concurrently reducing the overall consumption of traditional cement.

2. MATERIALS AND METHODS

2.1 Cement

In the research study, Ordinary Portland Cement (OPC) with a grade of 53 was utilized. The assessment of the physical properties of this cement was conducted following the guidelines outlined in IS12269:1987. The outcomes of the tests on the physical and chemical properties are presented in Table 1.

Table 1. Physical and chemical properties of cement

Chemical Properties	Physical Properties	Values
Alumina Iron ratio	-	1.2
Lime Saturation Factor	-	0.89
Insoluble Residue	-	1.72%
Magnesia	-	1.12%
Alkalies	-	0.58%
Sulphuric Anhydride	-	2.2%
LOI	-	3.12
-	Fineness modulus	7.9
-	Consistency	30%
-	Specific gravity	3.02
-	Initial and Final setting time	60 and 300 minutes

2.2 Fine and Coarse Aggregate

In this research, locally available river sand, reduced to a particle size of 150 micrometers after passing through a 4.75 mm IS sieve, was utilized. The specific gravity of the river sand, verified to be 2.65 and falling within Zone III as per IS 383:2016, was considered. The characteristics of the fine aggregate were calculated following the guidelines of IS 2386:1963. For the study, graded aggregates with a size of 20 mm were procured from nearby crusher plants. The specific gravity of the aggregate was found to be 2.75, and its additional properties were determined in accordance with IS 2386-1963, with detailed results recorded in Table 2.

Table 2. Physical properties of fine and coarse aggregate

Properties	FA	CA
Specific gravity	2.6	2.72
Bulk density	1680 kg/m^3	1608 kg/m^3
Loose density	1442 Kg/m^3	1482 Kg/m^3

Table 3. Chemical composition of Fly ash and Micro silica

S. No.	Chemical Composition	Fly ash (%)	Micro silica (%)
1	SiO_2	50.36	89.26
2	CaO	10.23	0.86
3	Al_2O_3	14.25	0.25
4	Fe_2O_3	4.6	0.36
5	MgO	0.23	1.11
6	SO_3	1.86	0.22
7	LOI	2.15	0.98

2.3 Fly Ash and Micro-silica

Fly ash was incorporated into the concrete as a pozzolanic component (Yuvaraj, 2016; Yellaiah and Gunneshwara, 2014; Golewski, 2023). Typically, fly ashes exhibit a finer particle size than cement. Originating as a byproduct of coal combustion, fly ash consists mainly of glassy, spherical particles, char, certain crystalline phases formed during cooling, and residual magnetite and hematite. For this study, Group F Fly ash obtained from the Ennore Thermal Power Plant in Chennai, Tamil Nadu, was utilized. The fly ash adheres to the standards outlined in ASTM C 618. It possesses a specific gravity of 2.4, and its chemical composition is detailed in Table 4. Micro silica is commonly used in high-performance concrete for various structures, including bridges, high-rise buildings, and marine environments. It is also employed in shot Crete, precast concrete, and other applications where improved strength and durability are essential. Micro silica is typically added to concrete mixtures in small proportions, and its dosage depends on the desired performance characteristics of the concrete in Table 3.

2.4 Bacteria

Gram-positive *Bacillus licheniformis*, commonly known as grass bacillus or hay bacillus, is a frequently encountered soil-dwelling bacterium (Kadian and Pannu, 2018; Pasnur and Jain, 2018). The pristine culture of bacteria *Bacillus licheniformis* used in this study was provided by the National Collection of Industrial Microorganisms (NCIM) based in Pune. The pristine culture was cultivated in a nutrient-rich medium before injecting into the concrete mixture. Bacterial growth can be facilitated using both liquid (broth) and solid (agar) media. For concrete preparation, the bacteria were put on probation in the water and incorporated into the mixture.

Additionally, yeast was introduced as an additional component, serving both as a material and a food source to support the bacteria's prolonged survival. Sub-culturing was performed every four weeks to maintain the bacterial culture's purity and vitality. The nutrient broth, comprising 13.0 grams suspended in 1000 ml of filtered water, provided the essential medium for the cultivation of *Bacillus licheniformis*.

2.5 Concrete Mix Design

In the mix design process adhering to IS 10262:2019 for conventional concrete of M25 grade, the materials obtained from the final trials for one cubic meter are detailed as follows: The water-to-cement (w/c) ratio is specified as 0.5, and the mix ratio is given as 1:1.77:2.94. Additionally, calcium lactate and bacteria are included in the mix, each constituting 0.57% of the weight of the cement, 0%, 10%, 20%, and 50% of Fly ash aggregate and micro silica. These additives are introduced to enhance the concrete's properties and facilitate bacterial activity.

To summarize:

Cement	350 Kg/m³
Fine Aggregate	1100 Kg/m³
Coarse Aggregate	1100 Kg/m³
Water-to-Cement (w/c) Ratio	0.5
Mix Ratio	1:1.77:2.94
Bacteria	0.57% by weight of cement

In the experimental procedure, the calculation of the amount of water required for the concrete mix involves considering the capacity of water absorption on Fine Aggregate (FAA) and the Water-to-Cement (W/C) ratio. Additionally, a bacterial mixture with a dosage of 5.4x10^6 cells/ml is introduced to the entire water volume at a rate of 0.5%. This bacterium-mixed water is then utilized to immerse fly ash aggregates for a duration of one hour (Rivera *et al*. 2015; Oner *et al.* 2005; Cao C *et al.* 2000). After the soaking period, the fly ash aggregates, treated as independent variables in this study, are extracted and incorporated into the concrete mixing process along with coarse aggregates. The water remaining after soaking is added to the concrete mixture. The variables in this experimental work are defined as follows:

- Independent Variables: Fly ash aggregates
- Dependent Variables: Self-healing performance and strength

This methodology aims to investigate the impact of fly ash and micro silica aggregates on the self-healing performance and strength of the concrete (Dvorkin *et al.* 2023; Erdogdu and Turker, 1998), taking into account the bacterial solution and water absorption characteristics. The one-hour soaking period serves as a critical step in preparing the fly ash aggregates for integration into the concrete mix.

Table 4. Mix propositions

Sample Name	Cement (Kg/m^3)	CA (Kg/m^3)	FA (Kg/m^3)	Micro silica (Kg/m^3)	Fly ash (Kg/m^3)	Bacteria (Lit/m^3)	Water Cement Ratio
S1	350	1100	1100	0	0	0	0.5
S2	350	1100	1100	10	10	0	0.5
S3	350	1100	1100	20	20	2.00	0.5
S4	350	1100	1100	30	30	2.00	0.5
S5	350	1100	1100	50	50	2.00	0.5

The concrete mixing process employed a pan mixer, beginning with the comprehensive blending of cement, fine aggregate, and coarse aggregate. This initial phase, recognized as a "dry mix," ensures a uniform distribution of these components. Following the dry mix, 65% of the required water was incorporated, taking care to initiate the blending process. The remaining water was then thoroughly mixed after introducing the bacteria. Subsequently, the entirety of the water, including the portion added earlier and the one mixed with bacteria, was introduced into the mixture. A careful approach was applied during the mixing process, continuing until homogeneity was achieved. Before adding the materials to the pan, a verification process was implemented to ensure the accuracy of the material amounts. This systematic and cautious approach to concrete mixing aims to maintain precision in component proportions, contributing to the overall quality of the final concrete product**.**

2.6 Proposed Mix and Development of RSM System

RSM was utilized to investigate the elements influencing a variation in results. RSM generates model equations for response prediction using input variables and analyzes and examines the impact of one or more variables on the response. Additionally, RSM creates experiments and evaluates the outcomes to generate statistical answers. Additionally, multi-objective optimization is done using the RSM. The optimization approach used in this study differs from existing methods by integrating Response Surface Methodology (RSM) with bacterial self-healing concrete, which allows for a more precise and data driven mix design compared to conventional trial and error or empirical approaches. Traditional self-healing concrete optimization primarily focuses on fixed bacterial concentrations or static replacement ratios for supplementary materials like fly ash and micro silica. However, RSM enables multi-variable interactions to be analyzed, optimizing bacterial dosage, pozzolanic materials, and their synergistic effects to enhance both strength and healing efficiency. This statistical approach reduces material wastage, improves efficiency, and minimizes costs by identifying the optimal mix proportions with the highest desirability score**.** The RSM analysis in the study was conducted using Design Expert 11 software, where 20 experimental runs with different fly ash and micro silica proportions were designed to assess compressive and split tensile strengths. Compared to conventional mix designs, this approach ensures that the bacterial self-healing mechanism is effectively optimized without excessive material usage, contributing to both cost effectiveness and sustainability. Responses may have a linear relationship or a relationship with higher-degree polynomials for the analytical frameworks between the defiant factors. In order to illustrate the response surface in all directions, a realistic model was selected. The RSM produced Twenty (20) experimental runs with various pairings of both the variables (micro silica and fly ash). Our statistical analysis was carried out using Design Expert software (version 11.0). Response surface methodology was used to calculate reliability. As a result, the variables and reactions have a better and better link. All things considered, our approach produced the most reliable outcomes. Table 5 displays the developed experimental mixes. After that, the mixtures were made in the lab and examined for the different qualities that were being considered. The mixes, which had different combinations of the factors, were named M1, M2, M3,... M20, as seen in Table 5. Table 5 displays each mix's primary component. Since mixes M1, M2, M4, M6, M7, M9, M10, M13, M18, and M19 are used to quantify the lack of fit in relation to the pure errors in the model's analysis of variance, they all include the exact amounts of fly ash and micro silica (repeating mixes).

Table 5. Testing variants using Design Expert software

Mix ID	Fly Ash (Kg/m^3)	Micro Silica (Kg/m^3)	Bacteria (Lit/m^3)	Compression Strength (Mpa)	Split Tensile Strength (Mpa)
M1	50	50	1.92	24	2.2
M2	25	25	1.92	28	2.5
M3	0	0	1.92	21	2
M4	25	25	0.96	24	2.3
M5	50	25	0.96	22	2.2
M6	25	25	0.96	23	2.3
M7	25	25	0.96	23	2.3
M8	25	0	0.96	21.5	1.9
M9	25	25	0	20.5	1.85
M10	25	25	0.96	23	2.3
M11	0	50	1.92	22.5	2.1
M12	0	0	0	20	1.8
M13	50	50	0	23	1.9
M14	0	25	0.96	20.5	1.9
M15	50	0	0	20.5	1.85
M16	0	50	0	20	1.85
M17	50	0	1.92	26	2.3
M18	25	25	0.96	25.5	2.3
M19	25	25	0.96	25.5	2.3
M20	25	50	0.96	21.5	2.25

2.7 Environmental Impact Assessment

While this study primarily focuses on mechanical and healing properties the environmental impact of using fly ash and micro silica in bacterial concrete was considered. Fly ash is an industrial byproduct. when used as a partial cement replacement helps reduce the carbon footprint associated with cement production by lowering CO_2 emissions. Additionally micro silica derived from the ferrosilicon industry enhances concrete durability. As a Result of extending the service life of structures and reducing the need for repairs and reconstruction. Although a detailed life cycle assessment (LCA) was not conducted, the study acknowledges the sustainability benefits of incorporating these pozzolanic materials, particularly in reducing environmental pollution and enhancing resource utilization efficiency. Future research should quantitatively evaluate the total carbon footprint reduction of the optimized mix through LCA analysis.

2.8 Statistical Analysis and Model Validation

To validate the optimization (Khed *et al*. 2022) models, Analysis of Variance (ANOVA) was performed to determine the statistical significance of the model parameters. The p-values (< 0.05) confirmed the significance of fly ash, micro silica, and bacterial content in influencing the compressive and split tensile strengths. The model fit was assessed using the Lack-of-Fit test, which yielded non-significant results ($p > 0.05$), indicating a good fit between the predicted and experimental data. Additionally, R^2, adjusted R^2, and predicted R^2 values were analyzed to ensure the reliability of the regression models.

2.9 Mechanical Properties of Concrete

After a curing period of 28 days, the specimen was removed from the curing tank and allowed to air-dry at room temperature. Various tests were then conducted on different types of specimens: The cube specimen underwent a compressive strength test using a Compression Testing Machine (CTM) with a capacity of 100 T (Bouzoubaa, 2001; Heikal *et al*. 2013; Wang *et al*. 2003). The test, following IS:5816-1999 guidelines, involved applying a vertical load to the cube at a rate of 0.75 N/mm²/min. For the cylindrical specimen, both split tensile strength and crack healing observations were performed. The cylindrical specimen was placed horizontally, and a load was applied along its length at a rate of 0.75 N/mm²/min, in accordance with IS:5816-1999. Another set of tests on the cylindrical specimen involved placing a dried and cleaned specimen between the CTM plates. Compression was distributed progressively at a rate of 1.2 N/mm²/min. These testing procedures are crucial for assessing the concrete's mechanical properties, including compressive strength and split tensile strength, as well as its ability to heal cracks. In the compressive strength test, 150 mm cube specimens were utilized for both regular and self-healing concrete. These cubes are standard in size and commonly employed for evaluating the compressive strength of concrete. For the split tensile test and inspection of fracture-crack healing, tubular-shaped specimens with a radium of 50 mm and a length of 200 mm were employed. The cylindrical shape is suitable for assessing the tensile strength of concrete and allows for visual observations related to crack healing.

2.10 Characterization Techniques

XRD, FTIR, and SEM are analytical techniques commonly used in material science and characterization. Here's a brief explanation of each:

XRD is used to analyze the crystallographic structure of materials. X-rays are diffracted by the crystal lattice, producing a diffraction pattern that can be used to identify crystal structures and phases. It is widely used to identify minerals and crystalline phases in materials and analyze the degree of crystallinity. FTIR is used to identify functional groups in a material based on its infrared absorption spectrum. Infrared radiation is passed through a sample, and the resulting spectrum provides information about chemical bonds and molecular structure. Used for material identification, polymer analysis, and studying chemical composition.

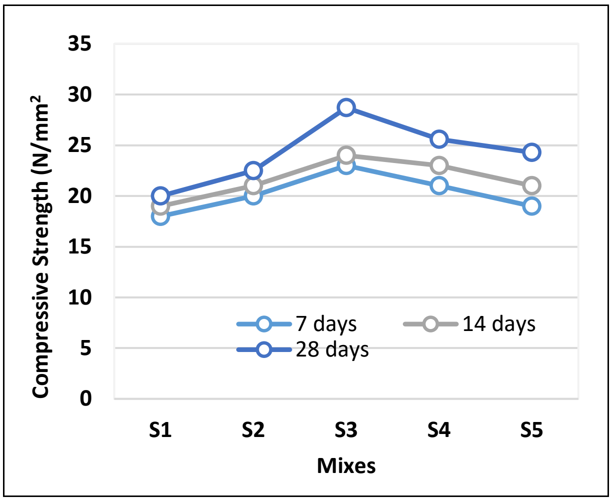

Fig. 1: Compressive strength of concrete

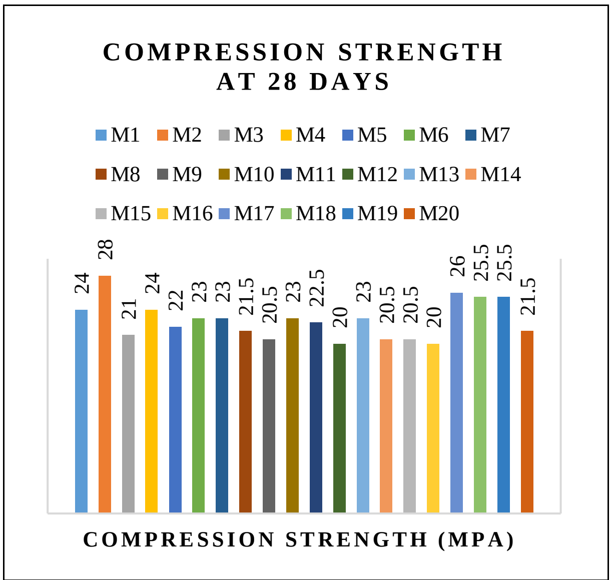

Fig. 2: Compressive strength of Concrete at 28 days (Design Expert – Mix combination)

3. RESULTS AND DISCUSSION

3.1 Mechanical Properties of Concrete

In the compression strength test, 150x150x150 mm cubical molds were utilized for casting cubes. These cubes were prepared both with and without a bacterial solution for varying fly ash and Micro silica contents, specifically 0%, 10%, and 50%. The cubes underwent testing at different intervals of 3, 7, and 28 days after the curing process in the compressive testing machine, as illustrated in Fig. 1. The test results, displayed in the graphs presented in Fig. 1, revealed interesting findings. According to the experiment's observations, both regular concrete and concrete with bacterial solution, where 30% of fly ash replaced cement, exhibited lower compressive strength compared to those with 10% fly ash substitution. This suggests that the incorporation of a higher percentage of fly ash (30%) had a negative impact on the compressive strength of the concrete specimens, whether or not a bacterial solution was used.

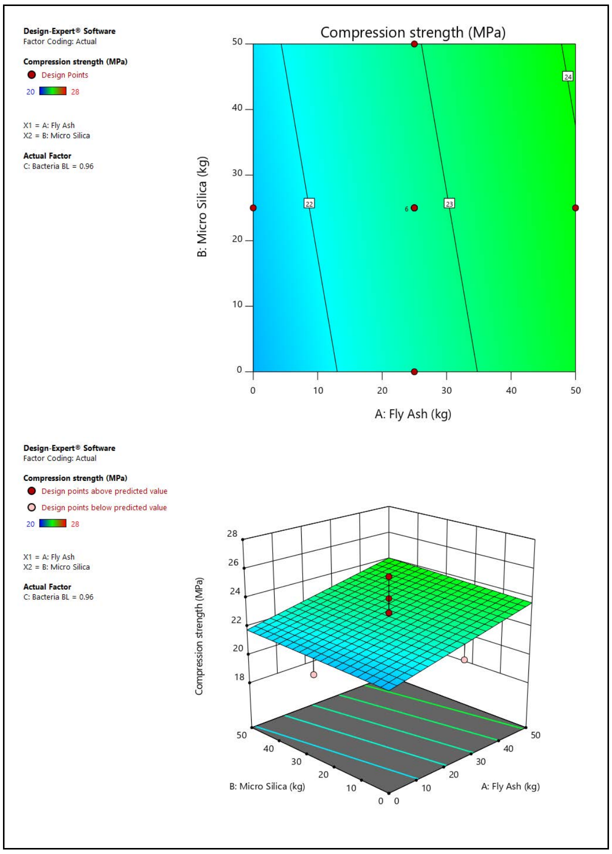

Fig. 3: Compressive strength of concrete for 28 days (Design Expert) – 3D surface

Compression strength = +22.75 + 1.15 A + 0.2000B + 1.75C …….(Annova equation 1)

During the curing period from day 14 to day 28, the compressive strength of concrete mixes S1, S2, S3, S4, and S5 exhibited percentage increases of 7 %, 9%, 15%, 11%, and 9.2%, respectively. This trend indicates an improvement in strength for these concrete mixes during this specific time frame. The increase in strength observed in concrete mixes that replaced fly ash and micro silica is attributed to the pozzolanic action of fly ash and micro silica in the mixtures. Pozzolanic materials, like fly ash, create more cementitious chemicals by reacting with calcium hydroxide that is produced during cement hydration. Concrete gains strength, durability, and other desirable qualities as a result of this reaction.

For tensile strength testing, cylindrical molds measuring 150 x 300 mm were employed. Cylinders were cast with varying fly ash concentrations of 10% to 50%, both with and without a bacterial solution. Tensile strength testing was performed on these cylindrical specimens after 7, 14, and 28 days of curing using the machine depicted in Fig. 2. The results for tensile strength are presented in graphs. According to the findings from the experiment, it was observed that the bacterial concrete, i.e., the concrete with the bacterial solution, demonstrated higher tensile strength compared to regular concrete. This suggests that the incorporation of bacteria in the concrete mix positively influenced its tensile strength characteristics.

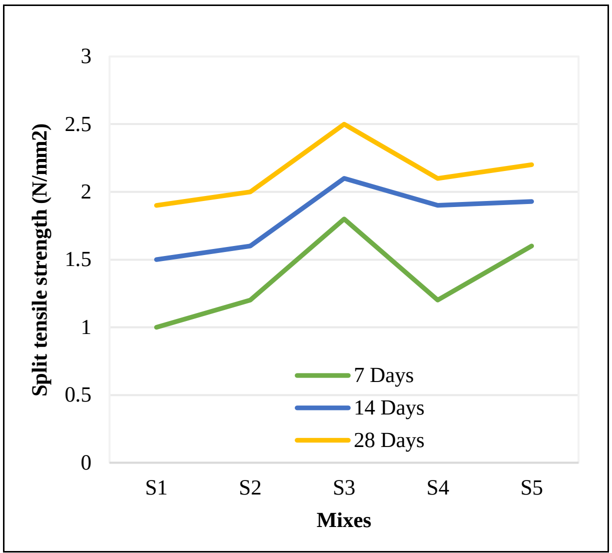

Fig. 4: Split tensile strength of Concrete

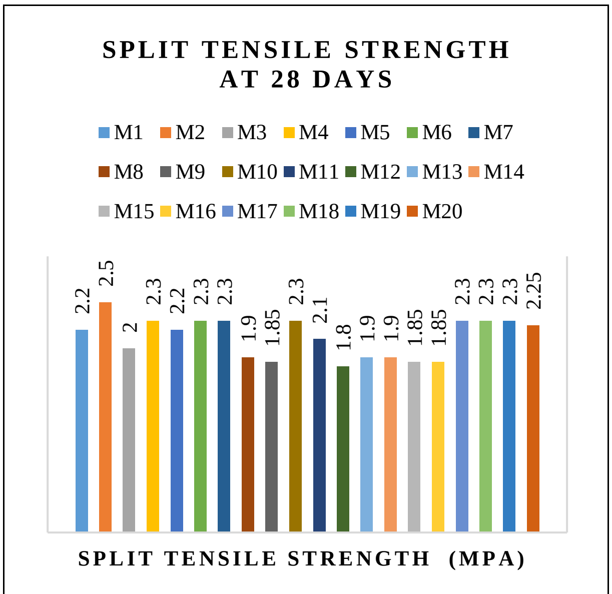

Fig. 5: Split tensile strength of Concrete at 28 days (Design Expert – Mix combination)

The experiment's results imply that the introduction of the bacterial solution has a strengthening effect on the tensile properties of the concrete, showcasing its potential as a beneficial additive in enhancing concrete performance. The concrete mixtures S1, S2, S3, S4, and S5 fall under the category of bacterial concrete as they contain bacteria. The compressive strength tests were conducted at two different durations: 14 and 28 days. Additionally, the strength increment from 14 to 28 days was calculated to evaluate the concrete's long-term performance. Split tensile strength tests were carried out on specimens after 14 days of curing and on the identical specimens after 28 days of curing, which represents the healing period. The actual split tensile strength measurements revealed a recovery in split tensile strength following crack healing. This observation indicates that the inclusion of bacteria in the concrete mixtures contributed to a healing mechanism, leading to improved tensile strength over time.

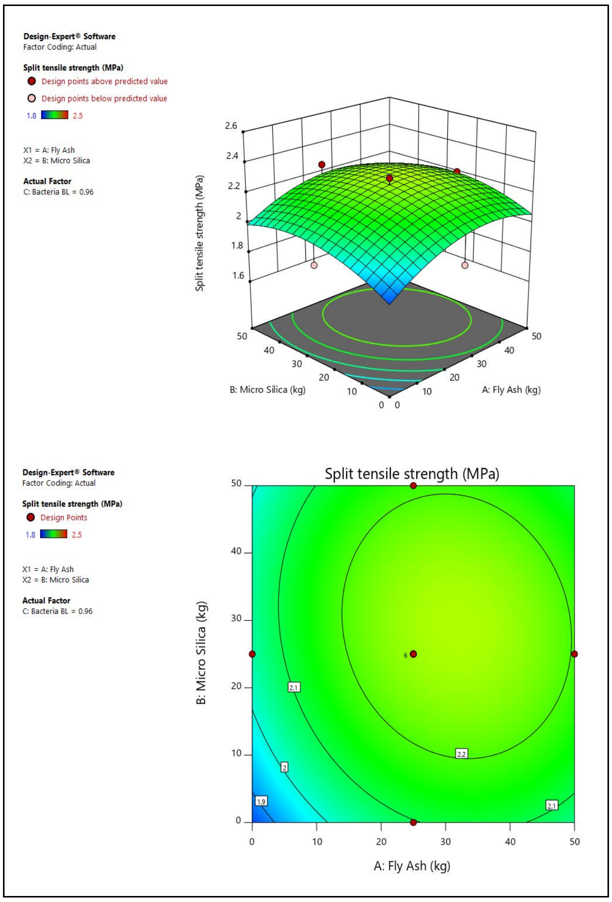

Fig. 6: Split tensile strength of concrete for 28 days (Design Expert) – 3D surface

Split tensile strength = +2.26 +0.0800 A +0.0450 B +0.1850 C -0.0250 AB +0.0375 AC -0.0125 BC -0.1409 A^2 -0.1159 B^2 -0.0159 C^2 ………………… (Annova equation 2)

Over 28 days of healing interval, specimens underwent split tensile (ST) loading to assess the split tensile strength of both fresh and cured specimens. The split tensile strengths of each blend were subsequently compared to the STS of the corresponding uncracked specimen. Remarkably, specimens with concrete mixes containing 50% fly ash and micro silica exhibited a significant degree of strength recovery.

Specifically, the strength recovery in S1 and S4 mixtures was higher, at 65% and 76%, respectively, while the recovery in S2 and S3 mixes was 82% and 62%, respectively. This indicates that concrete mixes S1, S2, S3, S4, and S5 were able to recover a substantial portion of their split tensile strengths following the healing period, demonstrating the effectiveness of the healing mechanism, particularly in mixes with higher fly ash content.

3.2 Evaluation of Variants for RSM Simulations

A framework was built to determine the strengths of the concrete using fly ash and micro silica material as elements that were statistically supported using the ANOVA result, as stated in Table 6. Each simulation was evaluated for predictive value using the accuracy interval, i.e., Prob > F less than 0.05 or ($p < 0.05$). All of the predictions have p-values below 0.05 and thus become significant. Each of the model variables was additionally evaluated for relevance in the model through $p < 0.05$. For the 28-day Compressive Strength and 28-day Split tensile test models, all of the parameters were significant, as a proportion of their F-values was below 0.05. Further validation of the model with regard to lack of fit was carried out. The lack of fit is defined as the measure of predicted models missing the observation. The lack of fit is tested for statistical significance using the confidence interval. From Table 6, the lack of fit for all the models had p-values more than 0.05 and consequently are not significant in proportion to their pure errors. This implied that the models fit the experimental data well and had high prediction accuracy. Standard statistically significant plots are tests obtained from the RSM method, which are used to explain and assess the suitability and sufficiency of the prediction in terms of a typical supply. At the outset, when preparing the evaluation, it is believed that the statistics are regularly dispersed. The usual distribution charts for the Compressive strength, split tensile strength, and Split tensile models are shown in Fig. 8 (a) and (b). For each of the simulations, the collected data values were matched up around the rising line. That implies the model and variables were consistently dispersed

Table 6. ANOVA fit summary for mechanical strength models

Responses	Source	Sum of Square	Mean Square	F Value	P Value	Significance
Split Tensile Strength 28 days (Mpa)	Model	0.7342	0.0816	5.52	< 0.0001	Significant
	A-Fly Ash	0.0640	0.0640	4.33	< 0.0001	Significant
	B-Micro Silica	0.0203	0.0203	1.37	< 0.0001	Significant
	C-Bacteria BL	0.3423	0.3423	23.16	< 0.0001	Significant
	AB	0.0050	0.0050	0.3384	< 0.0001	Significant
	AC	0.0112	0.0112	0.7613	< 0.0001	Significant
	BC	0.0013	0.0013	0.0846	< 0.0001	Significant
	A^2	0.0546	0.0546	3.70	< 0.0001	Significant
	B^2	0.0369	0.0369	2.50	< 0.0001	Significant
	C^2	0.0007	0.0007	0.0471	< 0.0001	Significant
	Residual	0.1478	0.0148		< 0.0001	Significant
	Lack of Fit	0.1478	0.0296		< 0.0001	Non-Significant
	Pure Error	0.0000	0.0000			
Compression Strength 28 days (Mpa)	Model	0.7342	0.0816	5.52	< 0.0001	Significant
	A-Fly Ash	0.0640	0.0640	4.33	< 0.0001	Significant
	B-Micro Silica	0.0203	0.0203	1.37	< 0.0001	Significant
	C-Bacteria BL	0.3423	0.3423	23.16	< 0.0001	Significant
	Residual	0.0050	0.0050	0.3384	< 0.0001	Significant
	Lack of Fit	0.0112	0.0112	0.7613	< 0.0001	Non-Significant
	Pure Error	0.0013	0.0013	0.0846	< 0.0001	Significant

The perturbation plot is used to analyze the effects of both fly ash and micro silica with Bacteria at a specific design point, i.e., at the core designed point, and to examine whether the reactions are responsive to the factors. Fig. 7 illustrates the perturbation plots for the Compressive strength and Split Tensile strength model.

From Fig. 8a, the Compressive strength has a reduced susceptibility to both fly ash (A) and Micro silica (B) since the slopes of A and B are minimal, i.e., they are softer. As demonstrated in Fig. 8b, the STS is not sensitive to change in fly ash due to the flat line of A, while it has less sensitivity to change in micro silica since the slope of B is not precisely straight, i.e., it as less slope.

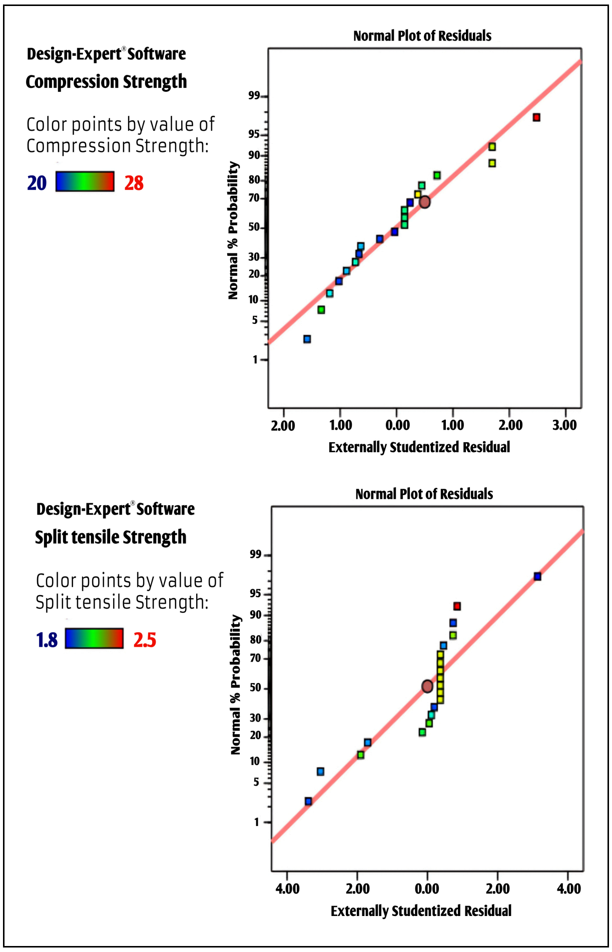

Fig. 7: Normal plot versus externally studentized residuals: (a) 28-day compressive strength and (b) 28-day splitting tensile strength

3.3 Quality and Purity of Bacterial Inoculants

To ensure the quality and purity of *Bacillus licheniformis* before incorporation into the concrete mix, the bacterial culture was obtained from the National Collection of Industrial Microorganisms (NCIM) in Pune. The bacteria were grown in a nutrient-rich broth and regularly sub-cultured every four weeks to maintain viability. Additionally, microbial purity was verified using Gram staining and optical density (OD) measurements at 600 nm to confirm active bacterial growth. Contaminant testing was conducted using standard plating techniques on selective media to ensure that no other bacterial strains interfered with the self-healing process.

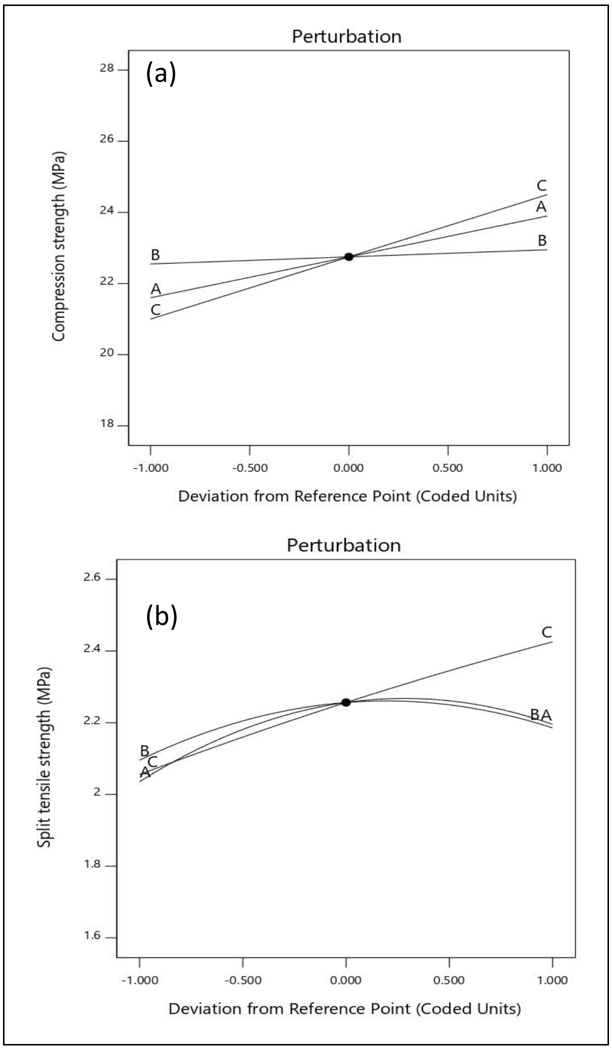

Fig. 8: Perturbation plots–CS & STS (a) 28-day compressive strength and (b) 28-day splitting tensile strength

3.4 Statistical Validation of Crack Healing, Strength and Durability

The effectiveness of crack healing, mechanical strength, and durability was statistically validated using Analysis of Variance (ANOVA). All response variables compressive strength, split tensile strength, and self-healing efficiency showed significant p-values ($p < 0.05$), confirming that fly ash, micro silica, and bacterial content had a measurable impact on performance.

The optimization process using Response Surface Methodology (RSM) was further validated through regression analysis, where R^2 values close to 1 indicated strong model accuracy. Additionally, the Lack-of-Fit test ($p > 0.05$) confirmed that the developed model effectively predicted experimental outcomes. The desirability function ($D = 1$) ensured that the optimized mix proportions were precisely aligned with the target properties.

3.5 Influence of Environmental Temperature on Self-healing and Strength Recovery

Temperature plays a critical role in bacterial self-healing and strength recovery, as microbial activity is directly influenced by ambient conditions. In this study, all mechanical and healing tests were conducted at a controlled temperature of 27 ± 2°C. This temperature range was maintained in a humidity-controlled curing chamber to simulate standard construction site conditions.

Bacterial self-healing is most effective in temperatures between 20°C and 40°C, as *Bacillus licheniformis* exhibits optimal calcite precipitation within this range. If temperatures drop significantly (e.g., below 10°C) bacterial metabolic activity slows, reducing the rate of calcium carbonate formation. Conversely, at excessively high temperatures (above 50°C), bacterial viability declines, compromising the healing efficiency. The controlled temperature environment ensured consistent bacterial performance, preventing external fluctuations from influencing the experimental outcomes.

3.6 Effectiveness and Empirical Validation

Subsequently examining a connection among the factors and suggestions, an optimization technique was used with RSM to figure out the most effective results for the trial combinations. The tuning process was executed by laying out specific requirements for the variables and reactions, as given in Table 7. For this present work, 20 clarifications have been collected for all the trial mixtures, as illustrated in Fig. 10. Every possible preferred value was derived based on the value portions indicated in the trial arrangement. All reactions cannot come up with an ideal value at the identical probable mixture. It is tough to analyze the two or three variables as their impact on the outcome of the trial would be quite distinct. From the ramp view of efficiency provided in Fig. 10, it can be noticed that the desirability factor is 1, i.e., the outcome is desirable, and the consequences of optimization are exact. From Table 8, it was seen that the variable limits are given along the appropriate values for the three primary responses in the experiment. Based on the multifaceted optimization, the optimal mix in terms of durability was attained by partly substituting 30% of cement with fly ash and Micro silica by weight of cementitious substances to produce the following desirable qualities at normal temperature: 7-day Compression strength of 23.22 MPa, 28-day Compressive strength of 28.72 MPa and 7-day Split tensile strength of 1.68 MPa, 28-day Split tensile test of 2.53 MPa. As shown in Fig. 10, the desirability is 1. This means that the best optimal combinations of the multiple set-up objectives for optimization were accomplished with little or no substantial error, implying that the projected values are the desired ones.

Table 7. Criteria setting for multi-objective optimization

Run	Lower Limit	Upper Limit	Bacteria	Compression Strength	Split Tensile Strength
1	30	40	0.6	22.44	2.17

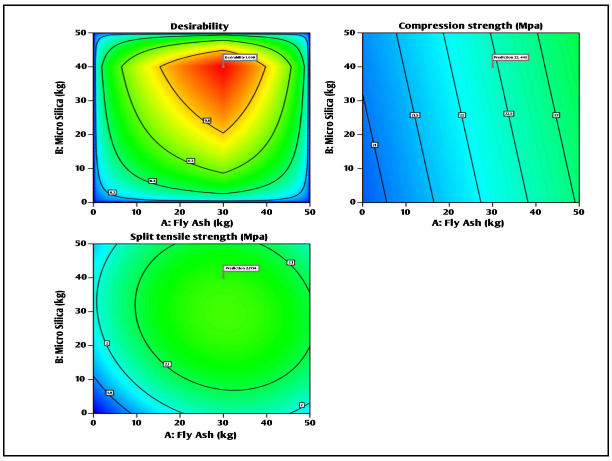

Fig. 9: Optimized solution with desirability

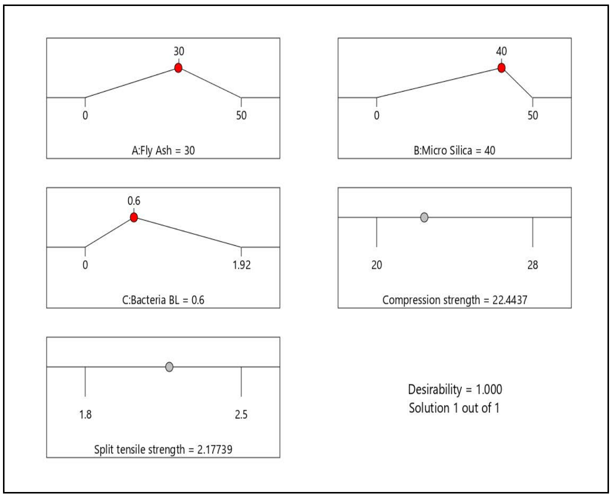

Fig. 10: Optimization ramp view

3.4 Characterization Techniques

3.4.1 X-Ray Diffraction Analyzing

The fly ash and silica fume XRD findings are shown in Fig. 11. With a significant percentage of quartz (SiO_2) is the main ingredient influencing the mechanical properties. Al_2O_3, the second most common element after quartz, is also advantageous for decreasing porosity and improving mechanical qualities. Peaks with high intensity, limited width, and a considerable amount of crystallinity show the material's crystallinity. X-Ray Diffraction (XRD) analysis revealed that the incorporation of fly ash and micro silica significantly altered the crystalline structure of the concrete matrix.

The presence of pozzolanic materials contributed to the formation of additional calcium silicate hydrate (C-S-H) gel, which enhances the overall strength and durability of the concrete.

Compared to conventional concrete, bacterial concrete with fly ash and micro silica showed higher peak intensities for calcite ($CaCO_3$) and quartz (SiO_2), indicating increased crystallinity. The secondary hydration reaction of fly ash further densified the microstructure by reducing porosity. Additionally, the amorphous nature of calcium lactate (identified in FTIR spectra) facilitated better bacterial-induced calcite precipitation, improving crack-sealing efficiency. These peaks are especially useful for creating aggregates since they exhibit a high degree of crystallinity. Fly ash and silica fumes are valuable components in the manufacturing of concrete because, according to the XRD study, its composition and crystalline structure contain ingredients that help to improve mechanical characteristics and reduce porosity.

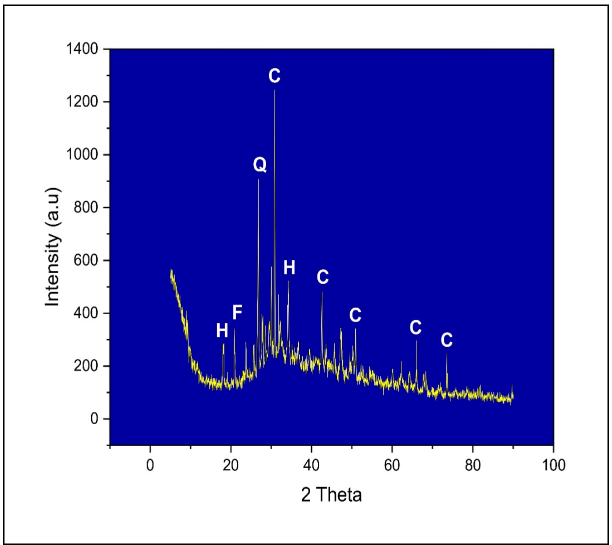

Fig. 11 XRD Analysis of concrete and bacteria mixtures (C-Calcite; F-Florite; H-Hematite)

X-ray diffraction Examination of concrete and bacteria compounds. The broad base width and low intensity of the peaks indicate that calcium lactate is an amorphous solid. This amorphous nature suggests a lack of a well-defined crystalline structure, and the XRD results indicate that calcium lactate includes a high concentration of calcite ($CaCO_3$) and quartz; in spite of its amorphous character, the presence of identifiable components such as calcite and quartz implies that these elements are included in this composition. The amorphous nature of calcium lactate makes it relatively simple to separate into its components, with calcite being used for calcium carbonate precipitation, contributing to specific cementitious properties. Quartz, on the other hand, is recognized for its ability to increase vitality. Additionally, the mention of the mineral fluorite (CaF_2) emphasizes the presence of calcium, further contributing to the material's beneficial properties.

3.4.2 FTIR Analysis

The FTIR spectra of several concrete mixtures with fly ash and nano-silica serving as the only cement substitutes are shown in Fig. 12 following 28 and 90 days of curing, respectively. The formation of hydraulic compounds such as C-S-H is credited with the discovery of Si-O asymmetric stretching bonds at 950 cm^{-1}. This finding emphasizes how important Si-O bonds are in the synthesis of important cementitious chemicals. There are three basic zones seen in the resulting infrared spectrum. The first zone covers wavelengths between 850 and 1200 cm^{-1} and is characterized by stretching vibrations. This range is essential for comprehending the material's vibrational properties and offers helpful details about the chemical makeup and bonding of concrete mixtures. In order to fully understand the composition and behavior of the concrete mixes during varying curing times, FTIR spectra analysis helps discover crucial chemical properties and molecules, such as Si-O bonds involved in C-S-H production.

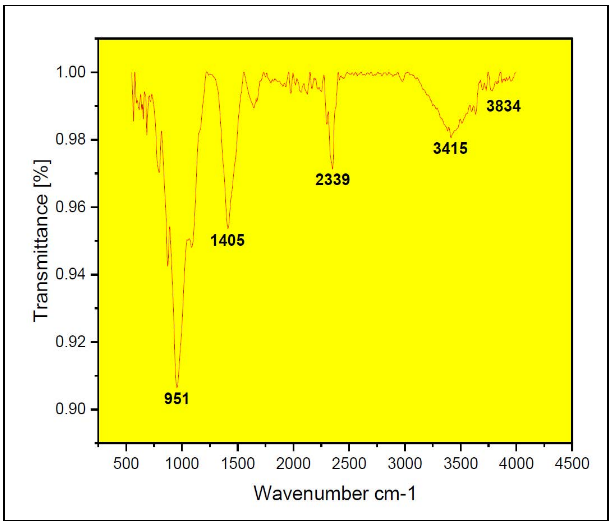

Fig. 12: FTIR Analysis of concrete and bacteria mixtures

When compared to the control mix, the FTIR results clearly show a change in the peaks, indicating the presence of fly ash and nano-silica. The hydration of Tricalcium silicate and Dicalcium silicate, crucial constituents of cementitious materials, causes a significant decrease in the strength of the Raman bands at 1405 and 3400–3800 cm^{-1} as the curing time increases. Bands found at 1405 and 950 cm^{-1} also shift toward the lower wavenumber side, particularly between 1450 and 1460 cm^{-1}, as the amount of nano-silica increases. The pattern suggests that the presence of nano-silica contributes to a decrease in carbonation, and these variations are linked to the degree of carbonation. Furthermore, the bands at 960 and 980 cm^{-1}, which correspond to ettringite, mono sulphate, and C-S-H molecules, likewise shift towards the lower wavenumber side as the concentration of both variables rises. This discovery indicates that the presence and concentration of fly ash and microsilica control the composition and characteristics of these cementitious compounds. The FTIR results shed light on the chemical reactions and changes that occur inside the concrete mixtures, demonstrating how fly ash and Mirco silica affect carbonation, hydration, and the formation of cementitious compounds.

4. CONCLUSION

This study focused on assessing the regaining capacity of concrete, particularly exploring the functionality of self-healing concrete. Mechanical and healing property tests were conducted to identify the most effective self-healing materials and to enhance the overall effectiveness of self-healing concrete. The specimens underwent testing for split tensile strength, compressive strength, and healing performance. The outcomes of these tests led to the following deductions:

The study results indicate that maximum strength is attained when using 100% natural aggregate, both in conventional and self-healing concrete. However, in cases where strength is lost after healing, a concrete mix containing 30% fly ash aggregates demonstrated good performance. The recovery of split tensile strength properties was remarkably enhanced in concrete mixes containing 30% and 50% fly ash and micro silica with bacteria.

While the mechanical qualities of the mix with 30% fly ash and micro silica with bacteria are slightly lower when compared to 100% fly ash and micro silica with bacteria, the overall performance is deemed satisfactory. This suggests that the inclusion of fly ash aggregates, even at a reduced percentage, can contribute to the improvement of specific properties, particularly in terms of self-healing capabilities and recovery of split tensile strength.

The findings underscore the importance of considering alternative aggregate materials, such as fly ash and micro silica, in concrete mixtures to achieve a balance between maximum strength and enhanced self-healing properties, even if there is a marginal decrease in mechanical qualities.

The generated models for evaluating the 28-day Compressive strength and Split Tensile tests performed with the RSM model were substantial with high precision.

Extended efficiency results indicated the most efficient blends included Micro silica and fly ash with bacterial variables of 28.7 MPa and 2.53 MPa, respectively. To achieve the maximum effectiveness of the blend as a constant, the environmental temperature with a desirability of 100%

XRD analysis identifies that calcium lactate, despite its amorphous appearance, has elements that influence the strength and other aspects of the material.

FTIR evidence provides insights into the chemical changes and interactions within the concrete mixes, highlighting the impact of fly ash and micro silica on the hydration process, carbonation, and the creation of cementitious compounds.

The findings from this study have significant implications for concrete infrastructure longevity and maintenance costs. The use of bacterial self-healing concrete can reduce the frequency of repair interventions by autonomously sealing cracks, thereby extending the service life of structures. Compared to conventional concrete the optimized mix demonstrated improved durability. which can reduce maintenance costs by minimizing crack propagation and preventing moisture and chloride ingress. This is particularly beneficial for critical infrastructure such as bridges, highways, and marine structures, where long-term durability is essential. Additionally, the integration of fly ash and micro silica not only enhances strength and sustainability but also contributes to lower overall material costs by reducing reliance on cement.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest

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