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Sustainable bio-concrete performance and durability using calcite precipitating bacteria along with fly ash

ABSTRACT

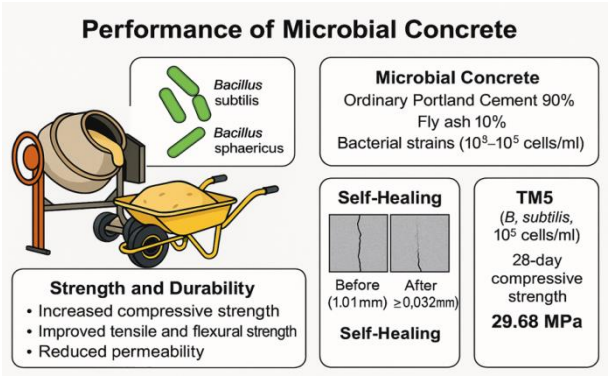
The effect of Bacillus subtilis and Bacillus sphaericus bacteria on the mechanical properties of M20 grade concrete mixed with 10% fly ash as a partial replacement for cement has been analyzed in this study. Total eight samples of concrete were produced with one control (TM1), one with fly ash (TM2) and rest of the six concrete samples with bacterial addition having concentration from 10^3 to 10^5 cells/ml. TM5 (B. subtilis, 10^5cells/ml) sample shows highest compressive (29.68 MPa), tensile (1.90 MPa) and flexural strength (2.60 MPa) due to microbial-induced calcite precipitation (MICP) that caused matrix densification. Durability characteristics were also found to be significantly improved in bacterial concrete. TM5 and TM8 have shown lowest permeability value (3.10 and 3.12 × 10^{-9} cm/s) and RCPT (1632 and 1507 coulombs) respectively due to lower chloride ion penetration in concrete. Resistance to sulphate was also increased; TM5 has shown least percentage weight loss (4.13%) after 90 days. SEM analysis showed that there is denser microstructure and deposition of calcite particles in bacterial concrete. Overall, the addition of B. subtilis bacteria (10^5cells/ml) with 10% fly ash improves the mechanical performance, durability and microstructure of bio-concrete.

Keyword: *Bacillus subtilis, Bacillus sphaericus, Strength, Durability, Self-healing*

1. INTRODUCTION

Self-healing concrete refers to an advanced material used in dealing with one of the most common problems associated with concrete, which is cracking, leading to reduced durability and lifetime of buildings [1]. This unique material has the ability to heal on its own through the integration of mechanisms that lead to self-healing of cracks [2]. There are various ways of achieving self-healing, such as using bacteria to produce calcium carbonate crystals, using capsules containing agents that will automatically activate upon cracking, and expansive minerals that seal cracks on their own [3]. This innovative material prevents crack formation and propagation, thus making the building more durable in harsh environments while minimizing the frequency of maintenance and repair actions [4]. Self-healing concrete greatly helps in creating sustainable constructions as it leads to lower usage of materials and reduced repair activities [5, 6].

Graphical Abstract



Self-healing concrete may typically be broadly categorized into two types: autogenous (intrinsic) and autonomous healing methods [7]. The autogenous self-healing method utilizes the natural property of concrete to heal small cracks through continuous hydration of the hydratable cement particles and recrystallization of the calcium carbonate. [8, 9] Such a natural process of self-healing may be assisted with the use of supplementary cementitious materials, like fly ash, silica fume, and appropriate polymers, which help

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to increase efficiency of crack sealing and decrease permeability. [10] In contrast to this, the autonomous self-healing method utilizes external healing agents, like bacteria, polymers, or minerals, which get activated once a crack occurs and trigger a reaction [11, 12].

From autonomous healing techniques, bacterial self-healing concrete is perhaps one of the most advanced technologies because of the capability of these organisms to biologically precipitate calcium carbonate crystals within the cracks [13]. In this case, bacterial spores and nutrients like calcium lactate are embedded in the concrete. Moisture ingress into the concrete results in the metabolic activation of the bacteria and conversion of nutrients into calcium carbonate crystals through microbial induced calcite precipitation (MICP) [14, 15]. This calcite precipitates and fills the cracks, making the concrete water-proof and more durable. Prior research has shown that the self-healing capacity of bacteria is better than other materials used for concrete [16, 17]. In addition, bacterial self-healing concrete reduces maintenance costs and makes concrete infrastructure sustainable [18, 19].

However, the use of supplemental cementitious materials (SCMs), especially fly ash, has greatly improved the effectiveness of bacterial self-healing concretes through the improvement of mechanical and durability properties. The fly ash, which is the byproduct of the burning of coal, has pozzolanic properties, thus it can react with the calcium hydroxide that is produced in the hydration reaction with the formation of extra calcium silicate hydrate (C–S–H). The result of such reactions is the refinement of the pore structure, the decrease in porosity, and the strengthening of the ITZ, consequently improving the strength and decreasing the permeability of the concrete [20, 21]. Additionally, due to the fine grain size of fly ash, the particle packing becomes better, forming a dense cement matrix that is more conducive to the survival of bacteria and the process of MICP.

In the same way, super absorbent polymers (SAPs) have received significant attention as internal curing agents, which can increase the self-healing potential of concrete. Super absorbent polymers absorb large amounts of water during mixing and release the accumulated moisture during cement hydration and cracking. Such availability of water results in an additional hydration of remaining cement grains, bacteria metabolism, and precipitation of calcite inside the cracks. As a result, SAPs increase the effectiveness of crack closure, reduce water permeability and improve the durability of self-healing concrete [22, 23]. However, despite encouraging results in laboratory conditions, the performance of bacterial concrete made with fly ash and SAPs in the field is not yet well-understood enough. Changes in temperature, humidity, wetting/drying cycles, sulphates or chlorides could affect the viability of bacteria, calcite precipitation and overall efficiency of self-healing. Hence, it is necessary to conduct more experiments to evaluate the durability and reliability of such sustainable materials under real-life conditions [24, 25].

Recent research indicates that sustainable bio-based techniques have gained much interest in the past few years as possible solutions to minimizing the environmental effects of concrete production [26]. Among these bio-based techniques, one of the most promising techniques is the use of microbial-induced calcite precipitation (MICP) which is used as an environmentally sustainable approach to produce concrete with improved durability. The technique uses calcite-producing bacteria to precipitate calcium carbonate in the pore spaces and cracks, hence increasing the efficacy of crack healing, density of the matrix, compressive strength, and resistance against aggressive environmental factors [27]. Bacterial mineralization occurs in an efficient natural way compared to the traditional concrete repair techniques and uses very low energy with reduced maintenance needs for the entire lifecycle of the concrete structure. Also, the use of supplementary cementitious materials like fly ash and GGBS reduces ordinary Portland cement which leads to reduced carbon dioxide emissions during cement production and improves sulphate resistance and resistance to chloride ion penetration [28, 29].

Although a lot of research works have been conducted on bacterial self-healing concrete, most of the previous studies mainly focused on the crack-healing efficiency or the application of a single bacterial species. Few studies systematically compare the combined effect of various bacteria species and bacterial concentrations in fly ash modified concrete. A comprehensive evaluation is needed that combines fresh concrete performance, mechanical properties, permeability, resistance to chloride ion ingress, resistance to sulphate attack, ultrasonic pulse velocity (UPV) and microstructural features into one experimental program. Moreover, the comparative effectiveness of Bacillus subtilis and Bacillus sphaericus in microbial-induced calcite precipitation and enhancement of performance of concrete is not yet established sufficiently. Thus, this lack of systematic investigation limits the determination of optimum bacterial species and concentration for the production of durable and sustainable bio-concrete.

Thus, the current research work focuses on the effect of the bacteria Bacillus subtilis and Bacillus sphaericus in different bacterial concentrations ranging from 10^3 to 10^5 cells/ml along with 10% fly ash used as the replacement material for the cement in M20 bio-concrete. The study focuses on finding out the optimal bacterial concentration that is required to increase the calcite precipitation and thus improve the properties of concrete.

To this end, eight concrete mixtures including a control mix, a fly ash reference mix and six bacterial concrete mixes were prepared and tested experimentally. Fresh concrete properties were assessed by slump and compaction factor tests while hardened concrete performance was assessed by compressive strength, split tensile strength and flexural strength tests. The durability performance was studied through permeability, RCPT, sulphate resistance and UPV tests. SEM was also used to study calcite formation and densification of the matrix as a result of bacterial activity. The results of this study provide an in-depth knowledge on the synergistic effects of fly ash and calcite producing bacteria and develop an optimized bio-concrete mixture that can improve the structural performance, durability and sustainability for resilient infrastructure applications.

This study's results are anticipated to aid in the development of sustainable self-healing concrete technology through the formulation of an optimized bacterially-based concrete that enhances not only its mechanical and structural properties but also its durability at minimal environmental cost compared to the traditionally used cement-based building materials.

2. MATERIALS AND METHODS

Self-healing concrete development for M20 grade targets an optimal combination of strength, durability, and crack-healing ability through novel materials and techniques. M20 concrete with a mix proportion of 1:1.5:3 (cement : sand : coarse aggregate) and a design compressive strength of 20 MPa is a typical reference standard for evaluating self-healing technologies [30]. 33 grade Ordinary Portland Cement (OPC) is employed as the main binder, providing consistent mechanical properties. Fine Aggregate with Clean and graded sand as per IS 383:2016 requirements is utilized. Coarse Aggregate with Crushed aggregates with maximum size of 20 mm and following IS 2386:1963 requirements is used. Potable water as per IS 456:2000 requirements is utilized for mixing and curing. Supplementary Cementitious Materials, Flyash are incorporated to enhance pozzolanic reaction, decrease permeability, and increase crack-sealing capacity. Superabsorbent Polymers (SAP): These are incorporated to promote self-healing by absorbing and holding water in the concrete matrix, inducing prolonged hydration. Bacteria (for MICP), Spore-forming bacteria like Bacillus subtilis and Bacillus sphaericus are incorporated with nutrients (e.g., calcium lactate) to induce precipitation of calcium carbonate under water. The concrete mix is proportioned according to IS 10262:2019 guidelines for M20 grade, ensuring proper workability and compressive strength. Fly ash substitutes 10% of the cement content to enhance durability and self-healing effectiveness.

Bacteria and nutrients are combined with the cementitious materials. Encapsulation techniques is a gel carriers encapsulate the bacteria during mixing and activation. Encapsulated Healing Agents, Microcapsules are consistently distributed throughout the mix to provide sufficient coverage. When cracks form, the capsules break and release their contents, sealing the cracks chemically or physically. The samples are cured under normal conditions (28 days) or exposed to wet-dry cyclic conditions to represent actual applications and trigger self-healing processes. The performance of self-healing concrete is assessed based on, mechanical behavior to check if the degradation meets the M20 grade condition. Durability Tests for the strength of self-healed cracks to withstand water leakage Crack Width Recovery from microscopic observation to determine the closure efficiency. Through the incorporation of novel materials and techniques, self-healing concrete for M20 grade unites mechanical strength with independent crack repair, opening doors to more sustainable and resilient construction materials. Figure 1 illustrates the methodology of this study. Table 1 illustrates the mix proposition of different mix.

Table 1. Mix proposition of various mix

Mix	Description	Cell Concentration
TM1	Conventional Concrete	-
TM2	90% OPC + 10% Flyash	-
TM3	90% OPC + 10% Flyash + Bacillus subtilis	10^3
TM4	90% OPC + 10% Flyash + Bacillus subtilis	10^4
TM5	90% OPC + 10% Flyash + Bacillus subtilis	10^5
TM6	90% OPC + 10% Flyash + Bacillus sphaericus	10^3
TM7	90% OPC + 10% Flyash + Bacillus sphaericus	10^4
TM8	90% OPC + 10% Flyash + Bacillus sphaericus	10^5

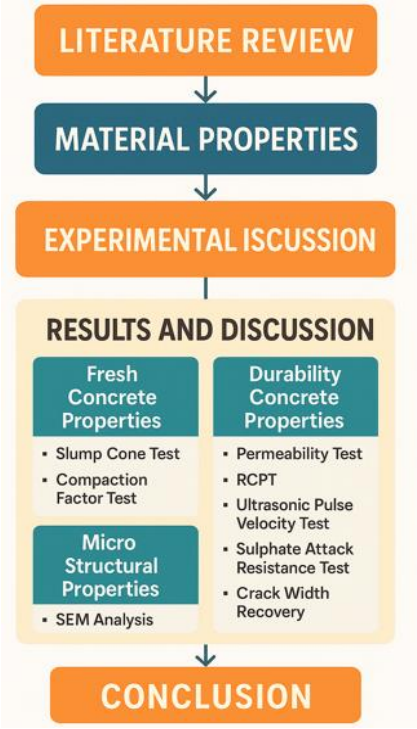


Figure 1. Methodology of this research

3. RESULTS AND DISCUSSION

3.1. Slump Cone Test

Slump test results show the effect of the presence of fly ash and bacteria on the workability of fresh concrete mixes. In the case of the control concrete mix TM1, a slump of 122 mm was observed, implying satisfactory workability and consistency of the concrete mix. In the case of 10% addition of fly ash in TM2, there is an increase in slump to 125 mm. This is because of the sphere shape and smooth surface nature of fly ash. This results in a "ball-bearing effect," whereby the internal friction between cement particles is reduced due to the smoothness, leading to increased flowability and cohesiveness of the concrete mix. [31].

The slumps of bacterial concrete with the bacterial species Bacillus subtilis (TM3-TM5) and Bacillus sphaericus (TM6-TM8) were between 119-124 mm, which demonstrates slight differences in comparison with the control and fly ash samples. [32] Inclusion of bacteria in concrete at concentrations of 10^3-10^5 cells/ml practically did not influence on the workability of fresh concrete. Moreover, even at the higher bacterial content of TM4, TM5, TM7, and TM8 the slumps were quite consistent, which means that the bacterial inclusion does not change the rheological properties of the concrete paste. The slight decrease in slumps could be explained by the fact that the bacterial cells with spores, nutrients slightly increase the amount of solids in the paste.

Thus, the test results demonstrate that simultaneous inclusion of fly ash and bacteria provides favorable conditions for further improvement of the concrete characteristics without decreasing its workability. The relative constancy of the slumps of all mixes proves that the inclusion of bacteria does not affect the performance of the fresh concrete. The corresponding slumps of all mixes are presented in figure 2.

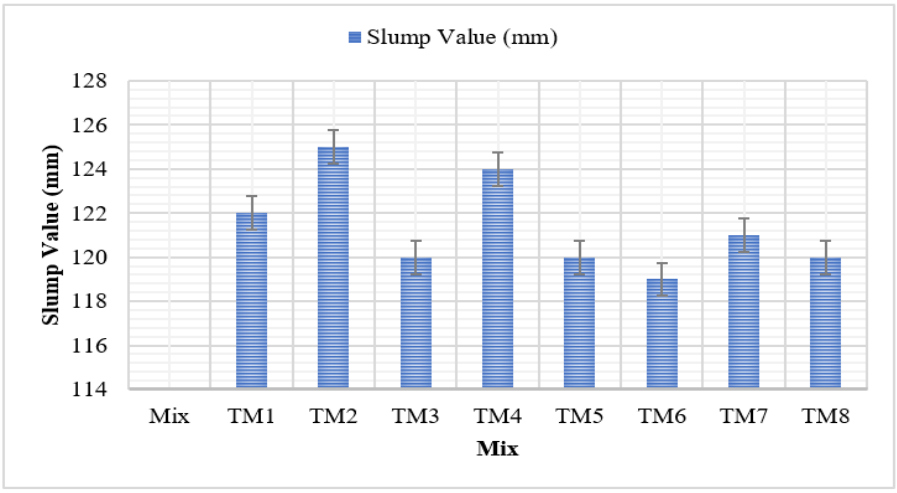


Figure 2. Slump value

3.2. Compaction Factor Test

The compaction factor test serves as an effective parameter for testing the workability and compaction properties of the concrete, especially those having lower or moderate workability. The values found from different mixes of concrete suggest that the use of fly ash and bacteria did affect the compaction property of fresh concrete. For instance, the control mix (TM1) showed a

compaction factor of 0.938, which suggests that the mix had moderate workability and compactability. With the addition of 10% fly ash in TM2, the value of compaction factor rose to 0.962. Such an increase in the compaction factor is because of the spherical shape and fine size of the fly ash particles, reducing the internal friction between cement particles [33].

The concrete mixes with Bacillus subtilis (TM3-TM5) had a compaction factor ranging from 0.923 to 0.954. Out of the three mixes, TM4 having 10^4 cells/ml gave the highest compaction factor of 0.954 implying that this bacterial cell density gives favorable conditions for good workability coupled with dispersion throughout the cement matrix [34]. However, there was a small reduction in the compaction factor for both lower (10^3 cells/ml) and higher (10^5 cells/ml) bacterial concentrations. This could be due to change in the solid content as well as interaction between the bacterial spores, nutrient and cement particle which slightly influences the fresh concrete flow characteristics.

On the other hand, the compaction factor of Bacillus sphaericus mixes (TM6-TM8) varied from 0.915 to 0.931. Out of these mixes, TM7 having 10^4 cells/ml gave the highest compaction factor of 0.931. From the above analysis, it is evident that the same trend was followed by the two bacteria with regard to compaction factor. In this case, an intermediate bacterial density proved to be more beneficial in improving workability of fresh concrete compared to both lower and higher bacterial densities.

In summary, the results clearly show that, although fly ash greatly improves the workability and compaction properties of the concrete, the incorporation of bacteria only brings about small changes which do not in any way compromise the properties of fresh concrete. This implies that the production of bio-concrete utilizing bacteria can be done by following the normal concrete mixing and compaction techniques without any loss in workability, as long as the bacterial content is properly controlled.

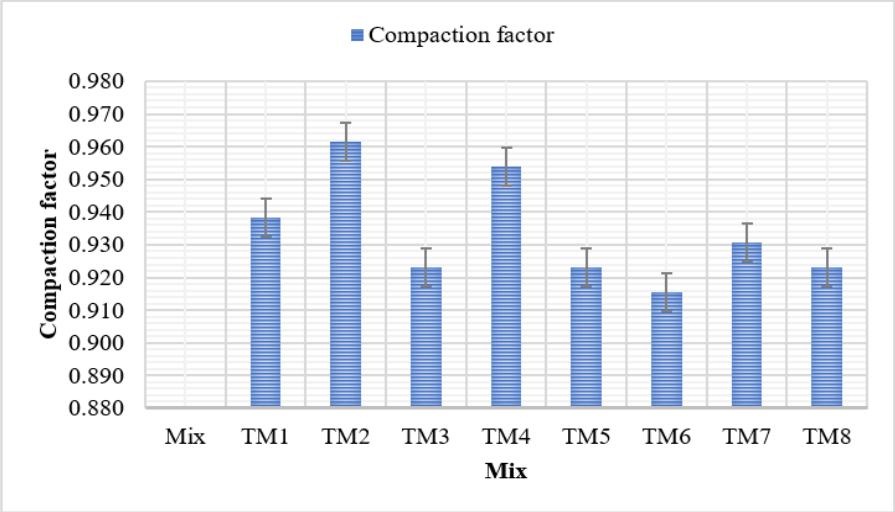


Figure 3. Compaction factor value

3.3. Compressive Strength Test

The performance of various concrete mixes at 7, 14 and 28 days is shown by the results of compressive strength test. TM1 (normal concrete) registered 25.57 MPa at 28 days while TM2 (10% fly ash) had a small increase to 27.11 MPa which is the beneficial aspect of fly ash in the increase in strength. The compressive strength of Bacillus subtilis mixtures (TM3-TM5) was higher than the control samples in all curing ages. Highest strength (29.68 MPa at 28 days) was observed for TM5 with the highest cell density (10^5 cells/ml). The gradual increase in bacterial concentration steadily increased strength, showing that the microbially induced calcite precipitation (MICP) was responsible for the enhanced bonding within the matrix. The Bacillus sphaericus (TM6–TM8) containing mixes also gave better performance than the control mix (TM1) and the fly ash mix (TM2).

TM8 at a cell concentration of 10^5 cells/ml presented 28.81 MPa at 28 days. But the relative difference in microbial efficiency in terms of the increase in strength over Bacillus subtilis mixes [35]. Typically, the addition of bacteria strongly improved the compressive strength, and higher cell concentrations gave better results. These results are indicative of the potential of microbial concrete to improve durability and sustainability. The value of compressive strength is shown in Figure 4.

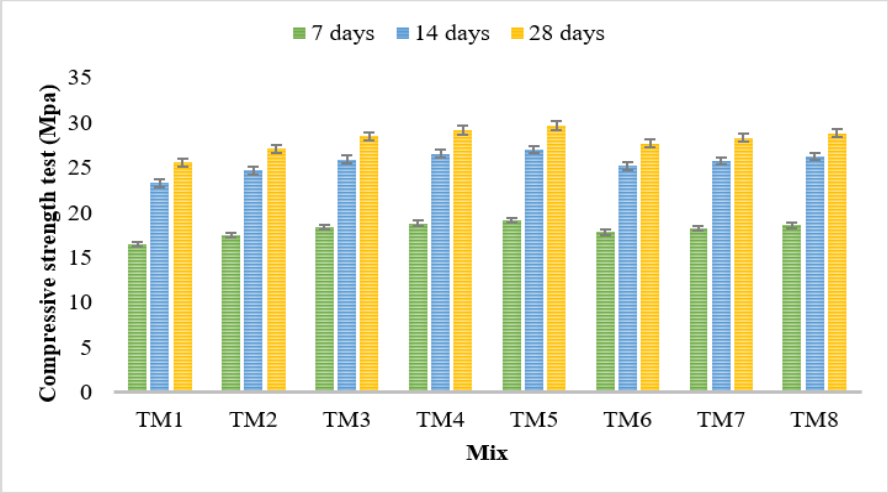


Figure 4. Compressive strength value

3.4. Split Tensile Strength Test

The results of the split tensile strength tests show the effect of various mixes on tensile strength at 7, 14, and 28 days. TM1 (normal concrete) showed 1.63 MPa at 28 days and TM2 with 10% fly ash showed improved 1.73 MPa due to increased pozzolanic activity and matrix densification. Concrete mixes containing Bacillus subtilis (TM3-TM5) were superior to the control and fly ash mixes for all ages of curing.TM5, which has the maximum cell density (10^5 cells/ml), achieved the highest tensile strength of 1.90 MPa at 28 days. This is due to microbial-induced calcite precipitation (MICP), which improves the bond between aggregates and cement paste.

Concurrently, Bacillus sphaericus mixes (TM6-TM8) showed higher tensile strength than TM1 and TM2, and TM8with 10^5 cells/ml recorded 1.84 MPa at 28 days. The improvement in strength was somewhat lower than in the Bacillus subtilis series, suggesting that there may be a difference in microbial action or mix compatibility [36]. Overall, microbial addition drastically improved tensile strength with increasing cell concentrations showing improved performance. These observations support the viability of microbial concrete to enhance tensile properties.Figure 5 is the split tensile strength result.

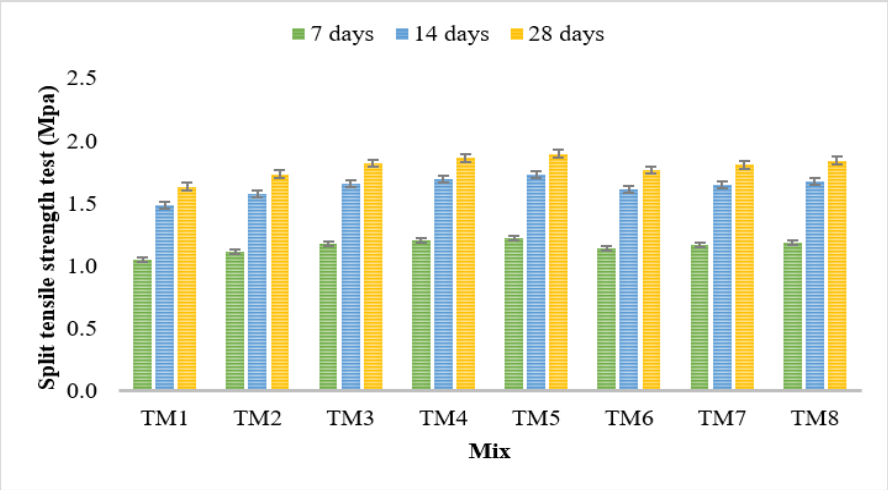


Figure 5. Split tensile strength value

3.5. Flexural Strength Test

The flexural strength tests show the performance of the concrete mixes when subjected to bending stress at 7, 14, and 28 days. TM1 (plain concrete) had a flexural strength of 2.24 MPa at 28 days. TM2, with 10% fly ash, was better at 2.37 MPa because it had improved matrix refinement as well as greater interfacial bonding. Mixes with

Bacillus subtilis (TM3-TM5) exhibited significant increases in flexural strength. TM5, having the highest bacterial density (10^5 cells/ml), also produced the highest strength of 2.60 MPa at 28 days, 16% higher than the control mix. The gain in strength is attributed to microbial-induced calcite precipitation (MICP), which involves matrix densification and bridging cracks.

Blends with Bacillus sphaericus (TM6-TM8) also showed enhanced flexural strength, with TM8 (10^5 cells/ml) having 2.52 MPa at 28 days. While the improvement in strength was less than in the Bacillus subtilis mixes, it nevertheless confirmed the viability of microbial addition in enhancing flexural performance [37]. The findings validate the enhancement of flexural strength by bacterial inclusion, where the most favorable improvements occurred at enhanced cell levels. These results indicate the efficiency of microbial concrete to enhance the resistance against bending stresses.Figure 6 represents the flexural strength value.

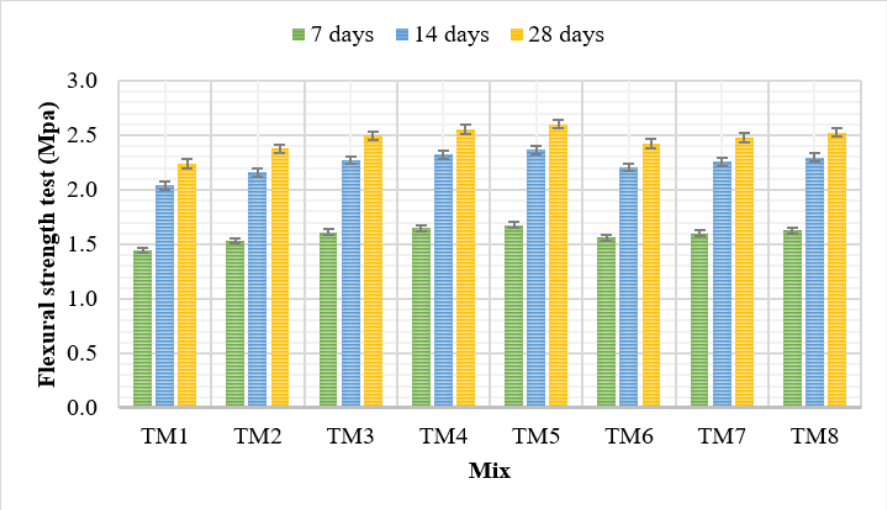


Figure 6. Flexural strength value

3.6. Permeability Test

The permeability test results show the capability of different concrete mixes to hold back water intrusion after 28, 56, and 90 days. TM1 (ordinary concrete) recorded permeability of 6.92×10−9 cm/sec6.92 \\times 10-9 {cm/sec} 6.92× x10−9 cm/sec after 90 days. TM2, with 10% fly ash, had lower permeability (6.80×10−9 cm/sec 6.80 \\times 10-9{cm/sec}6.80×10−9 cm/sec) as a result of the pozzolanic action and finer pore structure. The Bacillus subtilis (TM3-TM5) mixes showed uniformly lower permeability than the control and fly ash mixes. In fact, TM5 prepared at 10^5 cells/ml showed a lowest value of 6.65×10−9 cm/sec6.65 \\times 10-9{cm/sec}6.65×10−9 cm/sec at 90 days. It is considered that this reduction is caused by MICP, which has successfully filled micro-cracks and lowered porosity.

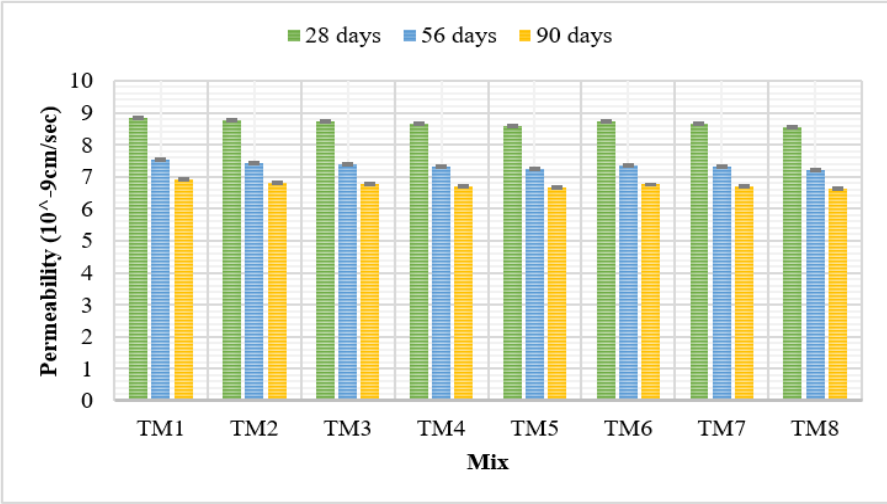


Figure 7. Permeability test value

In the same manner, Bacillus sphaericus (TM6-TM8) mixes showed better impermeability. TM8, which had the highest cell density, achieved a 6.62 × 10^{-9} cm/s value that was slightly better than TM5. Both bacterial strains are shown to improve impermeability, although Bacillus sphaericus performed slightly better at higher densities [38]. The findings affirm that permeability is greatly reduced with bacterial addition, making the compound more durable by preventing water intrusion and possible structural damage due to environmental exposure. Figure 7 depicts the permeability test value.

3.7. RCPT

The results of the Rapid Chloride Penetration Test (RCPT) indicate the chloride ion penetrability of different mixes of concrete at 28, 56, and 90 days. TM1 (normal concrete) had a chloride ion penetration of 1796 coulombs at 90 days, which is moderate permeability. TM2, with 10% fly ash, had less penetration to 1734 coulombs, reflecting the densification of the concrete matrix as a result of pozzolanic activity. Mixes containing Bacillus subtilis (TM3-TM5) had uniformly lower chloride penetration values than TM1 and TM2. TM5 (10^5 cells/ml) obtained the lowest value of 1632 coulombs at 90 days, proving the efficacy of microbial-induced calcite precipitation (MICP) in pore sealing and increasing resistance to chloride ingress.

Likewise, Bacillus sphaericus blends (TM6-TM8) showed enhanced resistance, with TM8 (10^5 cells/ml) having the minimum penetration value of 1507 coulombs at 90 days. This shows better performance by Bacillus sphaericus with increased concentrations, perhaps owing to its effectiveness in MICP and matrix densification [39]. Bacterial inclusion overall greatly reduced chloride permeability, with increased cell concentration showing better results. These results validate the ability of microbial concrete to enhance durability in environments with chlorides. The RCPT value is illustrated by Figure 8.

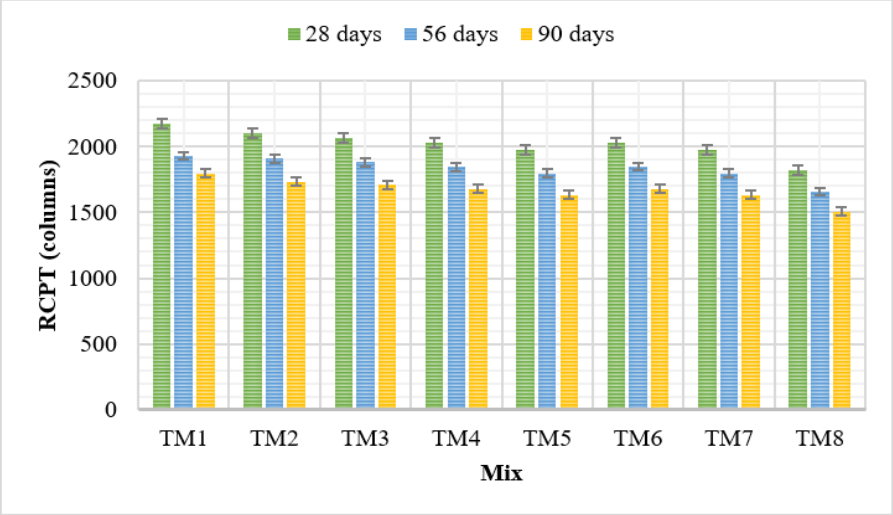


Figure 8. RCPT value

3.8.Ultrasonic Pulse Velocity Test

UPV values for various concrete mixes represent the internal density and compactness of different M20 concrete mixes after 28, 56, and 90 days. The normal concrete mix (TM1) displayed the highest value of UPV, which was 4.12 km/s at 28 days and gradually decreased to 3.83 km/s at 90 days. Fly ash concrete (TM2) contained 10% of fly ash, displaying lower UPV values (4.08 to 3.80 km/s). It implies that there were lower values of compactness, but improved matrix refining due to pozzolanic action.

In contrast to this, the microbial concrete containing Bacillus subtilis (TM3-TM5) demonstrated low values of UPV. For instance, TM5 having the largest number of bacteria cells (10^5 cells/ml) had an UPV of 3.42 km/s at 28 days and gradually lowered up to 3.10 km/s after 90 days. It may be concluded that even though the microbial action causes self-healing and densification with age, the initial values of pulse transmission are somewhat affected by the presence of bacteria due to bio-pores and retarded calcite deposition.

Additionally, blends with Bacillus sphaericus (TM6–TM8) presented decreased UPV relative to the control. TM6, with 10^3 cells/ml, recorded the highest UPV amongst microbial blends (3.69 km/s at 28 days, decreasing to 3.34 km/s at 90 days), which signifies improved internal consolidation at

lower dosages [40]. Greater dosages of bacteria (TM7 and TM8) yielded increasingly lower UPV measurements, which may be because of excessive microbial colonies hampering homogeneity in the concrete matrix.

In general, microbial incorporation yielded lower UPV values than regular concrete. The reduction is not necessarily negative because it could be counteracted by increased self-healing and durability properties. Figure 9 shows the UPV trends of all mixes and cure times.

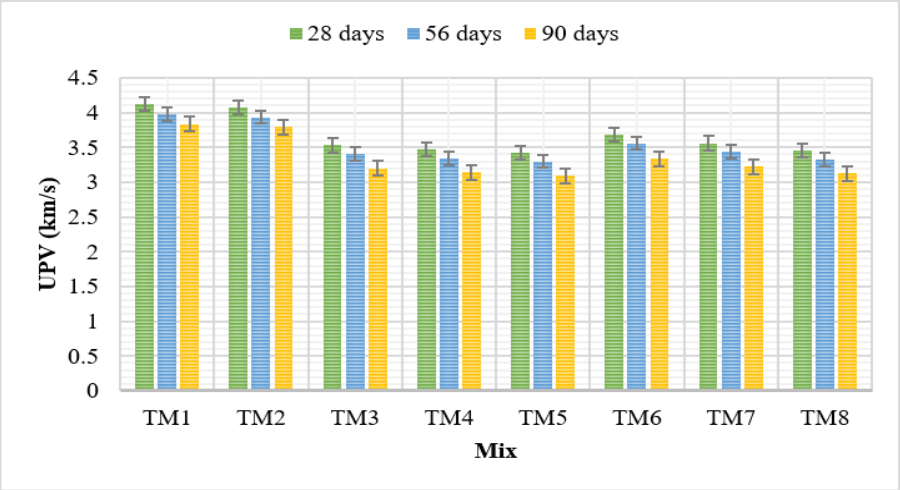


Figure 9. Ultrasonic pulse velocity

3.9. Sulphate Attack Resistance Test

The sulphate attack resistance of the concrete mixes was determined based on the percentage weight loss at 28, 56, and 90 days. TM1 (plain concrete) showed the highest sulphate-induced deterioration, with a weight loss of 5.48% at 28 days, decreasing to 4.61% at 90 days. This suggests moderate susceptibility to sulphate exposure in the long term. TM2 with 10% fly ash exhibited limited improvement with a slightly reduced weight loss of 4.58% at 90 days as a result of enhanced matrix densification by the pozzolanic reaction.

The incorporation of Bacillus subtilis in blends TM3–TM5 led to a gradual decrease in sulphate attack. TM5, having the highest bacterial content (10^5 cells/ml), indicated the least weight loss of 4.13% at 90 days, indicating improved resistance. The MICP caused by microorganisms is believed to have filled micro-pores and fissures, limiting access of sulphate ions and enhancing the durability.

Conversely, Bacillus sphaericus (TM6–TM8) mixes showed better performance than the control. TM8, with 10^5 cells/ml, had 4.32% weight loss at 90 days, showing a better protection, albeit less effective than Bacillus subtilis at the same level [41]. TM5, among all the mixes, was the best, verifying the positive effect of greater microbial concentration in withstanding sulphate attacks.

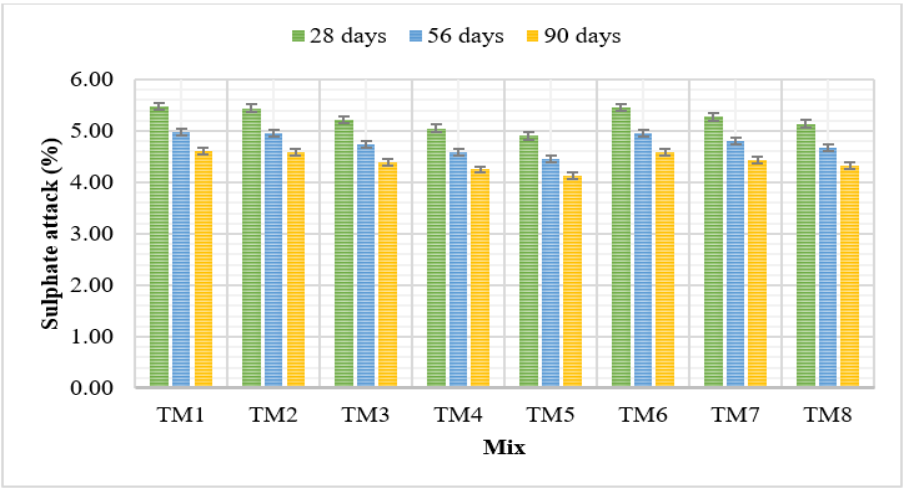


Figure 10. Loss of weight after sulphate attack test

Generally, the sulphate degradation of the mixes was reduced with increasing concentrations of bacteria. This is indicative of the effectiveness of MICP in preventing chemical deterioration and prolonging the durability of concrete in sulphate-bearing environments. Figure 10 presents the sulphate attack resistance of all mixes and periods.

3.10. Crack Width Recovery Test

The values of crack width indicate the self-healing capacity of different concrete mixtures after a period of 28 days. TM1 (normal concrete) and TM2 (with 10% fly ash) showed almost no reduction in crack width at 28 days with ultimate widths of 1.009 mm and 1.012 mm respectively, indicating low self-healing capacity. The mixtures with Bacillus subtilis (TM3–TM5) exhibited high reductions in crack width. TM5 showed the highest concentration of bacteria (10^5 cells/ml) and the least final crack width of 0.032 mm after 28 days, indicating good MICP for crack sealing. TM3 and TM4 also showed better healing with final widths of 0.045 mm and 0.038 mm respectively showing the effect of concentration of bacteria.

At the same time, there was an increase in the ability for self-healing by Bacillus sphaericus blends (TM6-TM8). For example, TM8, which had 10^5 cells/ml, reduced the crack width to 0.035 mm after 28 days; although it was slightly weaker than TM5, it was still a considerable improvement. The widths for TM6 and TM7 were 0.042 mm and 0.038 mm respectively [42]. On the whole, it can be stated that the addition of bacteria greatly improved the ability for self-healing of the material; higher cell concentrations produced better results.

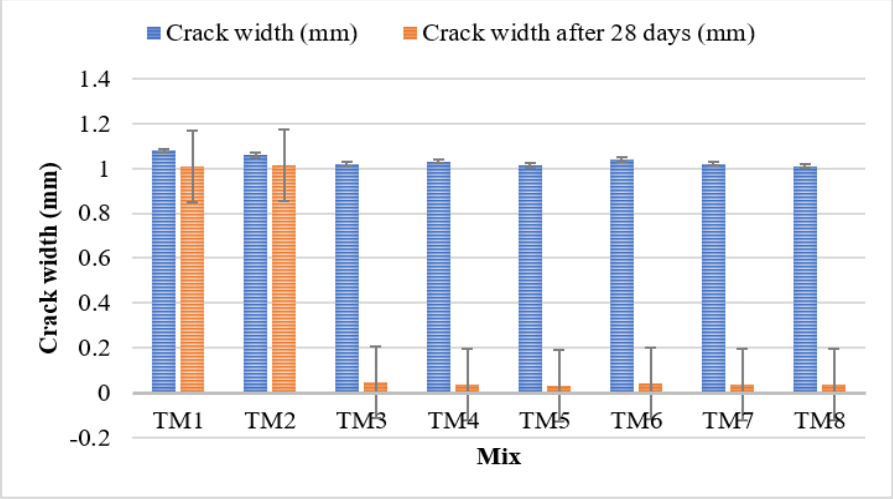


Figure 11. Crack width of concrete before and after healing

3.11. SEM Analysis

The microstructure development of the control mixes TM1 to TM8 was analyzed using Scanning Electron Microscopy (SEM), which would be helpful for understanding the influence of microbial activity and pozzolanic addition on the formation of hydration product morphology and densification of the pores. Figure 12(a) gives a microstructure of the control mix TM1 (traditional concrete), which possesses a highly porous and loosely-packed microstructure along with uneven distribution of the hydration products. Micro-cracks and unreacted cement particles can be observed, thus implying the insufficient hydration process and weaker matrix [43]. Figure 12(b) (TM2: 90% OPC + 10% fly ash) gives a finer microstructure but with tight packing due to the pozzolanic reaction products, with the presence of micro-voids.

Contrarily, the microstructures in bacterial concrete composites (TM3-TM8) have improved significantly. As can be seen from Figure 12 (c) (TM3: Bacillus subtilis, 10^3 cells/ml), the initial steps of MICP, such as pore filling and formation of needle-like structures similar to ettringite and the initial formation of C-S-H gel, have been realized. In the figures below (Figure 12(d)-Figure 12(e); TM4 and TM5; 10^4 and 10^5 cells/ml, respectively), as the concentration of Bacillus subtilis increases, there is an evident densification of the microstructure of the samples.

Similarly, there is enhancement in the morphology of suspensions containing Bacillus sphaericus (TM6-TM8). As shown in Figure 12 (f) (TM6: 10^3 cells/ml), the initial steps of biomineralization have been achieved, whereas Figure 12(g)-Figure 12(h) (TM7 and TM8; 10^4 and 10^5 cells/ml, respectively) depict highly crystallized forms of calcite within the C-S-H gel matrix. Importantly, TM8 is characterized by dense and homogeneous microstructure, and hence, increased cell concentration is effective in improving MICP and filling of capillary pores.

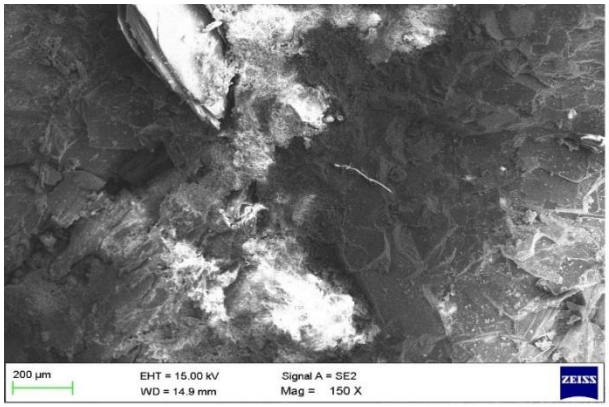


Figure 12(a). SEM of TM1 mix

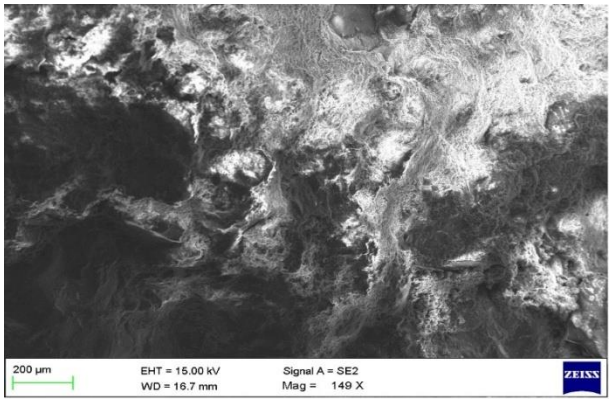


Figure 12(b). SEM of TM2 mix

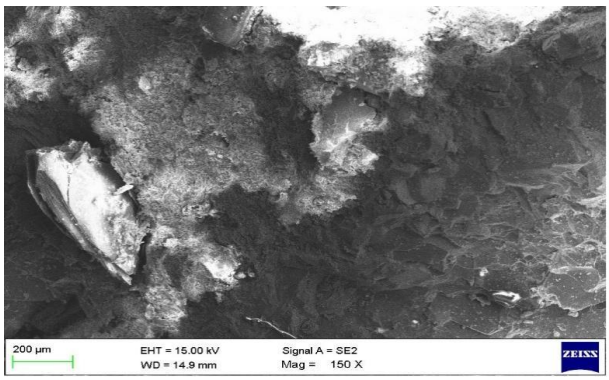


Figure 12(c). SEM of TM3 mix

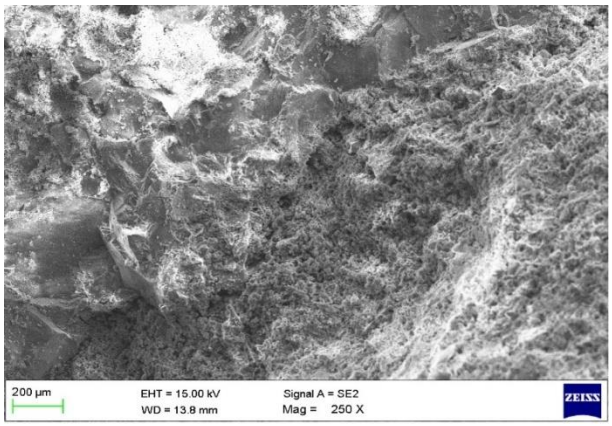


Figure 12(d). SEM of TM4 mix

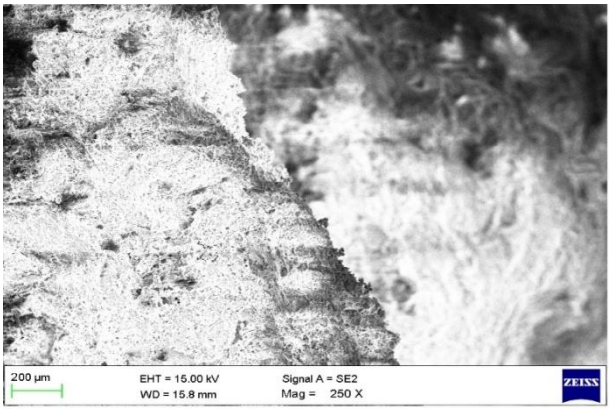


Figure 12(e). SEM of TM5 mix

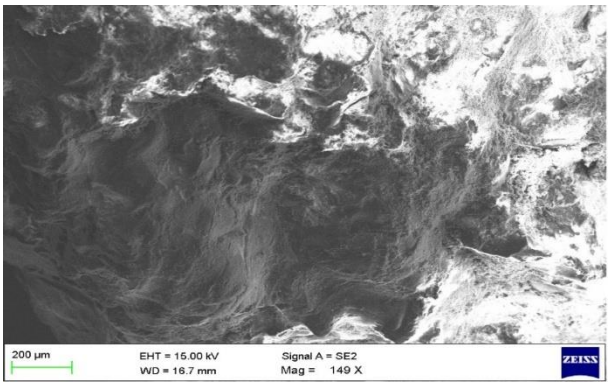


Figure 12(f). SEM of TM6 mix

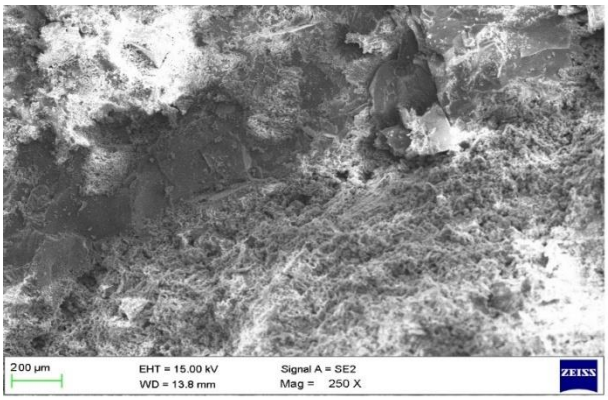


Figure 12(g). SEM of TM7 mix

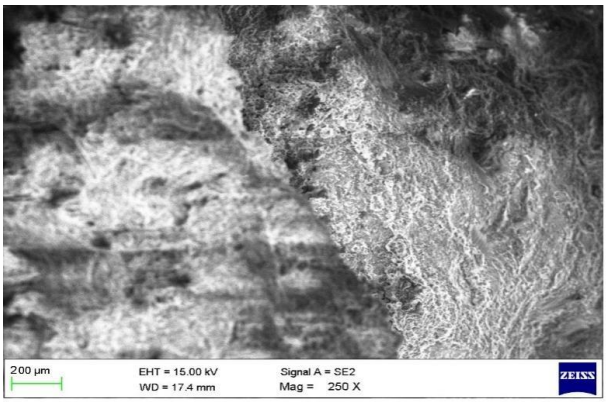


Figure 12(h). SEM of TM8 mix

In general, the SEM findings confirm that bacterial absorption, particularly when performed using high concentrations of bacteria (10^5 cells/ml), greatly contributes to improving the structural characteristics of concrete. This is attributed to the formation of large amounts of calcium carbonate and tightly packed C-S-H gel networks, resulting in superior strength, low permeability, and increased durability. It has been found that Bacillus sphaericus produces denser hydration products than Bacillus subtilis.

4. CONCLUSION

This experimentation studied the influence of using microorganisms, Bacillus subtilis, and Bacillus sphaericus, in a concrete mixture having a composition of 90% OPC and 10% fly ash. It was found that microbial concrete is highly beneficial to the efficiency of normal concrete in aspects such as workability, strength, durability, and impermeability.

Using bacteria had slight influence on the slump and compaction values, which are tolerable. Microbial concrete had great improvements in compressive strength, an efficiency parameter, when there was increased bacterial concentration. The highest 28-day compressive strengths of 29.68 MPa and 28.81 MPa were obtained by TM5 and TM8 containing 10^5 cells/ml of Bacillus subtilis and Bacillus sphaericus, respectively, compared to ordinary concrete (25.57 MPa). Equally, improvements were noted for split tensile strength and flexural strength, with TM5 and TM8 once more showing the best results.

The durability of microbial concrete was evidenced by reduced permeability and lower chloride ion penetration (RCPT). TM5 and TM8 exhibited the lowest permeability (6.62×10^{-9} cm/s to 6.65×10^{-9} cm/s, respectively) and the lowest RCPT values (1632 coulombs and 1507 coulombs, respectively, at 90 days). These improvements are attributed to microbial-induced calcite precipitation (MICP), which reduces porosity and seals cracks effectively.

The self-healing capabilities of microbial concrete further add weight to its credentials as an environmentally friendly building material. It is essential to decrease the crack width, and TM5 and TM8 reduced the initial crack widths from 1.01 mm to 0.032 mm and 0.035 mm, respectively, after 28 days.

Comparing between the two bacteria species, it is seen that Bacillus subtilis performed relatively well in increasing the strength and durability of concrete. Bacillus sphaericus also had distinct advantages in terms of impermeability and self-healing.

Maximum UPV was observed in TM1 (Control) which reflects maximum density while TM2 (10% fly ash) showed a slight decline. All other bacterial mixes (TM3-TM8) had a low value of UPV, with TM6 (10^3 cells/ml B. sphaericus) having the best one amongst the lot. Increase in bio-pores decreased the value of UPV due to an increase in bacterial content. Sulphate resistance of the mixes improved in the case of the bacterial mixes with TM5 (B. sphaericus) showing minimum weight loss (4.13%).

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IZVOD

PERFORMANSE I TRAJNOST ODRŽIVOG BIO-BETONA PRIMENOM BAKTERIJA KOJE TALOŽE KALCIT SA LETEĆEG PEPELA

*U ovoj studiji analiziran je uticaj bakterija *Bacillus subtilis* i *Bacillus sphaericus* na mehanička svojstva betona klase M20, sa dodatkom 10% letećeg pepela kao delimične zamene za cement. Izrađeno je ukupno osam uzoraka betona: jedan kontrolni (TM1), jedan sa letećim pepelom (TM2) i šest uzoraka sa dodatkom bakterija u koncentracijama od 10^3 do 10^5 ćelija/ml. Uzorak TM5 (*B. subtilis*, 10^5 ćelija/ml) pokazao je najveću čvrstoću pri pritisku (29,68 MPa), zatezanju (1,90 MPa) i savijanju (2,60 MPa), usled mikrobiološki indukovanog taloženja kalcita (MICP) koje je dovelo do zgušnjavanja matrice. Takođe je utvrđeno da su karakteristike trajnosti značajno poboljšane kod bakterijskog betona. Uzorci TM5 i TM8 pokazali su najniže vrednosti propustljivosti (3,10 i 3,12 × 10^{-9} cm/s) i RCPT vrednosti (1632 i 1507 kulona), usled manjeg prodiranja hloridnih jona u beton. Povećana je i otpornost na sulfate; uzorak TM5 pokazao je najmanji procenat gubitka mase (4,13%) nakon 90 dana. SEM analiza je pokazala gušću mikrostrukturu i taloženje čestica kalcita u bakterijskom betonu. Sveukupno, dodatak bakterija *B. subtilis* (10^5 ćelija/ml) uz 10% letećeg pepela poboljšava mehaničke performanse, trajnost i mikrostrukturu bio-betona.*

***Ključne reči**: *Bacillus subtilis*, *Bacillus sphaericus*, čvrstoća, trajnost, samozaceljivanje*

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