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Degradation Resistance of Bio-Concrete Containing Rice Husk Ash and *Bacillus Subtilis* under Acidic, Sulfate, and Elevated Temperature Exposure

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Abstract
This study investigated the degradation resistance of bio-concrete containing rice husk ash (RHA) and Bacillus subtilis when exposed to acidic, sulfate, and elevated temperature environments. Concrete mixes with 0%, 5%, 7.5%, and 10% RHA and B. subtilis [6 MacFarland] were tested for pore solution pH, residual compressive strength at 30 – 100°C, mass loss in 5% H_2SO_4 for 28 days, and strength loss in 5% Na_2SO_4 for 90 days. Results showed that RHA reduced pore pH from 12.6 to 11.3 due to pozzolanic consumption of $Ca(OH)_2$, but all mixes remained above pH 11.0, ensuring bacteria viability. Compressive strength increased up to 80°C for all mixes, with 10% RHA + 6 MF achieving 46 N/mm² at 80°C, 41% higher than the control. Acid attack mass loss decreased from 20% to 11%, while sulfate-induced strength loss decreased from 22% to 8% for the same mix. The improved performance is attributed to microbially induced calcite precipitation (MICCP) and secondary C-S-H formation, which densified the matrix and reduced permeability. The findings indicate that the synergy between RHA and B. subtilis enhances durability in aggressive environments, making 10% RHA + 6 MF bio-concrete suitable for infrastructure exposed to acid, sulfate, and moderate heat.

Keywords: *Bioconcrete, Bacillus subtilis, Rice Husk Ash, Acid Attack, Sulfate Attack, Elevated Temperature.*

1.0 Introduction

Concrete is susceptible to microcracking due to its low tensile strength, which reduces long-term durability when cracks allow ingress of aggressive agents (Ram *et al.*, 2023; Saridhe & Selvaraj, 2020). Bio-concrete addresses this limitation through microbially induced calcium carbonate precipitation (MICCP), where dormant bacteria spores are activated by moisture to precipitate calcite within cracks (Adetoye *et al.*, 2026a; Soda and Mini, 2023). This self-healing mechanism, combined with a lower carbon footprint, makes bio-concrete attractive for underground structures, water-retaining systems, and infrastructure in aggressive environments (Zhang et al., 2025).

Rice husk ash (RHA) is a silica-rich agricultural waste that acts as a pozzolan by reacting with $Ca(OH)_2$ to form additional calcium silicate hydrate (C-S-H) gel (Saad *et al.*, 2015). *Bacillus subtilis* is an alkaliphilic, spore-forming bacterium that survives the high-pH concrete matrix and precipitates $CaCO_3$, thereby reducing porosity and permeability. High temperature, acidic, and sulfate exposures are major causes of concrete degradation (Sani *et al.*, 2024; Mehta & Monteiro, 2017). Elevated temperatures accelerate hydration but cause moisture loss and microcracking above 80°C (Neville, 2011). Sulfuric acid dissolves $Ca(OH)_2$, while sodium sulfate reacts with C-A-H to form expansive ettringite and gypsum (Mehta & Monteiro, 2017). Although RHA and MICCP have been studied separately (Wang, 2017; Bilir & Yildirim, 2020), limited data exist on their combined effect on bio-concrete durability under simultaneous chemical and thermal attack. Microbial-induced calcite precipitation combined with pozzolanic activity of RHA enhances the densification of the concrete matrix, leading to improved durability (Adetoye et al., 2026b).

Therefore, this study evaluates the degradation properties of RHA - *B. subtilis* bio-concrete under acidic, sulfate, and 30 – 100°C exposures to determine optimum mix proportions for durable construction.

2.0 Literature Review

2.1 Elevated temperature effects on concrete

High temperature degrades concrete mechanical properties through dehydration of C-S-H and thermal incompatibility between paste and aggregate (Sani *et al.,* 2024). Although concrete has low thermal conductivity, prolonged exposure above 80 °C causes strength loss (Aydin, 2019). Mohammed *et al.* (2021) reported that a 10% ternary blend of RHA, metakaolin, and burnt brick retained 27.3% higher residual strength than control at 800°C. Wang (2017) found that optimum RHA concrete retained 50% more strength than conventional concrete at 800 °C, due to denser microstructure. Bilir and Yildirim (2020) showed that MICCP concrete had 13.76% higher residual strength than control at 400°C, attributed to $CaCO_3$ filling pores.

2.2 Chemical attack mechanisms

Acid attack dissolves $Ca(OH)_2$ and decalcifies C-S-H, causing mass loss and strength reduction (Mehta & Monteiro, 2017). Sulfate attack forms expansive gypsum and ettringite, leading to cracking and spalling (Mehta & Monteiro, 2017). RHA reduces free $Ca(OH)_2$ through pozzolanic reaction, limiting reactants for acid and sulfate attack (Saad *et al.,* 2015). MICCP deposits calcite in pores, which reduces permeability and slows ion ingress.

Existing studies have investigated RHA or MICCP separately under heat or chemical attack (Wang, 2017; Bilir & Yildirim, 2020). However, the combined effect of RHA and B. subtilis on pH balance, thermal stability, and chemical durability has not been systematically quantified using response surface methods. This study fills that gap.

3.0 Materials and Methods

3.1 Materials

Grade 42.5 Portland limestone cement conforming to ASTM C150 (2023) was used. RHA was produced by burning rice husk at 750°C and sieving through 150µm (Jafer *et al.,* 2020). Fine and coarse aggregates met BS 812 (2020) requirements, with specific gravity 2.65 and 2.68, respectively. Mixing water conformed to ASTM C1602 (2022). *B. subtilis* was cultured at ATBU Teaching Hospital and selected for its alkaliphilic spore-forming ability.

3.2 Mix design and specimen preparation

A Box–Behnken design was used to develop M25-grade bio-concrete with RHA at 0%, 5%, 7.5%, and 10% and *B. subtilis* at 2 to 6 MacFarland. Specimens were 100 mm cubes for compressive strength, 100 mm × 100 mm × 500 mm beams, and 100 mm × 200 mm cylinders.

3.3 Test methods

pH Test: Fresh concrete pH was measured using a calibrated probe.

Elevated Temperature: Specimens were heated at 30, 40, 60, 80, and 100°C for 1 hour and tested for residual compressive strength (Sani *et al.,* 2024).

Acid Attack: Specimens were immersed in 5% H_2SO_4 for 28 days and mass loss was recorded.

Sulfate Attack: Specimens were immersed in 5% Na_2SO_4 for 90 days and strength loss was measured.

4.0 Results and Discussion

4.1 Test results on aggregates

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The tests conducted on aggregates were aggregate gradation, specific gravity, bulk density, water absorption, fineness modulus aggregates crushing value and aggregate impact value. The result of the tests conducted on the physical properties of aggregates are shown in Table 1.

Table 1: Physical Properties of Aggregates

Property	Fine Aggregate	Coarse Aggregate
Specific gravity	2.65	2.68
Loose bulk density (kg/m^3)	1320	1232
Water absorption (%)	1.4	0.7
Aggregate crushing value (%)	-	16.5
Aggregate impact value (%)	-	5.6

Table 1 summarizes the key physical properties of the fine and coarse aggregates used in the study. These properties affect workability, strength, and durability of the concrete.

a. Specific Gravity: Fine and coarse aggregates of 2.65 and 2.68 values fall within the normal range of 2.5 – 2.8 for natural aggregates. This indicates dense, good quality materials with low porosity, which helps in producing concrete with good strength and low permeability.

b. Loose Bulk Density: Fine and Coarse aggregates of 1320 and 1232 kg/m^3 values reflect how much material fits in a unit volume when loosely placed. The higher density for fine aggregate is expected due to smaller particle size and better packing. Both are typical for clean, well-graded aggregates and are suitable for mix design calculations.

c. Water Absorption: It measures the porosity of the aggregates. Values below 2% are considered low and indicate aggregates with low moisture uptake. This means the aggregates will not significantly affect the water-cement ratio during mixing, and the concrete will have good dimensional stability.

d. Aggregate Crushing Value (ACV): Coarse aggregate only: 16.5% ACV measures the resistance of aggregate to crushing under gradually applied load. A value of 16.5% is well below the 30% limit for concrete works per BS 812, indicating the coarse aggregate is strong and suitable for structural concrete.

e. Aggregate Impact Value (AIV) for Coarse aggregate only: 5.6% AIV measures resistance to sudden impact. A value of 5.6% is low, showing the aggregate is tough and resistant to fragmentation. Values below 10% are considered excellent for concrete exposed to impact or heavy loading.

Both fine and coarse aggregates meet standard requirements for strength, density, and durability. The low water absorption, ACV, and AIV confirm that the aggregates are hard, dense, and durable, making them suitable for producing M25 grade bio-concrete with reliable performance.

4.1.1 Particle size distribution of aggregates

The gradation for fine aggregate was conducted and is shown in Figure 1. The result showed that it was within the permissible standard.

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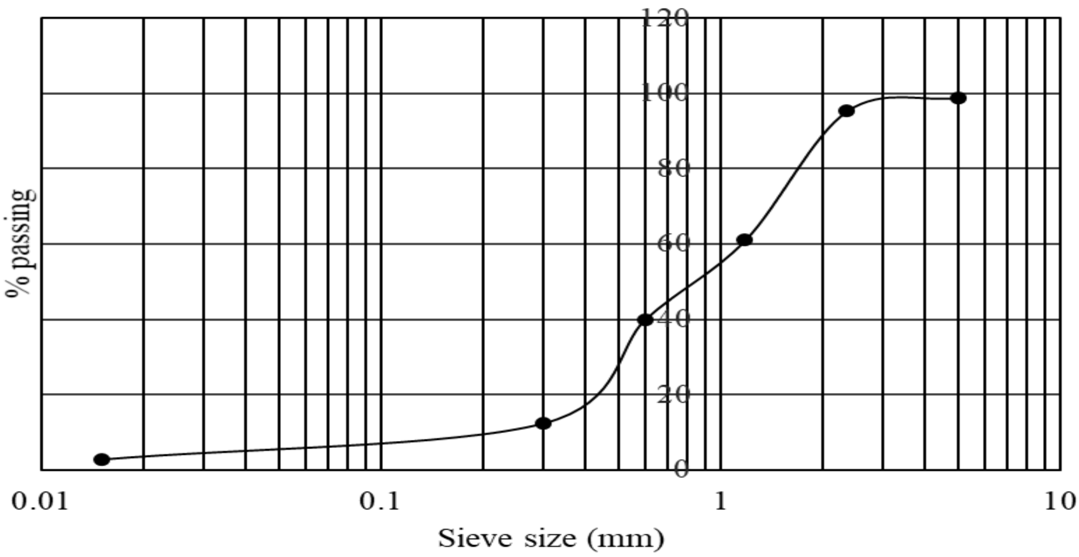

Figure 1: Percentage Passing of Fine Aggregates

4.2 Performance Evaluation Test Results

Tests were conducted on bioconcrete containing *bacillus subtilis* and rice husk ash in different environments such as pH, sulfate, acidic and the effect of elevated temperature from 30 – 100 ^oC.

4.2.1 pH of fresh bio-concrete

The results of the pH of fresh bio-concrete are presented in Table 2.

Table 2: Effect of pH on Bio-concrete

Bio-Concrete with RHA and *Bacillus Subtilis*	pH
Control	12.6
5% RHA, 2M	11.7
5% RHA, 4M	12.2
5% RHA, 6M	12.5
7.5% RHA, 2M	11.5
7.5% RHA, 4M	12.0
7.5% RHA, 6M	12.2
10% RHA, 2M	11.3
10% RHA, 4M	11.7
10% RHA, 6M	12.1

Note: 2MF = 6×10^8 CFU/mL, 4MF = 1.2×10^9 CFU/mL, 6MF =1.8×10^9 CFU/mL

B. subtilis is alkaliphilic and can form endospores that survive pH up to 13. All mixes in the Table 2 have pH ≥ 11.3, which is well within the range for spore germination and metabolic activity. The control mix (ordinary Portland cement) exhibited a pH of 12.6, consistent with typical values for hydrated cement. Incorporation of RHA (5-10%) reduced the pH values, likely due to the pozzolanic reaction consuming $Ca(OH)_2$ (Chindaprasirt *et al.*, 2009). Increasing the pH control from 2M to 6M increased the pH values, indicating reduced acid attack on the cement matrix (Ahmad *et al.*, 2019).

4.2.2 Residual compressive strength at elevated temperatures

The bio-concrete was subjected to elevated temperature of 30 to 100^oC. The heating was done under controlled temperature, as shown in Plate I. The result is presented in Figure 2 Appendix A.

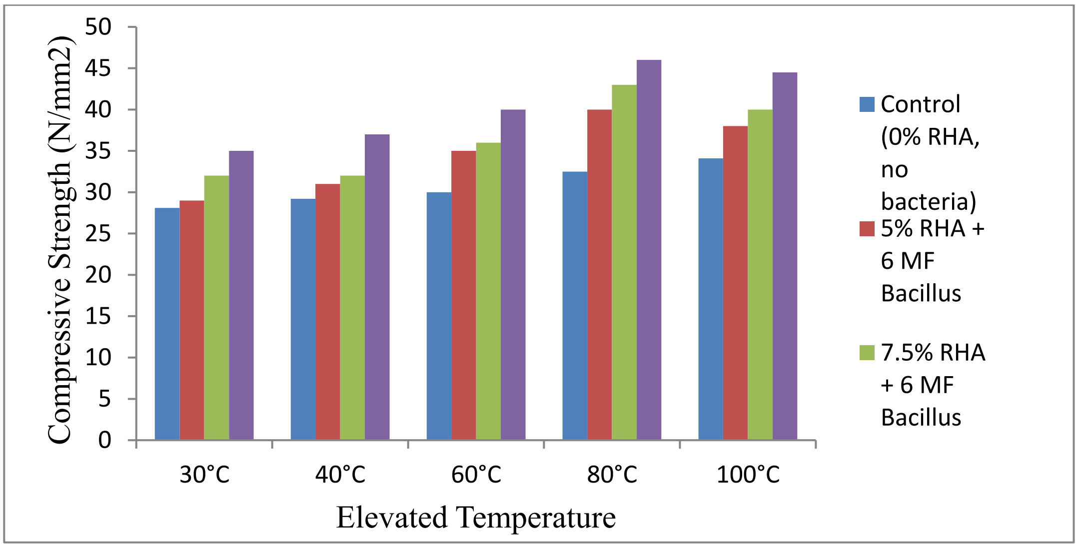

Figure 2: Effect of Elevated Temperature on Compressive Strength of Concrete

Figure 2 shows strength increased from 30 to 80°C for all mixes, then declined at 100°C. The 10% RHA + 6 MF mix achieved 46N/mm² at 80°C, 41% higher than control. The increase is due to accelerated hydration and pozzolanic reaction, while the decline at 100°C is due to rapid moisture loss and microcracking. MICCP reduced porosity and limited moisture loss, enabling higher residual strength than control. RHA formed additional C-S-H, improving thermal stability (Saad *et al.*, 2015).

Plate I: Burning of Bioconcrete at Elevated Temperature

4.2.3 Bio-concrete in acidic environment

When the concrete was exposed to sulfuric acid, it underwent acidic corrosion, where calcium hydroxide and other hydration products dissolve, leading to mass loss which is a key indicator of durability.

The bio-concrete was cured in sulfuric acid attack for 28 days. The result is presented in Figure 3 and Appendix B.

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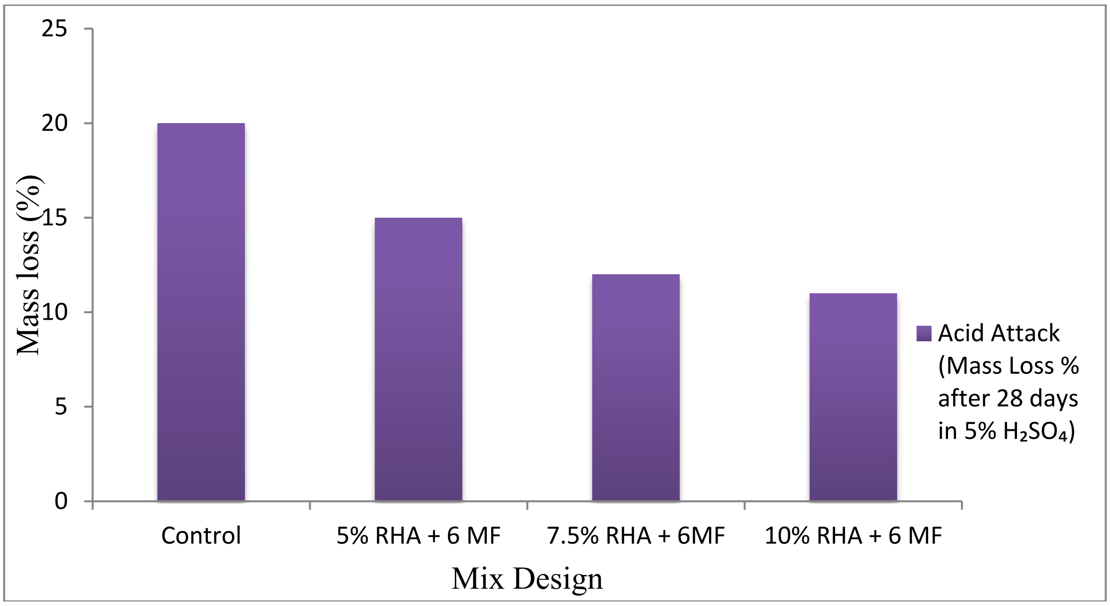

Figure 3: Mass Loss Versus Mix Design

Figure 3 shows mass loss after 28 days in 5% H_2SO_4 decreased from 20% for control to 11% for 10% RHA + 6 MF. RHA consumed $Ca(OH)_2$, limiting gypsum and ettringite formation (Saad *et al.,* 2015). Calcite precipitation by *B. subtilis* reduced permeability (Adetoye et al.,2026b).

4.2.3 Sulfate attack on bio-concrete

The bio-concrete was cured in sodium sulfate solution for 90 days. The result is presented in Figure 4 and Appendix C.

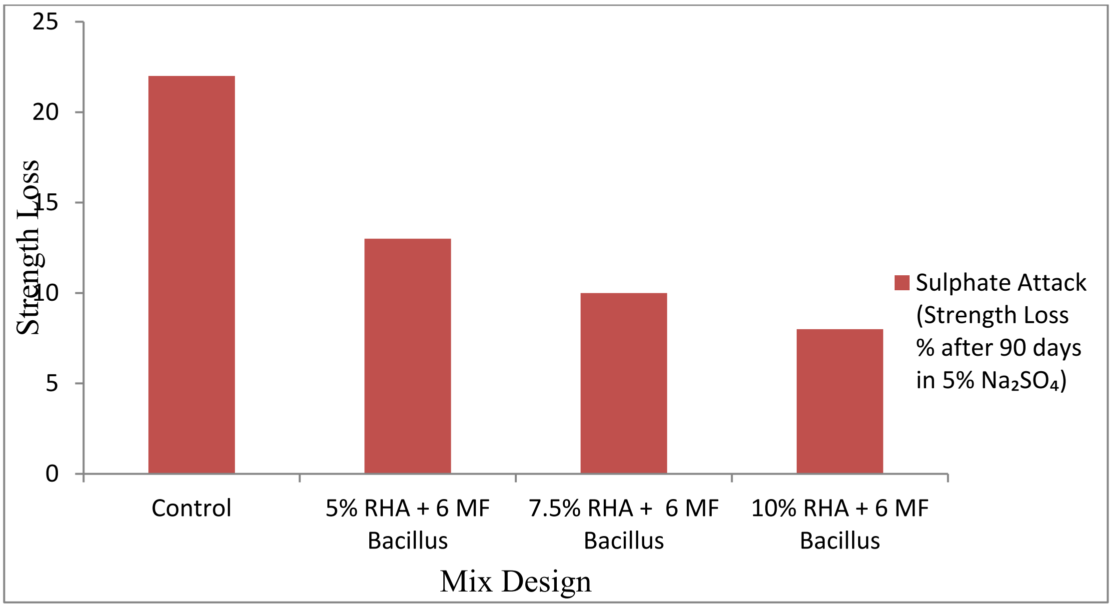

Figure 4: Strength Loss Versus Mix Design

Figure 4 shows strength loss after 90 days in 5% Na_2SO_4 decreased from 22% to 8% for 10% RHA + 6MF. RHA reduced free lime and refined pores, limiting ettringite formation. MICCP sealed microcracks, restricting sulfate ingress.

5.0 Conclusions

This study examined the degradation behavior of bio-concrete containing rice husk ash (RHA) and *Bacillus subtilis* under pH, acidic, sulfate, and elevated temperature exposures. The following conclusions were made:

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1. pH Balance: RHA reduced pH but all mixes remained > 11.0, supporting *B. subtilis* activity and steel passivation.

2. Thermal Stability: 10% RHA + 6MF achieved 46N/mm² at 80 °C, showing superior performance to control due to combined pozzolanic and MICCP effects.

3. Chemical Durability: The same mix reduced acid mass loss to 11% and sulfate strength loss to 8%, confirming a dense, low-permeability matrix.

4. The synergy between RHA and *B. subtilis* makes 10% RHA + 6MF bio-concrete suitable for structures in acidic, sulfate-rich, and moderately heated environments.

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APPENDICES

Appendix A: Residual Compressive Strength (N/mm^2) at Elevated Temperatures

Mix Design	Residual Compressive Strength at Elevated Temperature				
	30°C	40°C	60°C	80°C	100°C
Control (0% RHA, no bacteria)	28.1	29.2	30	32.5	34.1
5% RHA + 6 MF Bacillus	29	31	35	40	38
7.5% RHA + 6 MF Bacillus	32	32	36	43	40
10% RHA + 6 MF Bacillus	35	37	40	46	44.5

Appendix B: Acid Attack (Mass Loss % after 28 days in 5% H_2SO_4)

Mix Design	Initial Mass	Final Mass	Loss (kg)	Mass Loss %
Control	2.48	1.98	0.5	20
5% RHA + 6 MF	2.45	2.08	0.37	15
7.5% RHA + 6MF	2.43	2.14	0.29	12
10% RHA + 6 MF	2.42	2.15	0.27	11

Appendix C: Sulfate Attack (Strength Loss % after 90 days in 5% Na_2SO_4)

Mix Design	Initial Strength	Final Strength after Immersion	Strength Loss	Strength Loss %
Control	27.6	21.53	6.07	22
5% RHA + 6 MF Bacillus	29.6	25.63	4.00	13
7.5% RHA + 6 MF Bacillus	32.4	29.16	3.24	10
10% RHA + 6 MF Bacillus	34..5	31.74	2.76	8

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