Nigerian Journal of Engineering Science Research (NIJESR).
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ISSN: 2636-7114
Journal Homepage: https://nijesr.iuokada.edu.ng/

Durability Performance of Bio-Concrete Admixed with Rice Husk Ash Using Non-Destructive Testing Techniques and Box–Behnken Optimization

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Manuscript History
Received: 17/05/2026
Revised: 12/06/2026
Accepted: 28/06/2026
Published: 05/07/2026
https://doi.org/10.5281/zenodo.21278504

Abstract: *This study investigates the effect of Bacillus subtilis (B. subtilis) and rice husk ash (RHA) on the properties of grade M25 concrete. Grade 42.5 Portland limestone cement, RHA obtained by burning rice husk at 750 °C, and locally sourced fine and coarse aggregates were used. The chemical and physical properties of the materials were characterized according to ASTM and BS EN standards, and all were found to meet the required specifications. A Box–Behnken design was employed to evaluate the combined influence of B. subtilis concentration, RHA content, and curing age on water absorption, rebound hammer number, and ultrasonic pulse velocity. Factors investigated were bacteria dosage 2–6 McFarland (6×10^8 CFU/mL to 1.8×10^9 CFU/mL), RHA content 5–10%, and curing age 28–90 days. Concrete specimens were cast and tested at 28, 60, and 90 days. Results show that the addition of B. subtilis and RHA significantly reduced water absorption and improved surface hardness and internal quality. The optimal performance was achieved with 6 McFarland B. subtilis, 7.5% RHA replacement, and 90 days of curing, yielding the lowest water absorption of 0.6% and the highest UPV of 4800 m/s. The findings indicate that microbial-induced calcite precipitation combined with pozzolanic activity of RHA enhances the densification of the concrete matrix, leading to improved durability. This approach offers a sustainable method for producing high-performance bio-concrete.*

Keywords: *Bioconcrete, Bacillus Subtilis, Mechanical Properties, Rice Husk Ash, Optimization*

INTRODUCTION

Concrete exhibits relatively low tensile strength and durability compared to other construction materials, making it susceptible to microcracking during hydration and hardening. With time, these microcracks can propagate into larger fractures, compromising structural integrity and service life (Ram *et al.*, 2023; Saridhe and Selvaraj, 2020). Bio-concrete produced through microbially induced calcium carbonate precipitation (MICCP) presents a promising self-healing alternative. It enables autonomous crack healing, lowers carbon footprint, adds multifunctionality, and integrates readily with conventional casting practices (Zhang *et al.,* 2023). In MICCP-based systems, bacterial spores remain dormant within the matrix until activated by moisture, oxygen, and nutrients. Upon activation, the bacteria precipitate calcite that partially or fully seals cracks (Soda and Mini, 2023). Cracking poses particular challenges in underground structures, water-retaining elements, and nuclear facilities, where it may result from shrinkage, reinforcement corrosion, overloading, or ground movement. In such cases, crack locations are often inaccessible for inspection and repair. If left unaddressed, cracks accelerate deterioration and undermine long-term performance (Zhang *et al.,* 2025). The objective of this study is to evaluate water absorption and dynamic segregation in bio-concrete using non-destructive techniques, specifically ultrasonic pulse velocity (UPV) and rebound hammer testing.

Studies on *Bacillus subtilis* show that it enhances concrete properties and self-healing capacity through microbial calcite precipitation. Performance depends on bacterial concentration, nutrient medium, and curing conditions. *B. subtilis* consistently increases compressive, tensile, and flexural strength relative to control mixes. An optimal dosage of around 105 cells/ml yields up to 32% higher compressive strength, 14% higher split tensile strength, and 29% higher flexural strength at 28 days (Jena *et al.,* 2020). Priyom *et al.* (2020) reported similar benefits at a culture density of 0.637 OD600. Amusan and Adeagbo (2024) observed a 17.8% increase in compressive strength for 10% metakaolin–B. subtilis mixes, although a metakaolin–quicklime blend achieved a higher gain of 53%. Nagajyothi *et al.* (2017) also recorded strength gains when B. subtilis at 105 cells/ml was combined with 5–15% rice husk ash and quarry dust. Karimi and Mostofinejad, (2020) demonstrated that surface treatment with *B. subtilis* at 107 cells/ml reduced water, chloride, and CO_2 penetration by 24–58%, indicating lower porosity. Ultrasonic pulse velocity (UPV) is a non-destructive test used to monitor post-construction concrete performance. It provides a rapid, user-friendly method for detecting segregation and internal defects with acceptable accuracy (Bouabdallah *et al.,* 2024). Chereddy *et al.* (2021) screened several Bacillus species for self-healing efficiency, including B. subtilis, B. cereus, B. licheniformis, and B. halodurans. Among them, B. halodurans showed the best performance, with a UPV of 4.6 km/s and water absorption of 1.71%. Priyom et al. (2018) reported that microbial concrete with an optical density of OD600 = 0.637 exhibited better resistance to strength deterioration across curing conditions and ages, based on UPV testing. The rebound hammer test, commonly known as the Schmidt hammer test, is a widely adopted non-destructive testing (NDT) method for assessing surface hardness and estimating in-situ compressive strength of concrete. The method operates on the principle that the rebound distance of a spring-loaded mass striking the concrete surface is proportional to its hardness, which correlates with strength (Jedidi, 2020). Several studies have identified factors that influence rebound index values independently of compressive strength. Concrete age affects results, with 7-day-old concrete producing higher rebound values than 28-day-old concrete at equal strength levels. Moisture content also has a significant effect, as saturated specimens yield rebound indices approximately 5 points lower than dry specimens. Aggregate type further influences readings, with concrete made from crushed aggregates recording values about 7 points lower than those with natural aggregates at the same strength (Jedidi, 2020). Consequently, the rebound hammer is more reliable for comparative quality assessment, uniformity evaluation, and identification of deteriorated zones than for absolute strength prediction (Kumar & Singh, 2025). In practice, the rebound hammer is used to evaluate strength development during curing, assess in situ uniformity, and screen areas requiring further destructive testing. Its speed, simplicity, and non-destructive nature make it suitable for field applications, provided results are interpreted with consideration of influencing factors. Despite these advances, previously published research has paid limited attention to the durability performance of bio-concrete using NDT. In addition, optimization of bacteria concentration, RHA content, and curing age using Box-Behnken Design (BBD) remains limited.

MATERIALS AND METHODS

The materials used in this study included *Bacillus subtilis*, Portland limestone cement, rice husk ash (RHA), fine and coarse aggregates, and water. Grade 42.5 Portland limestone cement (Dangote brand) conforming to ASTM C150-23 was sourced from a certified vendor in Yelwa, Bauchi. Rice husk was obtained from a local rice mill in Sabo-Kaura, Bauchi. The husk was combusted in a kerosene-fired oven at the Industrial Design Laboratory, Abubakar Tafawa Balewa University (ATBU), Bauchi, at 750 °C.

The resulting ash was allowed to cool, ground to a fine texture, and sieved through a 150 µm sieve to obtain RHA. The fine aggregate used was natural river sand obtained from a stream at Bayara along the Bauchi–Dass road. The coarse aggregate consisted of crushed igneous rock with a maximum size of 20 mm, sourced from local suppliers in Bauchi. The coarse aggregate was hard, dense, durable, clean, and free from clay and organic impurities. All aggregates were well graded and met the requirements of ASTM C33.Water used for mixing and curing was obtained from the ATBU campus water supply in Yelwa, Bauchi. The water was potable and free from impurities, satisfying the requirements of ASTM C1602-22 for mixing water in concrete. The concrete mix design was carried out in accordance with the ACI 211.1 procedure and is presented in Table-1.

Table-1 Concrete Mix Design Proportions for M25 grade

Component	Value
Minimum Strength	25 N/mm²
Cement Type	OPC
Cement Grade	42.5
Slump (mm)	30 - 60
Maximum size of Aggregate (mm)	20
Water-cement ratio	0.55
Cement Content (kg/m³)	420
Fine Aggregate Content (kg/m³)	750
Coarse Aggregate Content (kg/m³)	915
Water-content (kg/m³)	220
Superplasticizer (kg/m³)	8.4
Mix ratio	1 :1.79: 2.26

Bacillus subtilis bacterium was produced at ATBU teaching hospital, Bauchi. It is considered the best studied gram-positive bacterium and a model organism to study bacteria chromosome replication and cell differentiation. *Bacillus subtilis* was employed in this study due to its ability to form endospores under unfavourable alkaline exposures. The bacteria in liquid (broth) grown in a media. The broth solution was prepared by suspending 13.0 grams of nutrient broth in 1000 ml of distilled water and growth medium that supports the growth of *Bacillus subtilis*, Luria-Bertani (LB) broth, which contains tryptone, yeast extract, and sodium chloride were prepared. The inoculated broth medium was kept in an incubation chamber at 37^0C for 24-48 hrs. *Bacillus subtilis* cells were harvested by filtration. The harvested cells were concentrated to 4 to 6 x10^⁶ CFU/mL and immobilized into concrete by mixing with RHA. It was added to the concrete mix during the mixing process, ensuring uniform distribution. Box- Behnken Design with 17 runs and control specimen were adopted in the design of experimental combinations. It was used to quantify the relationship between the controllable input parameters and the obtained response surfaces. The concrete samples, consisting of cubes of 100 mm x 100 mm x 100 mm, beams of 100 mm x 100 mm x 500 mm, and cylinder of 100 mm x 200 mm were produced with grade M25 were produced and cured by immersion in water for 28, 50 and 90 days. The water absorption test was carried out in accordance with BS EN 1881-122 on 100 mm x 100 mm x 100 mm cubes. The specimens were air-dried for 24 hours before being weighed and recorded. They were cured in water for 7 days before being air-dried and re-weighed to calculate the amount of water absorbed using Equation 1.

$$Water\ absorption = (W_2 - W_1) / W_1 \times 100\% \quad (1)$$

Where, W_1 = weight of dried cube (g), W_2 = weight of saturated cube (g).

Ultrasonic Pulse Velocity (UPV) tests were conducted using a PUNDIT Lab ultrasonic tester (Proceq, Switzerland) in accordance with ASTM C59 on the concrete specimens of 100 x 100 x 100 mm size at 90 days of curing. The equipment measures the transit time of an ultrasonic pulse through concrete, from which pulse velocity is calculated to assess homogeneity, detect internal defects, and estimate concrete quality. Prior to each day of testing, the UPV equipment was zero-calibrated using the manufacturer's certified calibration bar with a known transit time of 25.4 µs, as specified in ASTM C597. Petroleum jelly was applied between the transducers and the calibration bar to ensure full contact. The instrument was adjusted until the displayed transit time matched the certified value of the bar. Rebound hammer tests were carried out using a Schmidt Hammer Type N (Proceq, Switzerland) in accordance with ASTM C805. The hammer estimates the surface hardness of concrete, which correlates with in-situ compressive strength. The Schmidt hammer was calibrated at the start of each testing day using the steel test anvil supplied by the manufacturer. The anvil has a certified rebound value of 80 ± 2 for a Type N hammer. Ten consecutive impacts were taken on the anvil, and the average rebound number was recorded. The hammer was considered calibrated if the average fell within ±2 of the certified value. The plunger was also checked for free movement and full return. Where tests were conducted in orientations other than vertical downward, correction factors provided by the manufacturer were applied to the rebound numbers in accordance with BS 1881:202. All calibration records, including date, operator, and calibration values, were maintained to ensure traceability of test results.

RESULTS AND DISCUSSION

3.1 Test Results on Cement

The tests conducted on the physical properties of cement were specific gravity, consistency, initial and final setting time. The physical properties of the cement were tested in accordance with NIS 444-1 (2003) and EN 197-1 (2011). The results obtained are presented in Table-2.

Table-2 Physical Properties of Cement

Property	Value	Limit (BS EN 196)
Specific Gravity	3.15	3.10 – 3.15
Normal Consistency (%)	28	25 – 35
Setting time:		
(i) Initial	35 minutes	Minimum 45minutes
(ii) Final	380 minutes	Maximum 600minutes
Colour	Grey	
Loose Bulk Density (kg/m³)	1600	850 – 1650
Soundness (mm)	3.5	Max. 10

Table-2 presents the physical properties of the Grade 42.5 Portland limestone cement used in this study, alongside the requirements of BS EN 196. The cement had a specific gravity of 3.15, which falls within the BS EN 196 range of 3.10–3.15. Normal consistency was 28%, well within the typical range of 25–35% for Portland cement, indicating acceptable water demand for workable paste. The initial setting time was 35 minutes, and the final setting time was 380 minutes. While the initial set was below the BS EN 196 minimum of 45 minutes, the final set was within the maximum limit of 600 minutes, suggesting that the cement will develop stiffness within a practical timeframe for construction operations. The cement exhibited the characteristic grey colour of Portland limestone cement, confirming the absence of excessive impurities. The loose bulk density of 1600 kg/m³ also complied with the BS EN 196 range of 850–1650 kg/m³. Soundness, which measures volume expansion due to free lime and magnesia, was 3.5 mm, far below the 10 mm maximum specified in BS EN 196. This indicates dimensional stability and a low risk of post-hardening cracking. All tested properties of the cement met the requirements of BS EN 196, confirming its suitability for use in the bio-concrete mixes.

4.2 Characterization of Rice Husk Ash

The results of physical properties of RHA are presented in Table-3.

Table-3 Physical Properties of RHA

Property	RHA
Specific gravity	2.32
Colour	Grey
Shape Texture	Irregular

The specific gravity of RHA is lower than that of Portland cement, which is typically 3.10–3.15 (BS EN 196-3). This lower density is due to the porous, cellular structure of RHA formed during combustion of the rice husk. In concrete, replacing part of the cement with RHA reduces the overall density of the binder matrix. This contributes to lighter concrete and can also increase the paste volume for a given mass, which may improve workability. However, the high porosity of RHA also means it has high water demand.

4.3 Oxide Properties of Cement and Rice Husk Ash

The X-ray fluorescence results for oxides composition cement and rice husk ash are presented in Table-4.

Table-4 Chemical Properties of Cement and Rice Husk Ash

Oxide Composition	Cement	RHA
SiO_2	22.5	79
Al_2O_3	5.2	1.5
Fe_2O_3	3.4	0.9
CaO	59.7	4.2
MgO	1.3	1.3
SO_3	0.6	0.8
Na_2O	0.8	0.2
K_2O	1.2	2.9
P_2O_5	0.3	1.9
LOI	2.5	3.4

Table-4 presents the oxide composition of Grade 42.5 Portland limestone cement and rice husk ash (RHA), determined by XRF analysis. The results show how each material contributes to the chemistry and performance of the concrete matrix. In summary, the cement composition for Portland limestone cement and complies with standard requirements. The RHA composition, characterized by high SiO_2, low CaO, and low LOI, classifies it as a highly reactive pozzolan (Sani *et al.,* 2024). This confirms the suitability of the RHA used in this study for enhancing strength and reducing permeability in bio-concrete mixes.

4.4 Performance Evaluation Test Results

The results of the properties of hardened bio-concrete are shown in Table-5.

Table-5 Performance Evaluation Result of Bioconcrete

Run	Factor 1 A: *Bacillus Subtilis* McFarland	Factor 2 B: R.H.A %	Factor 3 C: Curing Age days	Response 1 Water Absorption %	Response 2 Rebound Hammer	Response 3 UPV m/s
Control	0	0	90	2.7	33	3800
1	4	7.5	60	1.5	39	4300
2	6	7.5	28	0.8	34	3500
3	4	7.5	60	1.5	37	4300
4	2	7.5	28	2	30	3550
5	2	5	60	1.7	32	3700
6	6	5	60	0.7	42	4450
7	4	10	90	1.7	44	4650
8	4	7.5	60	1.5	37	4300
9	6	7.5	90	0.6	45	4800
10	2	10	60	2	36	4000
11	4	7.5	60	1.5	37	4300
12	4	10	28	1.7	33	3800
13	4	7.5	60	1.5	37	4300
14	6	10	60	0.8	36	4100
15	2	7.5	90	1.9	36	4100
16	4	5	28	1.1	32	3700
17	4	5	90	1.2	43	4350

Table-5 shows the results from a Box-Behnken experimental design testing how three factors; *bacillus subtilis* affect Water Absorption, Rebound Hammer Number, and Ultrasonic Pulse Velocity (UPV). Water absorption ranges from 0.6% to 2.0%.: Absorption decreases with higher *B. subtilis* concentration and longer curing age. Best result: 0.6% at Run 9 - 6 McFarland, 7.5% RHA, 90 days. B. subtilis produces calcite that fills pores. Longer curing gives more time for this microbial precipitation and cement

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hydration, making the concrete denser and less permeable. Higher values of rebound hammer indicate harder, denser surface concrete. The range of 30 to 45 showed an increase with higher *B. subtilis*, higher RHA, and longer curing age. 6 McFarland, 7.5% RHA, 90 days was the best result recorded. It was as a result of Microbial calcite and pozzolanic reaction from RHA densify the matrix, increasing surface hardness. Ultrasonic Pulse Velocity (UPV) measures internal quality. Values > 4500 m/s are excellent, 3500-4500 m/s are good. The values recorded were from 3500 to 4800 m/s. UPV increases with longer curing and higher *B. subtilis*. The highest value of 4800 m/s was recorded with bioconcrete having 6 McFarland, 7.5% RHA, 90 days. It is because of fewer voids which allowed microcracks sound waves to travel faster. The strong correlation between UPV and rebound hammer confirms consistent densification. The optimal dosage for this study is 6 McFarland B. subtilis and 7.5% RHA cured for 90 days. The analysis of variance (ANOVA) for water absorption, rebound hammer and UPV are shown in Tables 6 – 8, and the diagnostic graphs are shown in Appendices A and B.

Table-6 Analysis of Variance for Water Absorption

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	3.04	2	1.52	93.86	< 0.0001
A- Bacillus Subtilis	2.76	1	2.76	170.36	< 0.0001
B-R.H.A	0.2813	1	0.2813	17.35	0.0010
Residual	0.2269	14	0.0162		
Lack of Fit	0.2269	10	0.0227		
Pure Error	0.0000	4	0.0000		
Cor Total	3.27	16			

Table-7 Analysis of Variance for Rebound Hammer

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	255.98	2	127.99	36.60	< 0.0001
A-Bacillus Subtilis	66.12	1	66.12	18.91	0.0007
C-Curing Age	189.85	1	189.85	54.29	< 0.0001
Residual	48.96	14	3.50	5.72	0.0537
Lack of Fit	45.76	10	4.58		
Pure Error	3.20	4	0.8000		
Cor Total	304.94	16			

Table-8 Analysis of Variance for UPV

Source	Sum of Squares	df	Mean Square	F-value	p-value
Model	1.699E+06	2	8.496E+05	21.78	< 0.0001
A-*Bacillus Subtilis*	2.813E+05	1	2.813E+05	7.21	0.0178
C-Curing Age	1.418E+06	1	1.418E+06	36.35	< 0.0001
Residual	5.462E+05	14	39011.13		
Lack of Fit	5.462E+05	10	54615.58		
Pure Error	0.0000	4	0.0000		
Cor Total	2.245E+06	16			

In Table-6, water absorption showed that the model is highly significant ($p < 0.0001$). Both factors are significant: *Bacillus subtilis* concentration (A, $p < 0.0001$, F = 170.36) has the strongest effect, followed by RHA content (B, p = 0.0010, F = 17.35). Lack of fit is zero due to no pure error, indicating the model fits the data adequately. In Table 7, Rebound Hammer model is significant ($p < 0.0001$). Curing age (C, $p < 0.0001$, F = 54.29) has a larger effect on rebound number than *B. subtilis* (A, p = 0.0007, F = 18.91). Lack of fit is not significant (p = 0.0537), confirming the model adequately describes the response. Table 8 showed result for Ultrasonic Pulse Velocity (UPV), the model is significant ($p < 0.0001$). Curing age (C, $p < 0.0001$, F = 36.35) is the dominant factor, with *B. subtilis* (A, p = 0.0178, F = 7.21) also significant. Lack of fit is zero, indicating good model fit. All three models are statistically significant. *B. subtilis* significantly influences water absorption, rebound hammer, and UPV. Curing age is the key factor for rebound hammer and UPV, while RHA significantly affects water absorption. The models are suitable for predicting and optimizing bio-concrete performance. The Fit statistics result is presented in Table-9 while the model Equations are presented in Equations 2 - 4.

Table-9 Fit Statistics Test Result

Parameter	W. A	R.H	UPV
Std. Dev.	0.1273	1.87	197.5
Mean	1.39	37.06	4129
C.V. %	9.13	5.05	4.78
R^2	0.9306	0.8394	0.7568
Adjusted R^2	0.9207	0.8165	0.7220
Predicted R^2	0.8874	0.7439	0.6078
Adeq Precision	28.9822	19.7178	14.665

Water absorption R^2 = 0.9306, Predicted R^2 = 0.8874, C.V.% = 9.13, and Adeq Precision = 28.98. The model explains 93% of variation and good for prediction. Rebound Hammer had a good fit with R^2 = 0.8394, Predicted R^2 = 0.7439, C.V.% = 5.05, and Adeq Precision = 19.72. The model is reliable for prediction. UPV had an acceptable fit with R^2 = 0.7568, C.V.% = 4.78, and Adeq Precision = 14.67, but lower Predicted R^2 = 0.6078 indicates weaker predictive power compared to the other two models. All three models are suitable for optimization within the tested ranges.

$$Water\ Absorption = 2.00662 - 0.29375 * A + 0.075 * B \quad (2)$$

$$Rebound\ Hammer = 21.9566 + 1.4375 * A + 0.157103 * C \quad (3)$$

$$Ultra - Pulse\ Velocity = 2{,}946.2 + 93.75 * A + 13.5767 * C \quad (4)$$

The effect of RHA and *B. subtilis* on water absorption of bioconcrete is presented in Fig. 1 while the effect of *B. subtilis and curing age* on rebound hammer of bioconcrete is presented in Fig. 2.

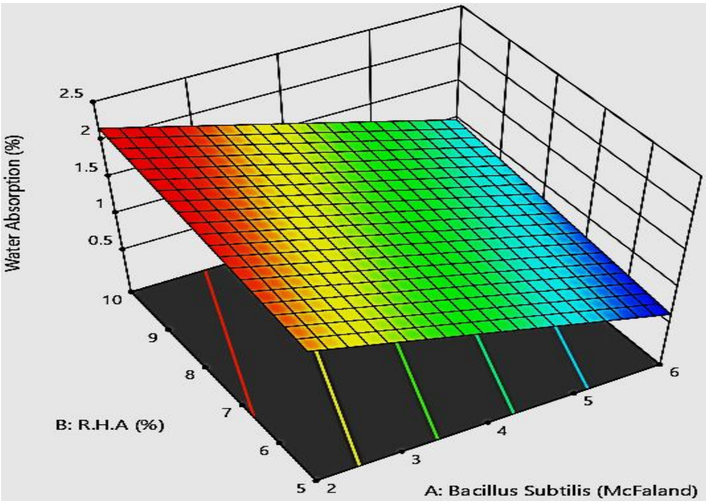

Fig.1 Effect of RHA and B. subtilis on Water Absorption of Bioconcrete

Fig. 1 illustrates the influence of *Bacillus subtilis* and RHA on water absorption of bio-concrete. Increase in the *bacillus subtilis* of bio-concrete from 2 to 6 McFarland causes significant reduction in water absorption of the bio-concrete, suggesting calcite precipitation for hardening.

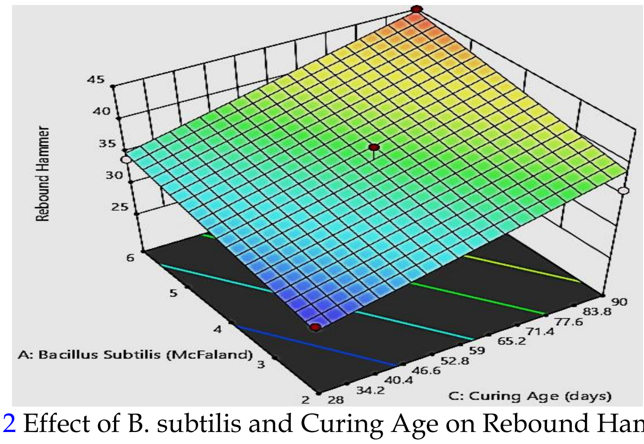

Fig. 2 Effect of B. subtilis and Curing Age on Rebound Hammer

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Fig. 2 illustrates the influence of *Bacillus subtilis* and curing age on the rebound hammer of bio-concrete. Increase in the curing age of bio-concrete from 28 to 90 days leads to higher rebound numbers while bacterial concentrations (2–6 McFarland) produced higher rebound numbers, suggesting optimal calcite precipitation for surface hardening.

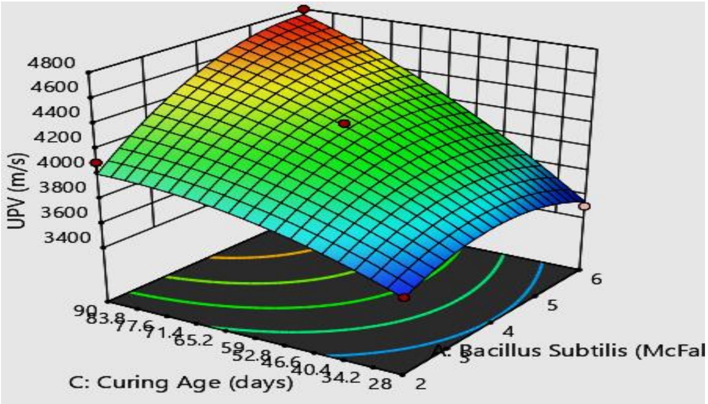


Fig. 3 3-D Response Graph of C.A, B.Sub. and UPV

Fig. 3 illustrates the influence of *Bacillus subtilis* and curing age on the UPV of bio-concrete. Bacteria concentrations (4–6 MF) tend to produce higher UPV values, suggesting optimal calcite precipitation for internal crack healing and densification. Longer curing ages (60–90 days) result in higher UPV values, as hydration and bacteria activity have more time to enhance internal structure.

4.5 Optimization of Bio - Concrete Mixtures by Numerical Method

The automatic optimization function for the optimal values of the factors for highest bio-concrete strength is presented in Fig. 4.

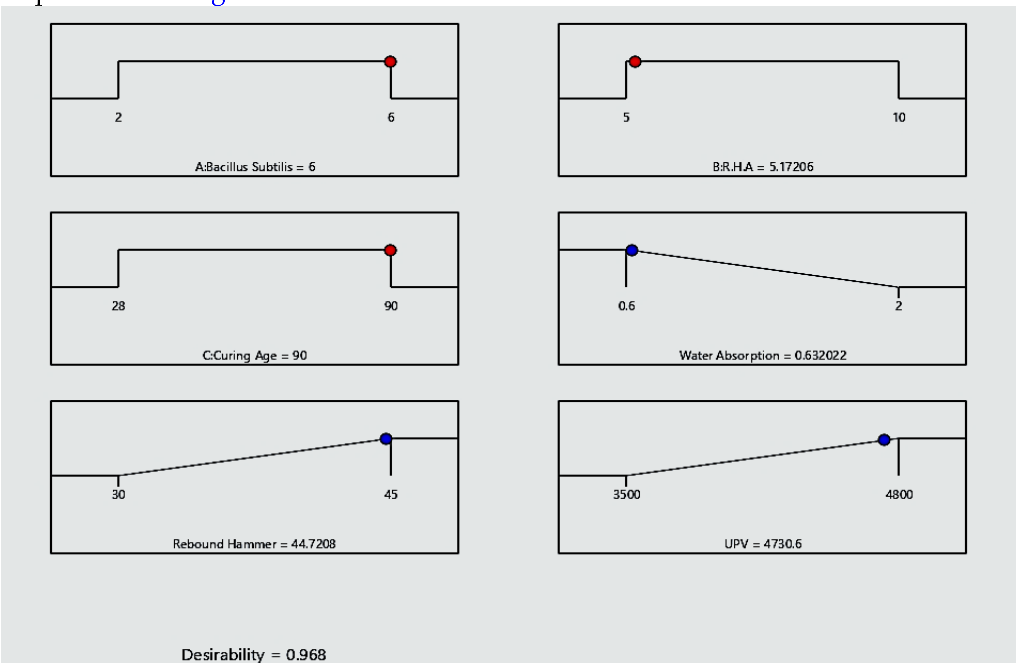


Fig. 4 Ramp Plot Showing the Optimal Values for Responses

The bacteria contribute to self-healing through calcite precipitation, while RHA provides pozzolanic activity and serves as a nutrient source for microbial growth. Together, they improve the overall strength of the concrete. Rebound hammer value of 45 indicates improved, strong and dense bio-concrete with faster ultrasonic wave travel (higher UPV) of 4700 m/s and lower water ingress of 0.6%.

CONTRIBUTION TO KNOWLEDGE

This study contributes to knowledge by demonstrating, through statistically optimized experiments, that the combined use of *Bacillus subtilis* and rice husk ash significantly improves the durability and non-destructive strength properties of Portland limestone cement concrete and provides predictive models for designing sustainable bio-concrete mixes.

CONCLUSION

The results show that bio-concrete performance was significantly enhanced by the combined use of RHA and *Bacillus subtilis*. Water absorption decreased with the addition of both materials, indicating a denser matrix resulting from pozzolanic activity and microbial-induced calcite precipitation. The lowest absorption of 0.6% was achieved at 7.5% RHA replacement, 6 McFarland bacteria dosage, and 90 days curing. At this same optimal condition, ultrasonic pulse velocity reached 4800 m/s, reflecting improved internal homogeneity, reduced voids, and excellent concrete quality. Rebound hammer numbers also increased, showing higher surface hardness consistent with the densification of the bio-concrete matrix. The optimal performance at 7.5% RHA, 6 McFarland bacteria, and 90 days curing confirms that microbial activity and pozzolanic reaction act synergistically to reduce permeability and improve both surface and internal properties. This validates the use of NDT methods for assessing bio-concrete performance and supports the approach as a sustainable route to producing more durable concrete.

CONFLICT OF INTEREST

There is no conflict of interest for this research work.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to the Department of Civil Engineering, Abubakar Tafawa Balewa University, Bauchi, for providing the laboratory facilities and technical support that made the study possible.

REFERENCES

Abdullahi Y. M., Sani A., and Adetoye O. (2024). Optimization of Concrete Containing Metakaolin and Cement Kiln Dust using Central Composite Design. *International Journal of Built Environment and Earth Science*. Vol. 5 (4), 1-12.

Amusan G. M. and Adeagbo G. A. (2024): Comparative Study of Metakaolin-Quicklime and Metakaolin Bacillus Subtilis as Self-Healing Agents in Concrete. Çankaya University Journal of Science and Engineering, CUSJE 21(01): 042-053, https://dergipark.org.tr/cankujse

ASTM C150 (2023). *Standard specification for portland cement;* ASTM International: West Conshohocken, PA USA

ASTM C1602 (2022). *Standard Specification for Mixing Water used in the Production of Hydraulic Cement Concrete.* ASTM International, West Conshohocken, PA, USA

ASTM International. (2020). Standard Test Method for Density of Hydraulic Cement (ASTM C188/C188M-20). West Conshohocken, PA: ASTM International. https://doi.org/10.1520/C0188_C0188M-20

Bouabdallah M. A., Mouli M. and Benmammar M. (2024). Impact of Admixtures on Segregation in Self-compacting concrete: A Comparative Study between Standardized and Ultrasonic Pulse Velocity (UPV) Methods. *The Journal of Engineering and Exact Sciences*, 10(6)

BSI. (2016). BS EN 196-1 (2016) Methods of testing cement. Part 1: Determination of strength. London: British Standards Institution.

Chereddy S., Nerella R., Madduru S. R., Mikkili I., Chava V., (2021). Comprehensive microbiological studies on screening bacteria for self-healing concrete. Materialia, 15, 101051. https://doi.org/10.1016/j.mtla.2021.101051

EN 197-1: (2011) Cement – Part 1: Composition, specifications and conformity criteria for common cements. Brussels: European Committee for Standardization.

Jena S., Basa B., Panda K.C (2020). Impact of Bacillus subtilis on the properties of concrete. *Materials Today: Proc.*, https://doi.org/10.1016/j.matpr.2020.03.129

Karimi, N., & Mostofinejad, D. (2020). Bacillus subtilis bacteria used in fiber reinforced concrete and their effects on concrete penetrability. *Construction and Building Materials*, *230*. https://doi.org/10.1016/j.conbuildmat.2019.117051

Nagajyothi, K., Pradesh, A., Chamberlin, K. S., Pradesh, A., Academics, A. D., Pradesh, A., Reddy, M. M., & Pradesh, A. (2017).

Experimental Study On Bacterial Rice Husk Ash Concrete By Incorporating Quarry Dust As Partial Replacement Of Fine Aggregate. *8*(4), 744–751.

Priyom S. N, Islam M., and Islam S., (2018). An Experimental Investigation on the Performance of Bacterial Concrete. 4th International Conference on Advances in Civil Engineering 2018 (ICACE 2018) 19 –21 December 2018 CUET, Chittagong, Bangladesh

Priyom, S. N., Islam, M., and Shumi, W. (2020). Utilization of Bacillus Subtilis Bacteria for Improving Mechanical Properties of Concrete. *Journal of the Civil Engineering Forum*, *1000*(1000). https://doi.org/10.22146/jcef.60216

Ram Yadav, R., Singh, P., Bishnoi, S., & Kumar Jain, A. (2023). Analyzing the mechanical properties of bacterial concrete. *Materials Today: Proceedings*. https://doi.org/10.1016/j.matpr.2023.07.084

Sani A., & Adetoye O. (2024): Optimization of Self-Compacting Concrete Incorporating Granite Dust and Rice Husk Ash Using Response Surface Methodology. *KIU Journal of Science, Engineering and Technology*. Vol. 3(1), 39-46. https://doi.org/10.59568/KJSET-2024-3-1-04

Sani A., Afolayan T. J., Adamu U. C. and Adetoye O. (2024). Effect of Elevated Temperature on Compressive Strength of Self-Compacting Concrete Produced with Rice Husk Ash. *Journal of Civil Engineering*, 16 (3), 19 – 34

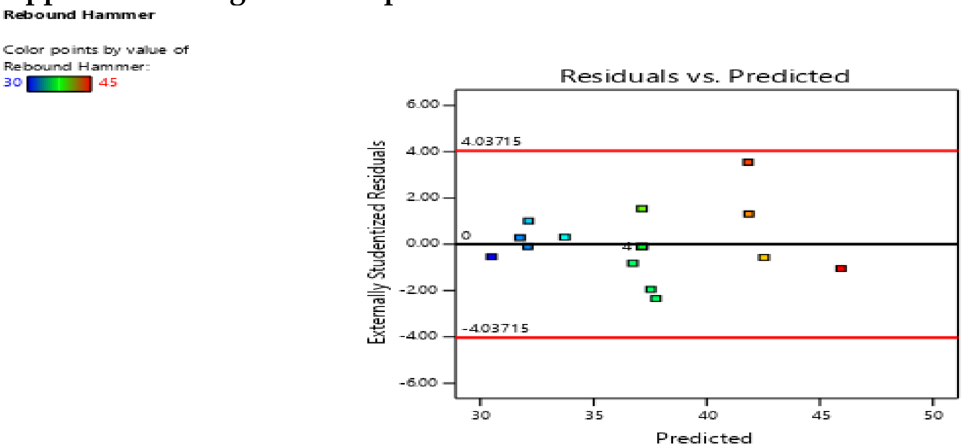
Saridhe, S. P., & Selvaraj, T. (2020). Microbial precipitation of calcium carbonate in cementitious materials - A critical review. *Materials Today: Proceedings*, *43*, 1232–1240. https://doi.org/10.1016/j.matpr.2020.08.762

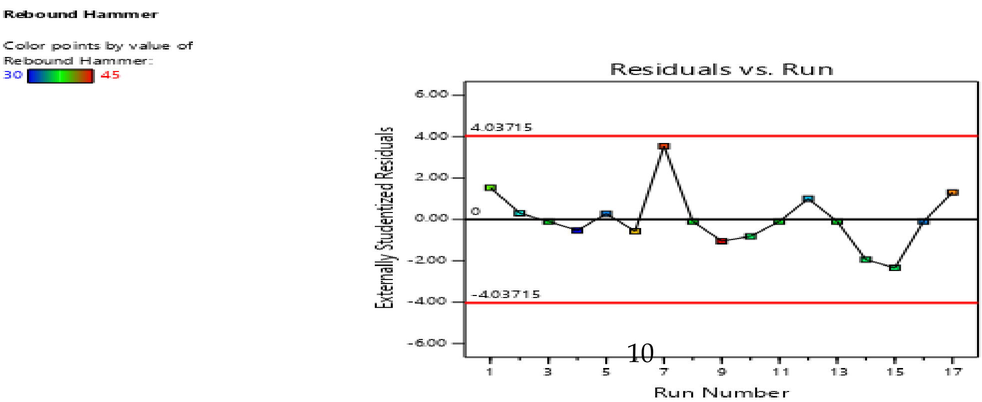
Soda, P. R. K., & Mini, K. M. (2023). A Critical Review on Assessment of Self Healing Performance of Bioconcrete. *KSCE Journal of Civil Engineering*, *27*(2). https://doi.org/10.1007/s12205-022-2161-3

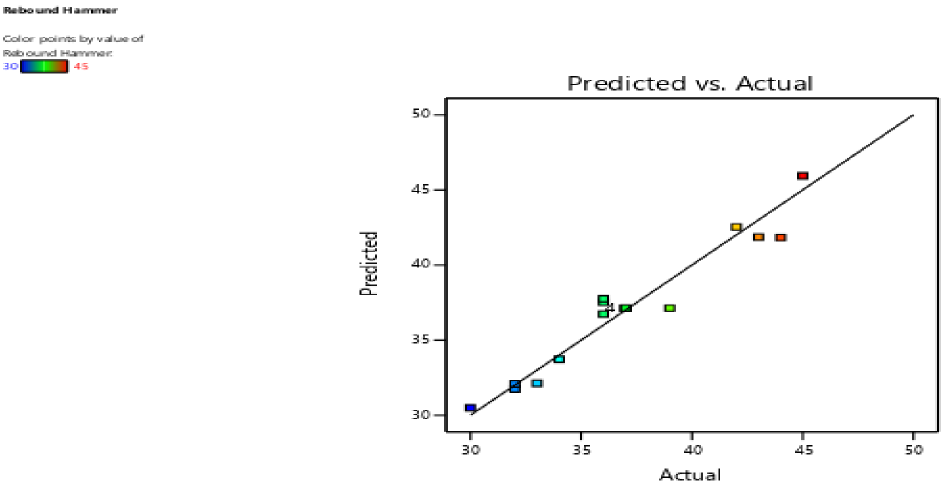
Zhang, B.; Zhu, H. (2023). Durability of seawater coral aggregate concrete under seawater immersion and dry-wet cycles. J. Build. Eng. 2023, 66, 105894. https://doi.org/10.1016/j.jobe.2023.105894

Zhang, C., Yuan, C., Cao, R., Wang, Y., Wang, Y., & Yang, J. (2025). Performance study of bacteria on improving fresh, mechanical, and durability characteristics of ultra-high-performance concrete. *Innovative Infrastructure Solutions*, 10 (xx), Article 22. https://doi.org/10.1007/s41062-025-02237-x

Appendix A. Diagnostic Graph for Rebound Hammer







Appendix B. Diagnostic Graph for UPV