



# Strength Optimization of Self-Compacting Concrete with Cement Kiln Dust Incorporating *Sporosarcina Pasteurii* Bacteria Using the Response Surface Method

Isah Garba<sup>1</sup>, Tasiu Ashiru Sulaiman<sup>1\*</sup>, Ibrahim Aliyu<sup>1</sup>, Yusuf Dada Amartey<sup>1</sup>, Jibrin Mohammed Kaura<sup>1</sup>, Yusuf Yau<sup>1</sup> and Bashir Usman<sup>2</sup>

<sup>1</sup>Department of Civil Engineering, Ahmadu Bello University, Zaria

<sup>2</sup>Department of Civil Engineering Nuhu Bamalli Polytechnic Zaria

\*Corresponding Author Email: [tasiuashirusulaiman@gmail.com](mailto:tasiuashirusulaiman@gmail.com)

## Abstract

Micro-crack formation significantly compromises the long-term durability and performance of concrete structures. This study investigates the effect of *Sporosarcina pasteurii* on the strength properties of self-compacting concrete incorporating cement kiln dust (SCC–CKD), while also developing predictive models and optimizing mix performance using response surface methodology (RSM). SCC mixtures were prepared with 10% cement replacement by cement kiln dust, a water–cement ratio of 0.43, and 2% superplasticizer dosage. The bacterial suspension was introduced at densities of 0,  $1.5 \times 10^8$ ,  $6.0 \times 10^8$ ,  $1.2 \times 10^9$ , and  $2.4 \times 10^9$  cells/mL, alongside a cementation reagent of 0.5 M concentration. The bacterial-to-cementation reagent ratio (B:C) was varied, and curing was conducted up to 56 days. Compressive, flexural, and split tensile strengths were evaluated, and microstructural changes were examined using scanning electron microscopy (SEM). The results indicate that strength properties improved with increasing bacterial density, reaching optimal performance at approximately  $1.2 \times 10^9$  cells/mL, beyond which a decline was observed. The developed RSM models demonstrated good predictive capability, with coefficients of determination ( $R^2$ ) of 94.70%, 79.46%, and 82.52% for compressive, flexural, and split tensile strengths, respectively. Optimization results revealed that a B:C ratio of 56:44, bacterial density of  $1.54 \times 10^9$  cells/mL and curing age of 52 days produced optimal strength values of 34.50 N/mm<sup>2</sup>, 10.09 N/mm<sup>2</sup>, and 5.07 N/mm<sup>2</sup> for compressive, flexural, and split tensile strengths, respectively. The findings demonstrate that the incorporation of *Sporosarcina pasteurii* enhances the mechanical performance of SCC–CKD through microbially induced calcite precipitation, and that RSM is an effective tool for predicting and optimizing its strength characteristics.

**Keywords:** Cement Kiln Dust, Compressive strength, Flexural Strength, Self-compacting Concrete, Split Tensile Strength, *Sporosarcina Pasteurii*

## 1. Introduction

Self-compacting concrete serves as an excellent alternative to traditional concrete. Its remarkable ability to flow effortlessly under its own weight in a freshly mixed state negates the requirement for external compaction. However, this enhanced workability may come at the cost of compromised strength and durability, which are vital design considerations in civil engineering projects. The excess water present in self-compacting concrete can weaken the microstructure of the aggregate–paste interfacial transition zone (ITZ) and increase the capillary porosity of the hardened paste, subsequently affecting the concrete's strength and durability characteristics [1]. The mechanical and durability performance of concrete has been improved through the incorporation of supplementary cementitious materials (SCMs), which also contribute to reducing CO<sub>2</sub> emissions associated with cement production [2]. When

producing concrete, various supplementary cementitious materials, including pozzolans [3], fly ash [4], ground granulated blast furnace slag (GGBFS) [5], and agricultural by-products such as rice husk ash (RHA) [6] have been successfully utilized.

The increasing production of cement has led to greater attention being given to cement kiln dust, a significant by-product that is often discarded as waste. Cement kiln dust is generated during the cement manufacturing process and can constitute 3–4% of the total cement output, depending on the raw materials and production methods employed. The use of cement kiln dust as a partial replacement for cement may produce cost-effective concrete with self-compactability in its fresh state and improved properties after hardening due to its pozzolanic characteristics, as demonstrated by numerous researchers. Microcracks are a fundamental aspect of concrete, and they are present in self-compacting concrete as well. The durability of self-compacting concrete is compromised when undesirable elements infiltrate the concrete matrix due to the expansion of microcracks under external stress and chemical reactions. While preventing cracking in concrete structures is challenging, it can be mitigated by improving the density of the concrete matrix. Concrete damage can be partially repaired using materials such as epoxy gel, mortar, polymer, or carbonated steel slag; however, these solutions tend to be expensive and require ongoing maintenance. Consequently, it is essential to explore alternatives that are natural and environmentally friendly to address such healing issues in concrete, and bacteria present a viable option compared to other substances.

Given favorable humidity levels and the presence of chemical or biological additives, concrete has the potential to autonomously heal macrocracks by sealing them, a phenomenon known as autonomous healing. Over the past decade, the utilization of microorganisms, such as bacteria, in concrete production has attracted the attention of numerous research groups, resulting in the development of “bacterial concrete” [7]. Various *Bacillus* species, including *Bacillus pasteurii* [8], *Bacillus pseudofirmus*, *Bacillus cohnii* [9], and *Bacillus alkalinitrilicus* [10], have proven effective in preventing cracks in concrete [11]. These bacterial cells enhance the pore structure of concrete through calcite precipitation [12]. The use of bio-concrete prolongs service life while lowering maintenance costs, reducing the need for construction work and conserving building materials and energy consumption [13].

Concrete has an inherent ability to heal itself, which is related to the deposition of calcium carbonate or the hydration of cement particles that help to close cracks. A study by [19] focused on optimizing the rice husk ash (RHA) content and the concentration of bacteria in self-compacting concrete (SCC). The findings indicated that the optimal rice husk ash content resulted in the highest strength at a bacterial concentration of  $10^5$  cells/mL, while the best durability was achieved at a concentration of  $10^7$  cells/mL. Taku [14] conducted a study on modeling the permeation properties of self-compacting concrete incorporating *Sporosarcina pasteurii*. The results demonstrated that the developed linear models, which were quadratic in form, were effective in predicting and optimizing water absorption and sorptivity of the bio-self-compacting concrete. Akhtar et al. (2023)[21] investigated the strength and durability characteristics of bacteria-based self-compacting concrete with mineral additives. The SCCMSBM and SCCMKBM mixtures exhibited ductile behavior under flexural loads, with increases of 5.55% and 10.52% at 28 days, and 5.26% and 10% at 56 days compared to the conventional mix. Improvements in split tensile strength were also recorded, with increases of 9.16% and 12.37% at 28 days, and 5.49% and 13.43% at 56 days.

The response surface methodology (RSM) is frequently utilized for modeling and accurately predicting the properties of concrete [15]. RSM comprises a range of statistical and mathematical techniques used to develop models for data fitting, enabling parameter optimization and understanding the interactions between variables [16]. Given the above context, this study aims to assess the strength properties and develop predictive mathematical models while optimizing self-compacting concrete incorporating cement kiln dust with varying suspension densities of *Sporosarcina pasteurii* using response surface methodology. In addition, a scanning electron microscope was employed to examine the morphological structure of the concrete. The main purpose of the study is to assess and optimize the strength of Self-Compacting concrete incorporating cement kiln dust using *Sporosarcina pasteurii* and response surface methodology.

## 2. Materials and Methods

### 2.1 Materials

#### 2.1.1 Portland Limestone Cement

Portland Limestone Cement (PLC) of grade 42.5N was used in this study and was obtained from a nearby dealer at Samaru market. Quality control and standard compliance tests were carried out on the cement.

#### 2.1.2 Cement Kiln Dust

The cement kiln dust in this study was sourced from Bua Company at Kalambaina Sokoto state.

#### 2.1.3 Coarse Aggregate

Crushed granite aggregate with nominal maximum size of 20 mm (passing 20 mm and retained on 10 mm sieve) was obtained from a local quarry site for control mixture preparation. The physical properties of the normal-weight coarse aggregate are presented in Table 2, with water absorption characteristics detailed in Table 3. Crushed granite with a maximum nominal particle size of 20 mm was sourced from a quarry opposite the Nigeria College of Aviation Technology (NCAT), Zaria.

#### 2.1.4 Fine Aggregate

The fine aggregate used in this study was locally sourced from a river at Area G behind Ahmadu Bello University, Zaria.

#### 2.1.5 Superplasticiser

The use of superplasticizer is essential in this study to maintain self-compacting mixtures. A polycarboxylate ether (PCE)-based superplasticizer was used and sourced from Sabon Gari market.

#### 2.1.6 Micro Organism

The microorganism used in this study is *Sporosarcina pasteurii*, which is commonly found in soil. This urease-positive microbe is rod-shaped, spore-forming, and Gram-positive, and was cultured from the Department of Microbiology, Ahmadu Bello University, Zaria.

#### 2.1.7 Cementation Reagent

The cementation reagent used in this study comprises 2.8 g of calcium chloride ( $\text{CaCl}_2$ ), 20 g of urea ( $\text{CO}(\text{NH}_2)_2$ ), 3 g of nutrient broth, 10 g of ammonium chloride ( $\text{NH}_4\text{Cl}$ ), and 2.12 g of sodium bicarbonate ( $\text{NaHCO}_3$ ) per litre of deionized water. The mass concentrations of urea and calcium chloride of 0.5 M were used in this study. The cementation reagent was prepared according to [12].

#### 2.1.8 Water

The water used in this research was obtained from the Department of Civil Engineering, Ahmadu Bello University, Zaria.

### 2.2 Methods

#### 2.2.1 Design of Experiment and Development of Mix Design Matrix

The experimental design was formulated using Design-Expert software. The mix design was conducted using a discrete multilevel categorical factorial design. The independent variables examined were the bacterial suspension density and the bacterial–cementation reagent ratio (B:C), along with curing age.

The bacterial suspension density levels were 0 (control),  $1.5 \times 10^8$ ,  $6.0 \times 10^8$ ,  $1.2 \times 10^9$ , and  $2.4 \times 10^9$  cells/mL. The bacterial–cementation reagent ratios (B:C) were 25:75, 50:50, and 75:25, where B denotes the bacterial suspension and C represents the cementation reagent. The curing periods were 7, 14, 28, and 56 days. The software generated a mix matrix consisting of 60 experimental runs with different combinations of the design variables to evaluate the strength properties of the self-compacting concrete

### 2.2.2 Mix Design

The self-compacting concrete mixtures were prepared in accordance with EFNARC (2005) guidelines and IS 10262 (2019), maintaining a constant water–cement ratio of 0.43. Portland limestone cement was partially replaced with cement kiln dust at an optimum level of 10%, and a superplasticizer dosage of 2% was used. Concrete mixes were produced using different bacterial suspension densities ( $0$ ,  $1.5 \times 10^8$ ,  $6.0 \times 10^8$ ,  $1.2 \times 10^9$ , and  $2.4 \times 10^9$  cells/mL) and varying bacterial–cementation reagent ratios (B:C = 25:75, 50:50, and 75:25). The cementation reagent was prepared at a concentration of 0.5 M

### 2.2.3 Bacteria

The bacteria used was classified as *Sporosarcina pasteurii*. This microorganism has the ability to precipitate calcite when provided with a calcium source and a suitable medium such as water. The physicochemical characteristics of the bacteria were examined at the Department of Water Resources and Environmental Engineering, Ahmadu Bello University, Zaria as presented in Table 1. Plate 1 shows the different suspension densities of *Sporosarcina pasteurii* cultured at the Department of Microbiology, Ahmadu Bello University, Zaria.

**Table 1:** Physiochemical Analysis of *S. Pasteurii*

Bacteria Species	PH	Electrical Conductivity(us/cm)
<i>S. pasteurii</i>	9.05	2850



**Plate 1:** Different Suspension Density *Sporosarcina pasteurii*

## 2.3 Determination of Static Strength for SCC-CKD with *S. Pasteurii* Bacteria

### 2.3.1 Compressive Strength

Compressive strength tests were conducted in accordance with BS EN 12390-3 (2009) standards on concrete cubes measuring  $100 \times 100 \times 100$  mm using a universal testing machine. The tests were performed at curing ages of 7, 14, 28, and 56 days. A total of 3 cubes were considered for an average for each curing age.

### 2.3.2 Flexural Strength

Flexural strength tests were carried out in accordance with [17] on concrete beam specimens measuring  $100 \times 100 \times 450$  mm. The specimens were tested using a universal testing machine with a maximum capacity of 10 tonnes under transverse loading at different curing ages. A total of 3 beams were considered for an average for each curing age.

### 2.3.3 Split Tensile Strength

Split tensile strength tests were conducted on concrete cylinders in accordance with [18] on specimens of size  $100 \times 200$  mm at curing ages of 7, 14, 28, and 56 days. A total of 3 cylinders were considered for an average for each curing age.

### 2.3.4 Mathematical Modelling Of SCC-CKD with *S- Pasteurii* Bacteria

The Design-Expert 13.0 software was used to model the strength properties of SCC–CKD incorporating *Sporosarcina pasteurii*. The relationships between independent variables and responses, as well as the statistical significance of these parameters, were established based on analysis of variance (ANOVA). Significant model terms were identified at a 95% confidence level ( $p < 0.05$ ). The software computed the sum of squares (SS), F-values, p-values, and coefficients of determination ( $R^2$ ) to evaluate the adequacy of the developed models. After fitting the experimental data, regression equations for predicting the strength properties were generated based on the general form of a multiple regression model.

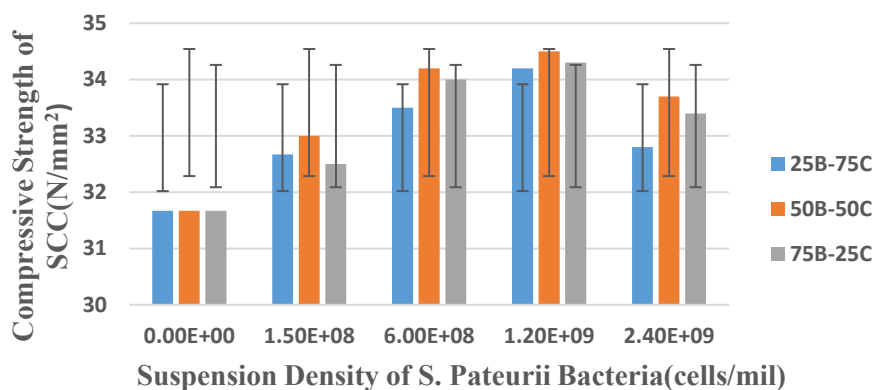
$$y = b_o + \sum_{i=1}^k b_i x_i + \sum_{i=1}^k b_{ij} x_i + \sum_{i=1}^{k-1} \sum_{j=i+1}^k b_{ij} x_i x_j + \dots + \varepsilon \quad (1)$$

Where  $y$  is the predicted response variable,  $b_o$ ,  $b_i$  and  $b_{ij}$  are respective intercept, model coefficients and linear and/or interaction terms, and are independent variables and symbol, represents errors.

## 3. Results and Discussions

### 3.1 Compressive Strength

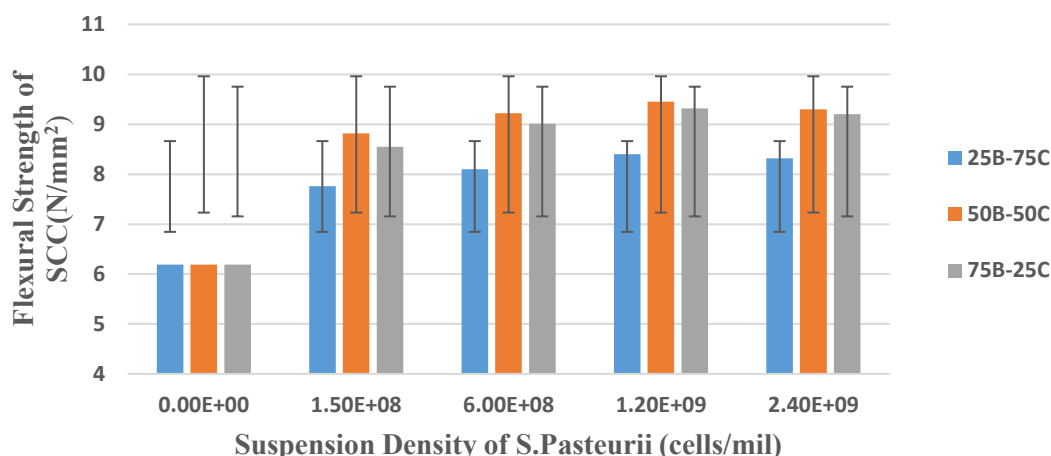
Figure 1 presents the compressive strength results of SCC–CKD with varying suspension densities of *Sporosarcina pasteurii*. The Figure illustrates how changes in bacterial suspension density influence the compressive strength of SCC–CKD across three different B:C ratios. As the bacterial density increases from 0 to  $1.20 \times 10^9$  cells/mL, all mixtures exhibit a consistent increase in compressive strength, reaching a peak at a concentration of  $1.20 \times 10^9$  cells/mL. This maximum improvement is particularly evident in the mix with B:C = 50:50, where the compressive strength reaches 34.5 N/mm<sup>2</sup>, while the B:C = 75:25 and B:C = 25:75 mixes also show notable improvements. However, when the bacterial suspension density is increased beyond this optimum level, a reduction in compressive strength is observed in all mixtures, with values falling below those recorded at the optimal dosage. These results suggest that moderate levels of *Sporosarcina pasteurii* enhance the microstructure of SCC, likely due to increased calcite precipitation, whereas excessive bacterial content may adversely affect the consistency and homogeneity of the concrete matrix.



**Figure 1:** Compressive Strength of SCC-CKD with Suspension Density of Bacteria

### 3.1.2 Flexural Strength

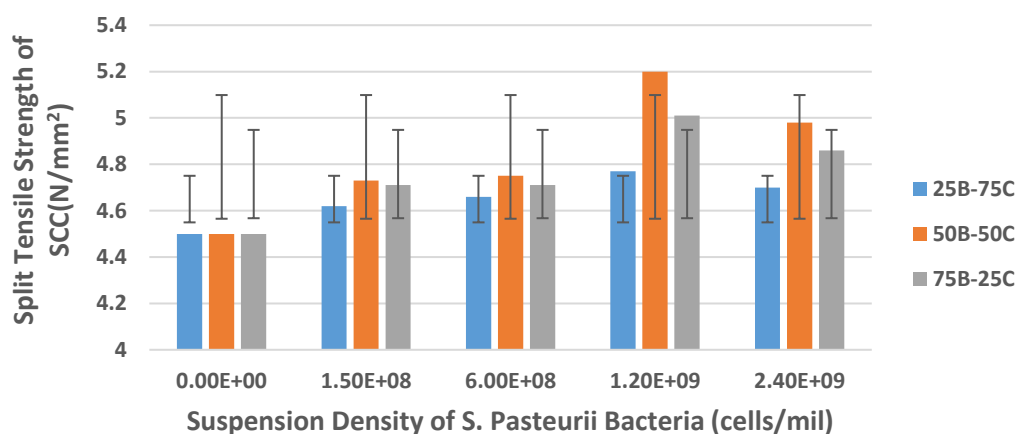
Figure 2 illustrates the flexural strength results of SCC–CKD with varying suspension densities of *B. pasteurii* bacteria. The plot demonstrates how the flexural strength of self-compacting concrete (SCC) varies with different concentrations of *S. pasteurii* across several mix ratios, denoted as B:C (B:C = 25:75, B:C = 50:50, B:C = 75:25). As the bacterial suspension concentration rises from zero to  $1.20 \times 10^9$  cells/mL, all tested mixes exhibit a notable increase in flexural strength, particularly the mix B:C = 50:50, which achieved a peak value of 9.45 N/mm<sup>2</sup>. Nevertheless, at the highest bacterial density of  $2.40 \times 10^9$  cells/mL, a slight decrease in flexural strength is observed in all mixes, though these values remain significantly higher than those without bacterial addition. This pattern underscores that incorporating an optimal bacterial suspension density can enhance the concrete's microstructure by promoting internal mineral formation, which improves its capacity to withstand bending and crack formation, thereby contributing to the exceptional improvement in both split and flexural strength of concrete [19, 20].



**Figure 2:** Flexural Strength of SCC-CKD with Suspension Density of Bacteria

### 3.1.3 Split tensile Strength

Figure 3 illustrates the results regarding the split tensile strength of SCC–CKD at varying bacterial suspension densities. The figure indicates how the split tensile strength of self-compacting concrete (SCC) is affected by the incorporation of different quantities of *S. pasteurii* bacteria, utilizing three distinct blend ratios, denoted as B:C (B:C = 25:75, B:C = 50:50, B:C = 75:25). An increase in bacterial suspension density from  $0.00 \times 10^0$  to  $1.20 \times 10^9$  cells/mL results in a significant enhancement in split tensile strength across all mixtures, with the mix B:C = 50:50 achieving a peak strength of 5.2 N/mm<sup>2</sup>. At the maximum suspension density of  $2.40 \times 10^9$  cells/mL, the mix B:C = 50:50 maintains its strength relatively well, while the other two mixtures show a slight decline. These findings suggest that a moderate level of bacterial inclusion enhances the concrete's resistance to cracking by improving internal cohesion, whereas an excessive bacterial suspension density may compromise the concrete's strength, potentially due to the introduction of defects or inconsistencies in the mix [19, 20].



**Figure 3:** Split Tensile Strength of SCC-CKD with Suspension Density of Bacteria

### 3.2 Analysis of Variance (ANOVA)

The ANOVA results for the compressive strength, flexural strength, and splitting tensile strength of SCC–CKD concrete are presented in Tables 2, 3, and 4, respectively. The analysis was carried out at a significance level of 5 percent to assess the importance of the experimental factors. Consequently, compressive strength, flexural strength, and splitting tensile strength were treated as dependent variables, while concentration, suspension density, and curing age were chosen as the independent variables. The variance analysis aimed to determine whether these factors significantly influenced the responses based on the predetermined confidence level. A 95% confidence level was selected, meaning that the P-value (probability level) should be below 0.05 ( $p < 0.05$ ). A P-value greater than 0.05 indicates that the model is not significant. As shown in

Tables 2 and 3, the models yielded P-values of  $< 0.0001$ , demonstrating a strong level of significance for these models.

**Table 2:** ANOVA Results for Compressive Strength of SCC-CKD at *S. Pasteurii*

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
<b>Model</b>	1365.71	6	227.62	157.70	$< 0.0001$	Significant
A-Concentration	2.43	1	2.43	1.68	0.2000	
B-Suspension density	23.68	1	23.68	16.41	0.0002	
C-curing age	1273.51	1	1273.51	882.32	$< 0.0001$	
A <sup>2</sup>	4.55	1	4.55	3.15	0.0816	
B <sup>2</sup>	63.07	1	63.07	43.70	$< 0.0001$	
C <sup>2</sup>	20.69	1	20.69	14.33	0.0004	
<b>Residual</b>	76.50	53	1.44			
<b>Cor Total</b>	1442.21	59				

**Table 3:** ANOVA Results for Flexural Strength of SCC-CKD at *S. Pasteurii*

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
<b>Model</b>	70.73	5	14.15	24.57	$< 0.0001$	Significant
A-Concentration	3.81	1	3.81	6.61	0.0129	
B-Suspension density	15.78	1	15.78	27.41	$< 0.0001$	
C-curing age	11.55	1	11.55	20.05	$< 0.0001$	
A <sup>2</sup>	3.40	1	3.40	5.91	0.0184	
B <sup>2</sup>	37.29	1	37.29	64.78	$< 0.0001$	
<b>Residual</b>	31.09	54	0.5757			
<b>Cor Total</b>	101.81	59				

**Table 4:** ANOVA Results for Split Tensile Strength of SCC-CKD at *S. Pasteurii*

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
<b>Model</b>	3.83	6	0.6375	41.71	$< 0.0001$	Significant
A-Concentration	0.0202	1	0.0202	1.32	0.2549	
B-Suspension density	1.28	1	1.28	83.86	$< 0.0001$	
C-curing age	1.51	1	1.51	98.57	$< 0.0001$	
A <sup>2</sup>	0.1599	1	0.1599	10.46	0.0021	
B <sup>2</sup>	0.8556	1	0.8556	55.98	$< 0.0001$	
C <sup>2</sup>	0.1532	1	0.1532	10.03	0.0026	
<b>Residual</b>	0.8100	53	0.0153			
<b>Cor Total</b>	4.64	59				

### 3.3 Statistical Analysis

Table 5 outlines the results of the fit statistics for the constructed models. These models focus on the responses of strengths of SCC–CKD. The values for the coefficients of determination ( $R^2$ ), along with the adjusted  $R^2$ , predicted  $R^2$ , and adequate precision metrics for each model, are included. The adequacy of the model can be assessed using the coefficient of determination ( $R^2$ ), which serves as a general measure of model efficacy [15]. The disparity between the predicted  $R^2$  and adjusted  $R^2$  was relatively small, with all differences remaining below 20%. Additionally, the adequate precision values, which evaluate the signal-to-noise ratio, exceeded 4. These results indicate that there are adequate signals, confirming that the models are suitable for predicting future outcomes. However, The relatively lower  $R^2$  value for flexural strength (79.46%) indicates that approximately 20% of variability is not captured by the model, likely due to the higher sensitivity of flexural behavior to microstructural heterogeneity, crack initiation, and testing variability. Despite this, the model remains acceptable for predictive purposes as supported by adequate precision values exceeding 4.

**Table 5:** Validity and Accuracy of Developed Predictive Mathematical models

S/No	Model	Coefficient of Determination $R^2$	Adjusted $R^2$	Predicted $R^2$	Difference: (Adjusted – Predicted) $R^2$	Adequate Precision > 4
1	Compressive strength	0.9470	0.9410	0.9303	0.0107	39.8746
2	Flexural strength	0.7946	0.7664	0.7629	0.0035	19.1746
3	Split tensile strength	0.8252	0.8055	0.7814	0.0241	24.7320

### 3.4 Regression Models of SCC-CKD with different Suspension density of *S. Pasteurii* Bacteria

Regression models were developed based on the strengths of SCC–CKD with varying suspension densities of *Sporosarcina pasteurii* using Design Expert software version 13, which derived regression equations for all the responses in both coded and actual terms. Quadratic models provided the best fit for all the responses. Insignificant terms were removed, and the final models were developed as shown below:

$$F_c = 30.71 + 0.8653S_d + 6.07C_a - 0.5840C^2 - 2.52S_d^2 - 1.46C_a^2 \tag{2}$$

$$F_f = 9.42 + 0.7062S_d - 0.5727C_a - 0.5050C^2 - 1.94S_d^2 \tag{3}$$

$$F_s = 4.96 + 0.2013S_d + 0.2089C_a - 0.10975C^2 - 0.2932S_d^2 - 0.1257C_a^2 \tag{4}$$

Where  $C_a$ ,  $S_d$ , and  $C$  represents the concentration of bacterial/cementation reagent, the suspension density of *S. pasteurii* bacteria, and curing age, respectively; while  $F_c$ ,  $F_f$ , and  $F_s$  denote the compressive strength, flexural strength, and splitting tensile strength, respectively.

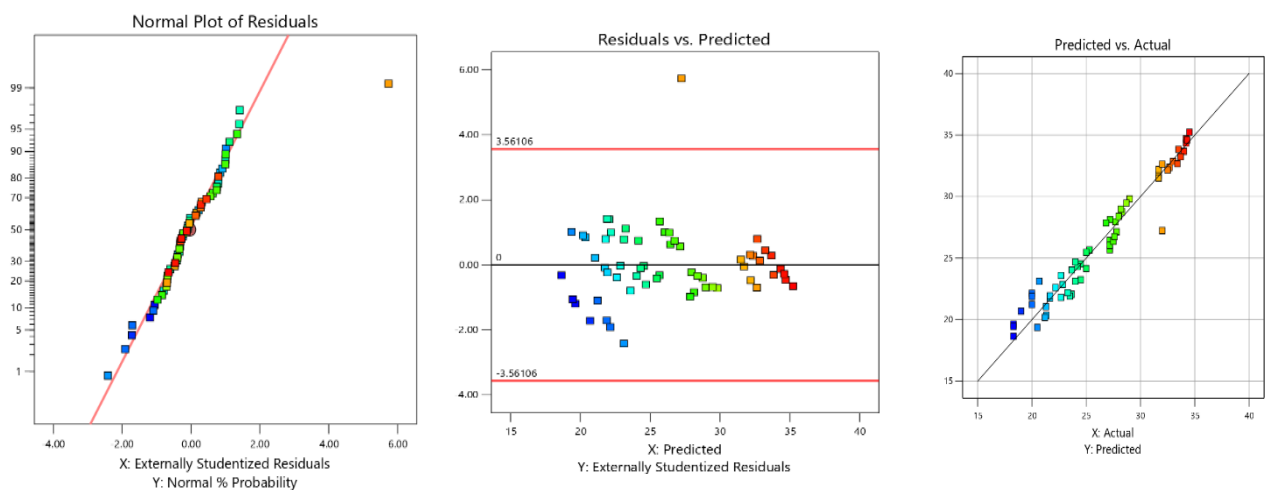
### 3.5 Diagnostics Plots Analysis

Figures 4 through 6 display diagnostic plots related to the models. The normal probability plots of residuals for the compressive strength of self-compacting concrete (SCC) with varying suspension densities of *Sporosarcina pasteurii* bacteria are shown in Figures 4a, 5a, and 6a. These plots indicate that most data points closely follow the straight reference line, suggesting that the residuals are approximately normally distributed. This outcome demonstrates that the regression models used to predict the strengths are statistically sound and free from significant bias or heteroscedasticity. The concentration of data along the line, with only a few outliers at the extremes, indicates that the

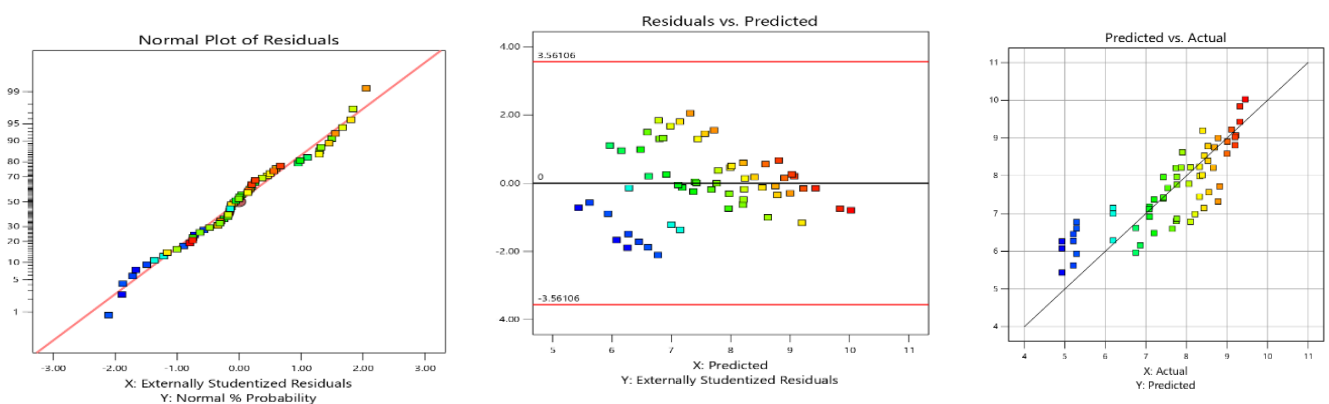
experimental results are stable and that the bacterial suspension exerts a systematic rather than random effect on SCC performance [21].

Furthermore, Figures 4b, 5b, and 6b illustrate the relationship between predicted values and their corresponding externally studentized residuals. The residuals versus predicted values plot shows that most points cluster around the zero line, indicating that the regression model produces reliable predictions with minimal error. The uniform distribution of residuals and the absence of a distinct pattern confirm that the model satisfies the assumption of constant variance, showing no evidence of heteroscedasticity. Only a few points exceed the  $\pm 3.56$  externally studentized residual limits, possibly due to experimental variability or unusual combinations of curing age and bacterial concentration.

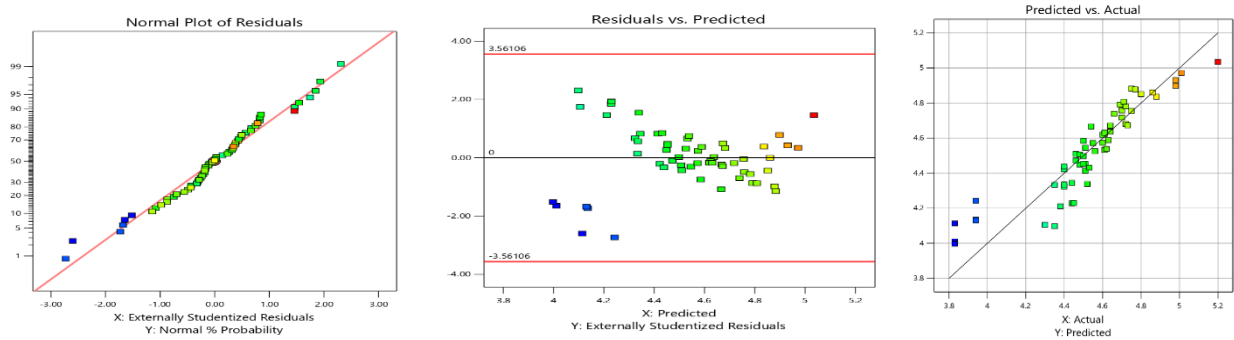
Lastly, Figures 4c, 5c, and 6c present the predicted versus actual plots for the strength of SCC containing various suspension densities of *S. pasteurii* bacteria. The close alignment of data points along the 45° line indicates a strong correspondence between predicted and actual values. This close clustering suggests that the regression model accurately represents the relationship between bacterial suspension density and concrete strength, demonstrating its strong predictive capability. Only a few points deviate slightly from the line, likely due to minor variations in curing conditions or bacterial concentrations beyond the optimized range.



**Figure 4:** Diagnostics plots of residuals for compressive strength: (a) Normal plot of residuals (b) Residuals vs predicted (c) Predicted versus actual



**Figure 5:** Diagnostics plots of residuals for flexural strength: (a) Normal plot of residuals (b) Residuals vs predicted (c) Predicted versus actual

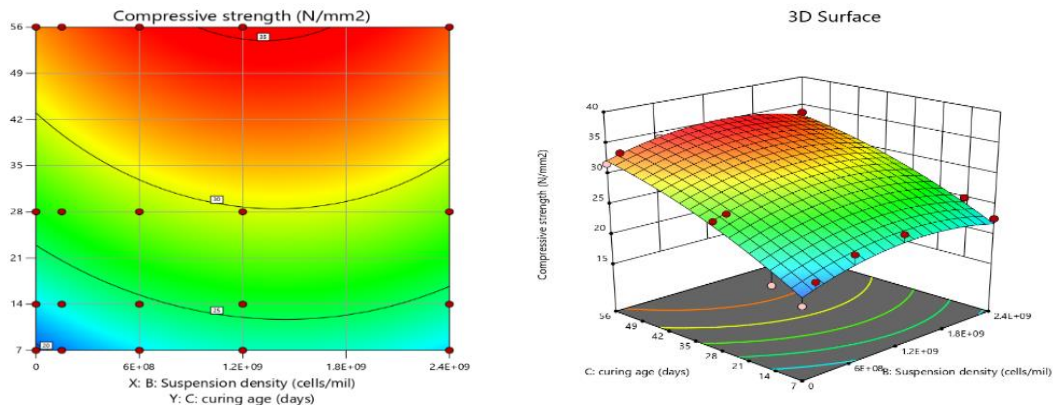


**Figure 6:** Diagnostics plots of residuals for split tensile strength: (a) Normal plot of residuals (b) Residuals vs predicted (c) Predicted versus actual

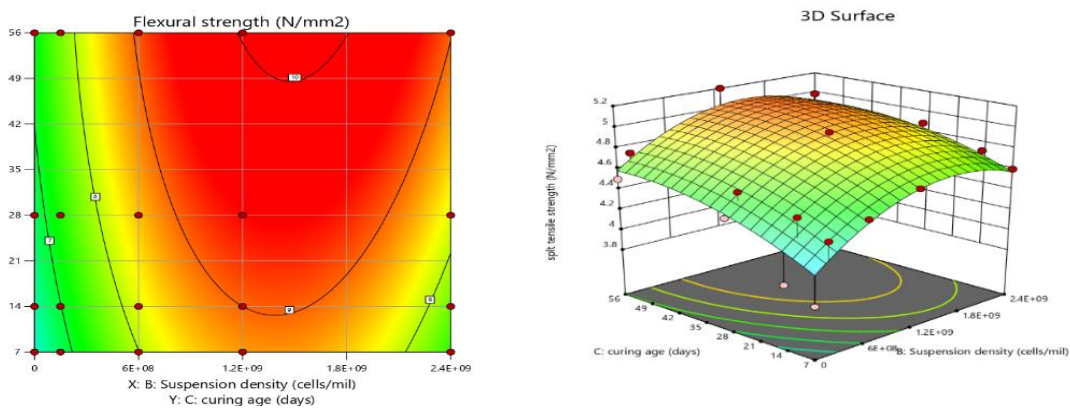
Additionally, Figures 7, 8, and 9 illustrate predictive model graphs (both contour and 3D surface plots) representing the compressive strength, flexural strength, and split tensile strength of SCC-CKD at varying bacterial suspension densities. Figures 7a and 7b, which show the 2D and 3D surface plots for compressive strength, reveal how SCC-CKD performance changes with different *Sporosarcina pasteurii* suspension densities and curing durations. Increasing bacterial concentrations and longer curing periods result in a notable enhancement of compressive strength, peaking at approximately 38–40 N/mm<sup>2</sup> at around  $2.0 \times 10^9$  cells/ml after 56 days of curing. This improvement is attributed to microbially induced calcium carbonate precipitation (MICP), which enhances the internal structure of the concrete by reducing porosity and strengthening matrix bonding [22, 23]. In contrast, mixes with bacterial densities below  $1.0 \times 10^9$  cells/ml and curing periods shorter than 28 days exhibited lower compressive strengths of about 18–25 N/mm<sup>2</sup>, likely due to insufficient biomineralization and incomplete hydration.

Figures 8a and 8b present the 2D and 3D surface plots for flexural strength. The plots indicate a gradual increase in flexural strength as both bacterial concentration and curing age increase, reaching a peak of approximately 9.5–10 N/mm<sup>2</sup> at around  $1.8 \times 10^9$  cells/ml and 56 days of curing. This enhancement is associated with MICP, whereby the bacteria produce calcium carbonate crystals that fill internal voids, reinforce the bond between aggregates and paste, and improve overall concrete [22, 23]. Lower bacterial densities and shorter curing times led to reduced flexural strengths of about 4.9–6.0 N/mm<sup>2</sup>, reflecting limited biomineralization and underdeveloped matrix formation.

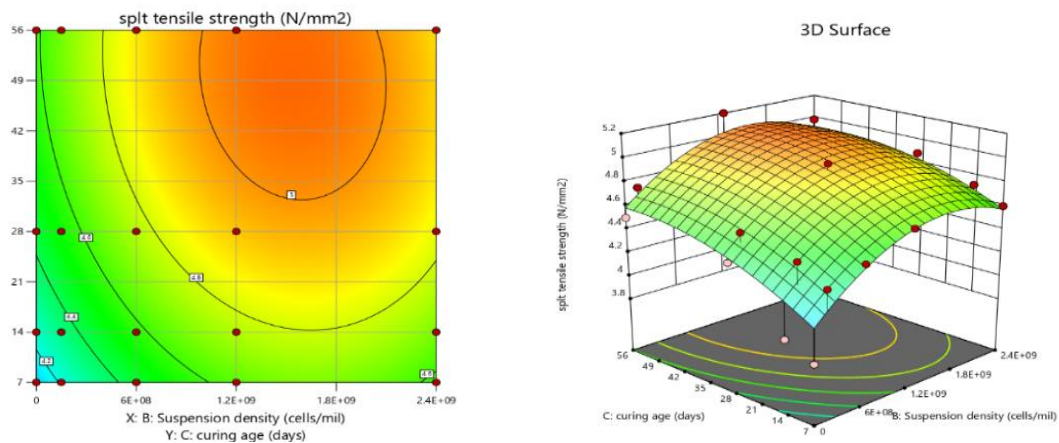
Similarly, Figures 9a and 9b show the 2D and 3D surface plots for split tensile strength. At low bacterial densities and short curing periods, split tensile strength remained modest, ranging from 3.8 to 4.2 N/mm<sup>2</sup>, as indicated by the blue-green regions of the plots. As both bacterial concentration and curing age increased, tensile strength improved, reaching an optimal level of approximately 5.0–5.2 N/mm<sup>2</sup>. This improvement is linked to MICP, where *S. pasteurii* facilitates calcite formation, filling micro-pores and binding particles more effectively, thereby strengthening the internal structure and overall tensile capacity of the concrete [24, 14].



**Figure 7:** (a and b) Contour and 3D plot of compressive strength of SCC-CKD with *S. Pasteurii*



**Figure 8:** (a and b) Contour and 3D plot of Split tensile strength of SCC-CKD with *S. Pasteurii*



**Figure 9:** (a and b) Contour and 3D plot of Split tensile strength of SCC-CKD with *S. Pasteurii*

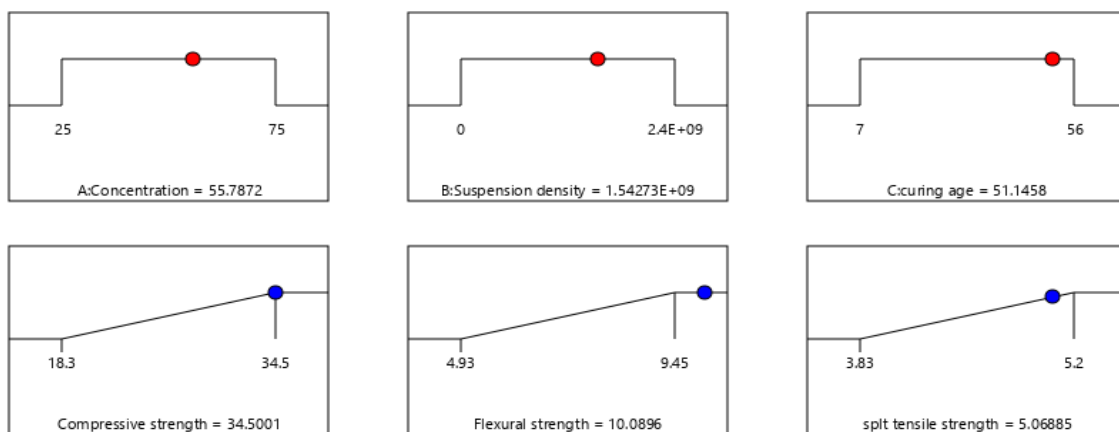
### 3.6 Optimization Result of SCC-CKD at different Suspension Density of *S. Pasteurii*

The constraints for the objectives related to the variables and responses are presented in Table 6. Figure 10 shows the ramps used for numerical optimization, illustrating the relationship between the independent variables and the responses. The figure highlights the optimized conditions that resulted in the best outcomes for the dependent variables. The analysis revealed that the optimal solution corresponds to a mix ratio of B:C = 56:44, a bacterial density of  $1.54 \times 10^9$  cells/mil, and a curing age of 52 days for hardened concrete. This combination of factors produced the best responses, yielding

compressive, flexural, and split tensile strengths of 34.5 N/mm<sup>2</sup>, 10.09 N/mm<sup>2</sup>, and 5.069 N/mm<sup>2</sup>, respectively. The optimization achieved a desirability value of 0.967, indicating a high overall effectiveness for strength enhancement.

**Table 6: Optimization Goals and Constraint**

Variables/Responses	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
A: Concentration	is in range	25	75	1	1	3
B: Suspension density	is in range	0	2.4E+09	1	1	3
C: curing age	is in range	7	56	1	1	3
Compressive strength	maximize	18.3	34.5	1	1	3
Flexural strength	maximize	4.93	9.45	1	1	3
split tensile strength	maximize	3.83	5.2	1	1	3



Desirability = 0.967  
Solution 1 out of 7

**Figure 10: Ramps Numerical Optimization Analysis**

### 3.7 Experimental Model Validation

The percentage errors are summarized in Table 7, with nearly all values falling below 5%. This indicates that the predictions from the developed models closely match the experimental results.

**Table 7: Experimental Model Validation**

Responses	Concentration (%)	S.pasteurii (cells/mil)	Curing Age (days)	Predicted Values	Experimental Values	Error (%)
Compressive strength (N/mm <sup>2</sup> )	56B-44C	1.8x10 <sup>9</sup>	52	34.502	34.62	0.34
Flexural strength (N/mm <sup>2</sup> )	56B-44C	1.8x10 <sup>9</sup>	52	10.090	9.50	-0.058
Split Tensile Strength (N/mm <sup>2</sup> )	56B-44C	1.8x10 <sup>9</sup>	52	5.069	4.88	-0.039

## 4. Conclusions

The following conclusions can be made from the results of this study:

- i. Incorporation of *Sporosarcina pasteurii* bacteria into SCC-CKD significantly improves its mechanical performance. Specifically, compressive, flexural, and split tensile strengths increase with rising bacterial suspension density, peaking at optimal concentrations before slightly declining at very high densities. The maximum observed strengths were 34.5 N/mm<sup>2</sup>, 9.45 N/mm<sup>2</sup>, and 5.20 N/mm<sup>2</sup> for compressive, flexural, and split tensile strength, respectively, at B:C = 50:50 and a bacterial density of  $1.20 \times 10^9$  cells/mil.
- ii. The mathematical models developed using Response Surface Methodology accurately predict the compressive, flexural, and split tensile strengths of SCC-CKD, with coefficients of determination ( $R^2$ ) of 94.70%, 79.46%, and 82.52%, respectively, indicating high reliability.
- iii. Numerical optimization identified the optimal mix parameters as B:C = 56:44, bacterial suspension density of  $1.54 \times 10^9$  cells/mil, and curing age of 52 days, yielding predicted strengths of 34.502 N/mm<sup>2</sup>, 10.090 N/mm<sup>2</sup>, and 5.069 N/mm<sup>2</sup> for compressive, flexural, and split tensile strength, respectively, with a desirability of 0.967.
- iv. The study confirms that moderate bacterial inclusion enhances concrete strength by promoting microbially induced calcium carbonate precipitation (MICP), which improves internal cohesion, reduces porosity, and reinforces the bonding between aggregates and paste. Excessive bacterial density, however, may slightly reduce strength due to potential inconsistencies or defects.
- v. The proposed models and optimization approach provide a reliable framework for designing SCC-CKD mixes with enhanced mechanical properties using bacterial reinforcement.

**Competing Interests:** The authors declare that they have no competing interests.

**Data Availability Statement:** The supported data associated with this researcher is available upon request from the corresponding author.

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