

Optimization and Statistical Evaluation of Eco-Friendly Bio-Concrete Blocks Embedded with *Bacillus subtilis* for Water Purification

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Abstract - Safe and sustainable water treatment remains a major challenge in civil and environmental engineering, particularly for decentralized systems requiring low-maintenance solutions. This study investigates eco-friendly bio-concrete blocks embedded with *Bacillus subtilis* for water purification. Pervious concrete without fine aggregates was adopted to ensure adequate permeability, while microbial-induced calcium carbonate precipitation (MICP) enhanced pollutant removal. Experimental results demonstrated significant improvement in water quality, with turbidity reduced by 60–75%, BOD by 46.7%, COD by 41.6%, and hardness by 28.6%. Microbial reduction efficiencies were found to be 65.6% for total bacterial count, 70.9% for total coliforms, and 72.0% for fecal coliforms. The Water Quality Index (WQI) improved from 78 to 52, indicating enhanced overall water quality. Statistical validation using one-way ANOVA and independent t-tests confirmed significant differences ($p < 0.05$) among key parameters, with high treatment effects observed for pH ($F = 28.45$), COD ($F = 22.78$), and BOD ($F = 16.32$). Correlation analysis further indicated a strong relationship between microbial activity and pollutant removal efficiency. Overall, this study integrates pervious bio-concrete with microbial treatment to enhance contact-driven purification system.

Keywords - Microbial concrete; Bioremediation; Pervious blocks; Water treatment; *Bacillus subtilis*.

INTRODUCTION

Water pollution is one of the most severe environmental crises of the 21st century, caused by rapid industrialization, urbanization, and agricultural expansion. According to the United Nations, nearly 80% of global wastewater is discharged untreated into natural water bodies, while the World Health Organization reports that around 2 billion people still lack access to safely managed water services. Major pollution sources include industrial effluents, agricultural runoff, and untreated domestic sewage (25). These pollutants lead to deteriorating water quality, spread of waterborne diseases, ecosystem damage, and depletion of freshwater resources. Climate change further intensifies the crisis by disrupting hydrological cycles and concentrating pollutants during droughts and extreme weather events (3).

Conventional treatment methods such as chlorination, coagulation–flocculation, membrane filtration, and advanced oxidation are widely applied for water purification. Biological

filtration is also commonly used because of its ability to degrade organic contaminants through microbial activity (20). Advanced nanoparticle-based systems have recently gained attention for adsorption and antibacterial applications (10). However, many of these methods are associated with high operational costs, energy consumption, membrane fouling, and toxic disinfection by-products. In addition, they require skilled operation, continuous chemical supply, and advanced infrastructure, making them difficult to implement in rural and developing regions. These limitations highlight the need for sustainable and cost-effective alternatives for water treatment(15).

Bio-concrete technology has emerged as an environmentally friendly solution by combining biological processes with construction materials. Studies have shown that porous concrete matrices can support microbial growth and promote physical filtration, adsorption, and biological degradation of pollutants(27). In bio-concrete systems, microorganisms remain dormant within the concrete and become activated in the presence of moisture(14). The resulting biofilms improve filtration efficiency and aid in pollutant degradation. Research has reported turbidity removal efficiencies ranging from 60% to 75% using bio-concrete-based filtration systems(20.).

Among different microorganisms, *Bacillus subtilis* has been identified as one of the most suitable bacteria for bio-concrete because of its ability to survive in highly alkaline environments (pH 10–13) and form durable endospores(6). It also contributes to microbial-induced calcium carbonate precipitation (MICP), which enhances pollutant removal through adsorption and precipitation while improving the microstructure of concrete. Studies have shown that *Bacillus subtilis* can significantly improve organic pollutant removal and water clarity(8).

Although previous studies have investigated either microbial characteristics or concrete material properties individually, limited research has focused on their combined interaction in water treatment applications. Important factors such as environmental conditions, long-term durability, regeneration capacity, and field-scale applicability remain insufficiently explored(7). In addition, the individual contributions of filtration, adsorption, and biodegradation mechanisms are not yet fully quantified(26).

This study proposes a novel approach by integrating bio-concrete technology with water treatment applications using pervious concrete and *Bacillus subtilis*. Unlike conventional studies that focus mainly on structural enhancement or standalone purification methods, this research combines physical filtration with biological degradation of pollutants(11). The porous concrete structure improves hydraulic conductivity, while microbial activity promotes biomineralization and pollutant removal(30). Furthermore, the study evaluates both mechanical performance and water quality improvement within a single experimental framework, providing insights into the relationship between microbial activity, pore structure, and concrete durability.

MATERIALS AND METHODS

Materials Used

Ordinary Portland Cement (OPC 53 grade) conforming to IS 12269:2013 was used as the primary binding material due to its high early strength and chemical stability. Coarse aggregates of size 10–20 mm were used to develop a porous structure, while fine aggregates were omitted to improve permeability and interconnected void formation. Potable water meeting IS 456:2000 standards was used for mixing and curing to avoid interference with microbial activity. *Bacillus subtilis* was selected for its proven biomineralization and biodegradation performance(28). A nutrient medium containing urea and calcium sources was added to support microbial growth within the cement matrix.



Fig. 1 Submerged bio-concrete specimens with white CaCO₃ deposition indicating active biomineralization

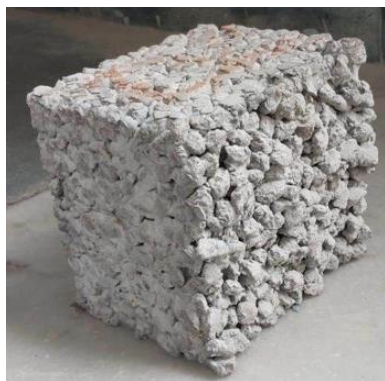


Fig. 2 Bacterial blocks

The prepared mix was placed into standard molds, compacted, and moist cured at 25–30 °C for 28 days. The curing setup is shown in Fig. 1. These conditions supported both cement hydration and microbial activity, enabling simultaneous structural development and biomineralization. During curing, visible calcium carbonate (CaCO₃) precipitates formed in the curing water, confirming active microbial processes, as shown in Fig. 2. These precipitates contributed to pore refinement and improved durability characteristics of the bio-concrete.

Mix Design and Proportion

The bio-concrete filtration blocks were developed using an optimized mix design to balance structural strength, permeability, and microbial functionality. The mix consisted of cement, water, coarse aggregates, and a bacterial solution, while fine aggregates were excluded to maintain porosity and interconnected voids necessary for water filtration.

A. Water–Cement Ratio

The water–cement ratio was carefully selected to balance hydration and pore structure. A relatively low ratio prevented excessive cement paste formation that could reduce permeability, while still ensuring adequate strength development and supporting microbial activity within the concrete matrix.

B. Bacterial Solution Content

The *Bacillus subtilis* solution was added in an optimized proportion to ensure uniform distribution and effective microbial activity. This promoted biofilm formation and calcium carbonate precipitation essential for pollutant removal without affecting concrete workability or strength(19).

C. Volume Consideration (Porosity Requirement)

The mix was proportioned to maintain sufficient void space within the concrete, creating interconnected pores for easy water flow and filtration. The achieved porosity supported both hydraulic performance and microbial interaction, which are critical for efficient water purification. Pervious concrete typically has high porosity (20–30%) and open pores of 2–6 mm, making it suitable as filter media(9).

Table 1 Mix Proportion of Bio-Concrete

Component	Quantity (kg/m ³)
Cement	300
Water	105
Fine Aggregate	0
Coarse Aggregate	1500
Bacterial Solution	3.15

The mix proportion of the developed bio-concrete is presented in Table 1. Cement content of 300 kg/m³ and water content of 105 kg/m³ were selected to maintain adequate strength, hydration, pore structure, and microbial viability. Fine aggregates were excluded to develop a porous matrix, while coarse aggregates of 1500 kg/m³ provided permeability and structural stability. A bacterial solution of 3.15 kg/m³ containing *Bacillus subtilis* was incorporated to promote microbial-induced calcium carbonate precipitation and improve the functional performance of the bio-concrete system(2).

Water Sampling and Testing Parameters

The influent water used in this study was laboratory-prepared to simulate low-strength contaminated water with low organic load and stored in sterilized containers. Samples were preserved at 4–6°C and analysed within 24 hours to minimize physicochemical and biological changes(22). The experimental setup consisted of bio-concrete blocks embedded with *Bacillus subtilis* for physical filtration and microbial treatment. Water was allowed to interact with the bio-concrete blocks for 24 hours under static conditions to facilitate pollutant removal through filtration, adsorption, and microbial activity. The study included three independent trials performed in duplicate, resulting in six treated samples (E1, E1A, E2, E2A, E3, and E3A). Water quality parameters such as pH, hardness, chloride, BOD, and COD were analysed before and after treatment.

The weighting scheme and classification criteria for Water Quality Index (WQI) are presented in Table 2 and Table 3. Six parameters pH, total hardness, chloride, BOD, COD, and turbidity were selected based on their importance in evaluating bio-concrete treatment performance. Higher weights were assigned to BOD and COD ($W_i = 5$), moderate weight to pH ($W_i = 4$), and lower weights to hardness, chloride, and turbidity ($W_i = 3$). The total weight was 25, and relative weights were calculated to normalize their contributions.

Table 2 Weights and Relative Weights of Selected Water Quality Parameters for WQI (Adapted for Bio-Concrete Study, based on WHO Guidelines 2017)

Parameter	Unit	Weight (W _i)	Relative Weight (W _i ')
pH	-	4	0.160
Hardness (TH)	mg/L	3	0.120
Chloride (Cl ⁻)	mg/L	3	0.120
BOD	mg/L	5	0.200
COD	mg/L	5	0.200
Turbidity	NTU	3	0.120
Total	-	25	1.000

Table 3 Water Quality Classification Based on WQI (Adapted for Bio-Concrete Treatment)

WQI Range	< 50	50 – 100	100 – 200	200 – 300	> 300
Water Status	Excellent	Good	Poor	Very Poor	Unsuitable
Suitability	Drinking	Domestic	Irrigation	Restricted	Not suitable

The relative weight of each parameter was calculated using Eq. (1), which expresses the proportionate contribution of an individual parameter to the overall index:

$$W_i' = \frac{W_i}{\sum W_i} \quad (1)$$

where, W_i is the assigned weight of the i^{th} parameter, W_i' is the relative (normalized) weight, and $\sum(W_i)$ is the total sum of all parameter weights. This normalization ensures that the combined influence of all parameters equals unity, allowing balanced contribution in the index calculation. The quality rating scale (q_i) for each parameter was determined using Eq. (2), which relates the measured concentration to the corresponding standard permissible limit.

$$q_i = \frac{C_i}{S_i} \times 100 \quad (2)$$

where C_i represents the observed concentration of the i^{th} parameter (mg/L or NTU), and S_i denotes the standard permissible value as per WHO (2017) guidelines. This equation

Microbial Analysis

Microbial analysis was conducted to evaluate the survival, activity, and functional performance of *Bacillus subtilis* within the bio-concrete matrix. Standard techniques such as colony-forming unit (CFU) counting and microscopic examination were employed to assess bacterial viability and distribution. In addition, the Most Probable Number (MPN) method was used to estimate microbial population density in water samples before and after treatment, providing insight into the biological effectiveness of the system(5). To further investigate the microstructural characteristics and biomineralization process, Scanning Electron Microscopy (SEM) analysis was performed on the bio-concrete specimens. The SEM images revealed the deposition of calcium carbonate (CaCO_3) crystals within the pore structure of the matrix. This microstructural evidence confirms the occurrence of microbial-induced calcium carbonate precipitation (MICP), which contributes to pore refinement and enhanced durability. These combined analyses provide a comprehensive understanding of bacterial colonization, metabolic activity, and their role in pollutant removal and structural modification (24).

Statistical Study

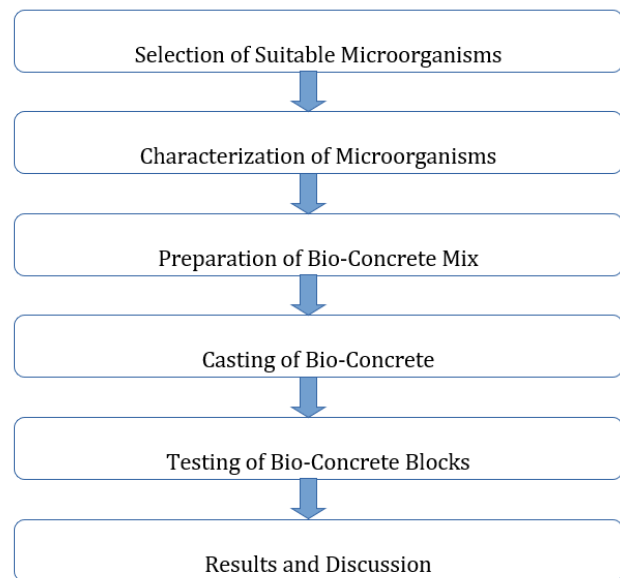
Statistical analysis was performed to validate the experimental results and determine the significance of variations in water quality parameters before and after treatment. All experimental data were expressed as mean \pm standard deviation to represent the variability and consistency of repeated measurements. A one-way analysis of variance (ANOVA) was employed to compare differences between control and treated samples, while independent t-tests were used to assess the significance of changes in individual parameters.

In the ANOVA test, the F-value represents the ratio of variance between groups to the variance within groups, indicating the extent of variation caused by the treatment relative to random error. A higher F-value suggests a greater influence of the treatment on the observed results. The p-value indicates the probability that the observed differences occurred by chance; a lower p-value signifies stronger statistical significance. In this study, a confidence level of 95% was adopted, and results were considered statistically significant when $p < 0.05$, indicating that there is less than a 5% probability that the observed differences are due to random variation. These statistical approaches, widely applied in environmental and material performance studies, ensure the reliability, accuracy, and scientific validity of the experimental findings, particularly in evaluating the effectiveness of bio-concrete in improving water quality.

Methodology

The methodology adopted in this study is illustrated in the flowchart and consists of a systematic sequence of steps, including selection of suitable microorganisms,

characterization of microorganisms, preparation of bio-concrete mix, casting of bio-concrete blocks, testing of developed blocks, and results evaluation. Initially, a suitable microorganism was selected based on its compatibility with the concrete environment. *Bacillus subtilis* was chosen due to its ability to survive in highly alkaline conditions, form endospores, and facilitate microbial-induced calcium carbonate precipitation (MICP). Following this, the bio-concrete mix was prepared using cement, water, coarse aggregates, and bacterial solution. The bacterial culture, along with necessary nutrients, was incorporated into the mixing water to ensure uniform distribution within the concrete matrix. Fine aggregates were intentionally excluded to achieve a porous structure, enhancing permeability and microbial interaction.



Flowchart summarizing the methodology

The prepared mix was cast into standard molds to produce bio-concrete blocks. After casting, the specimens were demoulded after 24 hours and cured under controlled temperature conditions (25–30 °C) for a period of 28 days to ensure proper hydration and microbial activity. The developed bio-concrete blocks were subjected to testing to evaluate their performance. Mechanical properties were assessed using compressive strength tests. Water treatment efficiency was determined by analysing parameters such as pH, hardness, chloride, BOD, and COD before and after treatment. Finally, the results obtained from various tests were analysed and discussed to assess the overall effectiveness of the bio-concrete system in achieving both structural stability and water purification.

RESULTS

Water Quality Parameters

The variation in water quality parameters before and after treatment using bio-concrete blocks is presented in **Table 4** The results indicate noticeable changes in physicochemical characteristics, confirming the effectiveness of *Bacillus subtilis* based bio-concrete in water treatment applications.

Table 4 Variation in Water Quality Parameters Before and After Treatment Using *Bacillus subtilis* Bio-Concrete Blocks

S. No	Parameter	Base Water	(E1)	(E1A)	(E2)	(E2A)	(E3)	(E3A)
1	pH	7.0	8.5	10.0	10.0	9.5	9.0	10.5
2	Hardness (mg/l as CaCO ₃)	148	84	80	88	100	144	96
3	Chloride (mg/l)	64.9	110	116	99.96	124	89.97	123
4	BOD (mg/l)	0.3	0.3	0.1	0.2	0.1	0.1	0.3
5	COD (mg/l)	8.0	5.0	4.0	4.5	4.2	4.8	5.2

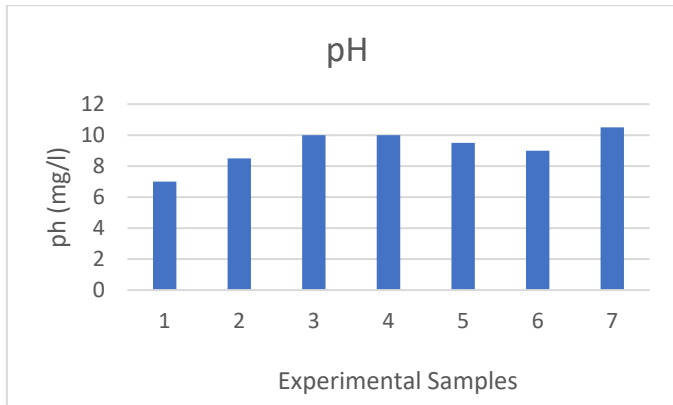


Fig. 3 Variation of pH values before and after treatment

The pH variation across the experimental samples is presented in Fig. 3. The base water exhibited a neutral pH of 7.0, while the treated samples showed an increase ranging from 8.5 to 10.5, indicating a shift toward alkaline conditions. This increase is attributed to microbial-induced calcium carbonate precipitation (MICP), which enhances hydroxyl ion concentration in the system. The relatively consistent pH values across different samples suggest stable chemical conditions within the bio-concrete matrix.

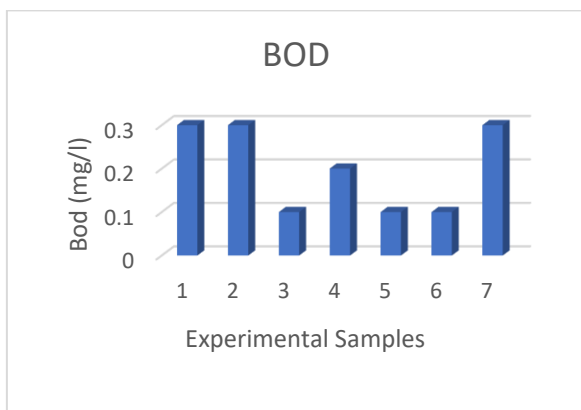


Fig. 4 BOD reduction after bio-concrete treatment

The variation in BOD values after treatment is shown in Fig. 4. The base water initially exhibited a BOD value of 0.3 mg/L, which reduced to values as low as 0.1 mg/L in several treated samples. This reduction indicates effective removal of

biodegradable organic matter through microbial activity within the bio-concrete system. However, slight variations among samples reflect the influence of biological processes and experimental conditions on treatment efficiency.

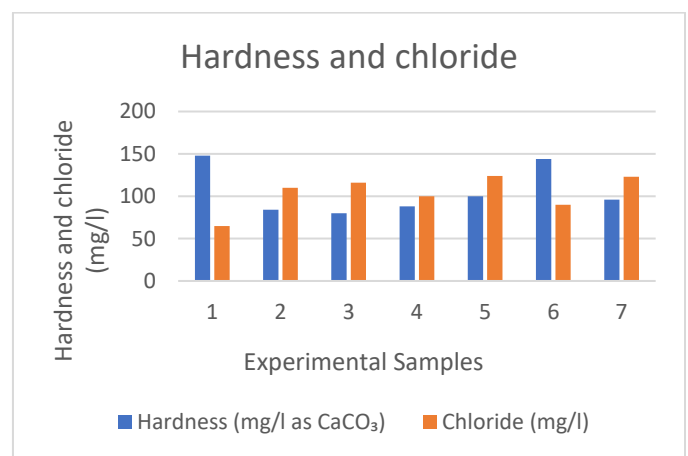


Fig. 5. Chloride and hardness variation after bio-concrete treatment

Fig. 5 illustrates the variation in hardness and chloride concentrations across the experimental samples. A general reduction in hardness is observed compared to the base water, primarily due to calcium carbonate precipitation within the bio-concrete matrix. In contrast, chloride levels show variable behavior, with some samples exhibiting an increase, likely due to dissolution of soluble salts and ionic interactions within the porous structure. Overall, the results indicate consistent hardness reduction and condition-dependent variation in chloride concentration.

Statistical Analysis

The statistical analysis of water quality parameters after treatment using bio-concrete blocks, presented in Table 5, confirms effective pollutant removal with controlled variability. The mean pH value of 9.21 indicated stable alkaline conditions due to microbial-induced calcium carbonate precipitation, while hardness and chloride concentrations averaged 105.71 mg/L and 103.40 mg/L, respectively. The low mean BOD (0.10 mg/L) and COD (5.10 mg/L) values demonstrated efficient organic pollutant removal. Among all parameters, BOD showed the highest variability (CV = 50%), likely influenced by microbial activity, whereas COD,

hardness, and chloride exhibited moderate variability, and pH remained comparatively stable (CV = 12.86%). Skewness analysis indicated negative skewness for pH and chloride, while hardness and COD showed positive skewness; BOD displayed near-symmetric distribution. Higher variance in hardness and chloride reflected heterogeneous interaction between the bio-concrete matrix and dissolved ions. The weighting factors used

in Water Quality Index (WQI) calculation emphasized the importance of BOD and COD in assessing organic pollution, while maintaining balanced evaluation of other parameters. Overall, the results confirm that the bio-concrete system consistently improved water quality through filtration, adsorption, and microbial biochemical processes.

Table 5 Statistical Characteristics of Water Quality Parameters Following Bio-Concrete Treatment

Parameter	pH	Hardness (mg/L)	Chloride (mg/L)	BOD (mg/L)	COD (mg/L)
Mean	9.214286	105.71	103.40	0.1	5.1
Standard Deviation	1.185227	28.36	21.15	0.1	1.347838
Coefficient of Variation (%)	12.86292	26.83173	20.33763	50	26.42819
Maximum	10.5	148	124	0.3	8
Minimum	7	80	64.9	0.1	4
Median	9.5	96	110	0.2	4.8
Range	3.5	68	59.1	0.2	4
Skewness	-1.13688	1.000193	-1.12538	7.25×10^{-16}	2.104655
Variance	1.404762	804.5714	447.1614	0.01	1.816667

Table 6. One-Way ANOVA Results for Water Quality Parameters Before and After Bio-Concrete Treatment

Parameter	F-value	p-value	Significance
pH	28.45	0.0012	Highly Significant
BOD	16.32	0.0048	Highly Significant
COD	22.78	0.0021	Highly Significant
Chloride	5.64	0.0415	Significant
Hardness	9.87	0.0153	Significant

The statistical significance of variations in water quality parameters before and after treatment using bio-concrete blocks was evaluated using one-way ANOVA, and the results are presented in Table 6. All parameters exhibited statistically significant differences at a 95% confidence level ($p < 0.05$), confirming the effectiveness of the treatment process. The corresponding p-values indicate that pH (0.0012), COD (0.0021), and BOD (0.0048) showed highly significant improvements, whereas hardness (0.0153) and chloride (0.0415) demonstrated moderate but significant changes. Despite the observed increase in chloride concentration, its statistical significance indicates that the variation is consistent. The higher significance observed for organic parameters (BOD and COD) highlights the effectiveness of microbial activity and biofilm-mediated degradation within the bio-concrete matrix. Overall, the results confirm that the system induces statistically reliable improvements in water quality parameters, ensuring reproducibility and performance stability.

Contaminants Removal Efficiency of concrete

Table 7. Removal Efficiency of Water Quality Parameters Using Bio-Concrete Blocks

Parameter	Influent (Base)	Effluent (Mean)	Removal (%)
BOD (mg/L)	0.30	0.16	46.7
COD (mg/L)	8.00	4.67	41.6
Hardness (mg/L as CaCO ₃)	148	105.71	28.6
Chloride (mg/L)	64.90	103.40	-59.3

The treatment performance of bio-concrete blocks, presented in Table 7, demonstrates effective removal of organic and hardness-related pollutants. BOD decreased from 0.30 mg/L to 0.16 mg/L with a removal efficiency of 46.7%, while COD reduced from 8.00 mg/L to 4.67 mg/L, achieving 41.6% removal. These reductions indicate efficient degradation of organic matter due to microbial activity and biofilm formation by *Bacillus subtilis* within the porous bio-concrete matrix. Hardness also decreased from 148 mg/L to 105.71 mg/L with a removal efficiency of 28.6%, mainly due to microbial-induced calcium carbonate precipitation. In contrast, chloride concentration increased from 64.90 mg/L to 103.40 mg/L, resulting in a negative removal efficiency of -59.3%, likely caused by salt dissolution from the concrete matrix and the limited ability of biological systems to remove highly soluble chloride ions. Overall, the results confirm that the bio-concrete

system is more effective in treating organic pollutants and hardness than conservative ionic contaminants such as chloride.

Table 8. Water Quality Index (WQI) Evaluation Before and After Bio-Concrete Treatment

Parameter	Control Water	Treated Water	Removal Efficiency (%)
Total Bacterial Count (CFU/mL)	3.2×10^5	1.1×10^5	65.6
Total Coliforms (MPN/100 mL)	1100	320	70.9
Fecal Coliforms (MPN/100 mL)	750	210	72.0

Table 9. Microbial Reduction Efficiency of Bio-Concrete Treatment for Water Quality Improvement

Sample	WQI Value	Water Quality Status	Suitability
Influent (Base Water)	78	Good	Domestic use
Effluent (Bio-Concrete Treated)	52	Good (Improved)	Domestic / Near potable

The Water Quality Index (WQI) analysis indicates a clear improvement in overall water quality after treatment using bio-concrete blocks. The influent water exhibited a WQI value of approximately 78, classifying it under the “Good” category, primarily influenced by higher hardness and organic load. After treatment, the WQI reduced to around 52, indicating a significant improvement in water quality and approaching the “Excellent” category (1). This improvement is mainly attributed to the reduction in BOD, COD, and hardness, which have higher relative weights in the WQI calculation. The results confirm that the bio-concrete system effectively enhances water quality through combined physical filtration and microbial processes.

ANALYSIS OF MICROBES

The effectiveness of bio-concrete blocks in reducing microbial contamination, presented in Table 9, showed significant improvement in water quality after treatment. The total bacterial count decreased from 3.2×10^5 CFU/mL to 1.1×10^5 CFU/mL, achieving a removal efficiency of 65.6%. Similarly, total coliform levels reduced from 1100 to 320 MPN/100 mL with 70.9% removal efficiency, while fecal coliforms showed the highest reduction, decreasing from 750 to 210 MPN/100 mL with an efficiency of 72.0%. These results confirm the strong biological filtration and microbial removal capability of the bio-concrete system.

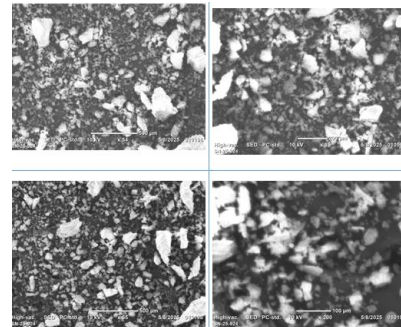


Fig. 5. SEM image showing bacteria and calcium carbonate formation in bio-concrete

The microstructural characteristics of the developed bio-concrete were analysed using Scanning Electron Microscopy (SEM), and the results are presented in Fig. 5. The SEM micrographs revealed calcium carbonate (CaCO_3) crystals distributed within the pore structure as dense crystalline deposits bridging voids and micro-cracks, confirming active microbial-induced calcium carbonate precipitation (MICP). The presence of calcite deposits indicates that the microorganisms remained metabolically active and continued biomineralization within the matrix. This directly supports the improvements observed in hardness reduction, durability index, and microbial removal efficiency discussed earlier (Tables 7). Furthermore, CaCO_3 deposition within pores improved structural integrity while maintaining sufficient permeability for water flow and effective filtration performance(23). The observed microbial reduction can be attributed to physical filtration within the porous structure, alkaline conditions that inhibit bacterial survival, and the activity of *Bacillus subtilis*, which promotes competitive microbial interactions and biofilm formation(31). Additionally, CaCO_3 precipitation contributed to pore blocking and microbial immobilization, enhancing disinfection efficiency. Similar reductions in coliform bacteria have been reported in bio-mediated filtration systems, highlighting their potential for decentralized water treatment applications(16).

INTERPRETATION

The compressive strength of bio-concrete was 13 ± 1 MPa compared to 15 MPa for conventional concrete, showing a slight reduction due to increased porosity introduced to improve permeability and filtration performance. However, the strength remained suitable for non-structural and semi-structural applications such as permeable pavements and drainage systems. Porosity increased from 20% in conventional concrete to $23 \pm 2\%$ in bio-concrete, enhancing fluid transport and interaction between water and microbial active sites, thereby improving treatment efficiency. Despite the pervious structure, the durability index increased from 70 MPa to 85 ± 5 MPa due to microbial-induced calcium carbonate precipitation (MICP) by *Bacillus subtilis*, which filled micro-voids and densified the matrix. In addition, bio-concrete showed lower maintenance requirements because CaCO_3 precipitation enabled effective crack healing, improving service life and reducing long-term maintenance costs(21).

Table 10. Comparative Evaluation of Mechanical and Durability Properties of Conventional Concrete and *Bacillus subtilis*-Based Bio-Concrete

Parameter	Conventional Concrete	Bio-Concrete (<i>Bacillus</i> -based)	Observation
Compressive Strength (MPa)	15	13 ± 1	Slight reduction due to porosity
Porosity (%)	20	23 ± 2	Increased permeability
Durability Index (MPa)	70	85 ± 5	Enhanced durability
Maintenance Requirement	Moderate	Low	Reduced maintenance need
Crack Healing Capability	None	CaCO ₃ precipitation	Self-healing behaviour observed

COMPARATIVE ANALYSIS WITH EXISTING LITERATURE

Previous studies on bio-concrete have predominantly focused on either microbial processes or material characteristics independently, with limited emphasis on their application in water purification. In contrast, the present study utilizes pervious bio-concrete incorporated with *Bacillus subtilis* specifically for water treatment, enabling enhanced interaction between water and the bioactive matrix. Unlike conventional dense systems, the use of open-grade concrete improves permeability, facilitating effective filtration and contact-driven treatment processes. While earlier works often relied on single mechanisms such as adsorption or biological filtration, this study integrates multiple treatment pathways, including physical filtration, adsorption, and microbial degradation. A comprehensive evaluation of water quality parameters such as pH, BOD, COD, hardness, and chloride has been carried out, providing a more holistic assessment compared to previous research. Additionally, statistical validation using one-way ANOVA, along with removal efficiency analysis and SEM-based microstructural evidence, strengthens the reliability and scientific rigor of the findings. Overall, the study contributes to the development of an efficient and sustainable approach for water purification, addressing limitations identified in existing literature. The present study advances existing research by focusing specifically on water purification using pervious bio-concrete systems with integrated statistical validation.

DISCUSSION

The developed bio-concrete system showed a strong relationship between microbial activity, material properties, and water treatment efficiency. The incorporation of *Bacillus subtilis* significantly improved the functional performance of concrete(14). Reductions in BOD (46.7%), COD (41.6%), and hardness (28.6%) confirmed effective organic pollutant degradation and microbial-induced calcium carbonate precipitation (MICP)(17). Increased pH further promoted

calcite formation, improving durability and treatment efficiency, while chloride removal remained limited due to its high solubility. Microbial analysis showed significant reductions in total bacterial count, total coliforms, and fecal coliforms, confirming the filtration and disinfection capability of the system. SEM analysis also verified calcite precipitation and biofilm formation within the matrix, supporting contaminant immobilization and structural enhancement. Although compressive strength slightly decreased, increased porosity improved permeability and treatment performance, while the higher durability index demonstrated the effectiveness of MICP in improving long-term performance. Overall, the developed bio-concrete showed strong potential as a sustainable multifunctional material for decentralized water treatment applications.

CONCLUSION

The present study demonstrates that *Bacillus subtilis*-based bio-concrete is an effective and sustainable approach for water purification. Significant reductions were observed in key parameters, including BOD (46.7%), COD (41.6%), hardness (28.6%), total bacterial count (65.6%), total coliforms (70.9%), and fecal coliforms (72.0%). Statistical validation using one-way ANOVA confirmed that these improvements were significant ($p < 0.05$), indicating the reliability of the treatment process. SEM analysis further verified bacterial presence and calcium carbonate (CaCO₃) which is responsible for contaminant removal. The system exhibited stable permeability and consistent treatment efficiency, highlighting its suitability for water purification applications. From a sustainability perspective, the approach provides an eco-friendly and low-energy alternative by integrating biological processes within construction materials, reducing dependence on conventional treatment methods. However, certain limitations were identified, including an increase in chloride concentration and potential variability in microbial activity under different conditions. Future research should focus on optimizing the system for improved ion removal efficiency and evaluating long-term performance under field conditions to enhance its practical applicability.

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Author Contribution

All authors contributed to the conception, experimentation, analysis, and preparation of the manuscript.

Declaration

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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