

# Application of Bio-Calcination Technology in Improving Strength and Durability Characteristics of Cement Mortar

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## Abstract

The construction industry is increasingly seeking sustainable technologies capable of enhancing the performance and service life of cement-based materials while reducing maintenance costs and environmental impacts. One promising approach is Microbially Induced Calcium Carbonate Precipitation (MICP), a bio-mediated process whereby selected bacterial species precipitate calcium carbonate within the pore structure of cementitious matrices. This study investigates the influence of *Bacillus coagulans* and nutrient broth medium on the compressive strength, water absorption, and microstructural characteristics of cement mortar. *Bacillus coagulans* cultures were introduced into mortar mixtures at cell concentrations of  $1.5 \times 10^8$ ,  $6.0 \times 10^8$ , and  $1.2 \times 10^9$  cells/mL, while nutrient broth medium was incorporated as a partial replacement of mixing water at levels of 30%, 40%, and 50%. Mortar specimens were prepared using a cement-to-sand ratio of 1:3 and a water-cement ratio of 0.50. Compressive strength tests were conducted after 3, 7, 14, and 28 days of curing, while water absorption and microstructural analyses were performed to evaluate durability and calcite formation. Experimental results revealed that bacterial incorporation significantly improved mortar performance compared with conventional mortar. The optimum mixture containing  $1.5 \times 10^8$  cells/mL *Bacillus coagulans* and 50% nutrient broth medium achieved a 28-day compressive strength of 28.02 MPa, compared with 24.08 MPa for the control specimen. Water absorption was reduced from 5.98% in the control mortar to 3.46% in the bio-treated mortar, indicating enhanced pore refinement and reduced permeability. Scanning Electron Microscopy (SEM) observations confirmed the formation of calcite crystals within pore spaces and microcracks, contributing to matrix densification and strength enhancement. The findings demonstrate that *Bacillus coagulans*-mediated bio-calcination represents an effective and environmentally sustainable strategy for improving the mechanical and durability properties of cement mortar. The technology offers significant potential for the development of self-healing and long-lasting construction materials suitable for modern infrastructure applications.

**Keywords:** Microbially Induced Calcium Carbonate Precipitation (MICP), *Bacillus coagulans*, Biocalcination, Cement Mortar, Compressive Strength, Durability, Calcite Precipitation, and SEM Analysis.

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## INTRODUCTION

### 1.1 Background of the Study

The construction industry remains one of the largest consumers of natural resources worldwide, with concrete and mortar serving as the most widely used construction materials due to their versatility, durability, and cost-effectiveness. Cement mortar, which is a mixture of cement, fine aggregates, and water, is extensively used in masonry construction, plastering, rendering, and repair works. Despite its widespread use, cement mortar is

vulnerable to several durability-related problems, including shrinkage cracking, permeability, chemical attack, and deterioration caused by environmental exposure. These challenges often compromise structural performance and significantly increase maintenance and rehabilitation costs.

The development of microcracks in cementitious materials begins at an early age due to plastic shrinkage,

thermal stresses, and drying shrinkage. As structures age, these microcracks can propagate and form interconnected networks that facilitate the ingress of water, chlorides, sulphates, and other aggressive agents. Such ingress accelerates reinforcement corrosion, freeze-thaw damage, sulphate attack, and carbonation, ultimately reducing the service life of concrete infrastructure (Neville, 2011).

In recent decades, researchers have explored innovative technologies aimed at improving the durability and sustainability of cement-based materials. Among these emerging technologies, biomineralisation has attracted considerable attention due to its environmentally friendly nature and its ability to enhance material performance. Biomineralisation refers to the formation of minerals through the biological activity of microorganisms. One of the most promising biomineralisation mechanisms is Microbially Induced Calcium Carbonate Precipitation (MICP), whereby microorganisms precipitate calcium carbonate ( $\text{CaCO}_3$ ) within the pores and cracks of cementitious materials (De Muyck, De Belie, & Verstraete, 2010).

MICP occurs when specific bacterial species metabolically produce carbonate ions that react with calcium ions in their surrounding environment, leading to the formation of calcium carbonate crystals. These precipitated crystals occupy voids and cracks, thereby densifying the cement matrix and improving mechanical and durability properties. The resulting calcium carbonate is chemically similar to naturally occurring calcite, which contributes to increased compressive strength, reduced permeability, and enhanced resistance to environmental degradation (Stocks-Fischer, Galinat, & Bang, 1999).

Among the bacterial species investigated for construction applications, *Bacillus* species have demonstrated exceptional potential because of their ability to form endospores that can survive harsh environmental conditions. *Bacillus coagulans*, in particular, has emerged as a promising candidate due to its high tolerance to alkaline environments, which are characteristic of cementitious systems. The bacterium can remain viable during cement hydration and facilitate calcite precipitation through metabolic pathways that support calcium carbonate formation.

Previous studies have reported significant improvements in compressive strength and durability properties following bacterial incorporation into cementitious materials. Ramakrishnan, Bang, and Deo (2001) demonstrated that bacterial treatment could improve crack healing and strength recovery in concrete. Similarly, Achal, Mukherjee, and Reddy (2011) observed enhanced compressive strength and reduced permeability in bacterial concrete specimens due to calcite precipitation. Chahal, Siddique, and Rajor (2012) further reported reductions in water absorption and chloride permeability in fly ash concrete treated with bacteria.

Despite these advances, the performance of bacterial mortar is influenced by several factors, including bacterial

species, cell concentration, nutrient availability, curing conditions, and calcium source. Nutrient media play a particularly important role because bacterial growth and metabolic activity depend largely on the availability of essential nutrients. Nutrient Broth Medium (NBM) provides proteins, amino acids, vitamins, and minerals required for bacterial proliferation and calcite production. Therefore, optimising nutrient concentration is critical for maximising the effectiveness of bio-calcination processes.

Bio-calcination technology presents a sustainable alternative to conventional chemical admixtures used for enhancing mortar performance. Unlike synthetic additives, bacteria are naturally occurring microorganisms capable of self-reproducing under favourable conditions. Their ability to precipitate calcium carbonate contributes not only to strength enhancement but also to self-healing capabilities that can prolong the lifespan of structures. This characteristic aligns with global sustainability goals aimed at reducing construction-related environmental impacts and promoting durable infrastructure development.

Consequently, investigating the influence of *Bacillus coagulans* concentration and nutrient broth medium replacement levels on the strength and durability of cement mortar is necessary to advance the practical application of bacterial technology in civil engineering.

## 1.2 Statement of the Problem

One of the major challenges affecting cement mortar and concrete structures is the formation of pores and microcracks during hydration and service life. These defects create pathways for moisture and aggressive ions to penetrate the cement matrix, resulting in progressive deterioration and loss of structural integrity. Conventional repair methods such as epoxy injection, polymer coatings, and chemical sealants are often expensive, labour-intensive, and environmentally unsustainable.

Although considerable research has been conducted on bacterial concrete and self-healing technologies, significant knowledge gaps remain regarding the use of *Bacillus coagulans* in mortar systems. Most studies have focused on other bacterial species such as *Sporosarcina pasteurii*, *Bacillus subtilis*, and *Bacillus sphaericus*. Comparatively fewer investigations have evaluated the effectiveness of *Bacillus coagulans* under varying bacterial concentrations and nutrient conditions.

Furthermore, the relationship between nutrient broth medium concentration and bacterial calcite precipitation remains inadequately understood. Since bacterial metabolism directly influences calcium carbonate production, improper nutrient levels may reduce bacterial activity and limit performance enhancement. There is, therefore, a need to determine the optimal bacterial concentrations and nutrient replacement levels capable of maximising compressive strength and durability improvements.

This study seeks to address these gaps by evaluating the effects of varying *Bacillus coagulans* concentrations and nutrient broth medium replacement levels on the engineering properties of cement mortar.

### 1.3 Aim of the Study

The aim of this study is to investigate the effect of *Bacillus coagulans*-induced bio-calcination on the strength development, water absorption characteristics, and microstructural properties of cement mortar.

### 1.4 Objectives of the Study

The specific objectives of the study are:

1. To evaluate the effect of *Bacillus coagulans* concentration on the compressive strength of cement mortar.
2. To determine the influence of varying percentages of nutrient broth medium on bacterial activity and mortar performance.
3. To assess the water absorption characteristics of bacterial cement mortar.
4. To investigate the microstructural changes resulting from bacterial calcite precipitation using scanning electron microscopy (SEM).
5. To identify the optimum bacterial concentration and nutrient replacement level for improved mortar performance.

### 1.5 Research Questions

The study seeks to answer the following research questions:

1. What effect does *Bacillus coagulans* concentration have on the compressive strength of cement mortar?
2. How does nutrient broth medium influence bacterial calcite precipitation within mortar?
3. Can bacterial bio-calcination reduce water absorption in cement mortar?
4. What microstructural changes occur as a result of bacterial incorporation?
5. Which combination of bacterial concentration and nutrient medium provides optimum performance?

### 1.6 Research Hypotheses

The following hypotheses were tested:

- H<sub>01</sub>: *Bacillus coagulans* has no significant effect on the compressive strength of cement mortar.  
H<sub>02</sub>: Nutrient broth medium replacement levels have no significant effect on mortar durability.  
H<sub>03</sub>: Bio-calcination does not significantly reduce water absorption in cement mortar.  
H<sub>11</sub>: *Bacillus coagulans* significantly improves the compressive strength of cement mortar.

H<sub>12</sub>: Nutrient broth medium significantly enhances bacterial performance and mortar durability.

H<sub>13</sub>: Bio-calcination significantly reduces water absorption in cement mortar.

### 1.7 Significance of the Study

This study contributes to the growing body of knowledge on bio-based construction materials and sustainable infrastructure development. The findings reveal insights into the role of *Bacillus coagulans* in improving mortar performance through microbial calcium carbonate precipitation.

The study is beneficial to:

- Civil engineers – by providing alternative techniques for enhancing durability and reducing maintenance costs.
- Researchers – by expanding knowledge of bacterial biomineralisation in cementitious materials.
- Construction Industries – by promoting environmentally friendly construction technologies.
- Policy Makers – by supporting sustainable infrastructure initiatives aligned with green construction objectives.
- Educational Institutions – by serving as reference material for future investigations in construction biotechnology.

### 1.8 Scope of the Study

This research focuses on the incorporation of *Bacillus coagulans* into cement mortar at concentrations of  $1.5 \times 10^8$ ,  $6.0 \times 10^8$ , and  $1.2 \times 10^9$  cells/mL. Nutrient broth medium was introduced at replacement levels of 30%, 40%, and 50%. Mortar specimens were prepared using a cement-to-sand ratio of 1:3 and a water-cement ratio of 0.50.

The investigation covers:

- Compressive strength development at 3, 7, 14, and 28 days.
- Water absorption characteristics.
- Microstructural analysis using scanning electron microscopy (SEM).
- Evaluation of calcite precipitation effects on mortar performance.

The study does not include field-scale applications, reinforcement corrosion studies, sulphate attack resistance, or long-term durability assessments beyond 28 days.

### 1.9 Operational Definition of Terms

Bio-calcination: The biologically induced formation of calcium carbonate through microbial activity.

Biomineralisation: A natural process in which living organisms produce mineral deposits.

Calcite: A crystalline form of calcium carbonate (CaCO<sub>3</sub>).

Compressive Strength: The maximum compressive

stress a material can withstand before failure.

**Durability:** The ability of a material to resist deterioration over time.

**Microbially Induced Calcium Carbonate Precipitation (MICP):** A biological process through which microorganisms precipitate calcium carbonate.

**Nutrient Broth Medium (NBM):** A growth medium supplying nutrients required for bacterial metabolism.

**Scanning Electron Microscopy (SEM):** An analytical technique used to examine material microstructure at high magnification.

**Water Absorption:** The amount of water absorbed by a material expressed as a percentage of its dry weight.

## 2. LITERATURE REVIEW

### 2.1 Introduction

The search for sustainable and durable construction materials has become increasingly important due to the growing demand for infrastructure development and the environmental challenges associated with conventional construction practices. Cement-based materials such as mortar and concrete remain indispensable in civil engineering; however, their susceptibility to cracking, permeability, and deterioration continues to pose serious problems. Researchers have therefore explored innovative approaches to improve the mechanical performance and durability of cementitious materials.

One of the most promising developments recently is the application of biotechnology in construction materials through microbially induced calcium carbonate precipitation (MICP). MICP is a biomineralisation process in which microorganisms precipitate calcium carbonate within the pore structures of cementitious materials. This process has been shown to improve strength, reduce permeability, and enhance durability.

This chapter reviews relevant literature on cement mortar, durability challenges, biomineralisation, bacterial calcite precipitation mechanisms, bacterial species used in construction, previous studies on bacterial mortar and concrete, and the theoretical and conceptual frameworks underpinning the present study.

### 2.2 Cement Mortar and Its Engineering Properties

Cement, fine aggregate, and water make up most of the mortar. It serves as a binding agent in masonry construction and as a finishing material in rendering and plastering applications. The composition, hydration characteristics, pore structure, and curing conditions largely influence the engineering performance of mortar.

According to Neville (2011), the strength of cement mortar develops through the hydration of cement compounds, particularly tricalcium silicate ( $C_3S$ ) and dicalcium silicate ( $C_2S$ ). These compounds react with water to form calcium silicate hydrate (C-S-H) gel and

calcium hydroxide, which provide structural integrity and strength.

Despite its widespread use, cement mortar possesses inherent weaknesses associated with porosity and cracking. During hydration, capillary pores are formed within the matrix. These pores create pathways through which water and aggressive chemicals can penetrate, accelerating deterioration processes such as carbonation, chloride ingress, sulphate attack, and freeze-thaw damage.

Mehta and Monteiro (2014) noted that permeability is one of the most important factors controlling the durability of cementitious materials. A reduction in pore connectivity directly improves resistance to environmental deterioration and increases service life.

### 2.3 Durability Challenges in Cementitious Materials

Durability refers to the ability of a material to withstand environmental and service conditions without significant degradation over time. Physical, chemical, and biological factors constantly influence the long-term performance of cement mortar and concrete structures.

#### 2.3.1 Cracking

Cracks are among the most common defects in cement-based materials. They may develop due to:

- Plastic shrinkage
- Drying shrinkage
- Thermal stresses
- Mechanical loading
- Chemical reactions

Even microscopic cracks can significantly increase permeability and facilitate the ingress of harmful substances.

According to Li and Herbert (2012), microcracks often serve as initiation points for structural deterioration because they create pathways for moisture and aggressive ions.

#### 2.3.2 Water Penetration

Water penetration contributes to numerous durability problems, including:

- Reinforcement corrosion
- Sulphate attack
- Alkali-silica reaction
- Freeze-thaw damage

Reducing water absorption is therefore essential for enhancing durability.

#### 2.3.3 Chloride Ingress

Chloride ions are particularly harmful in reinforced concrete structures because they initiate corrosion of

embedded steel reinforcement. Once corrosion begins, expansive rust products cause cracking and spalling of concrete cover.

### 2.3.4 Sulphate Attack

Sulphates present in soils and groundwater react with cement hydration products to form expansive compounds such as ettringite and gypsum. These reactions cause cracking, softening, and loss of strength.

The need to mitigate these durability problems has motivated researchers to explore biological approaches capable of reducing permeability and enhancing matrix densification.

## 2.4 Biotechnology in Construction Materials

Biotechnology involves applying living organisms or biological systems to solve engineering problems. Within the construction sector, biotechnology has emerged as a sustainable approach for improving material performance through microbial activities.

Applications of biotechnology in civil engineering include:

- Bioremediation
- Soil stabilisation
- Self-healing concrete
- Bio-cementation
- Crack repair
- Bio-calcination

Among these applications, MICP has attracted significant attention due to its ability to enhance cementitious materials through natural mineral formation.

De Muynck, De Belie, and Verstraete (2010) described microbial carbonate precipitation as one of the most promising technologies for sustainable construction because it utilises naturally occurring biological processes instead of synthetic chemical treatments.

## 2.5 Microbially Induced Calcium Carbonate Precipitation (MICP)

Microbially induced calcium carbonate precipitation is a biomineralisation process through which microorganisms produce calcium carbonate crystals in their environment.

The process generally involves three key components:

1. Active microorganisms
2. Calcium source
3. Carbonate source

When these components are available, calcium carbonate crystals precipitate according to the following:  $[Ca^{2+} + CO_3^{2-}] \rightarrow CaCO_3$   
The precipitated calcite fills pores and cracks within cementitious materials, resulting in improved mechanical and durability properties.

According to Stocks-Fischer, Galinat, and Bang (1999), bacterial cells serve as nucleation sites for crystal growth. Negatively charged bacterial cell walls attract calcium ions, facilitating localised calcite precipitation.

## 2.6 Mechanisms of Bacterial Calcite Precipitation

Several metabolic pathways can induce calcium carbonate precipitation.

### 2.6.1 Ureolytic Pathway

The most extensively studied pathway involves urease-producing bacteria.

In this process:

Urea is hydrolysed into ammonia and carbon dioxide.

The reaction increases pH and promotes carbonate formation.

Carbonate ions react with calcium ions to form calcium carbonate.

Whiffin (2004) demonstrated that ureolytic bacteria can rapidly produce large quantities of calcite under suitable conditions.

However, ureolysis produces ammonia, which may create environmental concerns.

### 2.6.2 Non-Ureolytic Pathway

Non-ureolytic bacteria produce carbonate ions through alternative metabolic activities, such as:

- Aerobic respiration
- Nitrate reduction
- Sulphate reduction
- Organic acid metabolism

*Bacillus coagulans* primarily functions through non-ureolytic pathways, making it environmentally attractive because it minimises ammonia production.

Xu, Yao, and Jiang (2014) reported that non-ureolytic bacteria can effectively precipitate calcium carbonate while avoiding some environmental drawbacks associated with urease activity.

## 2.7 *Bacillus* Species Used in Cementitious Materials

Several bacterial species have been investigated for construction applications.

### 2.7.1 *Sporosarcina pasteurii*

*Sporosarcina pasteurii* is one of the most commonly studied ureolytic bacteria.

Advantages include:

- High urease activity
- Rapid calcite production
- Strong crack-healing capability

However, ammonia generation remains a concern.

### 2.7.2 *Bacillus subtilis*

*Bacillus subtilis* possesses strong spore-forming capabilities and can survive highly alkaline environments. Jonkers (2011) reported successful crack healing in concrete using *Bacillus subtilis* encapsulated within lightweight aggregates.

### 2.7.3 *Bacillus sphaericus*

*Bacillus sphaericus* has demonstrated significant improvements in concrete strength and permeability resistance.

Studies indicate that the bacterium can remain viable within cement matrices for extended periods.

### 2.7.4 *Bacillus megaterium*

*Bacillus megaterium* is known for efficient biomineralisation and has been widely investigated in bacterial concrete technology.

Achal et al. (2011) observed substantial strength improvements associated with calcite precipitation by *Bacillus megaterium*.

### 2.7.5 *Bacillus coagulans*

*Bacillus coagulans* is a Gram-positive, spore-forming bacterium capable of surviving high-pH environments typical of cementitious systems.

Advantages include:

- High alkalinity tolerance
- Durable spore formation
- Non-pathogenic nature
- Effective calcite precipitation

These characteristics make *Bacillus coagulans* a suitable candidate for bio-calcination applications.

## 2.8 Nutrient Broth Medium and Bacterial Growth

The effectiveness of bacterial mineralisation significantly depends on nutrient availability.

Nutrient broth medium contains the following:

- Peptones
- Beef extract
- Amino acids
- Vitamins
- Minerals

These components support bacterial metabolism and reproduction.

In cementitious environments, nutrient supplementation ensures:

- Sustained bacterial viability
- Increased carbonate production
- Enhanced calcite precipitation

Researchers have reported that an inadequate nutrient supply can significantly reduce bacterial activity and limit biomineralisation efficiency.

Therefore, determining the optimum nutrient concentration is critical for maximising mortar performance.

## 2.9 Effect of Bacterial Calcite Precipitation on Compressive Strength

Numerous studies have reported improvements in compressive strength due to bacterial incorporation.

Ramakrishnan, Bang, and Deo (2001)

Observed strength increases ranging from 10% to 25% following bacterial treatment.

Achal, Mukherjee, and Reddy (2011)

Reported approximately 20–30% strength enhancement in bacterial concrete due to calcite deposition.

De Muynck et al. (2010)

Found that bacterial carbonate precipitation significantly reduced pore volume and increased compressive strength.

Chahal, Siddique, and Rajor (2012)

Demonstrated improved strength development in fly ash concrete treated with bacteria.

The consensus among these studies is that bacterial calcite acts as a pore-filling material that densifies the cement matrix and strengthens particle bonding.

## 2.10 Effect on Water Absorption and Permeability

Water absorption is a key durability indicator because it reflects pore connectivity within cementitious materials. Studies consistently show that bacterial calcite precipitation reduces water absorption.

De Muynck et al. (2010)

Reported substantial reductions in capillary water uptake following bacterial treatment.

Wang et al. (2012)

Observed lower permeability in self-healing concrete specimens containing bacteria.

Siddique and Chahal (2011)

Found that bacterial concrete exhibited significantly lower chloride permeability than conventional concrete.

These improvements are attributed to:

- Pore blockage
- Crack sealing
- Matrix densification

## 2.11 Microstructural Characterisation of Bio-Cement Materials

Microstructural analysis provides direct evidence of bacterial mineralisation.

Common analytical techniques include:

### Scanning Electron Microscopy (SEM)

Used to visualise:

- Calcite crystals
- Crack healing
- Pore filling

### X-Ray Diffraction (XRD)

Used to identify crystalline phases present in cement matrices.

### Energy Dispersive Spectroscopy (EDS)

Used to determine elemental composition of precipitated minerals.

SEM investigations conducted by Achal et al. (2011) revealed extensive calcite crystal growth surrounding bacterial cells embedded within mortar specimens.

## 2.12 Theoretical Framework

This study is anchored on the biomineralisation theory developed through the works of Lowenstam (1981) and later expanded by De Muynck et al. (2010).

The theory states that microorganisms can alter their surrounding chemical environment to induce mineral precipitation.

Within cementitious materials:

1. Bacteria metabolise nutrients.
2. Carbonate ions are generated.
3. Calcium ions become attracted to bacterial cell walls.
4. Calcium carbonate precipitates.
5. Calcite fills pores and cracks.

The resulting densification improves both strength and durability.

## 2.13 Conceptual Framework

Independent Variables:

- *Bacillus coagulans* concentration
- Nutrient broth medium replacement level

↓  
Microbial Activity

↓  
Calcium Carbonate Precipitation  
↓Pore Filling and Crack Healing↓

Dependent Variables

- Compressive strength
- Water absorption
- Durability
- Microstructural characteristics

## 2.14 Empirical Review

The empirical literature demonstrates a strong

relationship between bacterial mineralisation and improved cementitious material performance.

Most studies report:

- Strength increases of 10–35%
- Reduced permeability
- Reduced water absorption
- Improved crack-healing capacity
- Enhanced durability

However, very limited studies have specifically investigated *Bacillus coagulans* combined with varying nutrient broth medium replacement levels in cement mortar systems.

## 2.15 Research Gap

Although significant progress has been made in bacterial concrete technology, several gaps remain:

1. Limited studies on *Bacillus coagulans* compared to other *Bacillus* species.
2. There is insufficient understanding of how to optimise nutrient broth.
3. Lack of comparative evaluation of multiple bacterial concentrations.
4. Limited data on the interaction between bacterial density and nutrient availability.
5. Inadequate information on microstructural changes associated with *Bacillus coagulans*-induced bio-calcination.

The present study seeks to address these gaps by systematically evaluating the influence of *Bacillus coagulans* concentration and nutrient broth medium replacement on mortar strength, durability, and microstructure.

## 3. MATERIALS AND METHODS

### 3.1 Introduction

This chapter presents the materials, experimental procedures, laboratory methods, specimen preparation techniques, testing protocols, and analytical procedures employed to evaluate the effect of *Bacillus coagulans*-induced bio-calcination on the strength and durability characteristics of cement mortar. The study adopted a controlled laboratory experimental design to investigate the influence of bacterial concentration and nutrient broth medium replacement levels on compressive strength, water absorption, and microstructural development.

The methodology was developed based on established procedures reported in previous studies on microbial concrete and biomineralisation in cementitious materials (De Muynck et al., 2010; Achal et al., 2011; Chahal et al., 2012).

### 3.2 Research Design

The study employed a laboratory-based experimental

research design.

The experimental approach enabled the systematic manipulation of:

- Bacterial concentration
- Nutrient broth medium replacement level

while measuring their effects on:

- Compressive strength
- Water absorption
- Microstructural properties

A control mortar mix without bacterial incorporation was prepared and tested alongside bio-modified mortar specimens to provide a basis for comparison.

### 3.3 Materials Used

The materials used in this investigation included:

#### 3.3.1 Ordinary Portland Cement (OPC)

Ordinary Portland cement conforming to ASTM C150 specifications was used as the primary binder.

The cement possessed the following:

- Good workability characteristics
- Standard-setting behaviour
- Consistent hydration properties

**Table 3.1:** Relevant physical properties included:

Property	Value
Specific Gravity	3.15
Initial Setting Time	45 minutes
Final Setting Time	300 minutes
Fineness	320 m <sup>2</sup> /kg

#### 3.3.2 Fine Aggregate (Sand)

Natural river sand passing through a 4.75 mm sieve and conforming to ASTM C778 was used as fine aggregate.

The sand was:

- Clean
- Well graded
- Free from organic impurities

**Table 3.2** shows the aggregate properties.

Property	Value
Specific Gravity	2.63
Water Absorption	1.20%
Fineness Modulus	2.75
Maximum Size	4.75 mm

#### 3.3.3 Mixing Water

Portable drinking water free from contaminants was used for specimen preparation and curing.

The water satisfied ASTM C1602 requirements for concrete mixing.

- Potential for calcium carbonate precipitation
- The bacterial culture was obtained from a certified microbiological laboratory and maintained under controlled conditions before use.

#### 3.3.4 Bacterial Culture

The bacterial species used in this study was *Bacillus coagulans*.

The organism was selected due to:

- High alkalinity resistance
- Spore-forming capability
- Ability to survive cement hydration conditions

#### 3.3.5 Nutrient Broth Medium (NBM)

Nutrient broth medium served as the bacterial growth medium.

The medium contained:

- Peptone
- Beef extract
- Sodium chloride

- Essential minerals  
The medium provided nutrients necessary for bacterial growth and metabolic activity.

### 3.4 Preparation of Bacterial Suspension

The bacterial culture was grown in sterile nutrient broth medium under laboratory conditions.

The cultivation process involved:

1. Sterilisation of nutrient broth.
2. Inoculation with *Bacillus coagulans*.
3. Incubation at 37°C for 24–48 hours.
4. Monitoring bacterial growth.

**Table 3.3:** Cell concentration was determined using spectrophotometric and serial dilution techniques.

Sample Code	Bacterial Concentration
BC1	$1.5 \times 10^8$ cells/mL
BC2	$6.0 \times 10^8$ cells/mL
BC3	$1.2 \times 10^9$ cells/mL

These concentrations were selected based on previous studies demonstrating effective calcite precipitation within cementitious systems.

### 3.5 Experimental Variables

#### 3.5.1 Independent Variables

The independent variables investigated were:

- Bacterial Concentration
  - $1.5 \times 10^8$  cells/mL
  - $6.0 \times 10^8$  cells/mL
  - $1.2 \times 10^9$  cells/mL
- Nutrient Broth Replacement
  - 30%
  - 40%
  - 50%

#### 3.6.1 Control Mix

The control mortar consisted of:

**Table 3.4.:** Composition of the Control Mortar Mix

Material	Quantity
Cement	1 part
Sand	3 parts
Water	0.50 w/c

No bacteria or nutrient broth medium was added.

#### 3.6.2 Bio-Mortar Mixes

Nine bacterial mixtures were prepared.

#### 3.5.2 Dependent Variables

The dependent variables measured included:

- Compressive strength
- Water absorption
- Microstructural characteristics

#### 3.6 Mix Design

A mortar mix ratio of 1:3 (cement) was adopted throughout the investigation.

The water-cement ratio was maintained at: [w/c = 0.50]

This ratio was selected because it provides adequate workability while maintaining satisfactory strength development.

**Table 3.5:** Composition of Bacterial Mixtures Prepared with Different Bacterial Concentrations and Nutrient Broth Medium (NBM) Percentages

Mix ID	Bacteria	NBM (%)
BC1-30	$1.5 \times 10^8$	30
BC1-40	$1.5 \times 10^8$	40
BC1-50	$1.5 \times 10^8$	50
BC2-30	$6.0 \times 10^8$	30
BC2-40	$6.0 \times 10^8$	40
BC2-50	$6.0 \times 10^8$	50
BC3-30	$1.2 \times 10^9$	30
BC3-40	$1.2 \times 10^9$	40
BC3-50	$1.2 \times 10^9$	50

### 3.7 Casting of Mortar Specimens

The mortar specimens were prepared according to ASTM C109/C109M.

The procedure involved:

Step 1

Dry mixing of cement and sand until uniformity was achieved.

Step 2

Preparation of the bacterial solution by combining the bacterial suspension with water and a nutrient broth medium.

Step 3

Gradual addition of bacterial solution into the dry mix.

Step 4

Mechanical mixing for approximately five minutes.

Step 5

Placement into cube moulds measuring the following:

[50 × 50 × 50 mm]

Step 6

Compaction using a vibrating table.

Step 7

Surface finishing.

### 3.8 Curing Procedure

After casting, specimens were covered with plastic sheets to prevent moisture loss.

The specimens were demoulded after 24 hours and cured in water at the following temperatures:

[27 °C]

Curing periods were:

- 3 days
- 7 days
- 14 days
- 28 days

### 3.9 Experimental Testing

#### 3.9.1 Compressive Strength Test

The compressive strength test was conducted according to ASTM C109/C109M.

The test determines the maximum load a mortar specimen can withstand before failure.

The compressive strength was calculated as:

$$[f_c = \frac{P}{A}]$$

Where:

( $f_c$ ) = Compressive strength (MPa)

(P) = Failure load (N)

(A) = Loaded area (mm<sup>2</sup>)

Three specimens were tested for each mix and curing age.

The average value was reported.

#### 3.9.2 Water Absorption Test

Water absorption was determined in accordance with ASTM C642.

The procedure included:

1. Oven drying specimens at 105°C.
2. Recording dry mass.
3. Immersion in water for 24 hours.
4. Measuring saturated mass.

Water absorption was calculated using:

$$[WA = \frac{W_s - W_d}{W_d} \times 100]$$

Where:

WA = Water absorption (%)

WS = Saturated weight

Wd = Dry weight

Reduced water absorption indicates improved durability.

#### 3.10 Scanning Electron Microscopy (SEM)

SEM analysis was conducted to evaluate microstructural changes resulting from bacterial activity.

Specimens selected for SEM analysis included:

- Control mortar

- Optimum bacterial mortar

The analysis focused on:

- Crystal morphology
- Pore structure
- Calcite formation
- Matrix densification

Small fragments were extracted from 28-day specimens and coated with gold before imaging.

Images were obtained at various magnifications.

### 3.11 Mechanism of Bio-Calcination in Mortar

The bio-calcination process expected during experimentation can be summarised as follows:

Stage 1: Bacterial Survival

Spores survive within the cement matrix.

Stage 2: Nutrient Activation

Nutrient broth provides food for bacterial growth.

Stage 3: Metabolic Activity

Bacteria metabolise nutrients and generate carbonate ions.

Stage 4: Calcium Carbonate Formation

Carbonate ions react with calcium ions released during cement hydration.

$[Ca^{2+} + CO_3^{2-}] \rightarrow CaCO_3$

Stage 5: Pore Filling

Calcite crystals occupy pores and microcracks.

Stage 6: Strength Enhancement

Matrix densification improves mechanical performance.

### 3.12 Reliability and Quality Control

To ensure reliability:

1. Materials were obtained from the same source.
2. All specimens were prepared under identical laboratory conditions.
3. Testing equipment was calibrated before use.
4. Triplicate specimens were tested.
5. Average values were reported.
6. Standard ASTM procedures were strictly followed.

### 3.13 Statistical Analysis

Experimental data were analysed using descriptive and inferential statistical methods.

The following analyses were conducted:

Descriptive Statistics

- Mean
- Standard deviation
- Percentage improvement

Comparative Analysis

Comparison between:

- Control mortar

- Bio-modified mortar

Percentage Strength Improvement

Strength enhancement was determined using:

$$[SI = \frac{f_b - f_c}{f_c} \times 100]$$

Where:

(SI = strength improvement (%))

FB = bimodal strength

FC = control strength

### 3.14 Ethical and Safety Considerations

The bacterial strain used was non-pathogenic and safe for laboratory handling.

Laboratory safety measures included:

- Use of gloves and protective clothing.
- Sterilisation of microbiological equipment.
- Proper disposal of bacterial cultures.
- Compliance with institutional laboratory regulations.

## 4. RESULTS AND DISCUSSION

### 4.1 Introduction

This chapter presents the experimental results obtained from the investigation of the effects of *Bacillus coagulans*-induced bio-calcination on the strength and durability characteristics of cement mortar. The results are discussed in relation to the objectives of the study and compared with findings reported in previous research.

The parameters evaluated include:

- Compressive strength development
- Water absorption characteristics
- Percentage strength improvement
- Microstructural characteristics from SEM analysis
- Influence of bacterial concentration
- Influence of Nutrient Broth Medium (NBM)

The findings provide insight into the effectiveness of microbial calcium carbonate precipitation in enhancing mortar's performance.

### 4.2 Compressive Strength Results

#### 4.2.1 Compressive Strength Development of Control Mortar

The compressive strength values obtained for the control mortar are presented in Table 4.1.

**Table 4.1** Compressive Strength of Control Mortar

<b>Curing Age (Days)</b>	<b>Compressive Strength (MPa)</b>
3	12.56
7	17.42
14	21.65
28	24.08

The results indicate a continuous increase in strength with curing age due to the progressive hydration of cement compounds.

The 28-day compressive strength of the control mortar was 24.08 MPa and served as the benchmark for evaluating the performance of bacterial mortar. This trend agrees with Neville (2011), who reported that cement hydration continues beyond 28 days, resulting in

gradual strength development.

#### 4.2.2 Compressive Strength of Bio-Mortar

Table 4.2 presents the compressive strength values obtained for all bacterial mortar mixtures after 28 days of curing.

**Table 4.2** Twenty-Eight-Day Compressive Strength of Bio-Mortar

<b>Mix ID</b>	<b>Strength (MPa)</b>
Control	24.08
BC1-30	25.44
BC1-40	26.83
BC1-50	28.02
BC2-30	24.96
BC2-40	25.87
BC2-50	26.15
BC3-30	24.22
BC3-40	24.74
BC3-50	25.08

The results demonstrate that bacterial incorporation improved compressive strength relative to the control specimen.

The highest strength value of 28.02 MPa was achieved by mixture BC1-50 containing:

- $1.5 \times 10^8$  cells/mL bacterial concentration
- 50% Nutrient Broth Medium

This indicates that lower bacterial concentrations may be more effective than excessive bacterial loading.

#### 4.3 Percentage Strength Improvement

The percentage increase in compressive strength was determined using:

$$[SI = \frac{f_b - f_c}{f_c} \times 100]$$

where:

(f<sub>b</sub>) = Bio-mortar strength

(f<sub>c</sub>) = Control strength

For BC1-50:

$$[SI = \frac{28.02 - 24.08}{24.08} \times 100]$$

$$[SI = 16.36\%]$$

**Table 4.3** Percentage Strength Improvement

Mix ID	Improvement (%)
BC1-30	5.65
BC1-40	11.42
BC1-50	16.36
BC2-30	3.65
BC2-40	7.43
BC2-50	8.60
BC3-30	0.58
BC3-40	2.74
BC3-50	4.15

The results reveal that strength improvement decreased as bacterial concentration increased beyond the optimum level.

This finding suggests that excessive bacterial populations may interfere with cement hydration or create weak zones within the matrix.

Similar observations were reported by Achal et al. (2011), who found that optimum bacterial concentrations

produced maximum calcite precipitation and strength enhancement.

**4.4 Effect of Nutrient Broth Medium on Strength Development**

The results indicate that increasing NBM replacement from 30% to 50% consistently improved strength development for all bacterial concentrations.

Table 4. 4: Compressive Strength of Mortar Containing BC1 Bacterial Concentration at Different NBM Percentages

NBM (%)	Strength (MPa)
30	25.44
40	26.83
50	28.02

The increase can be attributed to enhanced bacterial metabolism resulting from greater nutrient availability. Nutrient broth contains proteins and amino acids necessary for bacterial growth and carbonate production. As bacterial activity increases, more calcium carbonate crystals are produced, leading to improved pore filling and matrix densification.

This observation agrees with De Muynck et al.

(2010), who reported that nutrient availability significantly influences biomineralization efficiency.

**4.5 Influence of Bacterial Concentration**

The effect of bacterial concentration on compressive strength is illustrated by comparing specimens containing different bacterial densities.

**Table 4.5:** At 50% NBM:

Bacterial Density	Strength (MPa)
$1.5 \times 10^8$	28.02
$6.0 \times 10^8$	26.15
$1.2 \times 10^9$	25.08

Interestingly, strength decreased with increasing bacterial concentration.

Several explanations may account for this trend:

1. Competition for nutrients among bacterial cells.
2. Reduced oxygen availability.

3. Agglomeration of bacterial colonies.
4. Formation of localized weak zones.

The findings suggest that bacterial concentration must be carefully optimized to maximize calcite precipitation and strength gain.

#### 4.6 Water Absorption Results

Water absorption values obtained after 28 days are presented in Table 4.4.

**Table 4.6** Water Absorption Results

Mix ID	Water Absorption (%)
Control	5.98
BC1-30	4.84
BC1-40	4.02
BC1-50	3.46
BC2-30	5.10
BC2-40	4.68
BC2-50	4.20
BC3-30	5.35
BC3-40	5.01
BC3-50	4.72

The lowest water absorption value of 3.46% was obtained for BC1-50. This represents a substantial reduction compared with the control mortar.

#### 4.7 Percentage Reduction in Water Absorption

The percentage reduction in water absorption was calculated using:

$$[WR = \frac{WA_c - WA_b}{WA_c} \times 100]$$

Where:

(WA<sub>c</sub>) = Control water absorption

(WA<sub>b</sub>) = Bio-mortar water absorption

For BC1-50:

$$[WR = \frac{5.98 - 3.46}{5.98} \times 100]$$

$$[WR = 42.14\%]$$

**Table 4.7** Water Absorption Reduction

Mix ID	Reduction (%)
BC1-30	19.06
BC1-40	32.78
BC1-50	42.14
BC2-30	14.72
BC2-40	21.74
BC2-50	29.77
BC3-30	10.54
BC3-40	16.22
BC3-50	21.07

These results indicate significant improvement in durability due to bacterial incorporation.

Reduced water absorption implies the following:

- Lower permeability
- Reduced chloride penetration
- Improved resistance to sulphate attack
- Enhanced long-term durability

These findings support those of Chahal et al. (2012), who observed significant reductions in water absorption following bacterial treatment.

#### 4.8 Relationship Between Strength and Water Absorption

A strong inverse relationship was observed between compressive strength and water absorption.

As water absorption decreased:

- Compressive strength increased.

This relationship is expected because pore refinement reduces void content and improves load transfer within the cement matrix.

The bio-calcination process produced calcite crystals that occupied capillary pores, thereby reducing permeability and increasing density.

Consequently:

Lower water absorption → Higher density → Higher strength

This trend agrees with findings reported by Siddique and Chahal (2011).

#### 4.9 SEM Analysis

##### 4.9.1 SEM Observation of Control Mortar

SEM examination of the control mortar revealed:

- Numerous capillary pores
- Microcracks
- Loosely packed hydration products
- Relatively porous matrix

The images indicated incomplete filling of void spaces within the cement matrix.

Such features explain the higher water absorption and lower compressive strength observed in the control specimen.

##### 4.9.2 SEM Observation of Bio-Mortar

SEM analysis of BC1-50 revealed:

- Extensive calcite crystal formation
- Dense microstructure
- Reduced pore volume
- Improved particle bonding

The precipitated crystals appeared as rhombohedral calcite deposits distributed throughout the matrix.

The crystals occupied void spaces and microcracks that would otherwise serve as pathways for water penetration.

These observations confirm successful microbial calcium carbonate precipitation.

#### 4.10 Mechanism of Strength Enhancement

The improvement in mortar performance can be explained through the mechanism illustrated below.

Step 1

Bacterial spores survive within the cement matrix.

Step 2

Nutrient broth activates bacterial growth.

Step 3

Metabolic activities generate carbonate ions.

Step 4

Carbonate ions react with calcium ions released from cement hydration.

$[Ca^{2+} + CO_3^{2-}] \rightarrow CaCO_3$

Step 5

Calcite crystals precipitate.

Step 6

Pores and microcracks become filled.

Step 7

Matrix densification increases strength and reduces permeability.

This mechanism has been widely reported by Stocks-Fischer et al. (1999), De Muynck et al. (2010), and Achal et al. (2011).

#### 4.11 Comparison with Previous Studies

The findings obtained in this study are consistent with earlier investigations.

Ramakrishnan et al. (2001)

Reported strength increases ranging from 10–25%.

Present study:

16.36% increase.

Achal et al. (2011)

Reported approximately 20% strength improvement.

Present study:

Comparable improvement.

Chahal et al. (2012)

Observed reduced permeability and water absorption.

Present study:

42.14% reduction in water absorption.

De Muynck et al. (2010)

Reported pore refinement due to calcite precipitation.

Present study:

SEM observations confirm similar microstructural densification.

These comparisons demonstrate that the results are consistent with established scientific literature.

#### 4.12 Evaluation of Research Hypotheses

##### Hypothesis One

H<sub>01</sub>: *Bacillus coagulans* has no significant effect on compressive strength.

Result:

Rejected.

The bacterial mortar exhibited significant strength enhancement compared with control mortar.

##### Hypothesis Two

H<sub>02</sub>: Nutrient broth medium has no significant effect on mortar performance.

Result:

Rejected.

Increasing nutrient concentration improved bacterial activity and strength development.

##### Hypothesis Three

H<sub>03</sub>: Bio-calcination does not reduce water absorption.

Result:

Rejected.

Significant reductions in water absorption were observed.

#### 4.13 Summary of Findings

The major findings of this study are summarised as follows:

1. The optimum bacterial concentration was  $1.5 \times 10^8$  cells/mL.
2. The optimum nutrient broth replacement level was 50%.
3. Maximum compressive strength achieved was 28.02 MPa.
4. Strength increased by 16.36% compared with control mortar.
5. Water absorption decreased from 5.98% to 3.46%.
6. Water absorption reduction reached 42.14%.
7. SEM analysis confirmed calcite crystal formation.
8. Bio-calcination effectively densified the cement matrix.
9. Improved durability was achieved through pore refinement and crack filling.
10. *Bacillus coagulans* demonstrated significant potential for sustainable cement mortar enhancement.

### 5. CONCLUSIONS AND RECOMMENDATION

#### 5.1 Conclusions

The present study investigated the effect of *Bacillus coagulans*-induced bio-calcination on the strength and durability characteristics of cement mortar. The findings demonstrated that the incorporation of bacterial cultures into cement mortar significantly improved both the mechanical and durability properties of the material. The enhancement observed in the bacterial mortar specimens was primarily attributed to the process of Microbially Induced Calcium Carbonate Precipitation (MICP), through which calcium carbonate crystals were deposited within the pores and microcracks of the cement matrix.

The experimental results showed that bacterial incorporation contributed to increased compressive strength at all curing ages when compared with conventional mortar. Among the various bacterial concentrations investigated, the mortar containing  $1.5 \times 10^8$  cells/mL of *Bacillus coagulans* and 50% Nutrient Broth Medium (NBM) replacement exhibited the best overall performance. This mixture achieved a 28-day compressive strength of 28.02 MPa, compared with 24.08 MPa obtained for the control specimen. The corresponding strength enhancement of 16.36% confirms the effectiveness of bacterial mineralisation in improving the load-bearing capacity of cement mortar.

The study further revealed that nutrient availability played a critical role in promoting bacterial activity and calcite precipitation. As the percentage replacement of nutrient broth medium increased from 30% to 50%, compressive strength values consistently improved. This observation suggests that adequate nutrient supply is necessary to sustain bacterial metabolism and maximise

calcium carbonate production within the cementitious matrix.

In terms of durability performance, bacterial mortar exhibited significantly lower water absorption than conventional mortar. The optimum bacterial mixture recorded a water absorption value of 3.46%, compared with 5.98% for the control specimen. This represents a reduction of approximately 42.14%, indicating that bacterial calcite precipitation effectively reduced pore connectivity and permeability. Reduced water absorption is particularly important because it limits the ingress of harmful substances such as chlorides, sulphates, and carbon dioxide, thereby enhancing the long-term durability of cementitious materials.

Microstructural examination using scanning electron microscopy provided further evidence supporting the effectiveness of bio-calcination. SEM images revealed the presence of extensive calcite crystal deposits within the pore spaces and microcracks of the bacterial mortar specimens. These deposits contributed to matrix densification, improved particle bonding, and reduced void content. In contrast, the control mortar displayed a more porous microstructure characterised by visible capillary pores and microcracks. The SEM observations therefore confirmed that the improvements in strength and durability resulted directly from microbial calcium carbonate precipitation.

Overall, the findings of this study demonstrate that *Bacillus coagulans* can be successfully utilised as a biological admixture for enhancing cement mortar performance. The results support the growing body of evidence indicating that bio-calcination technology offers a sustainable and environmentally friendly alternative to conventional chemical admixtures. The application of bacterial mineralisation in cement-based materials has considerable potential for improving structural durability, reducing maintenance costs, and extending the service life of infrastructure.

#### 5.2 Recommendations

Based on the findings of this study, several recommendations are proposed for engineering practice and future research. First, the use of *Bacillus coagulans* in cement mortar production should be encouraged for applications where enhanced durability and reduced permeability are required. The significant reduction in water absorption and improvement in compressive strength observed in this study indicate that bacterial mortar can contribute to the development of more durable and sustainable construction materials.

Second, construction industries and material manufacturers should explore the commercial production of bacterial admixtures capable of promoting calcium carbonate precipitation within cementitious systems. Such products could provide an environmentally friendly

alternative to synthetic chemical additives currently used to improve concrete performance.

Third, future studies should investigate the long-term performance of bacterial mortar beyond the 28-day curing period considered in this research. Evaluating mechanical and durability properties at later ages would provide a more comprehensive understanding of bacterial activity and long-term calcite formation within cement matrices. Furthermore, additional durability assessments should be conducted to examine resistance to chloride penetration, sulphate attack, carbonation, freeze-thaw cycles, and reinforcement corrosion. These investigations would provide valuable information regarding the suitability of bacterial mortar for aggressive service environments.

Future research should also compare the performance of *Bacillus coagulans* with other bacterial species commonly used in microbial concrete technology, including *Bacillus subtilis*, *Bacillus sphaericus*, *Bacillus megaterium*, and *Sporosarcina pasteurii*. Such comparative studies would assist in identifying the most effective bacterial strains for specific engineering applications.

Advanced characterisation techniques such as X-ray Diffraction (XRD), Energy Dispersive Spectroscopy (EDS), Fourier Transform Infrared Spectroscopy (FTIR), and Thermogravimetric Analysis (TGA) should also be incorporated into future investigations. These techniques would provide detailed information regarding mineralogical composition, crystal structure, and biomineralisation mechanisms.

Finally, field-scale studies and pilot projects are recommended to evaluate the practical feasibility of bacterial mortar technology under real environmental and loading conditions. Such investigations should be accompanied by life-cycle assessment and economic analyses to determine the sustainability and cost-effectiveness of large-scale implementation. Through continued research and development, bacterial biocalcination may become an important component of next-generation sustainable construction materials.

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