

Biom mineralization Biotechnology Utilizing *Lysinibacillus sphaericus* to Improve Mechanical Properties of Mortar

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ABSTRACT

Biom mineralization has notably enhanced the qualities of cement-based materials, particularly through bacterial-facilitated calcite precipitation via calcium lactate oxidation. However, existing research mainly targets self-healing aspects, with little focus on bio-based mortar properties. Consequently, this study provides a comprehensive examination of the enhancements in dry density, ultrasonic pulse velocity (UPV), and flexural strength, achieved through the application of a novel indigenous bacterial strain (*Lysinibacillus sphaericus* strain SKC/VA-1) from Indonesia, coupled with the incorporation of calcium lactate pentahydrate as an additive. A total of six mortar samples were prepared to investigate the influence of bacteria on the properties of mortar through biom mineralization. The samples included plain mortar (M), mortar mixed with calcium lactate pentahydrate (ML), mortar mixed with a 10% v/v bacterial inoculum (MB1), mortar mixed with calcium lactate pentahydrate and a 10% v/v bacterial inoculum (MLB1), mortar mixed with a 20% v/v bacterial inoculum (MB2), and mortar mixed with calcium lactate pentahydrate and a 20% v/v bacterial inoculum (MLB2). The employment of a distinct bacterial strain for oxidizing calcium lactate represents an innovative aspect of the current study. The presence of organic calcium was found to have no adverse effects on the mortar matrix. Optimal inoculum concentrations of bacteria (10% v/v), in conjunction with calcium lactate pentahydrate, yielded superior mechanical properties. Mineralogical characterization via X-ray diffraction and microstructural analysis through scanning electron microscopy substantiated the incidence of calcite precipitation, which facilitated pore infilling and consequently augmented both the ultrasonic pulse velocity and the flexural strength of the mortar.

1. Introduction

Biologically calcite precipitation, a biom mineralization process, has been extensively studied in various research domains, particularly geotechnics and construction engineering. Over the past two decades, numerous investigations have demonstrated the efficacy of this biological approach in enhancing the properties of cementitious materials, including concrete and mortar. Compared to conventional methods, this approach offers potential advantages such as lower cost and greater

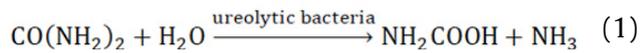
environmental friendliness, as highlighted by Achal and Mukherjee (2015). Notably, studies have shown that microbial metabolism during urea hydrolysis can significantly enhance the strength of concrete. For instance, Ramachandran *et al.* (2001) reported a remarkable 61% increase in cement mortar strength through microbially induced calcite precipitation facilitated by *Bacillus pasteurii*.

Based on the investigation, ureolytic bacteria can catalyze the hydrolysis of urea, leading to the production of ammonia. Notably, Achal *et al.* (2013) observed that *Bacillus* sp. CT-5 exhibited the potential to significantly improve the compressive strength of cement mortar by 40% during remediation

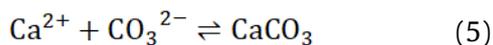
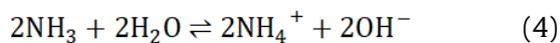
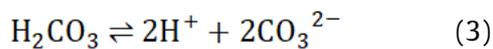
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processes. Additionally, as part of a subsequent reaction, the generation of bicarbonate acid is crucial as the primary component for facilitating calcite precipitation (Gollapudi *et al.* 1995; Burne and Chen 2000):

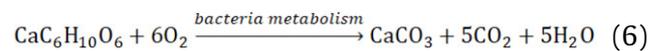


Additionally, in an aqueous environment, bicarbonate ions (HCO_3^-), ammonium ions (NH_4^+), and hydroxide ions (OH^-) are generated, leading to an increase in pH levels (Achal and Pan 2011). Furthermore, carbonate ions (CO_3^{2-}) are produced and bind with the available calcium source, facilitating the precipitation of calcium carbonate through the following reactions:



The presence of carbonic anhydrase (CA) activity is believed to play a crucial role in the urease hydrolysis mechanism, facilitating the production of carbonate ions (Eq. 3). Therefore, these ions can potentially combine and participate in the formation of calcite (Achal and Pan 2011; Castro-Alonso *et al.* 2019). This pathway has been widely utilized for its efficiency and effectiveness in promoting calcite precipitation (Dhami *et al.* 2013). However, when this mechanism is scaled up, it can significantly increase ammonium production (Li *et al.* 2015), potentially releasing ammonia and subsequent environmental contamination (Ivanov *et al.* 2019).

Numerous research efforts have been dedicated to identifying safer mechanisms, and the oxidation of organic calcium has emerged as a promising candidate due to its lack of environmental drawbacks (De Belie 2016). Among the various organic calcium options, calcium lactate has been identified as the optimal choice as it does not adversely affect the strength of cement-based materials (Jonkers *et al.* 2010). This oxidation mechanism has demonstrated the potential for calcite precipitation, as illustrated by previous studies (Jonkers *et al.* 2010; De Belie 2016) as follows:



Furthermore, previous investigations employing *Bacillus* sp. and calcium lactate have documented an approximate 12% improvement in concrete strength (Khaliq and Ehsan 2016; Vijay and Murmu 2019). Similarly, a relevant study has shown that the optimal combination of *Enterococcus faecalis* and calcium lactate resulted in an 18.9% increase in compressive strength and a 39.5% increase in flexural strength. However, limited research has explored the effects of calcite biomineralization on specific properties of cementitious materials using this pathway. The objectives of this study were as follows: (1) to assess the capability of a new bacterial strain (*Lysinibacillus sphaericus* strain SKC/VA-1) in enhancing the strength of bio-based mortar, specifically in terms of ultrasonic pulse velocity and flexural strength, by using a modified low-cost medium containing calcium lactate pentahydrate, (2) to evaluate the effects of adding calcium lactate pentahydrate and varying bacterial inoculum concentrations on the properties of the mortar, and (3) to investigate the possible mechanism of mineral formation by examining the microstructure and mineralogy of the specimens, incorporating bacteria and calcium lactate pentahydrate. The findings of this study have significant implications for the development of economically and environmentally friendly mortar materials.

2. Materials and Methods

2.1. Bacterial Culture

Lysinibacillus sphaericus strain SKC/VA-1, an indigenous bacterium, was isolated from a sample containing a mixture of crude oil and rust deposits from petroleum pipelines. A 250 ml Erlenmeyer flask containing 100 ml of nutrient broth medium was used to cultivate the bacterium. The medium consisted of 1.5 g/L of nutrient broth (Oxoid) and 1 g/L of calcium lactate pentahydrate ($\text{CaC}_6\text{H}_{10}\text{O}_6 \cdot 5\text{H}_2\text{O}$), which was obtained from a chemical store in Bandung, West Java, Indonesia (Syarif *et al.* 2019). Before bacterial inoculation (7×10^7 colony-forming units (CFU)/ml), the medium was sterilized by autoclaving at 121°C for 15 minutes. Subsequently, the culture was incubated under shaking conditions at 180 rpm and 28°C for 48 hours.

2.2. Fabrication of Mortar and Bio-mortar Specimens

Mortar samples were prepared using Ordinary Portland Cement Type 1 (SNI 15-2049-2004 2004), fine aggregate, and tap water. The Ordinary Portland Cement had a specific gravity of 3.15, while the fine aggregate had a fineness modulus of 3.39 and a specific gravity of 2.685. The control mortar without any additives was denoted as M. To investigate the influence of calcium lactate pentahydrate, this calcium precursor was added at a weight percentage of 0.5% into the mixture, and the corresponding specimen was labeled as ML. This composition was selected based on previous studies that confirmed its non-detrimental effect on cementitious materials (Jonkers *et al.* 2010; Vijay and Murmu 2019). On the other hand, the bio-mortar specimens were prepared by incorporating varying concentrations of bacterial inoculum (10% and 20% v/v) as a replacement for water. Bio-mortar specimens without calcium lactate pentahydrate were denoted as MB1 (10% v/v bacterial inoculum) and MB2 (20% v/v bacterial inoculum). Meanwhile, bio-mortar specimens with calcium lactate pentahydrate were marked as MLB1 (10% v/v bacterial inoculum) and MLB2 (20% v/v bacterial inoculum). The specific mixture compositions are presented in Table 1.

Thirty-six cylindrical specimens (18 for ultrasonic pulse velocity (UPV) and 18 for dry density measurement) and 18 beam specimens were prepared, with three replicates for each group (M, ML, MB1, MB2, MLB1, MLB2). The specimens were cast manually into two shapes: cylinders for UPV and dry density measurement and beams for flexural strength measurement. The cylindrical specimens were cut to heights of 9 cm (for UPV) and 3 cm (for dry density measurement). The cylindrical specimens

Component	Specimen code					
	M	ML	MB1	MLB1	MB2	MLB2
Cement (g)	600	600	600	600	600	600
Fine aggregate (g)	1,800	1,800	1,800	1,800	1,800	1,800
Water (cm ³)	300	300	270	270	240	240
Water/Cement ratio (W/C ratio)	0.5	0.5	0.5	0.5	0.5	0.5
Bacterium (cm ³)	0	0	30	30	60	60
Calcium Lactate Pentahydrate (g)	0	3	0	3	0	3

Bacterium (30 cm³ = 10% v/v bacterial inoculum; 60 cm³ = 20% v/v bacterial inoculum)

were cut to heights of 9 cm for UPV measurement and 3 cm for dry density measurement. The mixture was poured into PVC molds with a height of 17 cm and diameter of 4.5 cm in three layers, each compacted 32 times. As for the beam specimens, the mixture was poured into plywood molds with a length of 20 cm and a side of 5 cm in two layers, with each layer being compacted 25 times. The specimens were then covered with plastic wrap and stored at room temperature for 24 hours. Afterward, they were demolded and cured in tap water for 7 days before testing.

2.3. Measurement of Dry Density in Mortar and Bio-mortar Specimens

Dry density was determined following the standard procedure outlined in ASTM C642 (ASTM C642 2013). Three specimens from each group were cured in tap water for 7 days. After the curing period, the specimens were dried in an oven at 105°C for 24 hours, and their mass (M_s) was recorded. Next, the specimens were immersed in tap water for 24 hours, removed, wiped, and weighed to determine the mass at a saturated surface-dry condition. Finally, the specimens were wire-tied and submerged in tap water to measure their apparent mass in water.

2.4. Measurement of Ultrasonic Pulse Velocity in Mortar and Bio-mortar Specimens

The ultrasonic pulse velocity (UPV) measurement was conducted in accordance with the ASTM C597 standard (ASTM C 597 2016). Each group consisted of three specimens cured in tap water for 7 days. Subsequently, the specimens were tested using a PUNDIT device, a portable non-destructive digital ultrasonic tester (Ultrasonic tester E46). The instrument was calibrated using a calibration cylinder with a pulse propagation time of 56 μ s. The experiments were carried out in triplicate, ensuring the reported data represented the average values obtained from the three independent experimental runs.

2.5. Determination of Flexural Strength in Mortar and Bio-mortar Specimens

The flexural strength of the specimens was determined following the guidelines outlined in ASTM C293 (ASTM C293 2016). The test used a hydraulic compression tester servo-controlled (Hung Ta HT-8391) with a 1 MPa/min loading rate. Before

the test, all specimens were subjected to wet curing for 7 days. To ensure accuracy and reliability, the experiments were performed in triplicate, and the reported data represents the average values obtained from three independent experimental runs.

2.6. X-ray Diffraction (XRD) and Scanning Electron Microscopy with Energy Dispersive Spectroscopy (SEM-EDS) Characterization

The mineralogical composition of the specimens was determined through X-ray diffraction (XRD) analysis using a Bruker D8 Advance diffractometer with Cu-K α radiation in the range of 15° to 60°. Mortar from the flexural strength test specimens was collected and ground for the XRD analysis. Additionally, the microstructure of the specimens was examined using a scanning electron microscope-energy dispersive spectroscopy (SEM-EDS; JEOL JSM-J6510 A) according to ASTM standard (ASTM C1723 2010). The preparation process for SEM-EDS involved fixation, washing, serial dilution with acetone, and coating. Energy dispersive spectroscopy (EDS) was utilized to identify the precipitates resulting from bacterial metabolism within the microstructure of the specimens.

2.7. Statistical Analysis

All experiments were conducted in triplicate, resulting in 36 cylindrical specimens (18 for UPV and dry density measurements) and 18 beam specimens for the flexural strength test. A one-way analysis of variance (ANOVA), followed by Tukey's post hoc test, was employed to assess the statistical significance of differences among the specimens. The results were presented as mean values with their corresponding standard deviations.

3. Results

3.1. Dry Density Determination of Mortar and Bio-mortar Specimens

The results of the dry density measurements are presented in Figure 1. The addition of calcium lactate pentahydrate (ML) showed a negligible increase of only 2% in dry density compared to the control specimens (M), which was not statistically significant ($p^{ab} > 0.05$). However, the bacterial mortar without calcium lactate pentahydrate exhibited slightly higher dry density than the control specimens, although the differences were not statistically significant ($p^{ab} > 0.05$). The MB1 and MB2 specimens

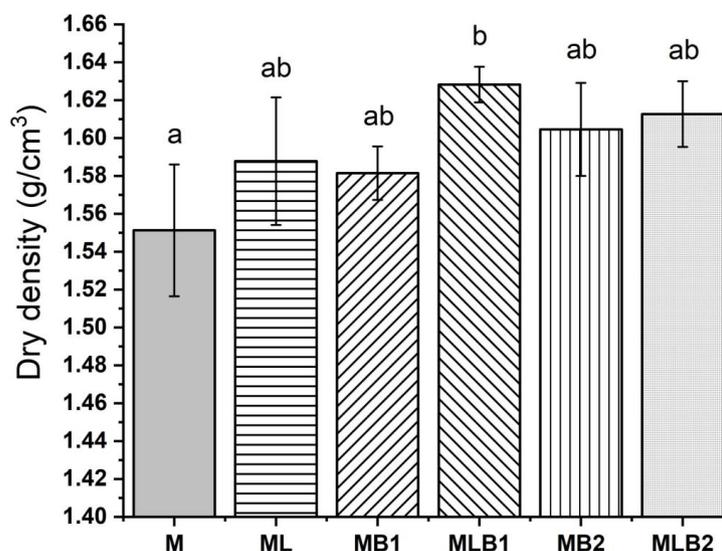


Figure 1. Comparative analysis of dry density in various mortar specimens. The specimens include plain mortar (M), mortar integrated with calcium lactate pentahydrate (ML), mortar incorporated with 10% v/v bacterial inoculum (MB1) or 20% v/v bacterial inoculum (MB2), and mortar containing calcium lactate pentahydrate combined with either 10% v/v bacterial inoculum (MLB1) or 20% v/v bacterial inoculum (MLB2). The mean values (represented as bars) are based on data from triplicate experimental runs ($n = 3$), and the standard deviations are shown as error bars. Statistical significance was evaluated using ANOVA and Tukey's post hoc test, with a significance level of 0.05

showed modest enhancements of dry density, with increases of 2% and 3%, respectively. In contrast, the bacterial mortar with calcium lactate pentahydrate (MLB1 and MLB2) demonstrated more significant increases in dry density, with enhancements of 5% and 4%, respectively. Notably, MLB1 specimens exhibited the most remarkable improvement in dry density compared to the control specimens, and the difference was statistically significant ($p^b < 0.05$).

3.2. Ultrasonic Pulse Velocity Analysis of Mortar and Bio-mortar Specimens

Ultrasonic pulse velocity (UPV) measurement is commonly used to assess the homogeneity and structural condition of mortar. The UPV results, presented in Figure 2, indicate notable changes. The ML, MB1, MLB1, and MLB2 specimens exhibited increases of 1%, 4%, 6%, and 1%, respectively, in UPV. However, the MB2 specimens did not show any significant improvement in UPV. The UPV analysis of the ML specimen confirms that the addition of calcium lactate pentahydrate (0.5% by weight of cement) does not adversely affect the structural

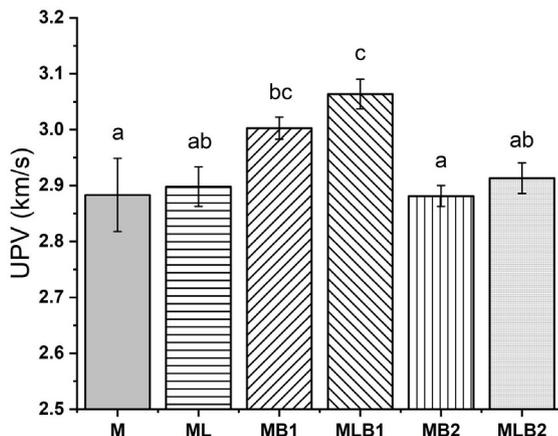


Figure 2. Comparative analysis of ultrasonic pulse velocity (UPV) in various mortar specimens. The specimens include plain mortar (M), mortar integrated with calcium lactate pentahydrate (ML), mortar incorporated with 10% v/v bacterial inoculum (MB1) or 20% v/v bacterial inoculum (MB2), and mortar containing calcium lactate pentahydrate combined with either 10% v/v bacterial inoculum (MLB1) or 20% v/v bacterial inoculum (MLB2). The mean values (represented as bars) are based on data from triplicate experimental runs ($n = 3$), and the standard deviations are shown as error bars. Statistical significance was evaluated using ANOVA and Tukey's post hoc test, with a significance level of 0.05

integrity of the mortar. Moreover, the inclusion of bacterial suspension contributes to the enhancement of UPV, with the most significant improvement observed in the MLB1 specimen ($p^c < 0.05$). However, the MLB2 specimen did not exhibit a substantial increase compared to the control specimens ($p^{ab} > 0.05$). The bacterial specimens without calcium lactate pentahydrate (MB1 and MB2) showed less improvement than the mortar specimens with organic calcium additives (MLB1 and MLB2). However, the addition of 20% v/v bacterial inoculum in MB2 did not significantly affect the UPV of the mortar ($p^a > 0.05$). The UPV of MB1 specimens was higher than the control specimen but did not differ significantly from MLB1 ($p^{bc} > 0.05$). In general, the specimens that received the optimal combination of bacterial inoculum and calcium source demonstrated improved uniformity in their properties.

3.3. Flexural Strength Analysis of Mortar and Bio-mortar Specimens

Figure 3 presents the flexural strength results of the specimens. The flexural strength of ML specimens

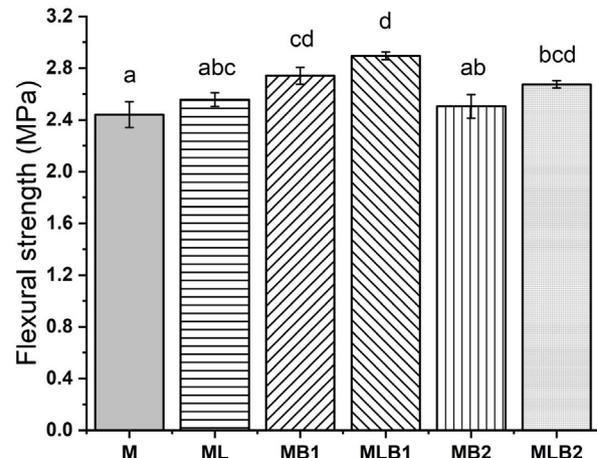


Figure 3. Comparative analysis of flexural strength in various mortar specimens. The specimens include plain mortar (M), mortar integrated with calcium lactate pentahydrate (ML), mortar incorporated with 10% v/v bacterial inoculum (MB1) or 20% v/v bacterial inoculum (MB2), and mortar containing calcium lactate pentahydrate combined with either 10% v/v bacterial inoculum (MLB1) or 20% v/v bacterial inoculum (MLB2). The mean values (represented as bars) are based on data from triplicate experimental runs ($n = 3$), and the standard deviations are shown as error bars. Statistical significance was evaluated using ANOVA and Tukey's post hoc test, with a significance level 0.05

showed no significant difference compared to the control specimens ($p^{abc} > 0.05$), despite a higher flexural strength value. This behavior indicates that adding calcium lactate pentahydrate did not negatively affect the mortar strength, as supported by the dry density and UPV results which showed a 5% increment. In the case of bacterial mortar without calcium lactate pentahydrate, MB1 specimens exhibited a significant difference ($p^{cd} < 0.05$) with a 12% enhancement in flexural strength compared to the control mortar (M). However, MB2 specimens only showed a 3% increment, which did not significantly differ from the M specimen ($p^{ab} > 0.05$). On the other hand, the flexural strength of MLB1 and MLB2 specimens with calcium lactate pentahydrate demonstrated significant differences compared to the M specimen. MLB1 exhibited the highest enhancement of 19% compared to the M specimen, showing a substantial difference ($p^d < 0.05$), although the flexural strength of MLB1 was not significantly different from MB1 ($p^{cd} > 0.05$) and MLB2 ($p^{bcd} > 0.05$). Adding 10% v/v bacterial inoculum at a concentration of 7×10^7 CFU/ml, along with calcium lactate pentahydrate (0.5% by weight of cement), resulted in the most optimal improvement in mortar properties in this study.

3.4. Mineralogical and Microstructural Analysis of Mortar and Bio-mortar Specimens

Figures 4 and 5 present the mineralogical spectra and microstructural analysis of the specimens, respectively. The mineral composition of the mortar primarily consisted of aggregate minerals such as labradorite and cristobalite, as well as cement minerals, including calcite (C), calcium silicate hydrate (CSH), and portlandite (P) (Figure 4). The primary minerals in the cement, tricalcium silicate (C3S) and dicalcium silicate (C2S) were also detected (Figure 4). The SEM-EDS images revealed the dominance of ettringite in the control specimen (Figure 5A). In contrast, the ML specimen exhibited the formation of CSH (Figure 5B). In the MB1 specimen, the presence of white precipitates and rod-shaped bacteria was observed (Figure 5C). These precipitates were identified as calcite, as confirmed by XRD analysis (Figure 4) and EDS spectra (Figure 5E). However, the calcite deposits did not fill certain voids. On the other hand, the incorporation of calcium lactate pentahydrate in the bacterial mortar resulted in the formation of more precipitates (Figure 5D). These precipitates were also identified as calcite based on XRD (Figure 4) and EDS spectra (Figure 5F).

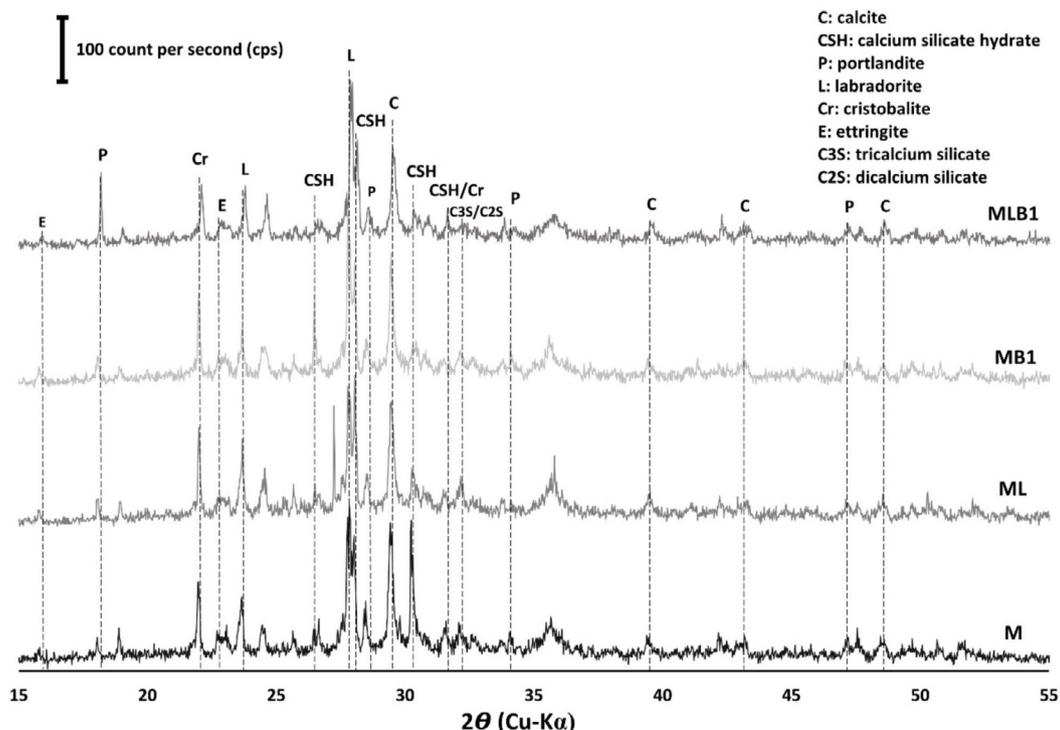


Figure 4. X-ray diffraction (XRD) spectra illustrate different mortar specimens' mineralogical composition. The specimens analyzed include plain mortar (M), mortar supplemented with calcium lactate pentahydrate (ML), mortar enriched with 10% v/v bacterial inoculum (MB1), and mortar composed of calcium lactate pentahydrate in conjunction with 10% v/v bacterial inoculum (MLB1)

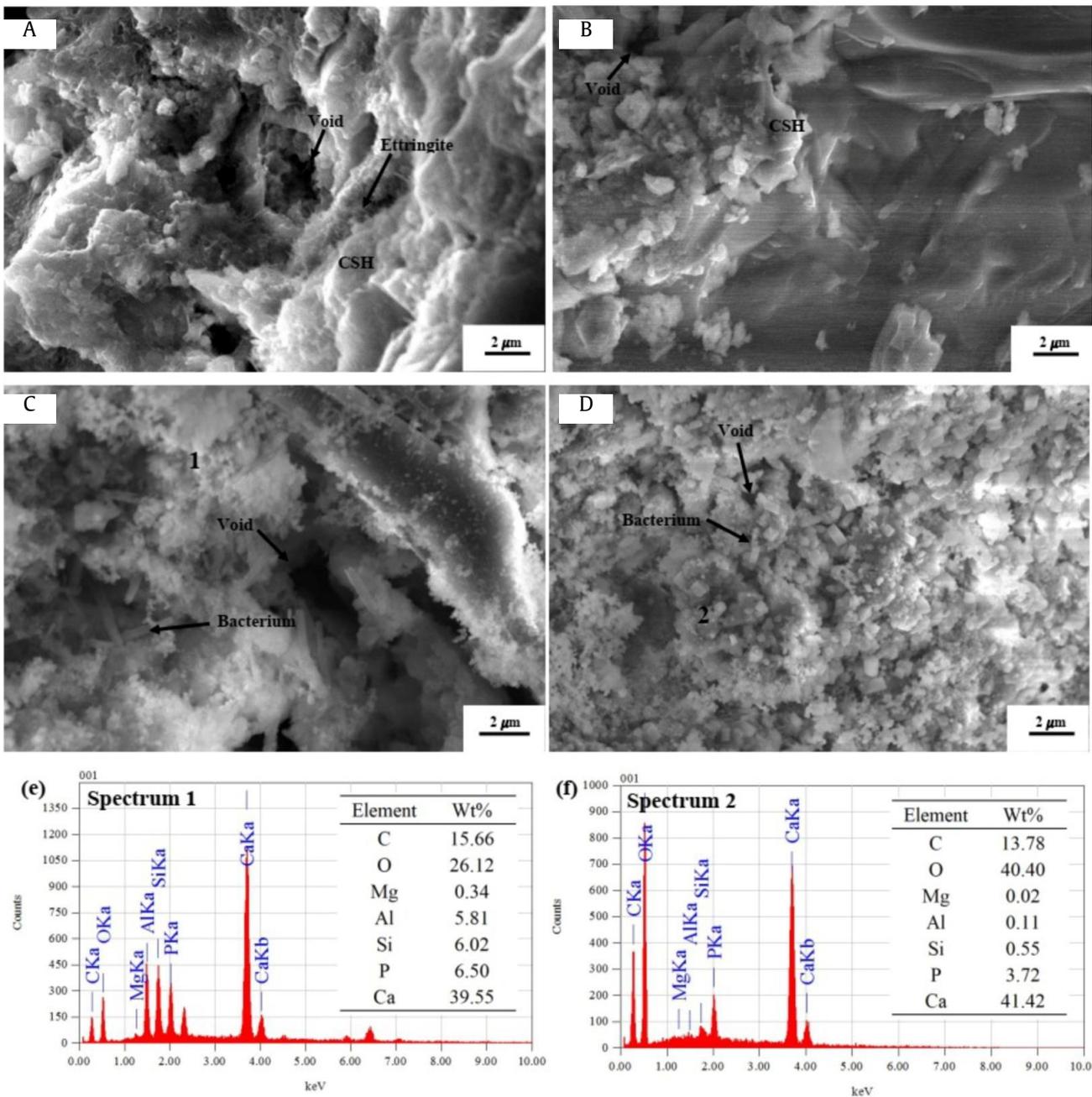


Figure 5. Scanning electron microscopy (SEM) images depicting the microstructural characteristics of various mortar specimens. The specimens include (A) plain mortar (M), (B) mortar supplemented with calcium lactate pentahydrate (ML), (C) mortar enriched with 10% v/v bacterial inoculum (MB1), and (D) mortar composed of calcium lactate pentahydrate in conjunction with 10% v/v bacterial inoculum (MLB1). Energy-dispersive X-ray spectroscopy (EDS) spectra provide evidence of bacteria-induced calcite (CaCO_3) precipitation, as demonstrated by analytical points 1 (MB1) (E) and 2 (MLB1) (F).

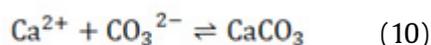
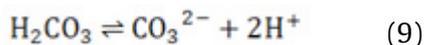
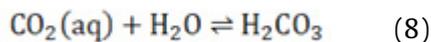
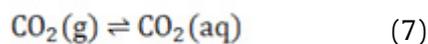
4. Discussion

Lysinibacillus sphaericus has been widely used to enhance cement-based materials through urea hydrolysis (Wang *et al.* 2016). However, this study demonstrated that the bacterium could also be

employed in an alternative pathway involving calcium lactate pentahydrate, leading to the precipitation of calcium carbonate within the mortar specimens. The selected composition of calcium lactate pentahydrate (0.5% by weight of cement) was found to have no adverse effects on

the mortar properties, consistent with the findings of Jonkers *et al.* (2010). The investigation of calcium lactate pentahydrate in the M specimens revealed its potential to facilitate the formation of additional calcium silicate hydrate (CSH) alongside the hydration reaction. The calcium sources provided by calcium lactate pentahydrate could react with water, producing portlandite. The subsequent pozzolanic reaction between portlandite and silicate minerals within the specimens contributed to the formation of calcium silicate hydrate. As a result, the ML specimens exhibited increased density, as evidenced by slight improvements in UPV and flexural strength (Figures 2 and 3). Regarding the UPV of the mortar specimens, those with the optimal combination of bacterial inoculum and calcium source demonstrated enhanced uniformity compared to previous studies (Andalib *et al.* 2014; Xu and Yao 2014; Bai and Varghese 2016).

In the absence of calcium lactate pentahydrate, applying *Lysinibacillus sphaericus* strain SKC/VA-1 improved UPV and flexural strength of the mortar specimens. The observed enhancement in the UPV and flexural strength of the mortar specimens can be attributed to the formation of calcite, which is likely facilitated by the activity of carbonic anhydrase (CA). This conclusion is consistent with findings from previous studies by Ozdemir (2009) and Achal and Pan (2011) through the following reactions (Eqs. 7-10) to describe the process.



The bacterium can sequester and convert available CO_2 into bicarbonate, forming carbonate ions in water. This process has been documented in previous studies by Bond *et al.* (2001) and Ramanan *et al.* (2009). In the presence of calcium oxide (CaO) in cement, the formation of calcite can be induced, as described by Achal and Pan (2014) and Mondal and Ghosh (2018). The presence of calcite can fill voids and improve mortar properties, although some voids may remain unfilled. This explains why incorporating bacteria alone can still result in calcite precipitation in a cementitious environment.

Furthermore, it has been observed that a bacterial inoculum concentration of 10% yields better results compared to 20%. Higher bacterial inoculation can lead to excessive calcite precipitation on the specimen surface, hindering hydration (Mondal and Ghosh 2018; Su *et al.* 2019). The presence of a deposited layer on the surface restricts water penetration into the mortar microstructure, resulting in reduced production of calcium silicate hydrate (CSH) gel and calcite, as reported by Ameri *et al.* (2019) and Vaezi *et al.* (2020). Consequently, higher bacterial inoculation is associated with lower mechanical properties. Moreover, the identification of silicate minerals, such as tricalcium silicate (C3S) and dicalcium silicate (C2S), as shown in Figure 4, plays a crucial role in the formation of calcium silicate hydrate (CSH) and portlandite (P) through the hydration reaction, as noted by Papadakis *et al.* (1992). The predominance of ettringite formation in the control specimens (Figure 5A) is attributed to the absorption of sulfate ions by calcium silicate hydrate (CSH) and subsequent propagation of microcracks and voids, as explained by Mehta and Monteiro (2014).

The addition of bacteria and calcium lactate pentahydrate increased the mechanical properties of the mortar compared to when calcium lactate pentahydrate was not present. The formation of calcite was more prominent, leading to the precipitation of calcite deposits that effectively filled the voids and improved the mortar properties. This calcite formation is believed to be facilitated by the bacterial metabolism in the oxidation mechanism, as proposed in previous studies by Jonkers *et al.* (2010) and De Belie (2016), as shown in Figure 6. It is possible that carbonic anhydrase (CA) activity, in addition to the bacterium's ability to promote CO_3^{2-} formation and the availability of Ca^{2+} in cement, also contributes to the cumulative production of calcite (CaCO_3), as depicted in Figure 6. Furthermore, the CO_2 produced from the oxidation mechanism could potentially be utilized by bacteria in carbonic anhydrase (CA) activity, further inducing calcite precipitation. Moreover, this CO_2 production can enhance the yield of calcite precipitation through the carbonation process of portlandite, as described by Jonkers *et al.* (2010) and Morandeanu *et al.* (2014) through the following reaction (Eq. 11). Further research is necessary to understand the contribution of these possible pathways fully.

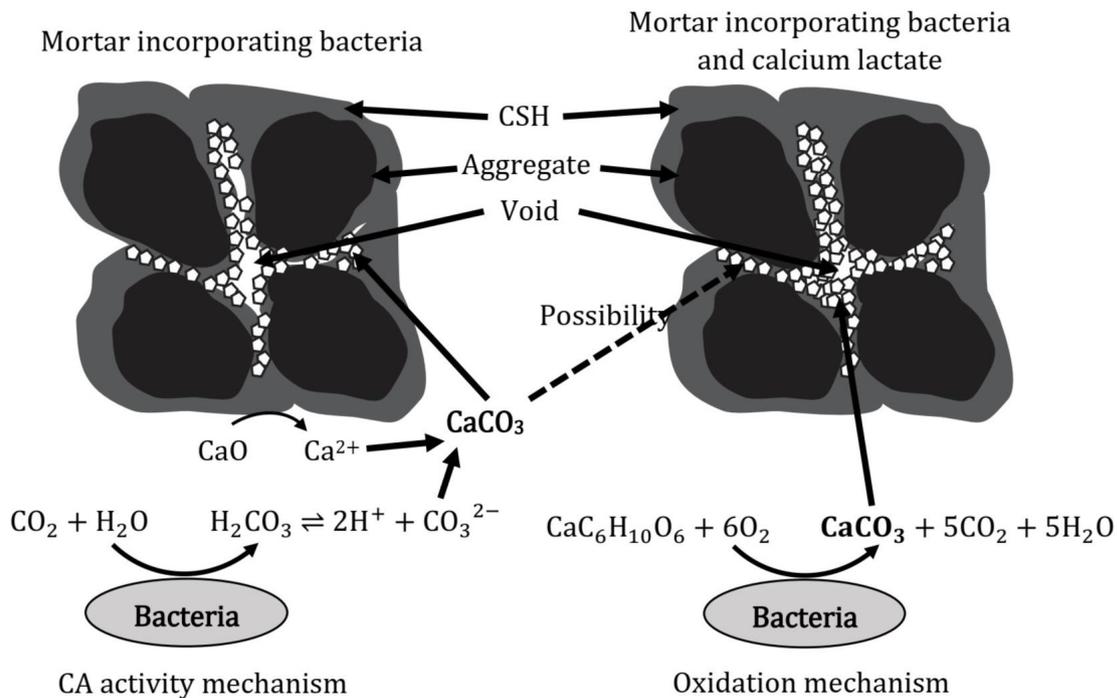


Figure 5. Hypothesized mechanisms of calcite formation in two bio-mortar specimens. The figure illustrates the potential biochemical pathways in a mortar specimen incorporated with bacteria (left) and a specimen that is a mixture of bacteria and calcium lactate (right)



Furthermore, the inclusion of bacteria and calcium lactate pentahydrate may potentially enhance the production of calcium silicate hydrate (CSH), as confirmed by XRD spectra. Previous studies have suggested that these additives could contribute to forming calcium silicate hydrate (CSH) (Chaurasia *et al.* 2019; Su *et al.* 2019). Consequently, incorporating bacteria and calcium lactate pentahydrate improved the ultrasonic pulse velocity (UPV) and flexural strength of the bio-mortar specimens, particularly the MLB1 specimen. The notable enhancement in mortar properties was achieved by incorporating a 10% inoculum solution of *Lysinibacillus sphaericus* strain SKC/VA-1 and calcium lactate pentahydrate at a dosage of 0.5% by weight of cement.

In this study, a 10% v/v bacterial inoculum, as used in the MLB1 specimen, provided a balanced nutrient ratio (C: N: P) that facilitated the biomineralization process of *Lysinibacillus sphaericus* strain SKC/VA-1. This balance enhanced mortar strength compared to the MB2 and MLB2 specimens, which contained a 20% v/v bacterial inoculum. The excessive organic matter from bacterial cells led to an overproduction of gases such as carbon dioxide and hydrogen, thus

resulting in internal pressure, creating voids or weak points in the bio-mortar, which could compromise its strength and structural integrity (Mondal and Ghosh 2018; Su *et al.* 2019). Several factors, including the type of bacteria, nutrient availability, and the overall composition of the bio-mortar, can influence the negative impact of an overabundance of bacterial cells on bio-mortar strength. *Lysinibacillus sphaericus* strain SKC/VA-1, a rod-shaped bacterium, can form endospores, enabling it to survive in harsh conditions. It also exhibits ureolytic activity, producing ammonia and carbonate ions, which are crucial for biomineralization (Patil *et al.* 2023).

Balancing the bacterial population to ensure effective biomineralization is essential for achieving the desired strength and performance of the bio-mortar. Furthermore, *Lysinibacillus sphaericus* tolerates alkaline pH conditions, a beneficial trait for bio-mortar applications due to the alkaline environment created by cement hydration. This bacterial ability to remain active in such conditions promotes extended biomineralization (Shirakawa *et al.* 2011). The unique characteristics of *Lysinibacillus sphaericus*, such as its shape, spore formation, ureolytic activity, alkaline pH tolerance, and environmental adaptability, make it a promising candidate for

bio-mortar applications. These characteristics can lead to improved interparticle bonding, enhanced strength, and increased durability of the bio-mortar. To optimize the effect of *Lysinibacillus sphaericus* on bio-mortar strength, controlling bacterial cell concentration and considering factors such as nutrient availability and environmental conditions is essential.

This study utilized a newly discovered indigenous bacterial strain from Indonesia (*Lysinibacillus sphaericus* strain SKC/VA-1) to assess its impact on the ultrasonic pulse velocity (UPV) and flexural strength of mortar specimens. The effects of calcium lactate pentahydrate and bacterial inoculum on these mortar properties were investigated. Mineralogical and microscopic analyses were conducted to elucidate the underlying reactions and the resulting compounds. Based on the experimental findings, the following conclusions can be drawn:

- (1) The utilization of the indigenous bacterium *Lysinibacillus sphaericus* strain SKC/VA-1 has significantly enhanced the mechanical properties of mortar. Remarkable improvements in dry density, ultrasonic pulse velocity (UPV), and flexural strength have been observed. These enhancements were achieved using a modified low-cost medium incorporating calcium lactate pentahydrate. The optimal combination of calcium lactate pentahydrate (0.5% by weight of cement) and bacterial inoculum concentration (10% v/v) was utilized to attain these improvements. Notably, applying this formulation had no adverse effects on the properties of the mortar specimens.
- (2) The utilization of calcium lactate pentahydrate in conjunction with *Lysinibacillus sphaericus* strain SKC/VA-1 presents a promising opportunity for enhancing the properties of mortar. However, ensuring that the bacterial inoculum concentrations employed in the specimens are optimized to achieve the desired improvements is crucial.
- (3) The potential mechanisms underlying the significant enhancement of mortar properties, such as ultrasonic pulse velocity (UPV) and flexural strength, when using *Lysinibacillus sphaericus* strain SKC/VA-1 with a 10% v/v inoculum and the addition of calcium lactate pentahydrate (0.5% by weight of cement) can be attributed to the following factors:
 - a. Oxidation mechanism: the bacterial metabolism is believed to oxidize the calcium lactate pentahydrate, resulting in the generation of CO₂. This CO₂ can then be utilized by the bacteria

in the carbonic anhydrase (CA) activity, leading to increased calcite production. This process helps fill the voids within the mortar, thereby improving its properties.

- b. Carbonic anhydrase (CA) activity: Even in the absence of calcium lactate pentahydrate, applying *Lysinibacillus sphaericus* strain SKC/VA-1 still yields improvements in UPV and flexural strength. This behavior suggests that carbonic anhydrase (CA) activity promotes cumulative calcite precipitation and the oxidation mechanism, thereby enhancing the mortar properties.

Furthermore, the inclusion of *Lysinibacillus sphaericus* strain SKC/VA-1 and calcium lactate pentahydrate may potentially enhance the production of calcium silicate hydrate (CSH), as evidenced by XRD spectra. This, in turn, contributes to a more pronounced improvement in UPV and flexural strength of the bio-mortar specimens, particularly in the case of the MLB1 specimen. However, further research is required to elucidate the contributions of these potential pathways fully.

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References

- Achal, V., Mukerjee, A., Reddy, M.S., 2013. Biogenic treatment improves the durability and remediates the cracks of concrete structures. *Constr. Build. Mater.* 48, 1–5. <https://doi.org/10.1016/j.conbuildmat.2013.06.061>
- Achal, V., Mukherjee, A., 2015. A review of microbial precipitation for sustainable construction. *Constr. Build. Mater.* 93, 1224–1235. <https://doi.org/10.1016/j.conbuildmat.2015.04.051>
- Achal, V., Pan, X., 2011. Characterization of urease and carbonic anhydrase producing bacteria and their role in calcite precipitation. *Curr. Microbiol.* 62, 894–902. <https://doi.org/10.1007/s00284-010-9801-4>
- Achal, V., Pan, X., 2014. Influence of calcium sources on microbially induced calcium carbonate precipitation by *Bacillus* sp. CR2. *Appl. Biochem. Biotechnol.* 173, 307–317. <https://doi.org/10.1007/s12010-014-0842-1>

- Ameri, F., Shoaie, P., Bahrami, N., Vaezi, M., Ozbakkaloglu, T., 2019. Optimum rice husk ash content and bacterial concentration in self-compacting concrete. *Constr. Build. Mater.* 222, 796–813. <https://doi.org/10.1016/j.conbuildmat.2019.06.190>
- Andalib, R., Abd Majid, M., Keyvanfar, A., Talaiekhoozan, A., Hussin, M.W., Shafaghat, A., Zin, R.M., Lee, C.T., Fulazzaky, M.A., Ismail, H.H., 2014. Durability improvement assessment in different high strength bacterial structural concrete grades against different types of acids. *Sadhana-Acad. Proc. Eng. Sci.* 39, 1509–1522. <https://doi.org/10.1007/s12046-014-0283-0>
- ASTM C 1723, 2010. Standard Guide for Examination of Hardened Concrete Using Scanning Electron Microscopy. ASTM International, West Conshohocken, PA.
- ASTM C 642, 2013. Standard Test Method for Density, Absorption, and Voids in Hardened Concrete. ASTM International West Conshohocken, PA.
- ASTM C 597, 2016. Standard Test Method for Pulse Velocity Through Concrete. ASTM International, West Conshohocken, PA.
- ASTM C 293, 2016. Standard Test Method for Flexural Strength of Concrete (Using Simple Beam With Center-Point Loading). ASTM International, West Conshohocken, PA.
- Bai, C.P., Varghese, S., 2016. An experimental investigation on the strength properties of fly ash based bacterial concrete. *Int. J. Innov. Res. Adv. Eng.* 3, 64–69.
- Bond, G.M., Stringer, J., Brandvold, D.K., Simsek, F.A., Medina, M.G., Egeland, G., 2001. Development of integrated system for biomimetic CO₂ sequestration using the enzyme carbonic anhydrase. *Energy and Fuels.* 15, 309–316. <https://doi.org/10.1021/ef000246p>
- Burne, R.A., Chen, Y.Y.M., 2000. Bacterial ureases in infectious diseases. *Microbes Infect.* 2, 533–542. [https://doi.org/10.1016/S1286-4579\(00\)00312-9](https://doi.org/10.1016/S1286-4579(00)00312-9)
- Castro-Alonso, M.J., Montañez-Hernandez, L.E., Sanchez-Muñoz, M.A., Macias Franco, M.R., Narayanasamy, R., Balagurusamy, N., 2019. Microbially induced calcium carbonate precipitation (MICP) and its potential in bioconcrete: microbiological and molecular concepts. *Front Mater.* 6, 1–15. <https://doi.org/10.3389/fmats.2019.00126>
- Chaurasia, L., Bisht, V., Singh, L.P., Gupta, S., 2019. A novel approach of biomineralization for improving micro and macro-properties of concrete. *Constr. Build. Mater.* 195, 340–351. <https://doi.org/10.1016/j.conbuildmat.2018.11.031>
- DeBelie, N., 2016. Application of bacteria in concrete: a critical evaluation of the current status. *RILEM Tech. Lett.* 1, 56. <https://doi.org/10.21809/rilemtechlett.2016.14>
- Dhami, N.K., Reddy, M.S., Mukherjee, M.S., 2013. Biomineralization of calcium carbonates and their engineered applications: a review. *Front Microbiol.* 4, 1–13. <https://doi.org/10.3389/fmicb.2013.00314>
- Gollapudi, U.K., Knutson, C.L., Bang, S.S., Islam, M.R., 1995. A new method for controlling leaching through permeable channels. *Chemosphere.* 30, 695–705. [https://doi.org/https://doi.org/10.1016/0045-6535\(94\)00435-W](https://doi.org/https://doi.org/10.1016/0045-6535(94)00435-W)
- Ivanov, V., Stabnikov, V., Kawasaki, S., 2019. Ecofriendly calcium phosphate and calcium bicarbonate biogrouts. *J. Clean. Prod.* 218, 328–334. <https://doi.org/10.1016/j.jclepro.2019.01.315>
- Jonkers, H.M., Thijssen, A., Muyzer, G., Copuroglu, O., Schlangen, E., 2010. Application of bacteria as self-healing agent for the development of sustainable concrete. *Ecol Eng.* 36, 230–235. <https://doi.org/10.1016/j.ecoleng.2008.12.036>
- Khaliq, W., Ehsan, M.B., 2016. Crack healing in concrete using various bio influenced self-healing techniques. *Constr. Build. Mater.* 102, 349–357. <https://doi.org/10.1016/j.conbuildmat.2015.11.006>
- Li, M., Fu, Q.L., Zhang, Q., Achal, V., Kawasaki, S., 2015. Bio-grout based on microbially induced sand solidification by means of asparaginase activity. *Sci Rep.* 5, 1–9. <https://doi.org/10.1038/srep16128>
- Mehta, P.K., Monteiro, P.J.M., 2014. *Concrete: Microstructure, Properties and Materials*, fourth ed. McGraw-Hill Publishing, New York.
- Mondal, S., Ghosh, A.D., 2018. Investigation into the optimal bacterial concentration for compressive strength enhancement of microbial concrete. *Constr. Build. Mater.* 183, 202–214. <https://doi.org/10.1016/j.conbuildmat.2018.06.176>
- Morandea, A., Thiéry, M., Dangla, P., 2014. Investigation of the carbonation mechanism of CH and C-S-H in terms of kinetics, microstructure changes and moisture properties. *Cem. Concr. Res.* 56, 153–170. <https://doi.org/10.1016/j.cemconres.2013.11.015>
- Ozdemir, E., 2009. Biomimetic CO₂ sequestration: 1. Immobilization of carbonic anhydrase within polyurethane foam. *Energy and Fuels.* 23, 5725–5730. <https://doi.org/10.1021/ef9005725>
- Papadakis, V.G., Fardis, M.N., Vayenas, C.G., 1992. Hydration and carbonation of pozzolanic cements. *ACI Mater. J.* 89, 119–130.
- Patil, P.P., Prabhu, M., Mutnuri, S., 2023. A novel and sustainable approach for biotransformation of phosphogypsum to calcium carbonate using urease producing *Lysinibacillus sphaericus* strain GUMP2. *Environmental Technology.* 44, 226–239.
- Ramachandran, S., Ramakrishnan, V., Bang, S., 2001. Remediation of concrete using micro-organism. *ACI Mater. J.* 98, 3–9. <https://doi.org/10.14359/10154>
- Ramanan, R., Kannan, K., Sivanesan, S.D., Mudliar, S., Kaur, S., Tripathi, A.K., Chakrabarti, T., 2009. Bio-sequestration of carbon dioxide using carbonic anhydrase enzyme purified from *Citrobacter freundii*. *World. J. Microbiol. Biotechnol.* 25, 981–987. <https://doi.org/10.1007/s11274-009-9975-8>
- Shirakawa, M.A., Cincotto, M.A., Atencio, D., Gaylarde, C.C., John, V.M., 2011. Effect of culture medium on biocalcification by *Pseudomonas putida*, *Lysinibacillus sphaericus* and *Bacillus subtilis*. *Brazilian Journal of Microbiology.* 42, 499–507.
- SNI 15-2049-2004, 2004.) Semen Portland. Badan Standardisasi Nasional (BSN), Indonesia.
- Su, Y., Feng, J., Jin, P., Qian, C., 2019. Influence of bacterial self-healing agent on early age performance of cement-based materials. *Constr. Build. Mater.* 218, 224–234. <https://doi.org/10.1016/j.conbuildmat.2019.05.077>
- Syarif, R., Rizki, I.N., Wattimena, R.K., Chaerun, S.K., 2019. Selection of bacteria inducing calcium carbonate precipitation for self-healing concrete application. *Curr Res Biosci Biotechnol.* 1, 26–30.
- Vaezi, M., Zareei, S.A., Jahadi, M., 2020. Recycled microbial mortar: effects of bacterial concentration and calcium lactate content. *Constr. Build. Mater.* 234, 117349. <https://doi.org/10.1016/j.conbuildmat.2019.117349>
- Vijay, K., Murmu, M., 2019. Effect of calcium lactate on compressive strength and self-healing of cracks in microbial concrete. *Front. Struct. Civ. Eng.* 13, 515–525. <https://doi.org/10.1007/s11709-018-0494-2>
- Wang, J., Ersan, Y.C., Boon, N., De Belie, N., 2016. Application of microorganisms in concrete: a promising sustainable strategy to improve concrete durability. *Appl. Microbiol. Biotechnol.* 100, 2993–3007. <https://doi.org/10.1007/s00253-016-7370-6>
- Xu, J., Yao, W., 2014. Multiscale mechanical quantification of self-healing concrete incorporating non-ureolytic bacteria-based healing agent. *Cem. Concr. Res.* 64, 1–10. <https://doi.org/10.1016/j.cemconres.2014.06.003>