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Construction biotechnology: improving mortar properties through calcium carbonate precipitation using a novel strain of the bacterium *Neisseria perflava*

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Abstract
In construction technology, there are significant efforts to reduce environmental emissions, particularly NH_3 and other pollutants. This study marks the first application of $CaCO_3$ biomineralization biotechnology in microbially induced calcium carbonate precipitation (MICCP) to enhance mortar properties using the non-pathogenic *Neisseria perflava* strain SKC/VA-3, which employs carbonic anhydrase mechanisms. The results demonstrated that *N. perflava* could significantly improve the physical and mechanical characteristics of mortar. Incorporating *N. perflava* and calcium lactate pentahydrate resulted in a 20% increase in compressive strength and a 14% rise in indirect tensile strength of the mortar. Examination through scanning electron microscopy-energy dispersive X-ray spectroscopy (SEM–EDS) revealed calcite formation within the microstructure of the bio-mortar. Additionally, self-healing assessments indicated that calcite precipitation, driven by bacterial metabolism, also occurred on the cracked surfaces of the bacterial mortar, suggesting potential for reduced maintenance and increased material longevity. This study provides the first report on the use of *N. perflava* for bio-mortar enhancement and represents a novel biotechnological approach to improving the properties of mortar and other cementitious materials. The utilization of *N. perflava* in bio-mortar represents a groundbreaking biotechnological advance, potentially enhancing mortar and other cement-based materials. This development contributes to sustainable, durable, and environmentally friendly construction technologies.

Keywords Bio-mortar · $CaCO_3$ biomineralization · Microbially induced calcium carbonate precipitation (MICCP) · *Neisseria perflava* SKC/VA-3 · Sustainable construction technology

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1 Introduction

Cement-based materials are integral to the construction industry. However, their high demand leads to significant cement production, contributing markedly to increased CO_2 emissions and energy consumption [1, 2]. Additional challenges include discontinuities arising from the composition of aggregates and water in the mix, which facilitate micro-crack formation [3]. To address these issues, various strategies have been employed, including the use of chemical admixtures (e.g., silica fume) and reinforcements (e.g., fibers) [4, 5]. Nonetheless, these conventional methods often incur relatively high costs. Consequently, researchers are exploring alternative approaches to reduce material and operational expenses, notably through biologically induced calcite biomineralization, commonly known as microbially induced calcium carbonate precipitation (MICCP) [6–12].

Bacillus spp. are the predominant calcite-precipitating bacteria employed in improving the properties of cementitious materials, as evidenced by numerous studies [6, 8, 11, 13, 14]. Additionally, the capability of other bacterial species such as *Shewanella* sp. [15], *Enterococcus faecalis* [16, 17], *Sporosarcina pasteurii* [18, 19], and *Acinetobacter* sp. [20] in enhancing the characteristics of cement-based materials has been documented. Despite these findings, the potential of other bacteria remains largely unexplored. Notably, certain *Neisseria* spp. may demonstrate the capability for calcium carbonate precipitation via carbonic anhydrase (CA) activity and the presence of calcium ions. It has been observed that *Neisseria gonorrhoeae* [21–24] and *Neisseria sicca* [25] can produce bicarbonate ions through CA activity. Carbonic anhydrase, a zinc-containing metalloenzyme, predominantly catalyzes the reversible hydration of carbon dioxide (CO_2) to bicarbonate ion (HCO_3^-) in many organisms, especially in the prokaryotic domain [26, 27]. Furthermore, CA activity plays a crucial role in biomimetic CO_2 sequestration, potentially leading to calcium carbonate formation in the presence of Ca^{2+} ions [13, 28, 29].

Correspondingly, *Neisseria perflava*, a member of the *Neisseria* genus, is identified as a Gram-negative, oxidase-positive, and catalase-positive bacterium. This species is commonly recognized as a commensal, coexisting symbiotically with humans, and is often found in the nasopharynx and oropharynx without being pathogenic or causing illness [30]. *Neisseria perflava* is part of the diverse non-pathogenic *Neisseria* species that make up the human pharyngeal microflora [30]. Studies have shown that *Neisseria perflava* is capable of producing carbonic anhydrase [31]. Furthermore, a study conducted by Sanders and Maren in 1967 [32] on the carbonic anhydrase activity in nine strains of *Neisseria perflava*, ascertained via the pH change technique, revealed that eight of these strains exhibited this enzymatic function. Nonetheless, the suitability of *Neisseria perflava* for application in MICCP remains unexplored.

Consequently, this study examined the capacity of the bacterium *Neisseria perflava* strain SKC/VA-3 to enhance mortar properties. The selection of *Neisseria perflava* strain SKC/VA-3 was based on its classification as a non-pathogenic bacterium [33, 34]. Calcium lactate pentahydrate served as the source of calcium and carbon due to its minimal negative impact on the strength of cement mortar, as documented by [35]. To analyze the microstructure of the bacterial mortar samples, scanning electron microscope-energy dispersive X-ray spectroscopy (SEM–EDS) was employed. Furthermore, visualization and characterization of self-healing, facilitated by bacterial metabolism, were also assessed using SEM–EDS.

2 Materials and methods

2.1 Microbial cultures and growth media

In this study, *Neisseria perflava* strain SKC/VA-3, isolated from a mixed sample comprising crude oil (sourced from PT. Pertamina, Sukabumi, West Java) and rust deposits from petroleum pipelines (Metal Laboratory, Faculty of Mechanical and Aerospace Engineering, Bandung Institute of Technology), was utilized. The 16S rRNA gene sequence of this strain is registered under the GenBank/EMBL/DDBJ accession number MT229315. Cultivation of this bacterial species was conducted in a medium consisting of 1.5 g/L nutrient broth (Oxoid) and 3.2 mM calcium lactate pentahydrate ($CaC_6H_{10}O_6.5H_2O$; sourced locally in Bandung, West Java, Indonesia), following the protocol outlined in Syarif et al. [36]. Sterilization of the media was carried out at 121 °C for 15 min before inoculating with the bacterial culture (10% v/v). The cultures were then incubated under agitation at 180 rpm for 48 h at room temperature, followed by storage in a refrigerator prior to their use in bio-mortar specimen preparation. The concentration of bacterial cells, measured

as colony-forming units (CFU) per milliliter, in the *Neisseria perflava* strain SKC/VA-3 culture, was determined using a serial dilution method, ranging from 10^{-5} to 10^{-9} [37]. These serially diluted samples were incubated on Nutrient Agar (NA) medium for a duration of 3 days, followed by a quantitative assessment of bacterial colonies. Utilizing this enumeration technique, the bacterial concentration of the *Neisseria perflava* strain SKC/VA-3 was estimated to be approximately 4.6×10^7 CFU/ml.

2.2 Preparation of mortar specimens

For the fabrication of all cylindrical mortar specimens, Ordinary Portland Cement (sourced from Indocement Ltd., Cirebon, Indonesia), fine aggregate (with a specific gravity of 2.685 and a fineness modulus of 3.39), and tap water were employed as the basic components. In this study, the mortar mix was formulated adhering to a ratio of 1:3 for cement to fine aggregate and a water-to-cement ratio of 0.5. Control specimens of ordinary mortar were produced using only the aforementioned mixture. In contrast, the bacterial mortar specimens in the experiment incorporated *Neisseria perflava* bacterial cells and calcium lactate pentahydrate ($CaC_6H_{10}O_6.5H_2O$), procured from a chemical supplier in Bandung, West Java, Indonesia. The bacterial cells, in their liquid form, were added as a partial substitute for water, constituting 10% v/v of the water content. The proportion of calcium lactate pentahydrate used was 0.5% of the cement's weight. Both sets of specimens were manually mixed and cast into PVC pipes with a diameter of 45 mm, followed by compaction using a tamping rod. Subsequently, they were immediately enveloped in plastic wrap and left to set for 1 day. Post 24 h, the specimens were demolded, cut to the desired dimensions, and submerged in tap water for a 7 day curing period. This study adopted a 7 day curing duration based on findings from previous research indicating that 70–90% of the compressive strength attainable in 28 days could be achieved within 7 days [38–40].

2.3 Ultrasonic pulse velocity measurement

For each set of control and bacterial mortar specimens, three cylindrical samples, each 4.5 cm in diameter and 9 cm in length, were prepared. Following a 7 day curing period in tap water, the specimens were removed, dried, and their dimensions measured in triplicate to calculate average values prior to testing. The testing procedure, adhering to ASTM C597 [41] standards, involved the use of a PUNDIT (Portable Unit for Non-Destructive Digital Indicated Testing) or an Ultrasonic tester E46. Before conducting measurements on the specimens, the PUNDIT was calibrated using a calibration cylinder with a known pulse propagation time of 56 µs. The average UPV for the three specimens in each of the control and bacterial mortar groups was then determined.

2.4 Porosity and water absorption measurement

The specimens utilized for porosity and water absorption tests were cylindrical, measuring 4.5 cm in diameter and 3 cm in length. Porosity was measured following the ISRM suggested method [42], and water absorption was determined in accordance with ASTM C642 [43]. After a 7-day immersion in tap water, three specimens from each of the control and bacterial mortar groups were extracted and oven-dried at 105 °C for 24 h to ascertain their oven-dry mass. These specimens were then submerged in tap water for another 24 h, removed, wiped, and weighed to obtain their saturated surface-dry mass. In the subsequent step, the specimens, secured with wire, were submerged in water to measure their apparent mass while immersed. The average values for porosity and water absorption for both control and bacterial mortar groups were then calculated.

2.5 Compressive and indirect tensile strength test

Compressive and indirect tensile strength tests were performed in triplicate, adhering to the methods suggested by ISRM [44, 45]. For compressive strength testing, specimens measuring 4.5 cm in diameter and 9 cm in height were utilized, while for the indirect tensile strength test (Brazilian test), specimens of the same diameter but 2.25 cm in height were employed. Following a 7-day period of wet curing, the specimens were removed, dried, and subjected to testing using a servo-controlled hydraulic compression tester (Hung Ta HT-8391). The loading rates applied were 6000 N/min for compressive strength and 2000 N/min for indirect tensile strength testing. The mean compressive and indirect tensile strengths for the three specimens in each group, both control and bacterial mortar, were subsequently calculated.

2.6 Microstructure observation

Microstructural and elemental analysis of the control and bacterial mortar specimens was conducted using Scanning Electron Microscope-Energy Dispersive X-ray Spectroscopy (SEM–EDS; JEOL JSM-J6510 A). Small fragments were extracted from both types of specimens and subjected to preparatory procedures before SEM–EDS analysis. This preparation involved chemical fixation using 2.5% glutaraldehyde for 24 h, followed by triple washing with phosphate buffer. Subsequent dehydration was carried out using a graded acetone series (25, 50, 75, and 100% concentrations for 15 min, 15 min, 24 h, and 15 min, respectively).

2.7 Self-healing visualization and SEM–EDS of the precipitates

Self-healing visualization was performed to examine the precipitates formed on the surfaces of the specimens. Post-indirect tensile strength testing, the fractured specimens from each batch were immediately visualized, marking this as the 0-day observation (Control shown in Fig. 5a and bacterial mortar in Fig. 5c). Detailed examination of specific areas (indicated by red rectangles in Fig. 5) was carried out using a trinocular stereomicroscope (SMZ-2 T, Nikon). Following the initial assessment, the specimens were submerged in tap water and re-examined after 21 days using the same method (Control depicted in Fig. 5b and bacterial mortar in Fig. 5d). The precipitates were then carefully extracted, chemically fixed, washed thrice with phosphate buffer, and dehydrated through a graded acetone series (25, 50, 75, 100%, each for 15 min, 15 min, 24 h, and 15 min, respectively), before being analyzed using SEM–EDS. The SEM–EDS analysis focused on verifying the presence of calcite precipitation on the surfaces of the bacterial specimens.

3 Results and discussion

3.1 Ultrasonic pulse velocity, porosity, and water absorption

Ultrasonic Pulse Velocity (UPV) measurements were conducted to assess the structural integrity and uniformity of the specimens. The results of these measurements are depicted in Fig. 1. Notably, the average UPV for the bacterial mortar specimens (incorporating *N. perflava*) was observed to be approximately 5% higher than that of the control, as indicated in Fig. 1. To evaluate the physical properties of the specimens, porosity and water absorption tests were performed. Figure 2a shows a marginal decrease (around 2%) in the average porosity of the bacterial mortar specimens, suggesting fewer voids compared to the control. This observation is consistent with the UPV data, where reduced void content correlates with decreased porosity and improved UPV. Additionally, a decrease in water absorption by approximately 7% was noted in the bacterial mortar, as illustrated in Fig. 2b.

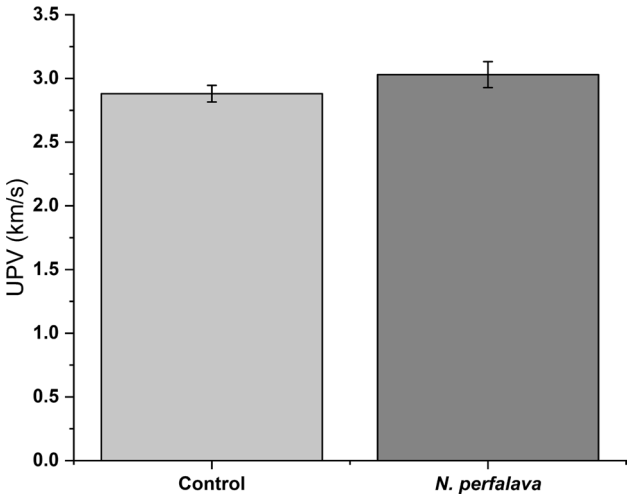

Fig. 1 Comparison of ultrasonic pulse velocity between control mortar and bio-mortar (containing *Neisseria perflava*)

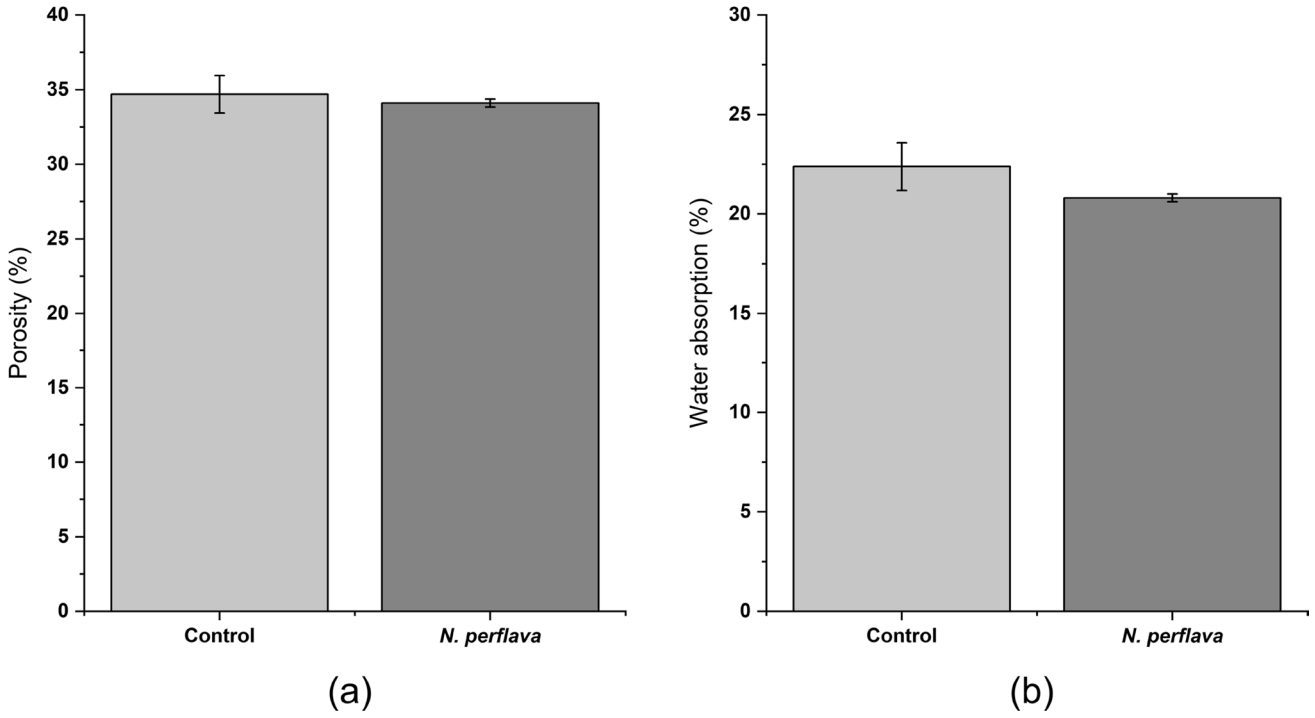

Fig. 2 Porosity (**a**) and water absorption (**b**) in control mortar and bio-mortar (containing *Neisseria perflava*)

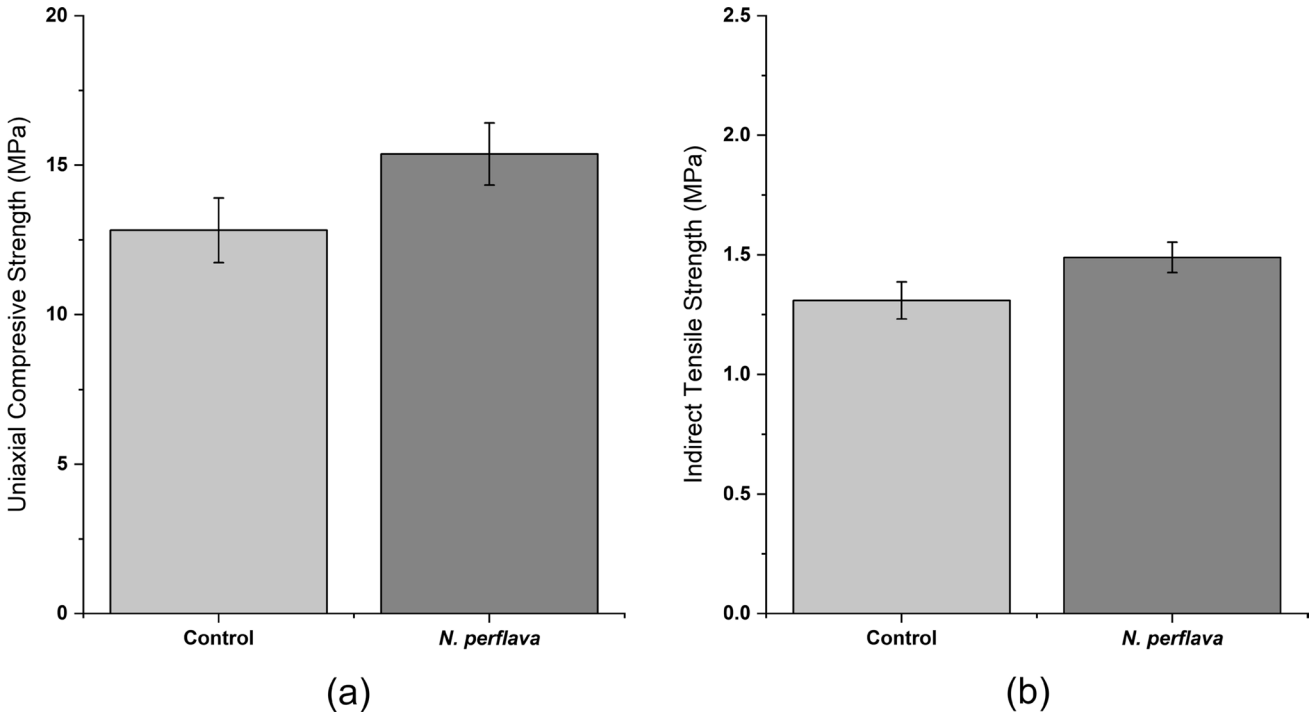

Fig. 3 Uniaxial compressive strength (**a**) and indirect tensile strength (**b**) of control mortar and bio-mortar (containing *Neisseria perflava*)

3.2 Compressive and indirect tensile strength

Compressive and indirect tensile strength tests were performed to evaluate the mechanical properties of the specimens, as depicted in Fig. 3. The results, illustrated in Fig. 3a, indicate a 20% increase in the average uniaxial compressive strength

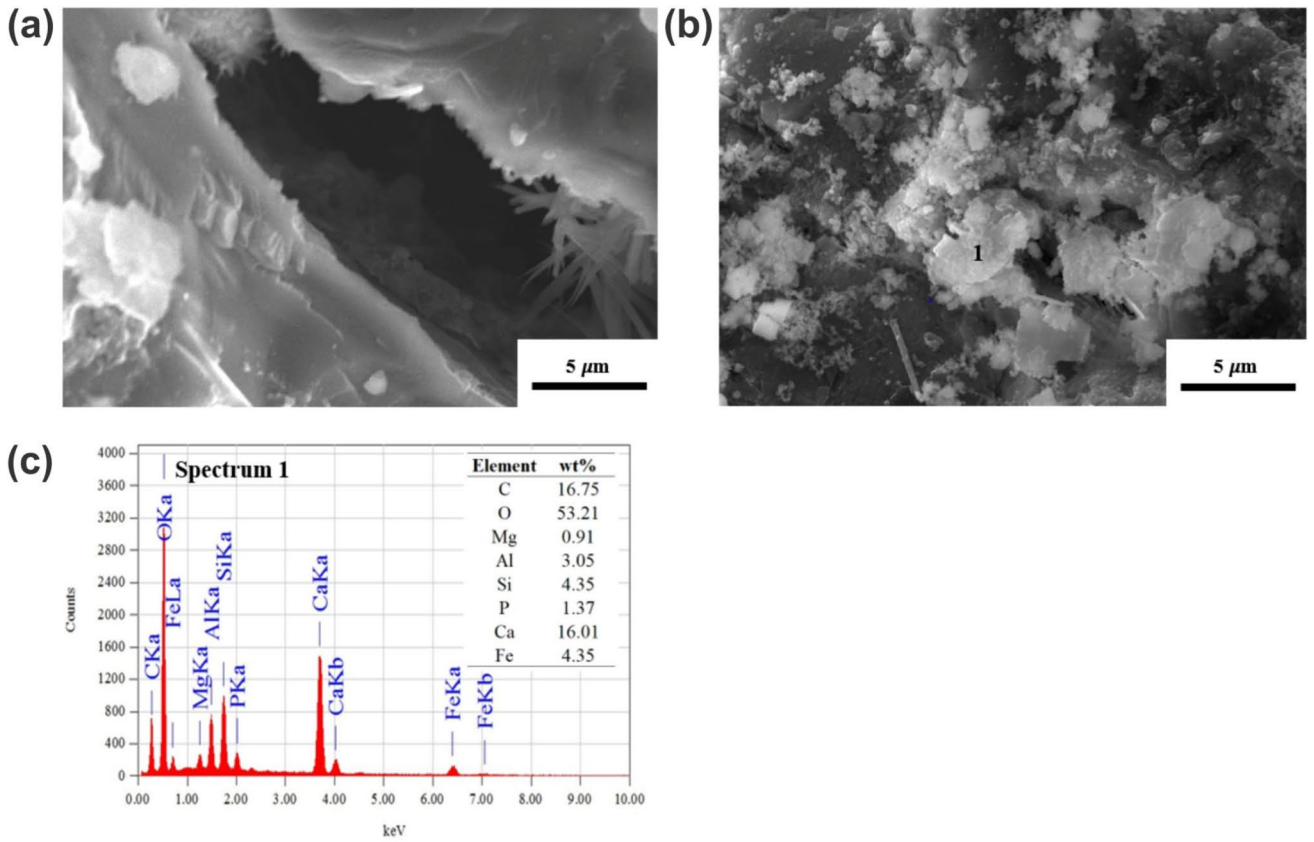

Fig. 4 Microstructural observations: (**a**) SEM image of control mortar, (**b**) SEM image of bio-mortar (containing *Neisseria perflava*), and (**c**) EDS spectrum (analytical point 1) of bio-mortar (containing *Neisseria perflava*)

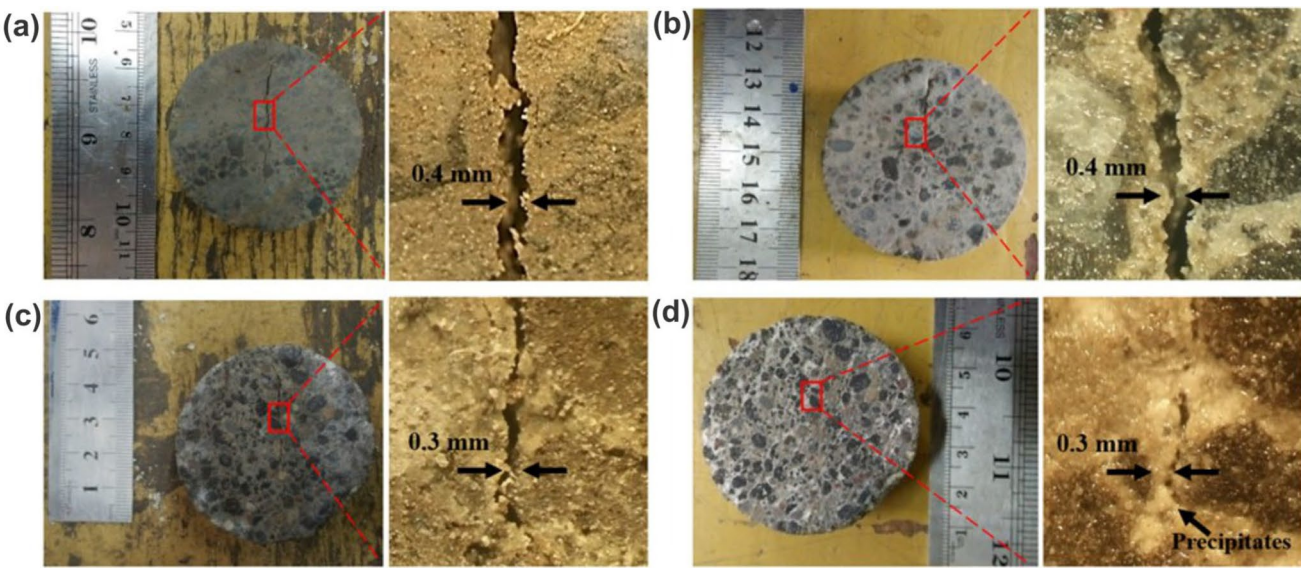

Fig. 5 Visualization of self-healing in cracked specimens from the indirect tensile strength test: (**a**) control mortar at day 0, (**b**) control mortar at day 21, (**c**) bio-mortar (containing *Neisseria perflava*) at day 0, and (**d**) bio-mortar (containing *Neisseria perflava*) at day 21

of the bacterial mortar specimens, a slightly higher enhancement compared to previous studies involving *Bacillus* spp. as the calcite-precipitating agent. Additionally, as shown in Fig. 3b, there was a 14% increase in the average indirect tensile strength of the bacterial mortar specimens. This improvement in strength correlates with the reductions in porosity and water absorption (shown in Fig. 2a, b) and aligns with the observed increase in ultrasonic pulse velocity, indicating enhanced mechanical properties.

3.3 The microstructure inside the specimens and self-healing phenomenon

Figure 4a, b illustrate the microstructure of the control and bacterial mortar specimens, respectively. Notably, in the bacterial mortar containing *N. perflava* (Fig. 4b), white precipitates were observed occluding the voids, which were tentatively identified as calcium carbonate based on the EDS spectrum analysis (Fig. 4c). Additionally, the examination of the cracked specimens post 21-day immersion revealed further instances of calcium carbonate precipitation on the surface of the bacterial specimen. The control mortar specimen's crack appeared unchanged from the initial state (Fig. 5a) to after the 21-day period (Fig. 5b). In contrast, the bacterial mortar specimen displayed white precipitates on its surface and within the crack after the 21-day immersion (Fig. 5d), which were confirmed as calcium carbonate by SEM (Fig. 6a) and EDS spectrum analysis (Fig. 6b).

3.4 Discussion

To date, no research has been conducted on the application of *Neisseria perflava* as a Microbially Induced Calcium Carbonate Precipitation (MICCP) agent. However, this study reveals that *Neisseria perflava* is capable of enhancing the mechanical properties of mortar, a prediction inferred from the carbonic anhydrase enzyme it produces. Smith and Ferry [26] reported that *Neisseria perflava* exhibits carbonic anhydrase activity, and Forkman [46] identified the location of carbonic anhydrase in the cell wall of *Neisseria flava*. Carbonic anhydrase (CA), a pivotal enzyme in various physiological processes, primarily catalyzes the interconversion of carbon dioxide (CO_2) and water (H_2O) into bicarbonate (HCO_3^-) and protons (H^+), and facilitates the reversible hydration of CO_2 to bicarbonate ions.

In the context of MICCP in this study, carbonic anhydrase aids in producing bicarbonate ions, which react with calcium ions to form calcite. This reaction contributes to an enhancement in the average ultrasonic pulse velocity (UPV) of bacterial mortar (incorporating *N. perflava*), approximately 5% higher than that of the control, as depicted in Fig. 1. The underlying mechanism involves CA activity from *N. perflava*, potentially leading to calcium carbonate ($CaCO_3$) precipitation. This precipitation fills the voids or micro-cracks within the mortar, thereby increasing its density and resulting in a higher UPV. This aligns with previous studies that incorporated *Bacillus* sp. into mortar mixtures [47, 48].

This study therefore provides direct evidence that *Neisseria perflava*, through its production of carbonic anhydrase, can be used for $CaCO_3$ biomineralization to enhance mortar properties (as demonstrated in Figs. 1 and 3). The enzyme catalyzes the reversible hydration of CO_2, an essential step in biomineralization, wherein organisms produce minerals [26]. The bacterial-induced precipitation of calcium carbonate leads to the formation of carbonate crystals, improving

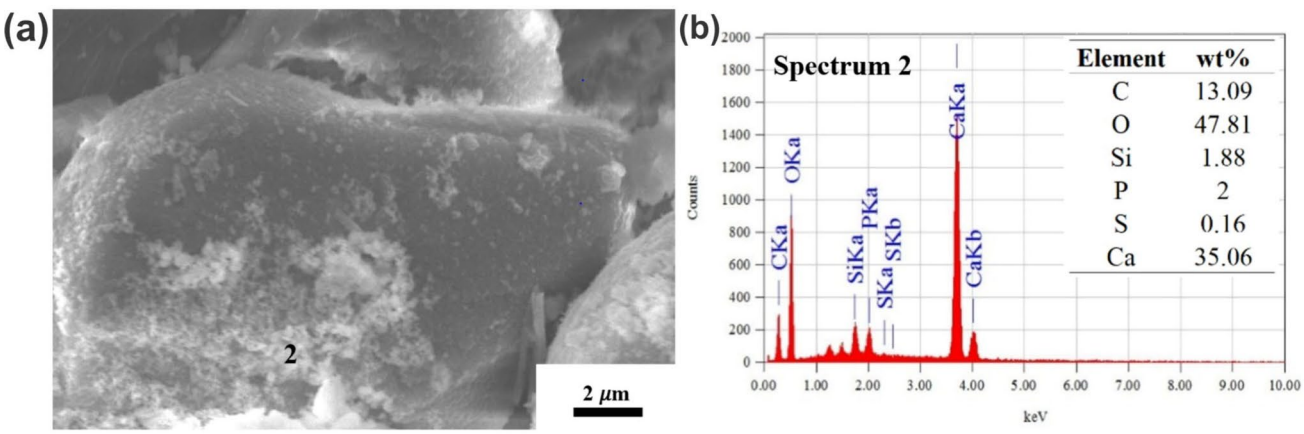

Fig. 6 (**a**) SEM image of precipitates from the surface crack of bio-mortar (containing *Neisseria perflava*) and (**b**) corresponding EDS spectrum (analytical point 2)

mortar's mechanical properties such as UPV and uniaxial compressive strength. In fact, bacterial mortar specimens showed a 20% increase in average uniaxial compressive strength compared to control specimens (Fig. 3). Due to CA activity stimulated by *N. perflava* metabolism, calcium carbonate deposits fill voids (as shown in Figs. 4, 5, and 6), reducing porosity and water absorption (Fig. 2). These deposits also impede water passage within the microstructure of bacterial mortar, decreasing water absorption [13, 18, 48, 49].

These findings suggest that *Neisseria perflava* exhibits a higher efficacy than previously studied *Bacillus* sp. in calcite precipitation. González et al. (2000) [50] reported only a 10% increase in uniaxial compressive strength using *B. pseudofirmus* after 7 days, whereas Vaezi et al. [51] observed a maximum 14% increase in compressive strength at the same age. Additionally, studies incorporating *B. subtilis* and calcium lactate in concrete reported only a 12% increase in compressive strength [52, 53].

Based on the results obtained from SEM–EDS analysis (Figs. 4–6), and considering that the pH of the mixture was maintained between 8 and 9 in this study [36], it can be deduced that the precipitation of calcium carbonate both within and on the surface of the bacterial mortar specimen is likely a consequence of the carbonic anhydrase (CA) activity resulting from the metabolic processes of *Neisseria perflava*. This deduction is further substantiated by the following chemical Eqs. (1–5):

$$CO_2\,(g) \rightleftharpoons CO_2\,(aq) \tag{1}$$

$$CO_2(aq) + H_2O \rightleftharpoons H_2CO_3 \tag{2}$$

$$H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \tag{3}$$

$$HCO_3^- \rightleftharpoons CO_3^{2-} + H^+ \tag{4}$$

$$Ca^{2+} + CO_3^{2-} \rightleftharpoons CaCO_3 \tag{5}$$

In summary, this study represents the first report of the capability of *Neisseria perflava* to produce carbonic anhydrase, which significantly enhances the mechanical properties of mortar through $CaCO_3$ biomineralization. This discovery holds substantial potential and offers advantageous prospects for MICCP, presenting an environmentally benign, sustainable, and cost-effective approach for addressing various engineering challenges, particularly in construction engineering and environmental applications. From a construction industry perspective, MICCP significantly enhances the durability, strength, and sustainability of concrete. This enhancement involves the role of specific bacteria, such as *Neisseria perflava* in this study, which precipitate calcium carbonate to seal micro-cracks within concrete structures. This biogenic precipitation not only improves the compressive strength and reduces the permeability of concrete but also extends the lifespan of the structures and mitigates environmental impacts compared to conventional concrete production methods, which are highly energy-intensive and major contributors to CO_2 emissions. Furthermore, MICCP offers cost efficiencies by decreasing the necessity for frequent repairs, thereby reducing the lifecycle costs associated with building materials. The technology is also applicable in hydraulic engineering to enhance the properties of geomaterials and prevent erosion [54]. However, translating MICCP from laboratory settings to full-scale industrial applications presents significant challenges. Future research is essential to tailor the conditions for varied environmental settings and to develop more sustainable substrates, enhancing the overall feasibility and sustainability of MICCP in the construction sector.

Given the non-toxic nature of the end-products, in contrast to ammonia produced by certain bacterial strains, MICCP employing carbonic anhydrase catalysis emerges as a preferable method. Employing *Neisseria perflava* in MICCP technology paves the way for the development of innovative construction materials, expanding its utility in construction engineering and environmental contexts. However, comprehensive research is imperative to fully comprehend the complexities of utilizing *Neisseria perflava* for biomineralization within the construction industry. Biomineralization processes are notably influenced by environmental conditions such as pH and temperature. For instance, the optimal stability of carbonic anhydrase, a vital enzyme in biomineralization, has been identified at a pH of 7.5–9.0 and a temperature range of 35–50 °C [55]. Additionally, the biomineralization process is subject to alteration by various environmental factors, including the concentrations of calcium and other ions [56]. To expedite the scalability of MICCP applications using *Neisseria perflava*, further research is necessary to optimize the specific conditions conducive to optimal biomineralization by this bacterium in construction-related applications.

Regarding the use of endospores in MICCP processes, the application of non-endospore-forming bacteria, such as *N. perflava*, for MICCP in biomortar or bioconcrete offers numerous advantages and substantial challenges. These bacteria are selected for their capacity to rapidly initiate MICCP, owing to their vegetative state, which eliminates the need for a germination phase typical of endospores. They are metabolically active, synthesizing enzymes critical for transforming biochemical compounds into carbonate ions, thereby effectively facilitating calcium carbonate formation and enhancing the structural properties of the material [57]. However, the application of these bacteria in concrete environments encounters challenges due to their reduced resistance to extreme conditions such as high pH, dehydration, and nutrient deficiency. Their active metabolic state also renders them more susceptible during storage and transportation and heightens their sensitivity to environmental stressors such as temperature variations and UV radiation, potentially impairing their functionality. Additionally, there is an increased risk of contamination [58] and a necessity for additional protective measures to sustain their viability within concrete, complicating and elevating the cost of the process [59]. Despite these obstacles, non-endospore-forming bacteria demonstrate considerable potential for advancing MICCP applications, contingent upon careful management of their limitations.

This study demonstrates that the bacterium *Neisseria perflava* can produce $CaCO_3$, enhancing mortar properties through biomineralization. This bacterium shows considerable commercial potential for use in MICCP within construction engineering. Although Gram-negative bacteria do not form endospores like Gram-positive bacteria, their outer membrane provides several advantages for survival in the heterogeneous concrete matrix, thus effectively contributing to MICCP and improving mortar durability. These advantages include: (1) acting as a protective barrier against environmental stresses such as pH fluctuations, temperature variations, and the presence of toxic compounds [60]; (2) facilitating the acquisition and utilization of nutrients from the concrete matrix, ensuring bacterial survival and functionality in MICCP [60]; (3) serving as nucleation sites for calcium carbonate precipitation, thereby enhancing MICCP efficiency [60]; (4) aiding bacterial adhesion to the concrete matrix and biofilm formation, which provide additional protection and facilitate nutrient uptake [61]. As there are no prior reports on using Gram-negative bacteria to enhance mortar properties through MICCP, further research should assess the viability of these bacteria post-curing to confirm their effectiveness within the concrete matrix during MICCP processes. Therefore, due to the benefits provided by the outer membrane of Gram-negative bacteria such as *Neisseria perflava*, these bacteria can effectively survive and contribute to MICP within the heterogeneous concrete matrix through various mechanisms, even without the presence of endospores.

The application of non-pathogenic *Neisseria perflava* for MICCP to enhance mortar properties presents multiple advantages over traditional MICCP mechanisms and established bio-concrete techniques. *Neisseria perflava* efficiently utilizes carbonic anhydrase to convert CO_2 into bicarbonate ions, significantly enhancing calcium carbonate precipitation. Its ureolytic activity facilitates the hydrolysis of urea into ammonia, which raises the pH and further promotes calcium carbonate precipitation. The calcium carbonate formed acts as a protective barrier, shielding the bacteria from environmental stresses and providing essential calcium ions for growth. Additionally, *Neisseria perflava*'s capacity to acquire and utilize nutrients from the concrete matrix enhances its survival and effectiveness in MICCP. It also forms synergistic relationships with other microorganisms within the concrete, boosting both its survival and functional contribution to MICCP. The scalability and cost-effectiveness of using *Neisseria perflava* make it suitable for large-scale construction projects. Moreover, the environmentally sustainable nature of the MICCP process with *Neisseria perflava* reduces reliance on traditional chemicals and minimizes the carbon footprint of construction projects by incorporating carbonic anhydrase for carbon capture sequestration. The process also enhances the self-healing properties of mortar, thereby reducing the need for maintenance and repairs. By leveraging these benefits, employing non-pathogenic *Neisseria perflava* for MICCP offers a promising strategy to enhance mortar properties while ensuring safety, sustainability, and cost-effectiveness.

During the study on the use of *Neisseria perflava*, a Gram-negative bacterium, to enhance mortar properties through calcium carbonate precipitation, several potential challenges were identified, including: (1) determining the optimal amount of bacterial inoculum to be added to the mortar; (2) identifying the optimal nutrient concentrations for bacterial growth; (3) the need for precise control of environmental conditions such as temperature, pH, and nutrient availability to support bacterial activity through carbonic anhydrase mechanisms; (4) potential increases in costs associated with large-scale bacterial cultivation and developing efficient methods for incorporating bacteria into mortar mixtures. These challenges can be addressed by future research aimed at confirming whether Gram-negative bacteria can provide benefits comparable to those of Gram-positive bacteria and potentially replace them in specific MICCP applications. For instance, in the crack repair process, Gram-negative bacteria may be more suitable than Gram-positive bacteria due to their ability to rapidly initiate MICCP processes. Unlike endospores, which require a germination phase, Gram-negative bacteria are in a vegetative state, making them metabolically active and capable of synthesizing enzymes that convert

biochemical compounds into carbonate ions, thereby effectively facilitating calcium carbonate formation and enhancing the structural properties of the material.

Therefore, this study assesses the efficacy of employing *Neisseria perflava* for MICCP to enhance mortar properties, marking a pioneering application of Gram-negative bacteria in construction engineering, contrasting with uses of Gram-positive strains documented in existing construction biotechnology research. Notably, Sarkar et al. [62] have shown that incorporating 30% class C fly ash and *Bacillus cohnii* endospores—a Gram-positive bacterium—into concrete not only boosts self-healing properties but also enhances durability and cuts carbon emissions by 39%, thereby marking a significant leap in sustainable construction practices. Similarly, Sarkar et al. [63] reported that the integration of *Bacillus Cohnii* endospores into an alkali-activated fly ash-based composite substantially improved crack healing and durability, decreased porosity, and enhanced resistance to environmental degradation, affirming its viability for modern construction. Moreover, Sarkar et al. (64) demonstrated that *Bacillus cohnii*, through microbial-induced calcite precipitation, effectively seals cracks and restores structural integrity, thereby increasing mechanical strength by up to 60% and enhancing durability, which confirms the dual sealing and healing functionalities of this biologically augmented concrete.

Furthermore, the potential scalability and feasibility of implementing $CaCO_3$ biomineralization biotechnology using *Neisseria perflava* strain SKC/VA-3 in construction projects present a promising outlook but also poses several challenges. Key issues include the scalability of bacterial cultivation, requiring optimization of growth conditions and cost-effective production methods that integrate into existing construction processes. Additionally, the MICCP technology must align with current construction practices without disrupting schedules or necessitating extensive training. Long-term viability and performance under varying environmental conditions, including freeze–thaw cycles and chemical exposure, need thorough evaluation. Regulatory and environmental considerations are crucial, particularly concerning the safety and ecological impacts of using bacterial agents. Economic viability is essential, with a detailed cost–benefit analysis needed to assess potential savings from reduced maintenance. Public perception and acceptance are vital; effective communication strategies are necessary to educate stakeholders about the benefits and safety of MICCP technology. Addressing these challenges through further research and pilot projects is essential for successful technology integration into the industry.

3.5 Conclusion

Based on the results of this study, several key findings regarding the improvement of mortar properties using the *Neisseria perflava* strain SKC/VA-3 can be concluded as follows:

- Enhancement in physical and mechanical properties: The incorporation of *Neisseria perflava* strain SKC/VA-3 with calcium lactate pentahydrate led to improved physical and mechanical properties of mortar. Specifically, there was a 5% increase in ultrasonic pulse velocity (UPV), indicating denser and more coherent material properties.
- Reduction in porosity and water absorption**:** The study noted a decrease in both porosity and water absorption rates of the mortar, by 2% and 7% respectively. This suggests a tighter microstructure and improved resistance against moisture penetration, which are crucial for the durability of construction materials.
- Increase in strengths: There was a significant enhancement in the compressive strength and indirect tensile strength of the mortar, by 20 and 14% respectively. These improvements in strength attributes are indicative of a more robust material, capable of withstanding greater loads and stresses.
- Microstructural evidence of calcium carbonate formation: Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM–EDS) analysis confirmed the formation of calcium carbonate within the mortar's microstructure. This biogenic calcification is critical for the healing and reinforcement of the material.
- Self-healing capability: The study observed self-healing capabilities in the mortar, with visible calcium carbonate precipitation on the surface cracks after 21 days. This feature enhances the longevity and sustainability of the mortar by reducing the need for manual repairs and maintenance.

Overall, the current study suggests that *Neisseria perflava* strain SKC/VA-3 holds significant potential for use in bio-mortar applications, highlighting its role in advancing eco-friendly construction techniques through microbial biomineralization.

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Author contributions R.S. carried out the experiments under the guidance of S.K.C. and R.K.W. All authors (R.S., R.I.C., S.K.C., S.H.P., R.K.W.) contributed to writing and reviewing the manuscript prior to submission. R.I.C. and S.K.C. thoroughly revised the manuscript.

Data availability Data is provided within the manuscript or supplementary information files.

Declarations

Competing interests The authors declare that they have no competing interests.

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