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Application of Bioengineering in Construction

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Abstract

Bio-cement and bio-concrete are innovative solutions for sustainable construction, aiming to reduce environmental impact while maintaining the durability and versatility of building materials. Bio-cement is an eco-friendly alternative to traditional cement, produced through Microbially Induced Calcium Carbonate Precipitation (MICP), which mimics natural biomineralization processes. This method reduces CO_2 emissions and enhances the strength and durability of construction materials. Bio-concrete incorporates bio-cement into concrete, creating a self-healing material. When cracks form in bio-concrete, dormant bacteria within the material become active in the presence of water, producing limestone to fill the cracks, extending the material's lifespan and reducing the need for repairs. The environmental impact of traditional cement production is significant, with cement generation accounting for up to 8% of global carbon emissions. Creative solutions are needed to develop more sustainable construction materials, with some efforts using modern innovations to make concrete ultra-durable and others turning to science to create affordable bio-cement. The research demonstrates the potential of bio-cement to revolutionize sustainable building practices by offering a low-energy, low-emission alternative to traditional cement while also addressing environmental concerns. The findings suggest promising applications in various construction scenarios, including earthquake-prone areas, by enhancing material durability and longevity through self-repair mechanisms.

Keywords

Sustainable Construction, Microbially Induced Calcium Carbonate Precipitation (MICP), Cement, Construction Industry, Microorganisms, Eco-Friendly, Sustainable Solution, Durability, Carbon Dioxide Emission (CO_2)

1. Introduction

The production of Ordinary Portland Cement (OPC), an essential binder in concrete, significantly contributes to global CO_2 emissions, with the built environment accounting for approximately 39% of worldwide greenhouse gas emissions [1]. Cement manufacturing alone was responsible for up to 8% of global CO2 emissions in 2016 [2], mainly during the clinker production phase. On average, producing one tonne of OPC requires 1.6 tonnes of raw materials, resulting in 0.8 tonnes of CO_2 emissions [3]. This has severe environmental effects, such as intensified greenhouse effect, increased frequency of natural disasters, and adversely affecting social development, health, and safety.

The global demand for OPC is expected to double by 2050 [4], so identifying sustainable alternatives is essential. Unlike OPC, microbial cement does not emit CO_2 during production and offers adjustable bonding performance through microbial activity and other variables [5]. This innovation could significantly mitigate the environmental impact of future construction activities. Bio-cement, also known as MICP (Microbially Induced Calcium Carbonate Precipitation), represents an innovative technology offering sustainable construction solutions [6]. Bio-cement utilizes microorganisms to generate calcium carbonate, which improves soil, fixes cracks in concrete buildings, and develops self-healing substances [7].

Soil stabilization, achieved by injecting bacteria and nutrients into the soil, enhances soil cohesion and strength, preventing erosion, improving load-bearing capacity, and reducing landslides [8]. Many Researchers have explored improving concrete strength and mediating cracks using MICP. However, the challenge lies in the high alkalinity of concrete mixtures, which can inhibit bacterial survival. Studies have identified bacillus cohnii and bacillus sphaericus as bacteria with high alkaline resistance, suitable for concrete mixtures [9]. This eco-friendly approach offers a cost-effective alternative to conventional repair methods. Furthermore, embedding bacteria and nutrients in bio-cement enables the creation of self-repairing materials to extend structural lifespan and reduce maintenance costs.

The debate over the financial feasibility of using bio-cement in construction often centers on its higher initial costs compared to conventional materials. These increased expenses arise from the requirement for specialized agricultural inputs, intricate manufacturing procedures, and limited production capacity [10]. Despite these upfront costs, the long-term advantages of bio-cement—such as enhanced energy efficiency, more excellent durability, and reduced maintenance needs—can offset these initial investments over the life of a building [11]. As mentioned before, the production of traditional cement is notably energy-intensive, emitting about 0.8 tons of CO_2 per ton of cement due to the calcination process and the high kiln temperatures required [12]. In contrast, bio-cement production can reduce carbon emissions by up to 70% through the use of agricultural by-products and lower temperature processes [10]. Additionally, bio-cement offers superior thermal insulation, which can significantly save heating and cooling costs. Buildings constructed with bio-cement can reduce energy expenses by up to 30% due to these improved insulating properties [11].

Furthermore, bio-cement tends to be more durable than traditional cement. It often requires less maintenance because it resists cracking and degradation more effectively. When reinforced with organic fibers, bio-cement structures show increased resistance to environmental damage, extending their lifespan and lowering long-term repair costs [12].

In summary, although the initial costs of bio-cement may be higher, its long-term economic benefits—including energy savings, reduced maintenance costs, and lower carbon emissions—make it a compelling choice for sustainable construction. To enhance its cost-effectiveness and encourage wider adoption, ongoing research, production scaling, and economic incentives are essential. This paper studies the various uses and benefits of bio-cement within the construction sector, emphasizing its potential to revolutionize sustainable building methods.

2. Analysis of Bio-Cemented Building Materials: Morphological and Chemical Insights

Studies on the morphology observed through Scanning Electron Microscopes (SEMs) of bio-cemented building materials have provided a detailed understanding of particle bonding within these materials. Alternatively, analysis of the chemical composition of bio-cemented building materials provides insights into how MICP works to strengthen them. Literature findings from energy-dispersive X-ray (EDX) and X-ray diffraction (XRD) analyses play a crucial role in substantiating the chemical components that define the bio-cementation technique. This section offers a thorough examination and discussion of the literature associated with these aspects.

Analysis of Bio-Cemented Sand and Mortar: Microstructural and Chemical Characterization

Zhan and Qian [13] employed Paenibacillus bacteria for Microbially Induced Calcium Carbonate Precipitation (MICP) to investigate the bonding of sand particles. The sand particles underwent bio-cementation through repeated spraying, up to seven in total. Applying bio-cement multiple times enhances the mechanical properties of sand materials by densifying their microstructures and effectively filling the void spaces during the bio-cementation process. **Figure 1** depicts the microstructural variations in the sand both before and after the bio-cementation process. **Figure 2(a)** shows loosely packed sand particles with noticeable gaps. **Figures 2(b)-(e)** show that the sand microstructures became denser, and the particles were more tightly packed after one, three, and five bio-cement applications.

The bio-cemented sand exhibited the most compact microstructure among all the samples in the study following the application of seven bio-cement layers. This is likely due to the increased calcium carbonate content and the reduced average porosity resulting from the incremental applications of bio-cement.

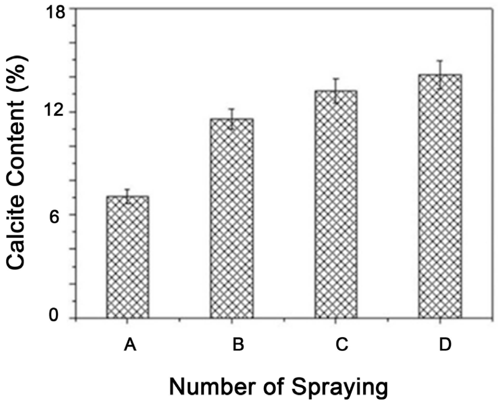

Figure 1. Variation in calcite levels in bio-cemented sand samples subjected to different applications (Application counts: (a) once, (b) three, (c) five, (d) seven times) [13].

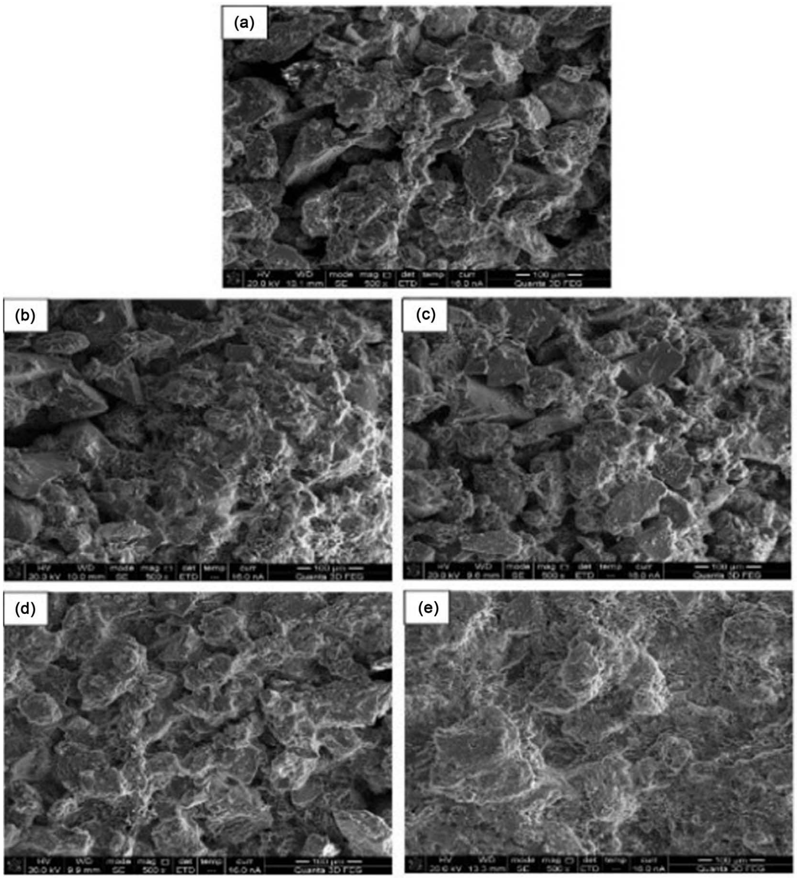

Figure 2. Effect of the number of sprayings on SEM photographs of the sample [13].

Zhan and Qian [13] observed that as the number of bio-cement layers increased, the calcium carbonate content in the sand rose from 7.08% with one application to 14.36% with seven applications, while the porosity decreased from 18.3% to 13.3%, respectively. The quantity of calcium carbonate in the bio-cemented sand was determined using the acid-washing technique.

Yu *et al.*'s research [14] revealed a consistent enhancement in the microstructures of bio-sandstone when treated with bio-composite cement injections up to six times. The study focused on strengthening the mechanical properties of bio-sandstone by using *S. pasteuri* to produce bio-composite cement. **Figure 3** demonstrates the bio-sandstone's microstructure after receiving different quantities of bio-composite cement injections. The irregular flake-like characteristics of the cement are shown in **Figure 3(b)**, **Figure 3(d)**, and **Figure 3(f)**. The data indicate that as the number of injections increased, the density of the bio-sandstone's microstructure also increased. After six injections (**Figure 3(e)** and **Figure 3(f)**), the microstructures were the most compact compared to those in other samples. The enhanced density results from calcium carbonate precipitation, which is induced by the bacteria and acts as a bonding agent to fill the pores within the bio-sandstone. However, the study lacked XRD analysis to quantify the calcium carbonate content in the treated bio-sandstone. Although the results were promising, additional research is needed to investigate how applying more than six injections of bio-composite cement affects the microstructures of bio-sandstone and to verify the efficacy of the bio-cementation technique.

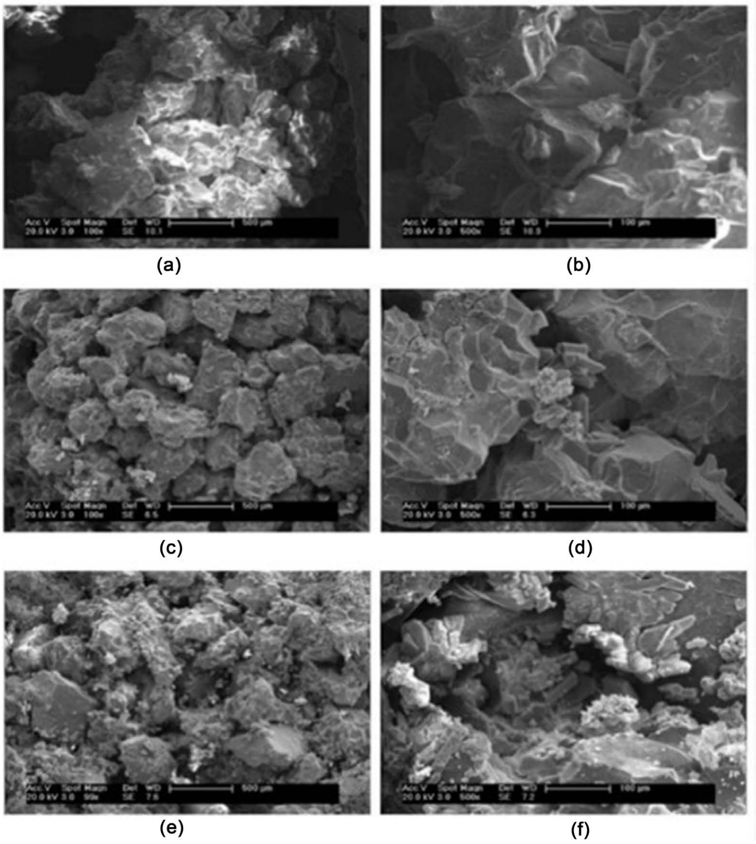

Figure 3. The microstructures of bio-sandstone are improved by the bio-composite cement after two, four, and six injections [14].

Zhang *et al.* [15] discussed how the choice of calcium source affects the microbial mortar microstructures formed through MICP induced by *Sporosarcina pasteurii* (ATCC 11859), a Gram-positive bacterium. In their bio-cementation process, they employed three distinct sources of calcium: calcium nitrate, calcium chloride, and calcium acetate. The process involved creating microbial mortars through three separate injections: a bacterial suspension with *S. pasteurii* and a culture medium, a fixative solution containing 50 mM of the chosen calcium source, and a nutrient solution that mixed the calcium source with urea at a concentration of 0.5 M. All applied using grouting techniques.

Figure 4 presents scanning electron micrographs of microbial cement samples. Specifically, Figure 4(a) highlights the chloride-treated sample, which displays a glossy surface with prominent hexahedral crystals of calcium carbonate. Figure 4(c) and Figure 4(d) depict the acetate sample, showing needle-like calcium carbonate crystals, typically identified as aragonite, one of the polymorphs of calcium carbonate alongside vaterite and calcite [16]. Calcium carbonate can crystallize in three different crystalline structures, referred to as polymorphs, all of which have the same $CaCO_3$ chemical formula. Figure 5 displays the X-ray diffraction (XRD) results for the acetate sample. The analysis indicated that the acetate sample was composed of 12% calcite and 88% aragonite, with the most intense aragonite diffraction peak observed at $2\theta = 26.223^{\circ}$. This confirmed that aragonite was the predominant calcium carbonate form present in the acetate sample's micrographs.

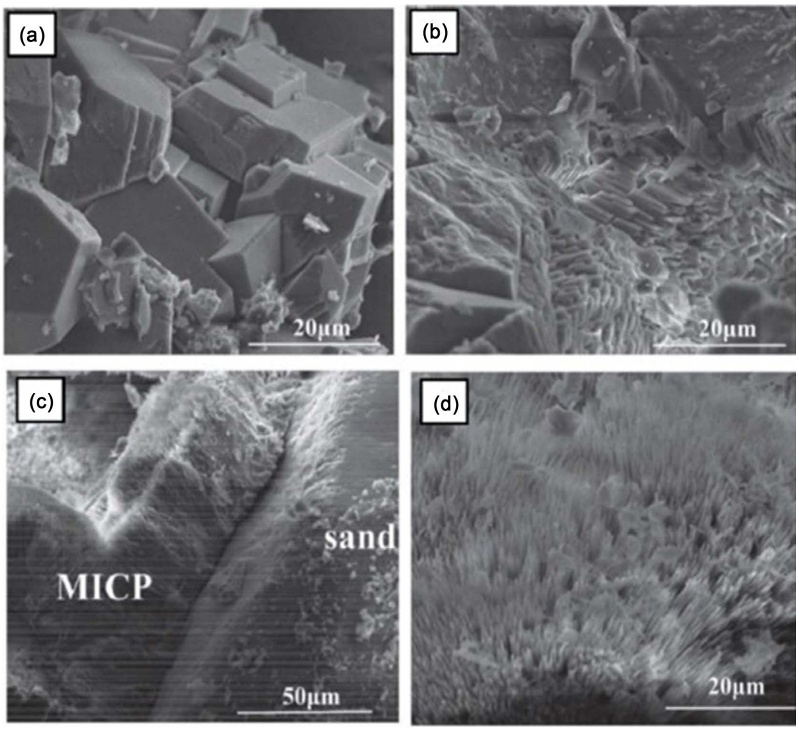

Figure 4. Investigating microbial mortars formulations utilizing various calcium sources: chloride, nitrate, and acetate samples involved in MICP Processes [15].

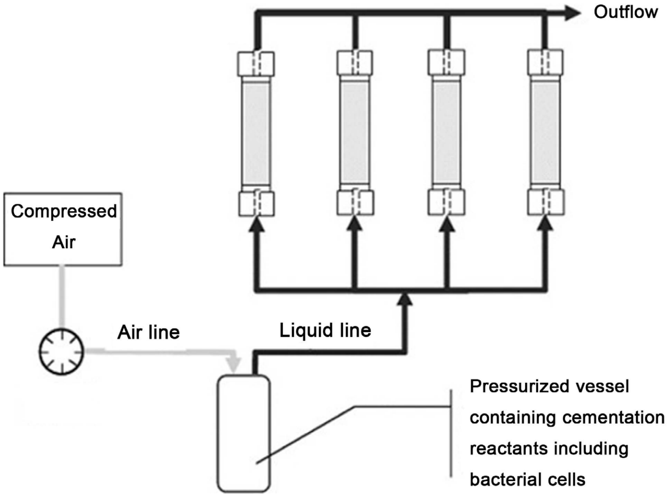

Figure 5. Preparation process of bio-cement [17].

3. Bio-Cementation

Bio-cementation is a technique where bio-cement is formed through the microbial-induced precipitation of carbonate. This process is utilized across several sectors, including erosion management, construction, environmental conservation, and petroleum. In the construction industry, bio-cement is employed for purposes such as wall and building coatings, soil reinforcement and stabilization, and anchoring sand in regions vulnerable to seismic activity.

Microorganisms play a crucial role in carbonate precipitation by generating an alkaline environment with high pH levels and increasing the concentration of dissolved inorganic carbon (DIC) through their metabolic processes. Three primary categories of microorganisms are known to facilitate carbonate precipitation: 1) photosynthetic microorganisms such as cyanobacteria and microalgae, 2) sulfate-reducing bacteria, and 3) bacteria involved in nitrogen transformations. Photosynthetic microorganisms can convert urea into bio-cement through bio-cementation processes, utilizing enzymes like urease or urea amidolyase to aid in this transformation alongside microalgae. In practical settings, bio-cement, which is essentially calcium carbonate, is often combined with materials such as sand. The patented process for bio-cement production, as shown in **Figure 3**, involves the use of *Bacillus pasteurii*, a heterotrophic bacterium that aids in urea hydrolysis. This process takes place in pipe columns filled with silica sand.

A mixture of urea and calcium solution, along with a bacterial solution, is injected multiple times into the sand core within a pressurized container until it is saturated. The bio-cementation process typically requires around 24 hours to complete, after which the resulting bio-cement is dried at 60˚C. Generally, mortar is a pre-mixed binder material composed of sand, binder, or aggregate. Biological mortar includes key ingredients such as limestone powder, nutrients, and bacterial paste. Research has explored the use of bio-cementation for repairing concrete cracks and creating bacterial concrete. Cracks treated with bio-cement exhibit a considerable enhancement in both strength and stiffness compared to those that have not been treated.

Bio-Concrete

The cement industry is widely recognized as one of the most environmentally harmful due to the presence of coal-fired power plants. This has led many scientists and engineers to develop alternative materials, like cement, to reduce environmental impact. Despite their efforts, it's rare to hear about success stories that go beyond the laboratory.

The Structural Technology Group has innovated and patented a biological concrete that promotes the rapid growth of pigmented organisms. Designed for use in building exteriors in Mediterranean regions, this material offers ecological, thermal, and aesthetic benefits over traditional construction options. It enhances thermal comfort and helps reduce atmospheric CO_2 levels. When two Dutch scientists from the University of Delft, Henk Jonkers, and Eric Schlangen, introduced their green, self-regenerating bio-concrete invention, many initially viewed it with skepticism. However, the two men were determined to move forward despite any negative feedback, as their sole focus was to build a complete structure using their miraculous biomaterial.

4. Evaluation of Calcium Precipitation Capability

Recent studies have demonstrated the potential of using various microbial strains to enhance the durability of concrete. Qbany *et al.* [18] explored how different environmental conditions influence the effectiveness of MICP. Their findings highlighted that key factors like pH levels, temperature, and nutrient supply significantly impact the precipitation process. The study evaluated various microbial strains, such as *Bacillus megaterium* and Bacillus cereus, under a range of conditions, offering crucial insights into enhancing MICP for practical use. This research emphasizes the necessity of customizing both microbial strains and environmental settings to optimize the efficiency of bio-cementation. Wang *et al.* [19] investigated the addition of different bacterial species into concrete mixes, observing notable improvements in both mechanical strength and longevity. They identified *Bacillus sphaericus*, *Bacillus pasteurii*, and *Bacillus subtilis* as particularly effective in inducing calcium carbonate precipitation, which serves to fill in cracks and voids within the concrete matrix. The study also described how these bacteria, when protected within encapsulating materials, were able to withstand the high alkalinity of concrete, thereby facilitating self-repair mechanisms. Vijay *et al.* [20] extensively assessed bacterial strains utilized in self-healing concrete applications. Their research highlighted the varying levels of urease activity among different bacteria, with *Bacillus pasteurii* demonstrating the most significant urease production, which is vital for bio-cementation. Both *Bacillus pasteurii* and *Sporosarcina pasteurii* were identified as highly effective in facilitating calcium carbonate formation. The study also highlighted the importance of selecting bacterial strains that not only exhibit high urease activity but also can endure and remain viable within the concrete environment.

In Zaghlol *et al.*'s studies [21] marine samples, including sediments and algae, were collected from the Abo Kier shore along the Mediterranean Sea in Alexandria, Egypt. These samples were cultivated on nutrient agar prepared with aged seawater at a concentration of 28 g/L. The medium was sterilized by autoclaving at 121˚C for 15 minutes and incubating at 37˚C for 24 hours. Colonies exhibiting diverse phenotypes were selected for further purification and tested for Gram reaction. To assess the urease production and urea utilization capabilities of the purified strains, Christensen's medium was prepared as described by Anitha *et al.* [22]. The composition of the medium included glucose (1.0 g/L), potassium dihydrogen phosphate (2.0 g/L), sodium chloride (5.0 g/L), peptone (0.2 g/L), urea (20.0 g/L), and phenol red (0.012 g/L) adjusted to a pH of 7. The medium was autoclaved at 121˚C for 15 minutes for sterilization, with urea separately sterilized using a 0.45 μm syringe filter. Agar (15.0 g/L) was added to create solid media. The inoculated isolates were cultivated in broth and streaked onto agar plates, which were then incubated at 37˚C for 24 hours. *Klebsiella pneumoniae* ATCC 13883 served as the positive control. Isolates that induced a color change to red or pink in the medium were identified as urease-positive and stored as glycerol stocks at 4˚C for future use.

To evaluate the calcium precipitation capability of marine bacterial strains, the methodology outlined by Stabnikov *et al.* [23] was followed. The procedure involved preparing a mixture containing 90 ml of 0.5 M anhydrous calcium chloride ($CaCl_2$), 90 ml of filter-sterilized 1 M urea, and 20 ml of culture liquid from each bacterial isolate. For controls, a negative control lacked any bacterial inoculation, whereas the positive control was inoculated with *Klebsiella pneumoniae*. The prepared cultures were incubated at 37˚C for 48 hours.

Calcium concentrations in the liquid phase were determined both before and after the bio-cementation process through EDTA titration, as per the APHA guidelines (1999). In this process, 1 ml of a sodium hydroxide buffer was added to 15 ml of the liquid sample to achieve a pH of 10. Subsequently, a few drops of a pH indicator solution (0.5% w/v Eriochrome Black) were added, and the mixture was titrated with 0.01 M EDTA until a distinct color change was observed.

Seven samples were gathered from the ocean and labeled as EZ11, EZ12, EZ14, EZ15, EZ16, EZ18, and EZ20. These samples underwent testing for urease activity and calcium deposition after a 48-hour incubation at 37˚C, with *Klebsiella pneumoniae* serving as the positive control. The findings revealed that *K. pneumoniae*, along with isolates EZ15 and EZ14, exhibited urease production, indicated by a color shift in Christensen's medium from yellow to pink. Isolate EZ15 displayed a lower precipitation rate at 35.7%, while EZ14 showed a much lower rate at 17.1%. Consequently, *K. pneumoniae* was the most proficient in producing calcium carbonate, followed by EZ15 and then EZ14. The other isolates did not demonstrate any urease activity. Table 1 presents the bio-cement production yield for selected isolates. *K. pneumoniae* produced 15 g/L, EZ15 yielded 13 g/L, and EZ14 resulted in 8 g/L. Additionally, the pH values rose from 7.0 to 9.3 for *K. pneumoniae*, 9.0 for EZ15, and 8.3 for EZ14, signifying that ureolytic bacteria decompose urea, which initiates MICP.

Table 1. Bio-cement production and pH of the medium after 10 days at 37˚C. Results are shown as average ± standard deviation from three separate trials.

Bacterium	Bio-cement yield (g/l)	pH
Control	0	7.0
K. pneumonia	15 ± 0.013	9.3
EZ14	8 ± 0.47	8.3
EZ15	13 ± 0.35	9.0

Identification using the VITEK 2 system revealed that EZ15 was isolated as *Staphylococcus epidermidis* with 98% confidence. Identification at the molecular level through 16S rRNA gene sequencing, followed by BLAST analysis on NCBI, confirmed this result, showing a 98% match of EZ15 to *S. epidermidis*. The sequence has been submitted to GenBank with the accession code MN795756. Figure 6 illustrates the phylogenetic tree of EZ15. Recognized for its potential, EZ15 was selected for extensive bio-cement production and subsequent studies, culminating in the creation of dried bio-cement crystals through this process.

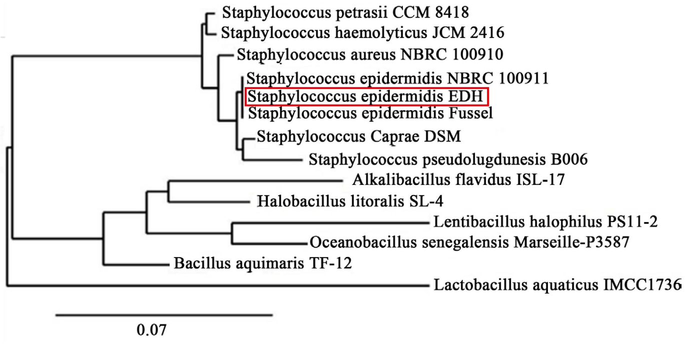

Figure 6. Evaluation of *Staphylococcus epidermidis* Strain EDH [21].

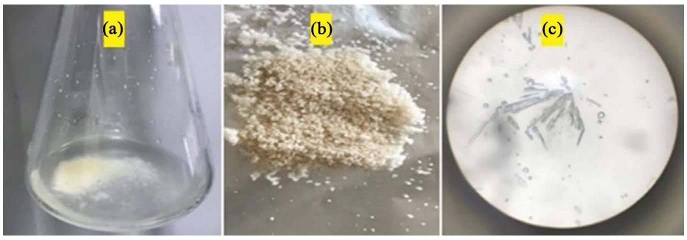

Figure 7. Bio-cement produced by *S. epidermidis* EDH (a), after being dried at 105˚C overnight (b), and the resulting bio-cement crystals observed under light microscopy (c) [21].

Dhami *et al.* [24] recently identified a bacterial community in Margaret River caves, Australia, capable of precipitating calcium from speleothems. The specific bacterial strains identified were *Sporosarcina pasteurii* UB, Bacillus cereus CB, Planococcus sp., Brevundimonas sp., and Methylobacterium sp. Additionally, the study revealed that the predominant bacterial types were Betaproteobacteria, Alphaproteobacteria, Bacteroidetes, and Gammaproteobacteria.

According to the research by Zaghlol *et al.* [21], *S. epidermidis* EDH, as depicted in **Figure 7**, is capable of using urea alone as a carbon source to support its growth and produce bio-cement. Similar bio-cement production capabilities were observed in other bacterial species like *S. pasteurii* UB, as reported by Gat *et al.* in 2016 [25]. Many microorganisms typically require only small amounts of organic carbon, making the ability to thrive in high urea concentrations a unique trait [22]. Thus, these bacterial species are promising candidates for cost-effective bio-cement production using urea-rich wastewater.

In general, three criteria affect the ability of bacterial isolates to induce MICP: The bio-cement strain needs to exhibit powerful urease activity, withstand high urea concentration, and tolerate high pH levels. In their study, Anitha *et al.* [22] discovered B. cereus KLUVAA in a high-urea rice field, which was able to produce urease on Christensen selective medium and endure urea concentrations of up to 10%, supporting our observations. Hait *et al.* [26] reported finding *K. pneumoniae* in sewage that also exhibited significant urease activity and could tolerate 10% urea concentrations. Furthermore, Dhami *et al.* [24] found that uratolytic microorganisms increased the pH of their growth medium from 8.7 to 10.8, whereas the control samples showed only slight pH changes, highlighting a clear relationship between urea breakdown and pH increase.

5. Conclusions

A thorough literature review highlights the significant contribution of microorganisms to bio-cementation across various building materials, including brick, concrete, and mortar. The success of these microorganisms in promoting bio-cementation is primarily influenced by the particular microbial species involved and the ideal conditions required for MICP.

Sporosarcina pasteurii is extensively acknowledged for its significant role in MICP research related to construction materials, primarily due to its high urease activity, swift growth, and effective calcium source utilization. Through MICP, calcium carbonate is produced, which functions both as a binder and a filler, thereby reducing the porosity of construction materials. Additionally, incorporating materials like rice husk ash, silica fume, fly ash, and chromium slag as partial cement replacements contributes further to reducing material porosity.

The mechanical properties of construction materials are notably improved through the porosity reduction achieved by MICP. This process enhances compressive strength, surface treatment, water resistance, bonding quality, and crack repair capabilities. Various studies utilizing scanning electron microscopy and

X-ray diffraction have verified the presence of calcite within bio-cemented materials.

Although current research shows favorable results, additional studies are necessary, especially on the resistance of bio-cemented materials to fire, to fully understand their performance in fire situations. Nonetheless, the potential for integrating bio-cementation into construction materials appears promising due to ongoing advancements in research and application.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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