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PREPARING OF BIO-CEMENT MORTAR BY USING BACILLUS LICHENIFORMIS BACTERIAL CELLS

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Abstract: The incorporation of microbial-induced carbonate precipitation (MICP) in cement mortar is a good idea toward sustainable development. This study discussed the effect of adding MICP (Bacillus licheniformis urease bacteria) to cement mortar in different ways. Spray and admixed treatment with different bacterial concentration (optical density). The results proved that the addition of these microorganisms resulted in 17–37% increase in the compressive strength of cement mortar compared with control mix; this increment can be attributed to the deposition of calcium carbonate in the pores of cement mortar matrix. The results showed an increment of approximately 37% and 21% in the compressive strengths of the cement mortar admixed with 1 O.D and 0.5 O.D, respectively, on sequential culturing in comparison with the control mix. Moreover, the treatment of the cement mortar with the bacterial culture spray at 1 O.D resulted in 17% improvement in the compressive strength when compared with that of the control mix.

Keywords: MICP, Cement Mortar, Bacillus Licheniforms, Compressive Strength.

1. Introduction

Portland cement is considered as the best construction material because of low cost of materials, construction and the low maintenance cost [1]. The rapid water–cement reactions generate three main products: hydrated calcium silicates (C_2SHx, C_3S2Hx) or calcium silicate hydrate gels (C-S-H), hydrated calcium aluminates (C_3AHx, C_4AHx), and hydrated lime Ca(OH) [2]. Biotechnology has been applied to create a new type of cement mortar [3]. In the MICP procedure, microorganisms assume a significant role in creating a high alkaline condition through urease activity, which may act as a nucleation site for $CaCO_3$ precipitation on bacterial surfaces [4]. When this process occurs in the pores of unbounded sand, the particles assemble with calcite. The resultant cementitious matter created is known as "Biocement", which is used as a sustainable alternative to cement. This product is created through a biological procedure without consuming electricity or using destructive substance processes [5].

MICP processes utilizing calcifying microorganisms have gained popularity as a portentous mean in the concrete research field [6]. Researchers are starting to utilize microorganisms such as bacteria, and algae to produce sustainable material through minimal environmental damage [7], [8]. The idea behind this phenomenon is that microbes catalyze chemical reactions and produce sediments that can coat and bind grains [9].

Preceding researches have exposed that adding particular organisms to cement mortar precipitate inorganic materials in the matrices' pores, which may be utilized as a filling substance for treating cracks within the structure. Similarly, it occurred that adding bacterial cultures with a substrate, composed of $CaCl_2$ and urea, to the cement material may lead to 20–35% increase in the compressive strength of the mortar in comparison with the control-mix. In contrast, no noteworthy enhancement was noted in the compressive strength resulting from the addition of only the growth media to the cement mortar without adding any bacteria [10]. In MICP, enhancement of the durability of cement materials has gained much attention in recent years. Obviously, the biomineralization procedure happens at a slow rate over the geological periods, similar to limestone and sandstone formation [11].

2. Materials and Methods

Microorganisms: Bacillus licheniformis is a gram-positive bacterial cell that is facultative and mesophilic. It grows at nearly 50°C and is, therefore, able to thrive at higher temperatures. The best enzyme-releasing temperature is 37°C. It has been recorded in a dormant spores form to bear severe environment. It is soil bacterial cell with a small size (1.3–4.0/0.5–1.2 μm) and the diameter of spores 0.8–1.3 μm, showing its urease-capability. This feature of B.lichenformis is significant to its application in the cement mortar [12].

Culture Media: B.licheniformis cells isolated from concrete specimen was used for its high urease activity (UA), which in turn gives high $\mathbf{CaCO_3}$ precipitation efficiency. This strain was cultivated in autoclaved nutrient broth (NB) (peptone 10 g/L, yeast extract 10 g/L, and sodium chloride 5 g/L). The cultures were grown in NB admixed with filter-sterilized 2% urea (w/v) (NBU) and 20 mM $\mathbf{CaCl_2}$ solution at **37**℃ under shaking condition at 130 rpm.

Mechanism of Biocementation: Biocementation is a process of producing binding materials based on MICP mechanism [13]. Biocementation is a series of subsequent steps started with attachment of the cell of urease-forming bacteria to the particle surface, formation of a micro-gradient in the pH concentration of carbonate/bicarbonate at the cell attachment site as a result of urea hydrolysis by urease; and [10] the creation of calcite crystals that adhere to the particles surface [11], [14], [15].

MICP is composed of a sequence of complex biological and chemical reactions, as given below [15]:

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad (1)$$

$$H_2CO_3 + H_2O \rightarrow HCO_3^- + H^+ \quad (2)$$

$$2NH_3 + 2H_2O \rightarrow 2NH_4 + 2OH^- \quad (3)$$

$$HCO_3^- + H^+ + 2NH_4 + 2OH^- \rightarrow CO_3^- + 2NH_4^+ + 2H_2O \quad (4)$$

Meanwhile, the charge of the wall of the bacterial cells is negative, the bacterial cell attracts positive ions from the surrounding media, including $\mathbf{Ca^{+2}}$, for the precipitation on their cell wall surface [15], as shown in Fig.1.

$$Ca^{+2} + \text{cell} \rightarrow \text{cell} - Ca^{+2} \quad (5)$$

$$\text{cell} - Ca^{+2} + CO_3^{-2} \rightarrow \text{Cell} - CaCO_3 \quad (6)$$

The formation of calcite is extremely impervious, it doesn't dissolve in water. The bacterial cells deposited calcite in the existence of substrate. $CaCl_2$ and urea were effective as nutrients.

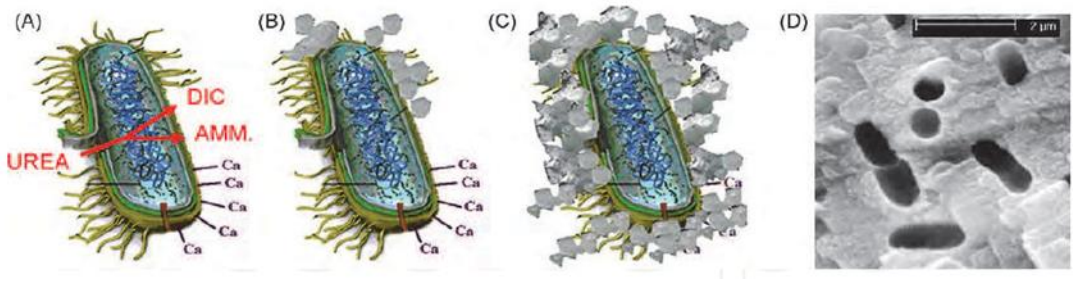

Figure 1. Illustration of calcite precipitation mechanism induced by urease microorganism [14].

Cement and Sand: The raw material utilized in this study was Portland cement produced by MASS Cement Factory, Sulaymaniyah, Iraq. Locally available sand of particle size < 4.75 mm with a specific gravity of 2.69.

Experimental Work: $\mathbf{CaCO_3}$ Precipitation Experiments: The selected bacterial isolate (B. LICHENIFORMIS) was tested for its ability to precipitate $\mathbf{CaCO_3}$. For measuring the amount of $\mathbf{CaCO_3}$ precipitation in the broth, NB was supplemented with 20 g/L urea/$\mathbf{CaCl_2}$, (NB U/Ca). A 250-mL volumetric flask containing 150 mL of NB supplemented with 2% filter-sterilized urea (20 g/L) and (20 g/L) calcium chloride at **37**℃ under shaking condition (at 130 rpm) and an initial pH of 7 for 7 days. The precipitate obtained was filtered through filter paper, washed several times with distilled water, dried at 60◦C in an oven for 3 h, and then weighed. The weight of $\mathbf{CaCO_3}$precipitation (Wp) was determined from the following equation:

$W_p = W_{ep} + W_e$ (7)

Where, Weep is the weight of filter paper containing precipitant and We is the weight of empty filter paper.

Microbial Cement Mortar Preparation: The cement mortar was prepared by using the cement/sand ratio of 1:2.75 (by weight) according to the ASTM [16]. Three types of cement mortar were prepared with the water/cement ratio maintained at a constant of 0.485 and casted in a cubic mold (dimensions: 50 mm × 50 mm × 50 mm).

Control Mix (CM): Cement and sand were carefully mixed with water. The fresh mortar pastes were casted into molds. After demolding, the control specimen was cured with tap water. After curing, the cubes with water were tested for their compressive test after 28 days of curing.

Bacterial Admixed Treatment (BAT): Cement and sand were carefully mixed, and grown cultures of B. LICHENIFORMIS. Water/cement ratio correspondent to bacterial concentration of OD600 of 0.5 and 1.0 for this strain. The fresh mortar pastes were casted into molds. After demolding, the bacterial admixed specimens were cured in a solution of 20 g urea/L and 20 mM $CaCl_2$. After curing, the cubes with water were tested for their compressive test after 28 days of curing.

Bacterial Spray Treatment (BST): Cement and sand were carefully mixed with water. The fresh mortar pastes were casted into molds. After demolding, the specimens were sprayed with a grown culture of B. LICHENIFORMIS at O.D 1, and then cured in a solution of 20 g urea/L and 20 mM $CaCl_2$. After curing, the cubes with water were tested for their compressive test after 28 days of curing.

Compressive Strength: The compressive strength of different mortar sample after 28 day of curing was measured according to ASTM [16] by Record the total maximum load indicated by the testing machine, and calculate the compressive strength as follows:

$UCS = P/A$ (8)

where: UCS: Unified compressive strength (MPa)

P: The total recorded maximum load (N)

A: Area of the loaded surface (mm2)

Scanning Electron Microscopy (Sem): The deposition of calcium carbonate inside the cement mortar by bacteria was analyzed by SEM (FEI Company Inspect S50; Holland) supplemented by an energy dispersive X-ray spectroscopy analyzer at an accelerating voltage of 0.2–30 kV. Before analysis, all samples were dried at 100◦C for 3 days.

3. Results and Discussion

Bacterial growth curve and carbonate precipitation: The B. licheniformis growth curve was obtained by this bacterial strain under optimum conditions after which Optical Density at 600 nm (O.D 600) [17]. for the bacterial strain was measured at different time intervals by UV spectrophotometer. As shown in Fig. 2. The amount of $CaCo_3$ precipitate was 7.82733 g/L after that the precipitate was analyzed by XRD to ensure its composition being pure $CaCo_3$ crystals.

In general, mineral identification for crystals formed by bacterial isolates was characterized by XRD, while referring to calcium carbonate or pure calcite powder as the standard. The positions of XRD spectra lines were close to those of pure calcite powder standard for all bacteria isolates, which indicates that calcite was the most common mineral carbonate $CaCo^3$ observed. Fig. 3 shows the result of mineral identification from the precipitation test for $CaCo^3$ crystals.

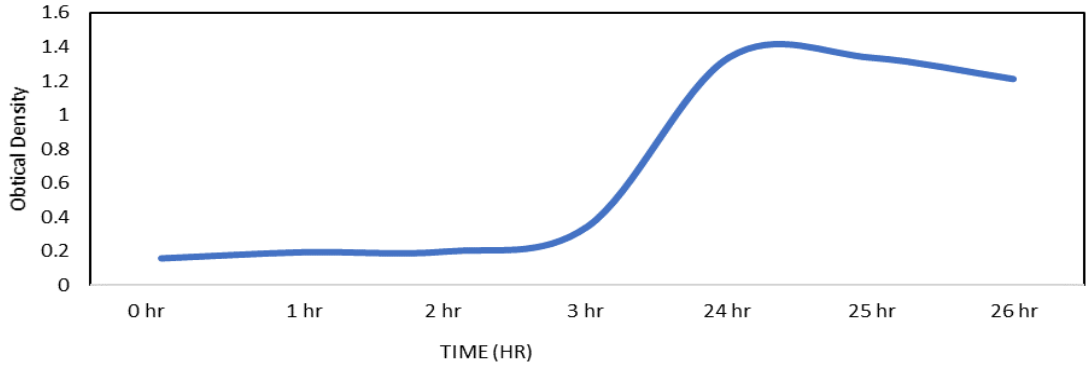

Figure 2. Bacterial growth curve.

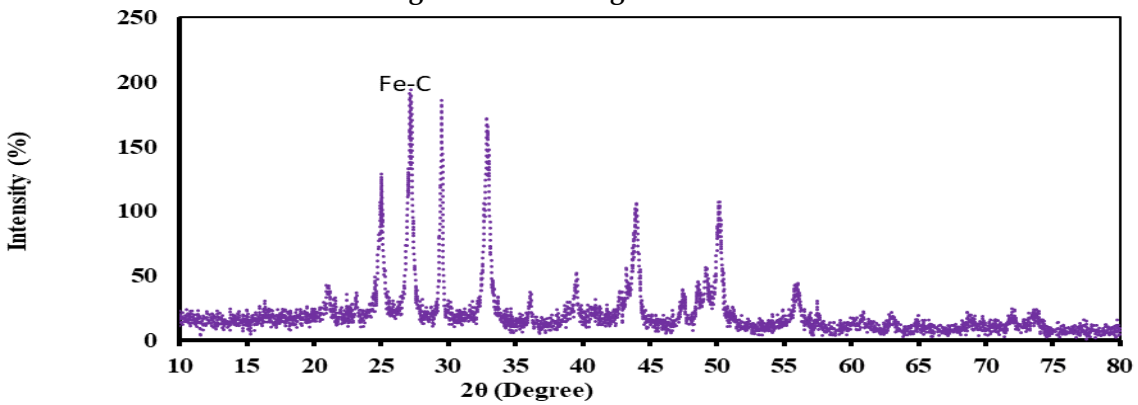

Figure 3. XRD diffraction of the precipitation.

Compressive strength: The impact of adding bacteria on the mortar properties was examined in the current study. The addition of bacterial cells to the mortar was performed either through casting stage or after casting via spray treatment.

A 38% increment in the mortar compressive strength was detected through the addition of bacterial culture along with N.B to the cement mortar throughout the casting stage, although, in BST samples, marginal increment of about 17% was detected in the mortar compressive strength [18]. The huge difference in the compressive strength of the resultant cement between the two methods can be due to the fact that spray treatment is a surface treatment and hence does not alter the bulk concrete properties to a large extent.

On the other hand, the mortar compressive strength increased within concentration of bacteria until 1 OD. The compressive strength of BAT with 1 O.D cells was increased by 38% compared with CM, while that of BAT with 0.5 O.D, was less than BAT with 1O.D because the quantity and size of calcite crystals deposited in BAT with 1 OD cells being greater than that precipitated by 0.5 OD cells. When the cells grow, calcium carbonate gets deposited on the wall surface of the cells in addition to the mortar matrices. As several pores in the matrices were plugged, the movement of the nutrient and O_2 to the bacteria diminished, finally the cell either died or formed endospore and represented as an organic fiber; this is accompanying by the rising of compressive strength, see fig.4 and table 1.

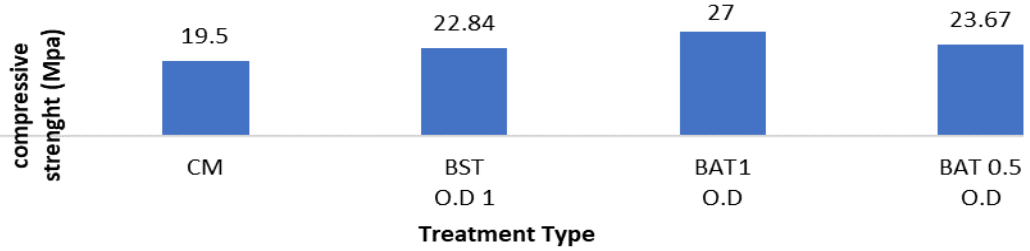

Figure 4. The compressive strength using different cement mortar at 28 day.

Table 1. Compressive strength of cement mortar.

Specimens	Average compressive strength For 3 specimens 28 day N/mm2 (MPa)
Control mix (CM)	**19.5**
Bacterial admixed treatment with 0.5 optical density (BAT 0.5 O.D)	**23.67**
Bacterial admixed treatment with 1 optical density (BAT 1 O.D)	**27**
Bacterial spray treatment with1 optical density (BST 1 O.D)	**22.84**

Scanning electron microscopy (SEM): The SEM images of cement mortar BAT 0.5 O.D, BAT 1 O.D cells and BST 1 O.D are shown in Fig. 5. From the SEM annotations, it can be seen that calcite deposited via bacteria may be illustrious with the pores of mortar matrices. Moreover, the content of precipitated calcite increased with the concentration of bacterial cells.

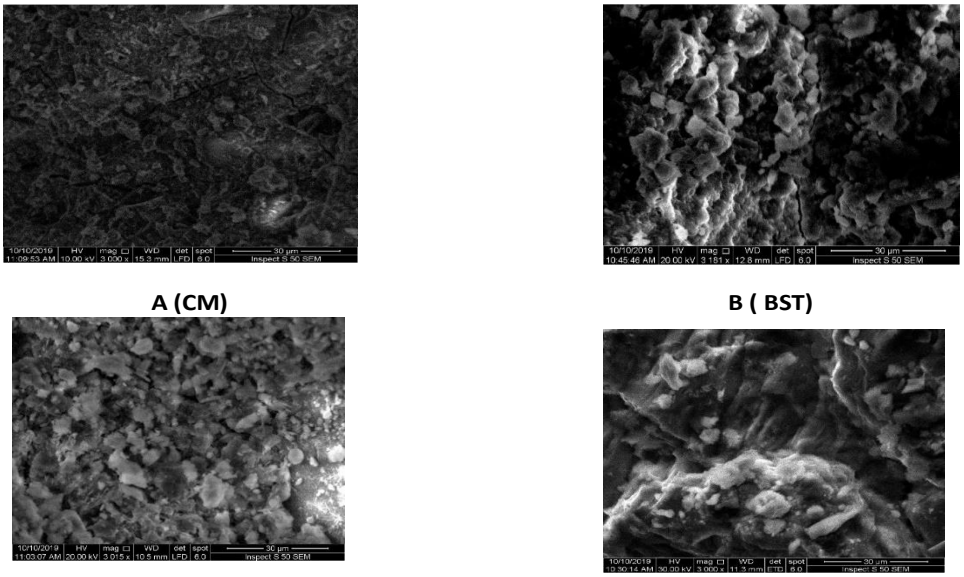

Figure 5. SEM photos of different cement mortar after 28 days of curing: (A) CM, (B) BST 1 O.D, (C) BAT 1 O.D, (D) BAT 0.5 O.D.

Clearly, SEM showed different morphologies of calcite crystals in the cement mortar samples prepared with different bacterial concentra-tions of B. lichiniforms. The SEM microimages of control CM or BAT with 0.5 and 1 OD bacterial cells up to 28 days are shown in Fig. 5. From the SEM images, the precipitation of calcite by bacterial cells can be distinguished within the pores of bacterial treated matrix; meanwhile, the calcite crystals cannot be observed in CM (fig.5.a) specimens prepared without the addition of any bacterial cells. On the other hand, the precipitated calcite increased with the concentration of bacterial cells. The amorphous calcite phase and the little amount of spherical calcite crystals were observed in BST with 1 OD (Fig. 5b), as described elsewhere [3]. The spherical calcite crystals precipitated by OD 0.5 of bacterial cells (Fig. 5d), which possess a smaller size than that of rod- shaped and spherical calcite crystals precipitated by BAT with 1 OD as shown in (Fig.5c).

4. Conclusion

The results showed that the two methods used for producing biocement (spraying and admixed urea bacterial culture) were successful and demonstrated a good enhancement in the compressive strength of the cement mortar when compared with that of the control mix. The reason for this increment is precipitation of the calcium carbonate inside the pores of the cement mortar matrices. The highest compressive strength was obtained from the BAT 1 O.D specimen, followed by BAT 0.5 O.D, and, finally, by BST 1 O.D specimen . The amount and size of calcite crystals precipitated inside cement mortar matrix by BAT 1 O.D was greater than that by BAT 0.5 O.D. Moreover, the degree of crystallinity of calcite crystals precipitated by BAT 1 O.D in cement mortar was higher than that by BST 1 O.D.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

I. R. Ghanim and S. E. Ebrahim; methodology, writing—original draft preparation, S. E. Ebrahim; writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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No Informed Consent Statement.

Data Availability Statement:

No Data Availability Statement.

Conflicts of Interest:

The authors declare no conflict of interest.

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