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*(Review)*

# A Literature Review of Bio-cement: Microorganisms, Production, Properties, and Potential Applications.

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## 1. ABSTRACT

This literature review provides an in-depth analysis of Bio-cement as an Eco-friendly substitute for conventional cement. Bio-cement is produced using microorganisms that produce calcium carbonate (CaCO3), which reduces the carbon footprint of cement production. The review covers the different types of microorganisms used, optimal conditions required for calcium carbonate production, the process of CaCO3 precipitation, and various test results. Additionally, the paper investigates the potential applications of Bio-cement in the construction industry, as well as the challenges and limitations associated with its use. The review highlights several studies on the use of different microorganisms, such as *Sporosarcina pasteurii, Bacillus sp. MCP11, and Bacillus sp. CR2,* to create Bio-cement. The mechanical properties of the final product are also analyzed, including compressive strength, tensile strength, and flexural strength. The paper provides valuable insights into the potential of Bio-cement as a

sustainable alternative to conventional cement, as well as the potential limitations and challenges that need to be overcome to fully realize its benefits. Overall, this literature review is an essential resource for researchers and individuals interested in the sustainable construction industry. It provides a comprehensive analysis of the different aspects of Bio-cement, including its production process, properties, and potential applications. The review serves as a starting point for further research and development of Bio-cement as a viable and Eco-friendly alternative to conventional cement.
***Keywords****:* Microorganism, $CaCo_3$, Emissions of carbon, MICP, Bio-cement.

## 2. Introduction

Bio-cement is a sustainable alternative to traditional cement that utilizes microorganisms to produce calcium carbonate. This process reduces the carbon footprint associated to traditional cement production and can improve the mechanical properties of the final product. In this paper, we will review the status of Bio-cement research and development, including the various microorganisms used, the conditions required for the optimal calcium carbonate production, the $CaCo_3$ Precipitations, and the results from various tests such as the XRD “X-Ray Diffraction” analysis test, SEM “Scanning electron microscope” examination test, The FTIR “Fourier-transform infrared spectroscopy” spectrum test, and compressive strength test, and Biocement potential applications in AEC “Architecture, Engineering, and Construction” industry. DE Muynck ET. Al. showed that bacterial carbonate precipitation bio-deposition affected the durability of mortar specimens with varying porosities. Depending on the porosity of the specimens, the surface deposition of calcium carbonate crystals reduced the water absorption by 85% [14]. Achal and Pan declared that chromium slag was utilized to produce bricks with MICP application of ureolytic bacteria *Bacillus sp. CS8* for surface treatment. The results of low water absorption of the treated bricks implied that they had a low permeability [5]. The compressive strength of concrete specimens injected with *B. megaterium* was marginally greater than that of *B. subtilis*-treated concrete samples. *Bacillus sphaericu*s was used to increase the compressive strength of concrete with a high strength (60 MPa) [4], according to Shanmuga Priya ET. Al. *Bacillus aerius* was added to concrete with 10% rice husk ash to replace some of the cement, increasing its compressive strength from 36 to 40 MPa [3]. According to Feng et al. sufficient calcium carbonate precipitation on the mortar surface after MICP, treatment by *S. pasteurii* could improve the features of recycled fine aggregates (RFAs) of mortars [1]. Iqbal et. al. in order to produce the bio-OPC cement samples, soil microbial solution with lentil seed powder (a source of protein) and sugar (a source of carbon) was employed in place of water The bio-bricks' maximum

compressive strength was found to be 2 MPa [4]. Using the identical kind Chahal, Siddique, and Rajor found that bacterial and fungal activities such as photosynthesis, ammonification, denitrification, sulphate reduction, and anaerobic sulphide oxidation could cause calcium carbonate to precipitate extracellularly [8]. Zaghloul, Ibrahim, and El-Badan concluded Bio cement provides an appropriate replacement for building projects to reduce the negative environmental effects of cement manufacturing [21].

## 3. Microorganisms used for Bio-cementation process

**Table 1.** Types of microorganisms used by Researchers in this Literature review paper:

| **Authors** | **Microorganism** |
|---|---|
| Heath, Leadbeater, and Callow (1995) | Mixture of *algae and cyanobacteria* collected from Ely Marina in Cambridgeshire, UK |
| Rivadeneyra et al. (1996) | *Deleya halophila, CCM3662 strain* |
| Ramachandran et al. (2001) | *S. pasteurii* |
| Mortensen et al. (2011) | *Sporosarcina pasteurii (ATCC 11859)* |
| Santomauro et al. (2012) | *Scenedesmus obliquus (algae)* |
| Al-Thawadi, Cord-Ruwisch, and Info (2012) | *Bacillus sp. MCP11 (DSM 23526)* |
| Stabnikov et al. (2013) | *S. pasteurii, especially S. pasteurii ATCC 11859* |
| Achal and Pan (2014) | *Bacillus sp. CR2.* |
| Seifan, Samani, and Berenjian (2016) | *B. sphaericus and Sporosarcina pasteurii* |
| Xu et al. (2017) | *Micro-bacterium sp. strain GM-1.* |
| Hait et al. (2018) | *Klebsiella pneumoniae* |
| Xiao et al. (2021) | *Sporosarcina pasteurii (DSM33)* |
| Esmail et al. (2022) | *Bacillus megaterium* |
| Ali, Mukhtar, and Dufossé (2023) | *Lysinibacillus spp. strain YL* |

## 4. Literature review of Biocement techniques and measured variable

Biocement, also known as microbial cement, is a sustainable alternative to traditional cement that has gained attention in recent years due to its potential environmental benefits. In this literature review, we will explore the current state of knowledge on Biocement, including its production, properties, and

potential applications. The paper will also examine the challenges and limitations associated with Biocement, as well as future research directions. Heath, Leadbeater, and Callow A mixture of *algae* and *cyanobacteria* was collected from the Ely Marina in Cambridge-shire, UK (OS Land Ranger 143, TL 546 798) and cultivated in calcified bio-films. Additionally, nine species of *cyanobacteria* and *algae* were extracted and identified using multiple keys in accordance with the Fritsch collection at the Freshwater Ecology Institute, Ambleside, UK. There are seven members, one of which is a member of the Diatomphyta, viz. Members of the genus Navicula and Cyanophyta viz. *Synechococcus sp.* More than one medium was used as follows: Isolate single cells using a pulled 1 mm glass tube inserted in a micro-manipulator. Cells that were isolated were transferred to BBM "Bold's Basal Medium" agar plates, twice-washed in sterile media, and then incubated at 25°C with continuous illumination (50 A mol m $^{-2}$ sl $^{-1}$). Colonies were moved to liquid BBM, where they were frequently subcultured. Additionally, silica was added to the medium at the final concentration of 200 mg $L^{-1}$ $Na_2SiO_3.5H_2O$, or 37.8 mg $L^{-1}$ Si, for the separation and development of diatom cultures. And, The calcified medium that provides favorable conditions for precipitation contains 400 mg $L^{-1}$ $Na_2CO_3$, 400 mg $L^{-1}$ $Na_2CO_3$, 100 mg $L^{-1}$ $MgSO_4$ $2H_2O$, 13.5 mg $L^{-1}$ $KH_2PO_4$, 25 mg $L^{-1}$ $KNO_3$, 0.34 mg $L^{-1}$ 1 $NaNO_2$, 2.5 mg $L^{-1}$ $H_3BO_3$, 1.0 mg $L^{-1}$ $(NH4)_6$ $Mo_7O_{24}$ $.4H_2O$, 1.5 mg $L^{-1}$ $MnC1_2.4H_2O$, 25 mg $L^{-1}$ $Na_2SiO_3$ $.5H_20$, 2.0 mg $L^{-1}$ 1.0 mg $L^{-1}$ of FeCl3 with EDTA (Na salt). The solution was filter sterilized and the pH "Potential of hydrogen" was changed to be pH 8.5. The medium is 1195 ps $cm^{-1}$ in conductivity, 5.79 meq $L^{-1}$ in alkalinity, and 1.34 in saturation index (SI) in relation to $CaCO_3$. The sample was made in the following way. To achieve a final chlorophyll awareness of 0.4 g $mL^{-1}$, axenic cultures for each alga from the early desk-bound portion were grown in 250 ml conical flasks with 100 ml of calcification media. The flasks were placed on a gyratory shaker and incubated at 25°C with constant light (50 mol $m^{-2}$ $s^{-1}$). Also created were control flasks with sterile calcification media. *Chlorococcum sp.* was used as a further control, with one group of flasks containing warm-killed algae (1000°C, 2 h), and the other group putting live algae in the dark. Three copies of each cure were made. For pH (Gallenkamp portable aggregate electrode), soluble calcium (titrated against 1 mm EDTA "Ethylenediaminetetraacetic" using glyoxal-bis-(2-hydroxy anil) as a hallmark), and chlorophyll measurements, every two days, a 5 mL sample was obtained from each flask. Additionally, the main calcification assay utilizing the green unicellular alga *Chlorococcum sp.* was used for inhibitor research. The inhibitors had been delivered to the *Chlorococcum* lifestyle 90 h after sub-culturing at 10 mg $L^{-1}$ until in any other case stated. Control flasks had been inhibitor unfastened, each cure was repeated three times and pH was determined, soluble calcium and chlorophyll. In which

inhibitors had been brought at several concentrations a good way to confirm the minimal awareness powerful at regulating precipitation and the dosage at which 100% inhibition changed until achieved. Additionally, inhibitor experiments using the Stigeoclonium variable were completed. The Olympus BH-2 microscope and transmitted light polarizing filters were used., samples have been checked for $CaCO_3$ precipitation during each test. Black and white Kodak TMAX 400 film has been used to shoot pictures. The sampling's outcomes were as follows: In all the flasks of calcification medium with no algae, precipitation was no longer seen, and the levels of soluble calcium and pH remained at initial levels. When cultured in calcification media, every type of algae extracted from calcified biofilms deposition $CaCO_3$. Tannic acid, $((NaPO_3))$,$(Na_4P_2O_7)$, Induction times PAA and a 92:8 mole % mixture of polyprotic acid and alanine (PAL) extended the experiments, and 50 hours after these compounds were given to the flasks, there were no signs of precipitation. Additionally, most of these flasks had deposited various quantities of $CaCO_3$ after 100 hours of use. The other substances may also have delayed precipitation, but this test was unable to detect it since there was a period between samplings during which conditions were no longer being watched. Additionally, polyaspartic acid MW 2000 (PA2000), tannic acid, PAL, PAA, $(KH_2PO_4)$, MDPA and PA250000 polyacrylic acid decreased the rate at which soluble calcium was eliminated from the medium during precipitation. In comparison to the controls, the rate at which the removal of soluble calcium from the medium was enhanced in the presence of $(NaPO_3)_6$ and $Na_4P_2O_7$. The pH of the flasks containing inhibitor, on the other hand, rose by at least 0.69 units above the pH at which precipitation initially appeared. They were successful in precipitating calcium carbonate. When $(NaPO_3)_6$ and $Na_4P_2O_7$ were introduced to the flasks at 100 mg $L^{-1}$, the $CaCO_3$ precipitation was completely inhibited. Furthermore, they observed that these algae are susceptible to high pH values, with death occurring when the pH reached 10.4. HEDP was the most efficient inhibitor of crystal nucleation. Chlorophyll was used to quantify growth, and the growth rates were determined by calculating the gradient of the slope that resulted from a plot of log chlorophyll vs. time. The slope of a plot of the concentration of soluble calcium against time was used to calculate the rates of soluble calcium elimination. Divide the rate of calcium elimination by the rate of growth to get, the ability of an alga to precipitate $CaCO_3$ was determined. The slope of a plot of the concentration of soluble calcium against time was used to calculate the rates of soluble calcium elimination. By dividing the rate of calcium removal by the rate of growth, the ability of an alga to precipitate $CaCO_3$ was determined. The research did not stop to this extent [11]. Rivadeneyra et. al. *Used Deleya halophila, CCM3662 strain*. With Medium: The bacteria were cultured on MH liquid medium that had 0.4% calcium acetate added to it. The

pH was then altered to 7.2 using 1 M KOH. The MH medium was modified to contain 1% (wt/vol) yeast extract (Difco), 0.5% proteose peptone No. 3, and 1% glucose in addition to a well-balanced combination of sea salts with a 7.5% (wt/vol) final salt concentration. In order to observe the crystallisation process, D. Halophila was also planted in liquid media in widemouthed vials that had previously been filled with vertically positioned glass coverslips. The bottles were incubated at 32°C, and after 120 hours, the coverslips were taken off for examination every 12 hours. The slides were preserved using $CaCl_2$ in a desiccator. Additionally, a liquid medium was used to grow microorganisms for 25 days to produce enough crystals to determine the mineralogical features. The crystals were collected, cleaned with distilled water, and allowed to dry naturally at 37 °C. The final outcomes were as follows: The major mineral in the crystals produced by *D. halophila* is aragonite ($CaCO_3$). This species' bio minerals emerged after 25 days of incubation, and magnesium levels were minuscule throughout the biolith growth process. Unlike other polymorphic forms of calcium carbonate, like calcite, very little magnesium substitutes for calcium in the crystalline network of aragonite. The sample was produced using a Link QX-200 equipment coupled to a Zeiss DSM-950 scanning electron microscope, and after completion, X-ray micro analyzer (EDX) tests were to be conducted on the sample. At 20 kV, 100 s of existence time, a spot size of approximately 200 nm, and a 45° tilt attitude, the samples were evaluated using pin-factor analysis. Dolomite (Ca 0.5-Mg 0.5-CO3) and high-purity calcite ($CaCO_3$) have been used as controls. These items have been coated with carbon and set up on a graphite pattern plate with carbon glue. And, the result of the test is as follows: calcium became the major alkaline-earth steel, and was followed by means of negligible quantities of magnesium. Since Halophila in particular mineralizes calcium, biomineralization via this species is fundamentally a calcification process. Despite the fact that the living medium included more of the latter metal (1094 ppm calcium compared to 2717 ppm magnesium), the biolithic created by this species had significantly more calcium than magnesium. After that, a light microscope was done and the result Within 48 h after seeding, Bio minerals were generated by *D. Halophila* in spherical forms. The investigation didn't end there; SEM analysis was also carried out, with the following findings: Morphological studies with SEM of the levels of crystal formation revealed that the first indications of biomineralization appeared after around 24 hours of incubation. However, with time, both the percentage of biolithic and the percentage of bio-minerals that reflect the very final ranges of the precipitation series increased. After 25 days of development, the XRD examination was completed, and the results revealed that the mineral section was mostly composed of aragonite (polymorphous calcium carbonate), with trace levels of magnesium calcite. It has been accomplished to reach: Due to the fact that *Deleya halophila* is a

Gram-negative encapsulated bacterium, the buildup of metal ions ($Ca^{+2}$, with a lesser percentage of $Mg^{+2}$) seen in our micro-analytical data most likely occurs within the cell wall and capsule. The adsorption of $Ca^{+2}$ and $Mg^{+2}$ cations at the cell surface by bacteria can operate as the center for the precipitation of carbonates, according to several authors. The process of carbonate precipitation involves the adsorption of $Ca^{+2}$ cations (and of a minor quantity of $Mg^{+2}$ cations), which is followed by a local explosion in their concentration. Along with the emission of $CO_2$ caused by the decomposition of organic materials, The local supersaturation and precipitation of calcium carbonate, primarily aragonite, may also result from the enhanced focus [15]. Research began in the 21$^{st}$ century; similarly, Ramachandran et al. demonstrated that embedding *S. pasteurii* cells into the cement matrix increased the compressive power of cement mortar. The calcium carbonate crystals produced by *Lysinibacillus spp. strain YL* under identical circumstances have not been subjected to X-ray diffraction investigation [22]. Katsuyama et al. prepare the sample as follows: waste cement sample utilized in this investigation (Tokyo, Japan). The sample is made up of tiny particles that were separated and pulverized as byproducts of a waste recycling factory for concrete. By using a light Scattering technique, the diameters of particles of the waste cement were examined and found to be spread throughout a range of 10-200 mm, peaking at roughly 25-40 m (area based) and 80 m (volume based). According to elemental analysis, calcium made up roughly 27.3% of the weight fraction. Based on thermogravimetric measurements made with the aid of a differential thermal analyzer, it was discovered that around 11% of the calcium had already carbonated (TGD-9600, Ulvac, Tokyo, Japan). Additionally, the extraction of calcium tests was carried out in a high-pressure stirring tank vessel reactor. The interior volume was 500 mL, and the container was built of the nickel-based alloy Hastelloy. With air-conditioning conditions, a known amount of waste cement particles and a set amount of ultrahigh-purity water were introduced into the reactor. The reactor was continually supplied with gaseous $CO_2$. The reaction temperature was precisely maintained while the reactor was immersed in a constant temperature bath with an accuracy of ±1 K. Additionally, the material in the reactor was stirred using a two-wing paddle-type fin, with the speed of the churning being adjustable between 0-1000 rpm. Throughout the extraction trials, small samples of the reactor's content were taken through a sintered metal filter with a mesh size of 5 μm at predetermined intervals. Inductively coupled plasma-atomic emission spectrometry was used to determine the calcium concentration of the filtered solution. As calcium carbonate may be the reason of the apparent extraction rate indicated above, consider the mismatch between the rate at which calcium ions are extracted from the waste cement and the rate at which they are precipitated with carbonate ions. The extraction rate will be significantly larger than the

precipitation rate, especially early in the reaction, when there is a lot of waste cement available for the extraction reaction and the C/W ratio is higher. This explains the hypersaturation that was seen during the first stages of the extraction process in the instances with higher C/W ratios. In the end, he predicted that as the extraction reaction advanced and the concentration of calcium ions in the aqueous phase grew, so would the amount of waste cement available for the extraction process. And, when figuring up the required C/W ratio, these trade-offs should be taken into consideration. Additionally, all the tests involving the precipitation process employed the aqueous solution created during the extraction procedure. After passing through a sintered metal filter with a mesh size of 5 m, the solution generated by the extraction reaction under the prescribed extraction conditions was passed to the precipitation reactor. Additionally, the precipitation reactor is a 300 mL reinforced glass jar. The precipitation process is triggered by either increasing temperature or decreasing $CO_2$ pressure. ICP-AES was used to determine the calcium concentration after a very little amount of the solution was filtered through a mesh size of 5 m. Following that, to measure the rate of calcium carbonate precipitation, A P-4010 (Hitachi) was used to quantify the concentration of calcium ions in the solution at regular intervals. The precipitation process was allowed to proceed for a certain period, and then the entire reactor contents were withdrawn and immediately filtered. Additionally using a differential thermal analyzer and the thermos gravimetric technique (DTG-60H, Shimadzu, Kyoto, Japan), the chemical makeup of the particles that remained on the filter was studied. According to the thermal gravimetric measurement, the precipitated CaCO3 concentration was greater than 98%. It should be emphasized considering these findings that the formation of the required high-purity $CaCO_3$ is contingent on the existence of seed crystals. Calcium carbonate has an 80% purity without seed crystals. As a result, during the precipitation experiments, the rate of calcium carbonate precipitation is almost precisely similar to the decrease in calcium concentration in the solution. After that, he kept looking into the impact of $CO_2$ changes in the concentration of calcium ions under various partial $CO_2$ gas pressures. The prescribed variables are a temperature of 323 K, stirring speed of 900 rpm, and 1.0 g of residual cement. The dotted lines on **Figure 1** represent the saturated calcium concentrations for the selected extraction conditions. Effect of $CO_2$ partial pressure on the calcium ion concentration of the solution over time. The starting solution volume was 200 mL, the precipitation temperature was 303 K, the stirring speed was 500 rpm, and the $CaCO_3$ seed crystal weight was 0.05 g. The **Figure 1** shows dotted, dashed, and solid lines, respectively, represents the estimated saturation concentration of $Ca^{+2}$ under these conditions and is based on thermodynamic principles.

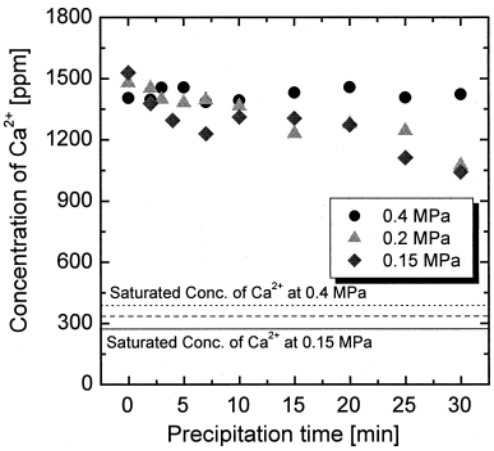


**Figure 1.** Temperature of precipitation influences the time course of calcium ion concentration in solution. Initial solution volume = 200 mL, CO2 partial pressure = 0.2 MPa, stirring rate = 500 rpm, CaCO3 seed crystal quantity = 0.05 g. The dotted, dashed, and solid lines on the graph represent the Ca2+ saturation concentrations determined using thermodynamic principles [12].

Finally in terms of cost and energy utilization, the proposed method for manufacturing calcium carbonate from waste cement using compressed $CO_2$ has substantial promise for flue gas desulfurization [12]. In a study by Mortensen et al. Biocement was made with the aid of *Sporosarcina pasteurii*. A urea-calcium-based cementation medium was used to culture the bacterium, which was then incubated for around 40 hours before being extracted. The researchers were able to delay the precipitation of calcium carbonate and produce a more uniform dispersion of cementation by raising the ammonium chloride content in the cementation medium. The tests were conducted using *Scenedesmus obliquus (strain 276-2)* [13]. A live organism Santomauro et al., It came from the Gottingen SAG cultural collection. In addition, $Zn^{+2}$, $Na_2EDTA$, $KH_2PO_4$, $(NH_4)Mo_7O_{24}$, and $MnCl_2$ were eliminated from the modified calcification medium on which the algae were grown in order to minimize chelate formation, and $FeCl_3$ was reduced to 0.01 mg/l. The solution was saturated for $Ca^{2+}$. $ZnSO_4.7\ H_2O$, which contains zinc, was added to the medium at three different concentrations (0, 3.27, and 6.53 mg $Zn^{+2}$/l).Additionally, all stock solutions — aside from $Na_2CO_3$ were combined, and sterilized in a Systec V-75 autoclave, and their pH levels were elevated to 6.3. The algae were washed in decriminalized water (Millipore), counted with a hem cytometer (Marienfeld, Lauda-Königshofen), and then added to the medium (around 4.0 x 108 cells/l) to reach the same cell content in all cultures. The culture was kept alive in an inform HT Multitron II rotary shaker at a constant 26 C under Gro-Lux 15W, 3500 lx fluorescent illumination. Due to photosynthesis, the pH rose over 9 over the course of many days. Daily pH adjustments were made using 0.1 M NaOH in both the organic, algae-containing solution and the algae-free solution. The 72-hour experiment was conducted.

All cultures thereafter adopted the lifestyle that was made apparent by their green colour. The results of the samples gathered were also reported. classification of poly-morphs and their crystallisation. The occurrence of precipitates in solutions containing algae was investigated at the start of the experiment and at intervals of 2 hours, 4 hours, 6 hours, 24 hours, and 72 hours. There were found to be two different types of crystals. According to the crystal form, we assume that the calcite-representing rhombohedric crystals and the aragonite-representing needle-like crystals are both minerals. *Scenedesmus obliquus* does not calcify when cultivated in the dark, and the pH only rises to a maximum of 9. Through light-dependent photosynthesis, the algae actively contribute to the alkalization of the medium in addition to acting as $CaCO_3$ precipitation nucleation sites. And for the pH, each culture's pH was adjusted to 8.5 before to the commencement of each experiment. The pH rose to 10.8 after 48 hours in the culture without zinc as a result of the algae's photosynthesis; following that, it practically stayed constant. The pH change was postponed by zinc, preventing it from rising to the high levels of the zinc-deficient culture. While the culture containing 3.27 mg $Zn^{+2}$/l reached pH 10.5 after 48 hours and stayed there, the culture containing 6.53 mg $Zn^{+2}$/l only reached pH 10.1 after 72 hours. This pH-shift is related to the amount of $Zn^{+2}$ present in the solution. Using a 0.1 M NaOH solution, daily pH changes were performed to the media devoid of algae in line with the pH readings taken from cultures containing algae, then for SEM analysis The outcomes were Only aragonite crystals are discovered in the medium, which also contains significant quantities of zinc and algae. On the other hand, calcite nearly always forms in environments with high zinc concentrations and low amounts of algae. The amount of aragonite produced is quite little. The findings of the XRD examination also showed, the presence of algae cells greatly changes the $CaCO_3$ precipitation within the medium as compared to medium without algal cells. Finally, we can state that the presence of living micro-algae has a considerable impact on the precipitation of calcium carbonate crystals. As a result of photosynthesis, the pH of the organic media containing the algae increased, and $CaCO_3$ crystals developed. In organic media, 3.27 mg $Zn^{+2}$/l or not, calcite and aragonite always form. In organic settings with 6.53 mg $Zn^{+2}$/l, even aragonite forms. In contrast, inorganic solutions devoid of zinc precipitate pure calcite. Both of the inorganic solutions containing zinc had significant calcite precipitation and a smaller amount of aragonite precipitation [16]. Similarly, in a study of Al-Thawadi, Cord-Ruwisch, Biocement was created using *Bacillus sp. MCP11*. The bacterium, which was cultivated in a medium containing yeast extract, urea, ammonium sulphate, sodium acetate, and calcium chloride, was isolated from soil and sludge samples. The scientists watched as originally spherical crystals formed, progressively fragmented, and then finally transformed into rhombohedral crystals. The production of

calcite and vaterite crystals was shown by X-ray diffraction examination of the calcium carbonate precipitated by MCP11 under comparable circumstances[8]. Then Stabnikov et al. did a study on the production of bio-cement using *S. pasteurii*, notably the *ATCC 11859 strain*. Pure cultures of UPB were gathered by the researchers from a variety of climates, including *halophilic* and *alkalophilic strains* from Singaporean and Ukrainian soil. The bacteria were grown in a medium that included trace elements as well as 82.5 g/l (0.75 M) of calcium chloride and 90 g/l (1.5 M) of urea. Using 1 M HCl, the pH of the mixture was brought down to 2.0. Ten grams of dirt were used to inoculate the medium, which was then shaken for six days at 150 rev/min at 30°C. After six batches of treatments with strains *VS1* and *VUK5*, the researchers found that the unconfined compressive strengths for dry bio-cemented sand samples were 765 and 845 kPa, respectively. The study demonstrated the potential of *S. pasteurii* for usage in building applications by proving its ability to create bio-cement [18]. Also, Achal and Pan, *Bacillus sp. CR2,* which was isolated from mine tailing soil in Urumqi, Xinjiang, China, was used to study the formation of calcium carbonate. Using 16S rRNA gene sequencing, a thorough molecular investigation was used to identify the bacteria. The bacteria were cultivated in Nutrient broth supplemented with urea and several calcium sources, such as calcium chloride, calcium oxide, and calcium acetate, in order to create calcium carbonate. The XRD spectra revealed that *Bacillus sp. CR2* primarily formed calcite in all media, with the most calcite peaks being seen in the calcium chloride-containing solution. In all media, with the exception of the one that used calcium acetate as the calcium source, aragonite and vaterite peaks were also seen. Calcite, vaterite, and aragonite were among the mineral poly-morphs that were discovered by SEM analysis. Many of the calcite crystals also included rod-shaped bacterial cells that were intimately linked to and emerging from the mineral surface. After growing bacterial cells in various calcium sources for 7 days, the cell pellets were examined using the FTIR spectra produced by infrared spectroscopic analysis. *Bacillus sp. CR2* generated the most calcium carbonate (2.32 mg) when calcium chloride was used as the calcium source, according to the research, making it the most favoured calcium source [5]. After that Seifan, Samani, and Berenjian used *Sporosarcina pasteurii* and *B. sphaericus* to aid in the formation of bio-minerals. The biological materials employed in this investigation were discovered to be mediated by a wide range of microorganisms, including bacteria, fungus, protists, and plants. Numerous objects, including shells, bones, teeth, and even limestone caverns, contain these bio-minerals. As the main calcium supply for the experiment, calcium carbonate, or $CaCO_3$-$H_2O$, was employed [17]. Similarly, in a study Xu et al., strain GM-1, a Micro-bacterium sp. isolated from active sludge, was used to induce calcium carbonate precipitation through

urea hydrolysis. The microorganism was incubated aerobically at 30°C in a unease-selective medium. Using the streak plate technique, the cells were inoculated on urease selective and control plates, and color change was seen after 2 days of incubation. Calcite was found to be the predominant calcium carbonate form in the XRD study of the white precipitate produced by the bacterium groups. According to the study, strain GM-1 may be useful in environmental bio-remediation and bio-recovery [20]. Also in Hait et al. completed a study employing *Klebsiella pneumoniae* to generate bio-cement. For the purpose of isolating the organisms that produce bio-cement, the researchers took soil, seawater, and sewage samples. The researchers used titrimetric analysis to check for the presence of $Ca^{2+}$ ions in hard water systems, such as saltwater and sewage water, in order to produce bio-cement. The calcium source employed was the original seawater's $Ca^{2+}$ concentration. The presence of calcium carbonate was further confirmed by the XRD examination of the synthetic bio-cement, which is compatible with earlier research. The signature peaks at 2θ = 29.8°(104) and 43.7 (202) clearly showed the presence of CaCO3. However, it is also clear from the pattern that calcite is not created in its pure form; peaks at 2 =16.7, 21.7, and 32.4 reflect different crystal structures of aragonite and dolomite, both of which are found in the bio-cement made by Klebsiella pneumoniae as shown in **Figure 2**.

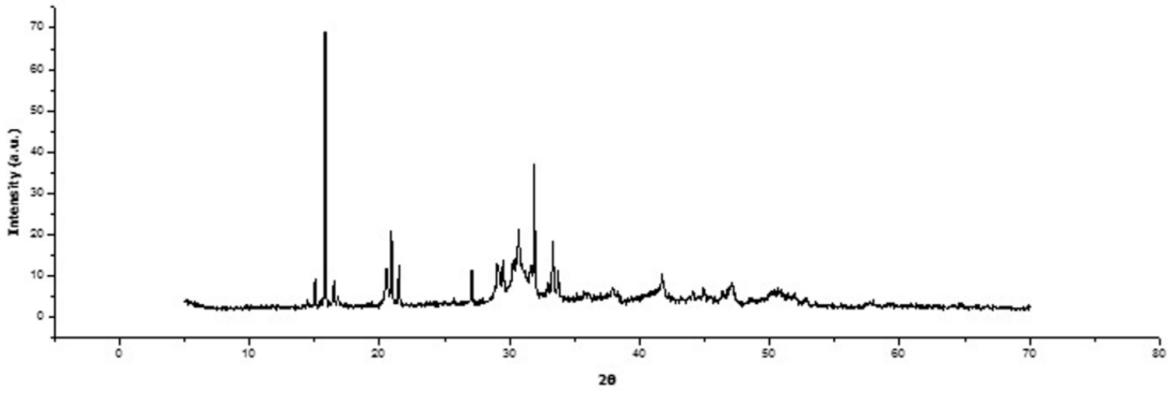


**Figure 2.** XRD analysis of white precipitate, obtained from isolate Klebsiella pneumoniae, [10]

The test organism's production of bio-cement was determined to be 0.46 g/1000ml in saltwater. The fact that there was less $Ca^{2+}$ in the filtrate proved that $Ca^{2+}$ was used for $CaCO_3$ precipitation [10]. Similar to this, Sporosarcina pasteurii (DSM33) was employed in a recent work by Xiao et al. to investigate calcium carbonate precipitation. The bacteria were obtained from Leibniz Institute DSMZ and was raised in a liquid medium comprising 0.13 M Tris buffer, 20 g of yeast extract, and 8.1 g of $NH_4Cl$. The bacteria culture become murky after 20–24 hours of incubation at 30 °C, signifying the development of the

bacterium. For the XRD analysis, fragments were crushed and sieved through a 75 µm mesh. The analysis revealed the formation of calcium carbonate crystals, and the XRD scan was conducted using Cu Ka radiation (40 kV, 30 mA) with a scanning rate of 0.017° 2θ/step from 5° to 80° 2θ as shown in **Figure 3** [19].

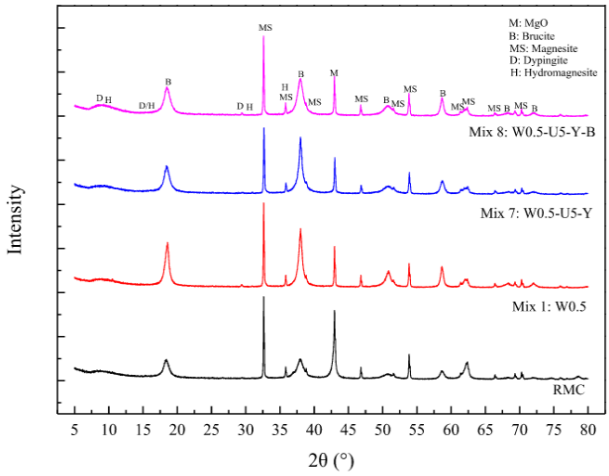


**Figure 3.** XRD patterns of RMC powder, control RMC paste (mix l), nutritious RMC paste (mix 7), and bio-RMC paste (mix 8) at 28 days, [19].

After that Esmail et al. did research on using *Bacillus megaterium* to make bacterial concrete. Using MALDI-TOF Biotyper, the bacterium was isolated from alkaline soil samples collected in Wadi EL-Nitron, Behera governorate, Egypt. The culture was grown in aerobic incubation in 2L Erlenmeyer flasks using a rotary shaking incubator at 150 rpm for 7 days at 30°C using autoclave Nutrient broth (NB) with a pH of 7.5. Two stages of work were carried out with Bacillus megaterium for their investigation. In the first step, calcium lactate, calcium formate, and calcium acetate were introduced as nutrition to bacteria at 0.25%, 0.125%, and 0.5% of cement weight, respectively, to concrete mixes in two ratios (0.5% and 0.25% of cement weight). *Bacillus megaterium* was introduced to concrete mixes in the second stage in two ratios (0.5% and 0.25% of cement weight), with calcium lactate provided as nourishment to bacteria in the ratios of 0.125% and 0.250% of cement weight. In concrete mixes containing 1% cement, superplasticizer was utilized. According to SEM imaging, the addition of bacterial concrete specimens' nutrients caused calcite crystals to develop in a variety of forms. According to earlier research, bacterial concrete has a better compressive strength than regular concrete. In comparison to normal concrete, the inclusion of *Bacillus megaterium, Bacillus subtilis***,** and their consortia enhanced compressive strength by

22.5%, 14.3%, and 15.8%, respectively. Additionally, when fly ash concentrations were 10%, 20%, and 40%, respectively, bacterial cells increased the compressive strength of mortar by 19%, 14%, and 10% in comparison to control specimens. In comparison to the standard concrete strength of 44 MPa, the employment of *Bacillus subtilis* at a concentration of 108 cells/ml resulted in a compressive strength of 52 MPa, while Bacillus megaterium at a concentration of 105 cells/ml produced a compressive strength of 57 MPa. Due to the calcification process, the strength of the highest grade of bacterial concrete (50 MPa) increased by 24% compared to the lowest grade (30 MPa) by 12.8%. The presence of bacteria improves the compressive strength of silica fume (SF) concrete at all ages, with the greatest strength of bacterial concrete being recorded at 56 days with 10% SF and being around 12% higher than that of the concrete with the same silica fume replacement. The deposition of calcite in the pores, followed by pore reduction, compact micro-structure formed, rendered concrete thick and so improving the strength, may be the cause of the increase in concrete strength with bacteria. The negatively charged groups on the surface of bacterial cells, which attract divalent ions like $Ca^{2+}$ and $Mg^{2+}$, serve as a location for nucleation. Urea is hydrolyzed by the urease enzyme into ammonia and carbonate, which then react with $Ca^{2+}$ to generate calcium calcite precipitate on the bacterial surface. The concrete mix surrounding the pores and micro-cracks is better packed and compacted as a result of the calcite, giving the specimens a significantly greater strength than controlled concrete examples. Calcium carbonate also accelerates hydrolyzation and serves as a catalyst for cement hydration, boosting the concrete's compressive strength [9]. And finally, Ali, Mukhtar, and Dufossé used *the Lysinibacillus spp. strain YL* to bio-precipitate calcium carbonate crystals. The microorganism was isolated from tropical beach sand and raised in a medium with calcium sources such as acetic acid or calcium acetate. An efficient and cost-effective method for bio-precipitation, according to the study, would involve employing a low concentration of $Ca^{2+}$ as low as 30 mM as shown in **Figure 4**. also showed that non-ureolytic *Bacillus cohnii* may be used to perform microbially induced calcium carbonate precipitation (MICCP), which might boost concrete's compressive strength by 49% [6].

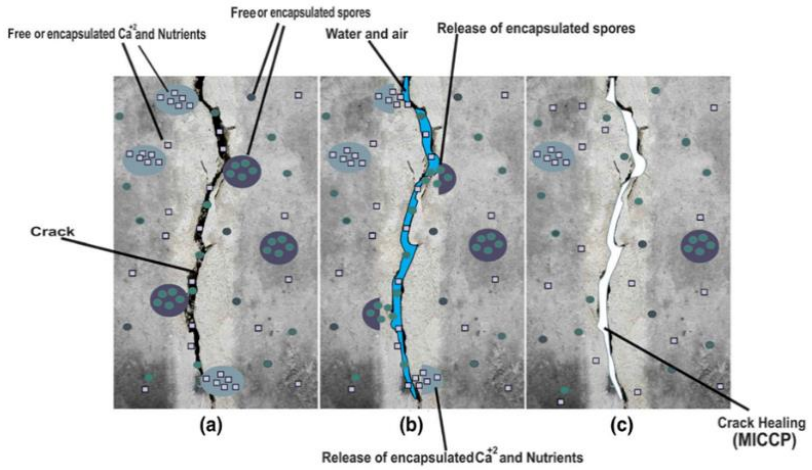


**Figure 4.** Schematic of MICCP crack healing - cracks form in bio-cement with spores and Ca+2. Water and air seepage release encapsulated spores and Ca+2 [6]

## 5. The effects of different parameters on the biocementation process

Firstly, the choice and concentration of nutrients introduced into concrete mixes are of paramount importance. In the study, calcium lactate, calcium formate, and calcium acetate were employed as nutrients at different levels, both in absolute concentration and as percentages of cement weight. The concentration of nutrients directly influenced the formation of calcite crystals within the concrete matrix. The results revealed that the choice and concentration of these nutrients significantly affected the size, morphology, and distribution of calcite crystals. This underscores the potential for tailoring the biocementation process to achieve specific material properties by optimizing nutrient composition. Furthermore, the concentration of the introduced bacterial strain is a key parameter. *Bacillus megaterium*, at different concentrations, showed varying effects on the compressive strength of the resulting concrete. The study demonstrated a remarkable enhancement in compressive strength, ranging from 14.3% to 22.5% compared to conventional concrete. At a concentration of 10^5 cells/ml, *Bacillus megaterium* achieved a notable compressive strength of 57 MPa. This emphasizes that the concentration of the bacterial strain can be fine-tuned to attain desired concrete properties, presenting a strategy to engineer concrete materials with superior mechanical characteristics. Additionally, the research delves into the impact of fly ash concentrations on the compressive strength of concrete. The incorporation of fly ash further improved the compressive strength of mortar specimens, with enhancements of up to 19% compared to control specimens. This parameter demonstrates the potential for using supplementary

materials to enhance the biocementation process, thereby offering eco-friendly alternatives in concrete production while optimizing mechanical performance.

## 6. Different strategies used for the biocementation process

One notable strategy involves the use of *Bacillus sp. strains,* such as *Bacillus megaterium* and *Bacillus subtilis,* which have exhibited exceptional abilities to induce calcium carbonate precipitation. These bacterial species are capable of altering the material properties of concrete through their metabolic activities. By introducing *Bacillus sp.* strains to concrete mixes at varying concentrations, the biocementation process can be fine-tuned to enhance compressive strength significantly. The incorporation of these bacterial strains into concrete mixes has resulted in concrete materials with compressive strengths ranging from 30 MPa to 50 MPa. This strategy not only offers a sustainable approach to concrete production but also provides an opportunity to engineer materials with superior mechanical performance. Another strategy employs microorganisms like *Sporosarcina pasteurii, Klebsiella pneumoniae,* and *Lysinibacillus spp.* to initiate the precipitation of calcium carbonate in concrete matrices. These microorganisms are isolated from various sources, including soil, sewage, and tropical beach sand. Each microorganism's unique characteristics and metabolic processes influence the resulting material properties. The use of different microorganisms demonstrates the adaptability and versatility of the biocementation process. In addition to microbial species, the choice of nutritional composition and supplementary materials plays a pivotal role in optimizing the biocementation process. Nutrient selection, such as calcium sources like calcium lactate, calcium formate, and calcium acetate, directly impacts the formation and distribution of calcite crystals within the concrete matrix. This offers the possibility of tailoring material properties to meet specific requirements in construction applications. Furthermore, the inclusion of supplementary materials like fly ash showcases the strategy of incorporating eco-friendly materials into the biocementation process. Fly ash concentrations, when combined with microbial activity, further enhance the compressive strength of concrete, presenting sustainable alternatives for concrete production with improved mechanical properties.

## 7. Utilization of microbial-induced calcite precipitation for various applications

Microbial-induced calcite precipitation (MICCP) has emerged as a versatile and promising technology with a wide range of applications across various fields. This biologically mediated process is being extensively explored for its potential to revolutionize the construction industry, environmental

remediation, and materials science. A notable application of MICCP within the construction industry is the enhancement of concrete properties. Research endeavors involving bacterial strains like *Bacillus megaterium* and *Bacillus subtilis* have demonstrated impressive results. The introduction of these bacteria into concrete mixes led to substantial improvements in compressive strength, ranging from 14.3% to 22.5% when compared to traditional concrete formulations. The use of fly ash in combination with bacterial cells further boosted the compressive strength of mortar specimens. *Bacillus subtilis* at a concentration of 10^8 cells/ml resulted in a compressive strength of 52 MPa, while *Bacillus megaterium* at a concentration of 10^5 cells/ml produced a remarkable compressive strength of 57 MPa. The highest-grade bacterial concrete exhibited a 24% increase in strength over the lowest grade, highlighting the efficacy of the calcification process in enhancing concrete strength. The benefits of MICCP in the construction sector extend to silica fume (SF) concrete, with the greatest strength observed at 56 days when 10% SF was incorporated. The improved compressive strength recorded at this juncture was approximately 12% higher than that of the control concrete with the same *silica fume* replacement, further underlining the potential of bacterial influence on concrete properties. Also, MICCP has garnered attention for its eco-friendly application in environmental remediation. Notably, the biocementation process facilitates the stabilization and immobilization of contaminants in soils. The precipitation of calcium carbonate in soil matrices can enhance soil cohesion and reduce permeability, thus reducing the mobility of harmful substances. This method provides an innovative approach for the treatment of contaminated sites, including those with heavy metal or radionuclide pollution. MICCP can significantly decrease the mobility of these contaminants, contributing to the sustainable remediation of soil and groundwater. Notably, in a study by Esmail et al., *Bacillus megaterium* successfully facilitated bacterial concrete production. The ability of this bacterium to enhance compressive strength makes it a promising candidate for creating bio-cement with environmental applications, including soil stabilization. In materials science, MICCP offers unique prospects for the production of biocement and biominerals. The research conducted by Xiao et al. showcased the feasibility of producing calcium carbonate crystals using *Sporosarcina pasteurii.* These crystals were formed in controlled environments, and their utilization for various applications was explored. The biomineralization process can be engineered to produce specific mineral phases and morphologies, opening doors for tailored materials with a multitude of applications in the fields of medicine, dentistry, and materials engineering. The cost-effectiveness of MICCP for biomineral production is exemplified by the use of low concentrations of Ca^2+, as low as 30 mM. The study suggests that this innovative approach may revolutionize the production of biominerals for diverse

industrial and medical applications. MICCP is a multifaceted and promising field with the potential to address pressing challenges in construction, environmental protection, and materials science. The quantifiable benefits, including enhanced concrete strength, reduced soil permeability, and cost-effective biomineral production, underscore the significance of MICCP as a sustainable and innovative technology with broad-ranging applications. As research in this area continues to advance, the full extent of its potential and practical implications is yet to be fully realized, making it an exciting area of exploration in scientific and engineering disciplines.

## 8. Conclusion

In conclusion, Biocement is an Eco-friendly substitution for traditional cement that utilizes microorganisms to produce calcium carbonate and reduce the carbon footprint of cement production. The literature review explored the various microorganisms, also the optimal conditions for calcium carbonate production, $CaCO_3$ precipitations, and potential applications of Biocement in the construction industry. The current study also examined the challenges and limitations associated with Biocement and highlighted the need for further research. The reviewed studies showed that Biocement has the potential to improve the mechanical properties of the final product and using this technique could decrease the environmental impact of cement production. **Table [1]** provides an overview of the researchers who have conducted studies in this area, along with the specific type of bacteria or algae that was used. This literature review study is a helpful for researchers and individuals interested in the sustainable construction sector.

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- **Conflict of Interest**

A declaration of conflict of interest.

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