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# The Role of Microorganisms in Bio-cement Production: An Extended Review

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## Abstract

Bio-cement is an innovative material with the potential for replacement of conventional cement through microorganisms-influenced process. The major method uses bacterial, fungal, or algal activity to produce Microbial-Induced Calcium carbonate Precipitation (MICP). This review aims to understand the microbial aspect of bio-cement production explaining the process through MICP that is enhanced by ureolytic bacteria with a focus on *S. pasteurii* through the provide urease. Bio-cement has many environmental advantages such as lower $CO_2$ emission in comparison with common cement and opportunities to utilization of waste products. In construction, it is used in self-healing concrete, crack repair, and soil stabilization among others to demonstrate its flexibility in the construction industry due to its available solutions to many structural and geotechnical problems. The review also includes directions for basic, applied, and translational research, targeted genetic modifications for enhanced microbial performance, bio-cement, and more effective microbial strains, and the convergence of bio-cement with 3D printing. Even though bio-cement is an environmentally friendly approach used for soil stabilization, the negative impacts that surround the environment, for further research in making the bio-cement more bio-deteriorate and energy efficient.

**Keywords:** Bio-cement • Microbially Induced Calcite Precipitation (MICP) • Soil stabilization • Ureolytic bacteria

## Introduction

### Overview of bio-cement production

Bio-cement is a new green product that can be classified as a new generation of cement materials that are made using the biological activities of microorganisms such as bacteria, fungi, or algae [1]. In the case of bio-cement, the major constituent is Calcium Carbonate ($CaCO_3$) which is built by microorganisms by the natural mechanism of Microbially Induced Calcium Carbonate Precipitation (MICP). In this process, bio-cement settles in between particles and binds them together like the way traditional cement binds construction materials [2]. Ordinary cement, particularly Portland cement is manufactured through the heating of limestone and other materials at high temperatures of about 1450 ℃ [3]. This process consumes significant energy and produces a significant volume of carbon dioxide ($CO_2$), cement production accounts for the largest portion of greenhouse gas emissions [4]. The cement industry in its conventional method contributes to an estimated 8% of global $CO_2$ emissions. Whereas bio-cement is made at room temperature, thus using one-third the energy needed to produce conventional cement [5]. Microorganisms are used to catalyze the cementation process and thus bio-cement aligns well with sustainable formwork materials since it has reduced $CO_2$ emissions [3]. Its application in construction is considered as revolutionizing the fight against ecological abuses, to which construction activity greatly contributes without compromising on the solidity of the material on offer as well as its service delivery [6].

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### Importance of microorganisms in bio-cement production

Bio-cement is produced mostly through microbial activity where bacteria that can precipitate calcium carbonate are used most frequently. These microorganisms are essential to the MICP process where they catalyze the transition of urea to carbon dioxide and ammonia that eventually results in the production of calcium carbonate [7]. However, one of the most common microorganisms, used in the treatment of polluted soils, is *Sporosarcina pasteurii*, the bacterium with the highest urease enzyme activity which is necessary for the decomposition of urea [8]. Bio-cement essentially undergoes natural microbial growth and the saturation of calcium ions that interact with soil particles or any other substrate forms a strong matrix with the portions of another substrate [9]. It is in this respect that the newly adopted role of biotechnology in construction is central to the search for more sustainable construction materials. Application of microorganisms to construction materials is an area where biotechnology is just starting to surface and which holds great promise for both as an attempt at biological solutions to problems otherwise addressed by high energy use cement production. The incorporation of microorganisms in the preparation of the bio-cement shows that construction materials could be environmentally friendly and sustainable [10]. The method of production uses less power, generates less waste, and produces an option for self-healing concrete; a concrete with microorganisms within it that wake up when it is wet to seal any crack [11]. This not only stems from the growth in demand for new structures or architectures and infrastructures but also decreases repair need and time, thus decreasing material and energy use in the long run. Furthermore, bio-cement creates opportunities for resource efficiency in terms of feedstock by using either waste materials or by-products industrial effluents or agricultural residues as the relevant microbial substrate [12]. This follows the circular economy where after a process is carried out waste turns into the input for a new process making bio-cement more sustainable.

## Microbial Mechanisms in Bio-cement Production

### Overview of Microbial Induced Calcium Carbonate Precipitation (MICP)

Microbially mediated calcium carbonate crystallization is the primary process that has been implicated in the manufacture of bio-cements. Calcium carbonate is a process known in biochemistry whereby microorganisms contain the ability to set concrete-like structures through a biochemical process, through concretion, which is a process that forms the mineral by encasing soil or sand-like particles in calcium carbonate [13]. The main concept of MICP is based on the ability of particular microorganisms to produce specific chemical environments in which calcium ions ($Ca^{2+}$) are capable of reacting with carbonate ions ($CO_3^{2-}$) to form $CaCO_3$ and, consequently, develop hardness in the material [14]. It starts with particular bacteria that secrete enzymes to degrade organic elements; for instance, urease. In MICP, one of the most frequently embedded compounds is urea which is hydrolyzed by the bacteria class. This hydrolysis elevates the pH of the environment and raises carbonate ion availability to facilitate the deposition of calcium carbonate [2]. The precipitated $CaCO_3$ is found to fill up the interstices of the particles and effectively act like cement that holds up the structure [15]. MICP has emerged as the center of research in bio-cement as it is a cost-effective and environmentally friendly process compared to the conventional cement manufacturing process which is a known carbon emitter [1]. As compared with other methods of material hardening, the major advantage of MICP is the ability to control microbial activity and direct calcium carbonate precipitation for engineering requirements [16].

**Role of ureolytic bacteria in MICP:** The most studied and commonly used bacteria in MICP are ureolytic bacteria. These bacteria possess the enzyme urease, which catalyzes the breakdown of urea into ammonia and carbon dioxide. This reaction results in the formation of carbonate ions, which are a key to calcium carbonate precipitation [17].

$(NH_2)_2CO+2H_2O \rightarrow 2NH_4^{+}+CO_3^{2-}$

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Once carbonate ions are present in the environment, they combine with calcium ions to form calcium carbonate, as shown below:

$CO_3^{2-}+Ca^{2+} \rightarrow CaCO_3$

$CO_3^{2-}+Ca^{2+} \rightarrow CaCO_3$

One of the most effective ureolytic bacteria is *S. pasteurii*. This bacterium's high urease activity makes it particularly efficient in hydrolyzing urea and accelerating the calcium carbonate precipitation process [18]. The calcium carbonate produced by these bacteria serves as the primary binding material in bio-cementation, reinforcing soil or other substrates by filling gaps and solidifying the structure [19] (Figure 1).

## Types of microorganisms used in bio-cement

Several types of microorganisms can induce calcium carbonate precipitation, although ureolytic bacteria are the most commonly researched. Each microorganism type offers different advantages and potential applications in bio-cementation.

**Bacteria:** Of all the microorganisms, bacteria are the most considered in the production of bio-cement especially with a high urease activity shown by *S. pasteurii*. These bacteria can capture and hydrolyze urea remarkably effectively to produce calcium carbonate, and the bacteria are easy to cultivate and apply in practice [21]. Some other bacteria including species of *Bacillus* and *Pseudomonas* have also been investigated for bio-cementation; however, their urease activity and cementation potential are not as high as that of *S. pasteurii* [22]. Some kinds of bacteria such as *S. pasteurii* are efficient due to the fast formation of carbonate ions in big numbers required for the fast deposition of calcium carbonate [23]. As such, these commodities are suitable for different construction uses such as stabilization of soils, repairing cracks, and preparation of self-healing cement [24].

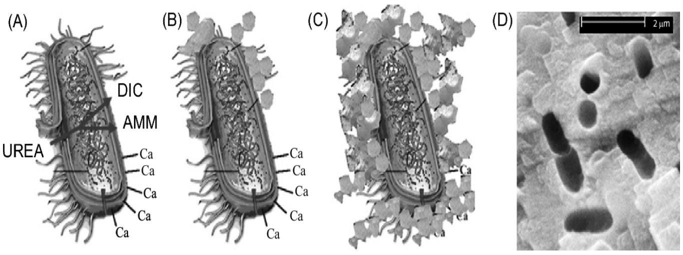


**Figure 1.** The Microbial-Induced Calcite Precipitation (MICP) process at the cellular level, highlighting how bacteria facilitate calcium carbonate ($CaCO_3$) formation, **A)** the beginning of the MICP process, where bacteria hydrolyze urea, producing ammonium and carbonate ions that can react with calcium; **B)** the initial precipitation of calcium carbonate ($CaCO_3$) as it begins to form around the bacterium; **C)** the final stage of calcite precipitation, where extensive crystallization occurs, possibly leading to the bacterial cell becoming encased in the precipitated minerals and **D)** a real-life microscopic image of bacteria interacting with $CaCO_3$ crystals [20].

**Fungi:** Bacteria and fungi have been used in the last few decades for bio-cementation but fungi are less applied as compared to bacteria. Thus, calcium carbonate precipitation using certain fungal species occurs through biogenic mechanisms, which are usually associated with one or several organic acids [1]. There are several organic acids that are released by these fungi, and interact with calcium ions which, in turn, form calcium carbonate analogously to bacterial bio-cementation. Fungi were also chosen because research shows that they are hardier than some bacteria and can colonize surfaces which may be of interest in bio-cementation [25]. It is still unknown whether it is possible to employ them in synthesizing bio-cement appropriate for use in natural conditions for instance in soil reinforcement or bioremediation [26]. However, due to the lower rate of precipitation at its current position, they are still a much less favored choice.

**Algae:** Algae, especially micro-algae, are also being investigated for bio-cement applications including limestone precipitating ureaphic algae and cyanobacteria. Besides bridging and algae in the proceeding section, algae itself can participate in the bio-cementation process by depositing calcium carbonate during photosynthesis [27]. Microalgae sometimes excrete calcium carbonate as waste and are thought to be suitable for bio-cement production as biotechnology [28]. However, due to their tolerance to various growth conditions and growth media, they can be a promising material for further investigations on bio-cement in marine or coastal structures [29].

## Enzymatic pathways

Bio-cementation is also encouraged by enzymes since they catalyze the biochemical reactions required for the formation of calcium carbonate particles. The dominant catalyst instrumental to the creation of MICP is the enzyme urease but other enzymes may contribute to bio-cementation in specific environments [9].

**Urease enzyme and its critical function in bio-cement formation:** Urease is the enzyme that brings about the degradation of urea to ammonia and carbon dioxide. This reaction plays the primary role in MICP as it leads to an increase in the pH of the environment and the formation of carbonate ions ($CO_3^{2-}$) [30]. In the absence of urease, it is apparent that the rate of urea hydrolysis would be slower which in turn slows the rate of calcium carbonate precipitation [31]. The urease enzyme is synthesized in substantial amounts by ureolytic bacteria such as *S. pasteurii* [18]. Urease activity is positively correlated with the rate of bio-cement formation as increased enzyme activity helps in the precipitation process leading to the formation of denser and more compact bio-cement structures [32].

**Other enzymes that may assist in cementation:** It should also be noted that the bio-cementation process can involve other enzymes in the bacterial as well as the fungal system depending on the microbe involved. Certain bacteria and fungi can produce enzymes called carbonic anhydrase for calcium carbonate precipitation [33].

**Carbonic anhydrase:** This enzyme makes carbon dioxide and water transform to bicarbonate ($HCO_3^-$), the ions with which calcium can form calcium carbonate. Carbonic anhydrase is useful where there is a lot of carbon dioxide for instance in soil or water systems [34].

**Organic acid metabolism:** In fungi, oxalic acid or other organic acid may react with calcium ions to form calcium oxalate, which may as well be changed over to calcium carbonate under certain circumstances. Although slower, it is beneficial in situations where ureolytic bacteria cannot thrive; In this case, they would not be able to inhabit the environment [35].

## Factors Influencing Microbial Bio-cement Production

There are sound principles, presumably holistic in scope, that govern the efficiency and success of microbial bio-cement production based on factors that define microbial action and the biochemical reactions that help cement surfaces. They include; the physical and chemical characteristics of the growth environment, the kinds of substrates and growth media employed, and the rate and efficacy of cementation. Knowledge of these factors is crucial in improving the microbial bio-cement process concerning real-life applications.

### Environmental factors

Microbial activity, especially in bio-cement production, is highly sensitive to environmental conditions. Factors such as temperature, pH, and moisture significantly affect the ability of microorganisms to produce calcium carbonate and form bio-cement [36].

**Temperature:** Temperature is one of the most important factors that affect almost every aspect of microbial activity, including the metabolism of the biochemical reactions that occur during bio-cement synthesis [37]. Many ureolytic bacteria, for instance, *S. pasteurii* prefer specific temperature conditions of operation; the normal range being between 25 ℃ and 40 ℃. Conditions beyond such an optimal temperature environment cool the bacteria's growth and diminish the urease activity and the precipitation of calcium carbonate [38].

**High temperatures:** High temperatures can alter the nature of the enzyme namely urease and this can affect the rate of hydrolyzing of urea and precipitating calcium carbonate. This can make the bio-cementation efficiency to be lower [39].

**Low temperatures:** The low temperatures also reduce the activity of the bacteria and the enzymes, the time taken for bio-cement development hence being much longer. In colder climates, this can become a big problem unless thermophilic bacteria are used in the process [40].

**pH:** Another critical factor that determines the bacterial activity and calcium carbonate precipitating action is the pH of the environment [41]. To enhance the precipitation of calcium carbonate, the solution should exhibit a pH above 8 because at higher pH values, carbon dioxide in the water forms carbonate ions ($CO_3^{2-}$) [42].

**Optimal pH:** The pH optimal for microbial bio-cement has been reported to be between 8 and 9. This pH range ensures the optimization of urease activity and remarkably enhanced calcium carbonate deposition [43].

**Imbalance pH:** Its frequent range is from 6 to 8; if it decreases and becomes less than 7, the activity of microorganisms is suppressed, and although calcium carbonate is not soluble in any acid, it does not precipitate as it should. On the other hand, a high pH, say greater than 10 has other side reactions or may suppress the growth of bacteria necessary for biodegradation [44].

**Moisture:** Relative moisture also plays a big role in microbial activity and bio-cement formation as well. These microorganisms have to use water to be able to absorb nutrients, elaborate enzymes, and transport calcium ions. But the second factor, moisture content should not be too high [45].

**Optimal moisture:** Primary, sufficient amount of moisture is required to support bacterial metabolism and the need for nutrient carriage and calcium ions for cementation [46].

**Excessive moisture:** Also, if the quantity of moisture is too high the nutrient and calcium ions are also weakened which reduces the effectiveness of bio-cementation. It can also impose a negative influence on the growth of some bacteria disturbing their growth rates [47].

**Low moisture:** During dry and warm states, microbial activity is controlled by dehydration thereby restraining bacterial-induced nucleation of calcium carbonate [25].

**Nutrient availability:** The nutrients for the bacteria like urea and calcium ions have a direct influencing effect on the rate and effectiveness of MICP. For microorganisms to be active metabolically and precipitate calcium carbonate, they require adequate nutrients.

**Urea concentration:** Consequently, urea is utilized for the urease activity that underpins MICP. A low concentration of urea would decrease the level of bacterial growth and reduce the deposit of calcium carbonate. However, high levels of urea might lead to the formation of ammonia, which is toxic to the bacteria present in the system [48].

**Calcium ion availability:** Calcium ions are essential in the formation of calcium carbonate. Calcium ion concentration has to be regulated because, on one hand, a lack of calcium ions can hinder the cementation process, but on the other hand, the presence of a large amount of calcium ions will cause uncontrollable precipitation [49].

### Substrate and growth medium

Microbial growth and bio-cement production are influenced by the selection of the substrate and the growth medium. The kind of substrate offers the physical base for the microorganisms whilst growth media offer them a nutrient base in which the microbial action can happen [19].

**Types of substrates:** Bio-cement is quite versatile in its application and it can be used to retrofit any of the following: soil, sand, and concrete. The influence of each substrate on microbial development and calcium carbonate formation is colorless.

**Soil:** Bio-cement is employed in soil stabilization. Also, the analysis of the particle size and composition of the soil shows the impact on calcium carbonate precipitation effectiveness [50]. Relative to others, sandy soils for instance create superiority in terms of permeability, uniformity of microbial deposition, and cementation [51].

**Sand:** Sand is another support that offers interconnected pathways for microorganisms to thrive and thus is widely used in bio-cementation. Its small uniform particle size means that it can be penetrated readily by nutrients and calcium ions and hence enhances the efficiency of bio-cementation [52] (Figure 2).

**Effect of nutrient concentration on bio-cement efficiency:** In essence, the nutrient concentration in the growth media greatly determines the efficiency of the bio-cement production [54].

**Optimal nutrient levels:** In the study, it was evident that appropriate nutrient concentrations are required for the growth of microbes and their rate of urease synthesis [52]. Practical concentrations include enough calcium ions and urea to let the bacteria effectively precipitate the calcium carbonate and biosynthesize bio-cement [55].

**Excess nutrients:** Excessive nutrients may cause microbes to grow out of the required amounts or could give undesirable byproducts, which may affect the process of bio-cementation [56].

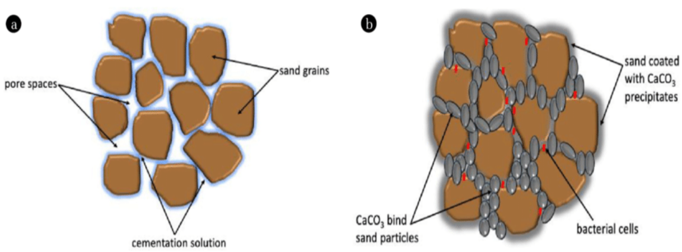


**Figure 2. a)** Microbial-Induced Calcite Precipitation (MICP) used in soil stabilization and bio-cementation, the initial state where the sand particles are separated with spaces filled by the solution and **b)** The process after bio-cementation, where bacteria produce $CaCO_3$, which binds the sand particles together [53].

**Low nutrient levels:** Low nutrient availability decreases microbial metabolism, retards the synthesis of urease, and decreases the deposition of calcium carbonate so lowering the cementation potentiality [57].

### Cementation rate and efficiency

The formation rate of bio-cement and cementation efficiency depends on such factors as the microbial growth rate, the rate of calcium carbonate precipitation, and other features of the environment and the substrate.

**Microbial growth rate and cementation speed:** The rate that microorganisms particularly the ureolytic bacteria grow is essential in controlling the speed and rate of calcium carbonate precipitation. Bacterial growth is however faster under conditions of increased nutrient concentrations to also enhance the synthesis of urease consequently increasing the rate at which the urea is hydrolyzed and the formation of calcium carbonates [19].

**Rapid growth conditions:** Only when conditions are ideal (temperature, pH, and nutrients) do bacteria increase in number, thus elaborating large amounts of urease and accelerating deposition of calcium carbonate [25].

**Slow growth conditions:** At average temperatures, the rate of bacterial growth decreases causing a decrease in urease production and a decrease in degree of cementation [58].

**Factors enhancing cementation efficiency** [40]

**Controlled nutrient supply:** Mineral nutrition by maintaining a constant feed of nutrients such as calcium ions and urea in the aquatic environment can improve bacterial reactions and cementation adequacy.

**Optimal environmental conditions:** Minerals, nutrients, some ions, the right temperature, pH, and moisture enhance the rate of bacterial growth and calcium carbonate deposits.

**Bacterial concentration:** The presence of more bacterial density in the substrate enhances the increased rate of calcium carbonate precipitation and enhances cementation efficiency.

**Factors hindering cementation efficiency** [59]

**Toxic by-products:** High concentrations of ammonia in solution generated from urea, through hydrolysis, can produce toxic conditions favorable for bacteria, thereby, limiting cementation.

**Imbalanced nutrients:** The introduction of excessive or inadequate concentration of cementation-promoting ions or molecules such as calcium ions and urea interferes with the cementation process and hence the complete formation of cement.

**Unfavorable environmental conditions:** The microbial growth of bio-cement is affected by extreme temperatures, non-optimal pH, and moisture extremes.

## Applications of Microbial Bio-cement

Microbial bio-cement is fast becoming an innovative material with wide-ranging uses in construction, technology, and environmental management. Since microorganisms possess the special property of precipitating calcium carbonate the use of bio cements is manifold, which includes repairing structures, stabilizing soil, and reducing carbon dioxide emissions.

### Construction and repair of structures

One of the most exciting uses of microbial bio-cement is in the construction of self-healing concrete. Here the bacteria belonging to the group of *S. pasteurii* are introduced into the concrete mix during the concrete preparation step. These microorganisms remain inactive until the appearance of cracks and when water penetrates in, the bacteria become active [60]. Once activated, the bacteria bring into play the bio-cementation process where the cracks are closed off by the calcium carbonate buildup [61].

**Mechanism:** When water infiltrates the structure, it dissolves the nutrients that are into the concrete matrix which is calcium lactate, and bacteria utilize the calcium lactate to form calcium carbonate [62]. This mineralized calcium carbonate displaces at the cracks and corners and restores mechanical properties of the concrete [63].

**Application in repairing cracks in existing infrastructure:** Besides self-healing concrete, microbial bio-cement is also used to repair cracks in existing structures. The former involves the use of bio-cement through the injection of cracks in concrete, where various microorganisms are used to generate calcium carbonate to fill the gaps as an enhancement mechanism of the concrete material [2].

**Process:** Microorganisms especially when mixed with a nutrient solution featuring urea and calcium ions are injected into the cracks. The bacteria then stimulate calcification that deposits mineral in the crack and toughens up the outer part of rock formation.

**Advantages:** This method is especially applicable when it comes to repairs of the crack in the areas which are difficult to reach, and in structures that are impossible or very expensive to replace like bridges, tunnels, and other historical structures [64]. They provided a green approach to several repairs, which are commonly achieved through energy-consuming processes or the utilization of synthetic materials [65].

### Soil stabilization

Bio-cement is widely applied in the context of soil stabilization with a focus on enhancing the mechanical characteristics of the soil needed for construction undertakings. MICP enhances the aggregation of the soil particles through the deposition of calcium carbonate along the particle surface resulting in improvements in the shear strength of the soil and decreased permeability [66].

**Application:** Bio-cement is typically applied in geotechnical practice where weak and soft foundation materials that are unable to bear loads needed for construction are present [67]. Compared to traditional Portland cement and Chemical cement, bio-cement makes it safer to construct on tricky and weak ground that otherwise may demand modification or reinforcement [68].

**Prevention of soil erosion and increasing ground stability:** Besides improving the strength of the soil, microbial-mineral bio-cementation is also useful in preventing soil slips and landslides and strengthening slopes, embankments, and coasts. This can be done basically by clogging the loose and free-moving particles of soil to ensure that they do not easily wash away by forces of wind, water, or people using some tools [69].

**Application:** Bio-cement has applications in reinforcing and stabilizing slopes on and near highways, stabilizing earth fill embankments near water bodies, and preventing coastal erosion [70]. It is most advantageous in areas that are ordinarily affected by landslides or erosion by the effect of rainfall or storm surges by the sea [71].

### Environmental and sustainability benefits

Another striking environmental factor influencing microbial bio-cement is that microbial bio-cement even though resource intensive has a lower value of $CO_2$ emissions compared with regular cement production [72]. The conventional process of cement making is combined with high energy consumption due to the calcination of limestone at a high temperature, about 1450 ℃, causing a big emission of $CO_2$ [73]. While the above cements are manufactured at high temperatures, often with the use of energy-laden processes, bio-cement is made at ambient conditions through microbial-induced calcium carbonate precipitation [74].

**Carbon footprint:** For bio-cement production, no fossil fuels need to be burned and no carbon is released during limestone calcination hence much lower $CO_2$ emissions associated with bio-cement [75]. This makes it a greener product and an appropriate solution for reducing the carbon footprint, which is a significant goal of the construction industry, and which takes up to 8% of the world's $CO_2$ emissions share [76].

**Sustainability:** Consequently, microbial bio-cement mitigates the built environment by diminishing the amount of traditional cement needed, for which the construction industry is currently hard-pressed to find ways to minimize $CO_2$ emissions globally [38].

**Waste management and recycling applications:** In the same way, bio-cement has applicability prospects in the waste management and recycling segment of the construction industry [12]. The process can be optimized to use all kinds of wastes for microbial growth and use the end product to produce constructional materials from industrial wastes or agricultural raw materials [77].

**Recycling waste:** For example, in bio-cement processes, waste material can be used as a source of calcium for microbial precipitation including fly ash (from coal combustion), slag (from steel production), and rice husk ash (from agricultural waste) [78]. This not only keeps waste out of landfills but also offers a cheap source of feedstock in bio-cement manufacturing [79].

**Circular economy:** It is a green technology because material waste is converted into useful input the manufacture of bio-cement through the circle economy [80]. This creates less dependence on new raw materials and helps in cutting down the hazards of the construction business as well as the waste disposal industry [81].

## Future Directions and Research Gaps

With microbial bio-cement being a relatively young field of study, several directions for further research and development can further improve the efficiency and economy of bio-cement production. Increasing its efficiency in terms of the current limitations and researching new opportunities will be important for bio-cement to become a commonly used product in construction and the environment.

### Genetic engineering of microorganisms

Among all the strategies identified as potentially enhancing bio-cement production in the future, the idea of optimizing microorganisms through genetic manipulation has been underlined. Though using naturally occurring bacteria like *S. pasteurii* is beneficial with a high rate of bio-cementation, an even higher rate can be produced by genetically manipulating the bacteria.

### Advancements in genetic engineering for cementation efficiency

**Enhanced urease activity:** The key enzymatic process in the MICP process is urease and it was discussed that the expression of it can be genetically enhanced to produce a higher yield. If the genetically engineered bacteria are built to have higher urease activity then the peptide sequence and their ability to hydrolyze urea enhanced the precipitation of calcium carbonate [32].

**Tolerance to environmental stress:** Engineering microorganisms to tolerate extreme ecological pressures including high or low temperature, pH, or salinity would also increase the applications of bio-cement [37]. This would make microbial bio-cementation feasible in different climates and geographical regions all over the world [82].

**Targeted cementation:** Other modifications may also help the microorganisms to form calcium carbonate more selectively and to cement only those parts of a structure or substrate that are desired to be reinforced with the substance. It may be especially beneficial in such areas where crack repair or soil stabilization is needed [83].

### Development of new microbial strains

One area for future work is the identification of new microbial species, which include bacteria, fungi as well as algae that can promote bio-cementation. Although previously, ureolytic bacteria such as *S. pasteurii* have been in focus of researchers in the construction of bio-cementing materials, other microorganisms might have potential benefits that have not been explored yet.

### Exploration of bacteria

**Non-ureolytic bacteria:** Some of the other bacteria that do not precipitate calcium carbonate through urea hydrolysis like the organic acidic bacteria could be useful in bio-cementation [84]. These bacteria might minimize the formation of ammonia which is an undesirable by-product of urea hydroxylation and therefore they are environmentally friendly [85].

**Anaerobic bacteria:** For bio-cement production, it is therefore possible that anaerobic bacteria could be produced in environments that contain negligible amounts of oxygen. Studies of these microorganisms might lead to a vast expansion of uses of the species for construction work in underwater or underground structures [86].

### Fungi

**Fungal species:** Some fungi have shown the capability of copper precipitation by secreting organic acids. Fungi could be used on bio-cement applications in which bacteria may not thrive, perhaps in more acidic environments or in substrates where organic matter is likewise plentiful [87].

### Algae

**Microalgae:** Specifically in aquatic environments, microalgae can precipitate calcium carbonate as part of their metabolic processes [88]. However, the use of microalgae in marine or coastal bio-cementation could represent new ways to protect coastal areas, underwater construction, and reforestation of coral reefs [89].

### Integration with other technologies

Other than bio-cementation, researchers have looked at how to combine bio-cementation with other sustainable construction technologies for a further enhanced impact. Adding complementary materials and processes to bio-cement could improve the overall sustainability of construction projects.

### Combining bio-cement with other sustainable materials

**Green concrete:** Other eco-friendly construction materials, like green concrete (which employs recycled aggregates and industrial by-products), can be combined with bio cement to further reduce the environmental impact of construction [90]. Adding bio-cement to these materials would create even more sustainable building solutions.

**Recycled materials:** Fly ash or slag can be used as substrates for bio-cement. Future research could focus on finding new recyclable ways of industrial and agricultural by-products with bio-cement to create low-impact, high-performance building materials [91].

### Potential for integration with 3D printing in construction

**This is an exciting frontier in sustainable construction:** The integration of bio-cement with 3D printing technologies is already changing construction by reducing material waste and energy consumption and leveraging this to further revolutionize eco-friendly construction 3D printing [92].

**Structures with bio-cement:** 3D printing buildings and infrastructure layer by layer would be low environmental impact difficult if not impossible, by using bio-cement in the process [26]. 3D printing could be used as a complementary precision and customizability for the sustainable properties of bio-cement and enable the production of complex and efficient structures with progressively lower emissions of carbon [93].

### Addressing environmental concerns

Although microbial bio-cement has high environmental benefits over conventional cement production, there are still environmental issues that need to be solved to make this solution sustainable in the long haul.

**Ongoing research on environmental impact:** Understanding the wider environmental impact of the bio-cementation process is one of the pivotal areas for research. Although bio-cement production results in lower $CO_2$ emissions, it is important to investigate the potential long-term environmental effects of large-scale microbial use, including

**Ecosystem disruption:** Releasing large amounts of microorganisms into the environment might disrupt the local ecosystem [94]. There is still work to be done on the risk and what ways there might be to mitigate any potential bad outcomes.

**Ammonia emissions:** Ammonia is a by-product of urea hydrolysis in the MICP process. Providing bio-cement production enables ammonia emissions, from bio-cement production to be expressed, which can lead to environmental pollution, so future research should work toward reducing or mitigating ammonia emissions during bio-cement production [95].

**Enhancing biodegradability and reducing energy consumption:** Additionally, it has been shown that bio-cement has encouraged biodegradability and that its energy consumption can be further reduced to render the process more environmentally sound.

**Biodegradable substrates:** Biodegradable substrates that can be used in microbial bio-cement production would develop to minimize waste and environmental impact [12].

**Reducing energy use in microbial cultivation:** Although bio-cement production is less energy inefficient than traditional cement production at first, cultivating microorganisms still eats energy. More energy-efficient cultivation methods, such as using renewable energy sources or growing conditions optimal for bio-cementation could also further reduce the carbon footprint of bio-cementation [96-100].

## Conclusion

Natural Microorganisms (MICP) are critical to bio-cement generation through natural processes, thereby replacing conventional cement with an eco-friendly process. Calcium carbonate precipitation capabilities of bacteria, fungi, and algae provide sustainable solutions to construction addressing reduced carbon emissions and energy consumption. As a concrete repair, self-healing material, and also as a stabilizing agent for soil, bio cement holds great promise for industry adoption. But, there are hurdles to overcome scaling production, bringing down costs, and answering concerns about ammonia emissions and possible ecosystem effects. The future of bio cement looks bright, ongoing research related to bio-cement is focused on the genetic engineering of microorganisms for increased efficiency, identifying new microbial strains, and integration of bio-cement with next-generation advanced construction technologies such as 3D printing. With the world's construction industry pursuing sustainability, bio-cement has become a revolutionary sort of material that could revolutionize building practices and help propel environmental sustainability.

## Ethical Approval

This article does not contain any studies with human participants or animals performed by the author.

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