

Recent Advancements in Bioconcrete Technology

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Abstract: *The present paper is an elaboration of the current study of bioconcrete technology with the applications of urease-producing microbes to produce stronger and more durable bioconcrete in the future. This is performed through microbiologically induced calcite precipitation (MICP) to restore concrete structures with the help of biomineralization of CaCO₃ using microbes. The main objective of the review is to discuss the applications of microbes to improve the quality of cement concrete. The review also discusses the biotechnology of bioconcrete production, as well as the physicochemical and bioengineering properties of bioconcrete in the light of recent research carried out in this field.*

Keywords: bioconcrete, microbes, biomineralization, self-healing, calcite precipitation

INTRODUCTION

The present paper deals with the study of bioconcretes prepared with the applications of urease-producing microbes like bacteria, fungi, and microalgae to produce rather more powerful and strengthen calcium carbonated bioconcretes in the future. This is also called microbiologically induced calcite precipitation (MICP). This could be a novel strategy to restore concrete structures with the help of biomineralization of CaCO₃ using microbes in the future.

Though cement concrete is a strong, relatively cheap, and one of the most commonly used building materials, it has been deteriorated well before the end of its service life, mostly due to cracks formed in them. These cracks are formed as a result of tensile forces like drying, shrinkage, and some undesirable abiotic external forces. These cracks weaken the concrete mechanically, allowing water to percolate and deteriorating them slowly (Jonkers 2011).

Keeping this view in mind, as nature is doing it, numerous investigations are being conducted to create concrete the way nature does with microorganisms. In natural environments, biomineralization of minerals takes place naturally. This is observed in both prokaryotic and eukaryotic microorganisms, as well as in materials like leaves containing latex, cystoliths, calcium oxalates, carbonates, silicates, and so on. Hence, bioconcretes may be regarded as a suitable alternative to cement concrete (Stock-Fischer et al. 1999).

The added microbes into concrete produce copious amounts of minerals required to plug the freshly formed cracks (Ferris and Stehmeier 1992). Simultaneously, it autonomously decreases the permeability and increases the durability of the concrete matrix. It would thus save not only the time and labour of manual inspection and repair but also wealth in the future (Jonkers 2011).

This is an interdisciplinary research comprising the study of bioconcrete technology, incorporating living microbes with some abiotic materials to improve the quality of concrete and to fill the cracks successfully in the future. Currently, this microbial concrete technology has become very popular because of its eco-friendly nature.

The self-healing capabilities and durability of building materials have increasingly become popular in the fields of bio-geo-civil engineering to improve the desired infrastructures of construction and concrete technology.

Furthermore, it appears that this is one of the most important multidisciplinary works done so far in the fields of microbiology with civil engineering. This could be a novel strategy to restore concrete structures with the help of biomineralization of CaCO_3 using microbes in the future. Attempts are being made to apply this eco-friendly microbial technology to obtain more sustainable, cheap, and durable building materials. We hope this technology will soon provide us with high-quality building materials that will not only be cost-effective but also environmentally safe. However, more work is required to improve this technology from both an economical and practical point of view.

The present review is an attempt to describe the role of various microbes like bacteria, fungi, and microalgae in bioconcretes. It discusses the scope of calcite mineralization in bioconcretes, designing the new bioengineering properties of bioconcretes for the evaluation of concrete quality in the future. It also describes the biotechnology of bioconcrete production and their

physicochemical and bioengineering properties in the light of recent research carried out so far in the same field.

METHDOLOGY

The present paper is prepared on the basis of researches done so far in the field of bioconcrete technology. Several research papers were consulted in order to explore the facts found therein and have been discussed in the light of recent researches.

DISCUSSION

As environmental activities influence soil in one way or another, the various microbes present in soil are able to convert the soil texture, thereby improving its strength by precipitating carbonates to bind the soil particles. Biogenic microorganisms such as bacteria, fungi, and microalgae are omnipresent in nature and are capable of precipitating calcium carbonate. *Sporosarcina pasteurii* is one of the most widely studied bacteria in this context. In addition, myxobacteria, which are usually found in soil, can also efficiently precipitate calcium carbonate (Fujita et al. 2000, Rodriguez et al. 2003, Chu et al. 2012, Sel et al. 2015) [5–8].

Further, diversified zones such as caves are known for their high humidity, calcium ions, and CO₂. These ingredients contribute to the biomineralization of calcium carbonate with the help of microbes. This is increasingly possible by introducing urea as a nutrient source, which promotes the precipitation of CaCO₃ in large amounts with the help of bacteria in caves (Li et al. 2010, Johnston et al. 2012, Zhu et al. 2013, Gherman et al. 2014, Okaya and Rodrigues 2015) [9-13].

In freshwater bodies, calcite precipitation can also take place with the help of cyanobacteria. Cyanobacterial sheaths act as lithified crusts around pebble-sized oncoid nodules on stream beds (Pedley 1993, Dupraz et al. 2009, Dittrich 2016)[4,14,15]. Although the mechanism of microbiologically induced calcite precipitation (MICP) in hypersaline lakes is still not very clear, extensive carbonate precipitation associated with microorganisms is quite common in such environments (Glunk et al. 2011) [16]. Cyanobacteria and sulphate-reducing bacteria (SRB) have been found to be involved in calcium carbonate precipitation in marine environments (Visscher et al. 2000, Schoon et al. 2010)[17, 18]. Similarly, travertine is a common form of limestone deposition in hot springs; however, its mechanism of formation is still debated (Fouke 2011). Nevertheless, cyanobacteria in combination with SRB have been found to be associated with this process (Tekin et al. 2000, Okumura et al. 2012) [20,21].

Microorganisms with their geochemical activities are found to be responsible for the deposition of minerals throughout the history of the Earth. Biomineralization is defined as the formation of minerals as a result of biological activities (Weiner and Dove (2003). Biologically mediated biominerals are formed as:

1. Biologically Controlled Mineralization (BCM)

(Where entire biomineralization processes are under the strict control of genes) .

2. Biologically Induced Mineralization (BIM)

(Where biological activity induces the formation of biominerals).

3. Biologically Influenced Mineralization or Organomineralization

(Where microorganisms are not directly involved, and precipitation is mediated by the organic fractions found in soils and sediments).

The various microorganisms found in nature are at least involved in one type of biomineralization (Madigan et al. 2015). Similarly, among minerals, calcite has been regarded as the most commonly encountered mineral (Skinner 2005). The aim of this discussion is to review the latest advances in the field of CaCO_3 microbial biomineralization.

Microbial carbonate precipitation (MCP) of biological origin plays a significant role in the precipitation of CaCO_3 are precipitated as a byproduct of metabolism of various microorganisms. The microbial cell walls as being the appropriate nucleation sites with negatively charged in nature attracted a variety of metallic ions. And, carbonates are easily precipitated, if and when Ca^{2+} ions are in adequate amounts (Warren et al. 2001, Fein 2006) [25,26].

As the mostly studied calcifications are either through photosynthesis or ureolysis, the photosynthetic microbes particularly the cyanobacteria have been found to be major microbes to produce CaCO_3 in nature. This is accomplished by the exchange of HCO_3^- and OH^- ions across the cell membranes (Miller and Colman 1980) [27]. Cyanobacterial stromatolites are found to be distributed in various environments (Rodriguez et al. 2012) [28].

Similar is the case with ureolytic microorganisms to hydrolyze urea to produce ammonia and carbon dioxide. The subsequent hydrolysis of ammonia produces OH^- that increases the pH. Further, the dissociation of carbonic acid takes place to produce bicarbonates. Finally, the presence of Ca^{2+} ions generated the CaCO_3 . Some of the various ureolytic microorganisms using urea as a nitrogen source are briefly discussed as under:

(a) Bacteria

Several bacteria have been found to be involved in the precipitation of CaCO_3 in bioconcretes. Some of them are given as under (Table 1) :

Table 1: Some of the bacteria involved in the biomineralization of calcite

Bacterial genus	Species involved in calcite biomineralization
Bacillus	<i>Bacillus subtilis</i>
Bacillus	<i>Bacillus sphaericus</i>
Bacillus	<i>Bacillus sp. CT-5</i>
Bacillus	<i>Bacillus pseudofirmus</i>
Bacillus	<i>Bacillus megaterium</i>
Bacillus	<i>Bacillus licheniformis</i>
Bacillus	<i>Bacillus flexus</i>
Bacillus	<i>Bacillus cohnii</i>
Bacillus	<i>Bacillus pasteurii</i> (<i>Sporosarcina pasteurii</i>)
Lactobacillus	<i>Lactobacillus reuteri</i>
Lactobacillus	<i>Lactobacillus animalis</i>
Lactobacillus	<i>Lactobacillus fermentum</i>
Staphylococcus	<i>Staphylococcus epidermidis</i>
Streptococcus	<i>Streptococcus mitis</i>
Streptococcus	<i>Streptococcus salivarius</i>
Streptococcus	<i>Streptococcus thermophilus</i>
Geobacillus	<i>Geobacillus thermoglucosidasius</i>
Shewanella	<i>Shewanella sp.</i>
Myxobacteria	<i>Myxococcus xanthus</i>
Proteus	<i>Proteus mirabilis</i>
Proteus	<i>Proteus vulgaris</i>
Actinomycetes	<i>Streptomyces aureofaciens</i>

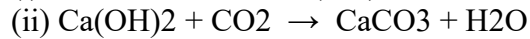
(Rodriguez et al. 2003, Ghosh et al. 2005, De Muynck et al. 2007, Ramakrishnan 2007, Jonkers et al. 2010, Jimenez et al. 2007, Naoto et al. 2010, Achal et al. 2011, Taliaekhzan et al. 2014, Andalib et al. 2016, Fatma et al. 2016, Zhu and Dittrich 2016, Anitha et al. 2018, Kunamneni and Meena 2018, Mohammad Rizwan et al. 2018) [6, 15, 29–41].

Bacterially Induced Crack Repair Technique

Bacterially induced crack repair has been developed as an alternative to conventional cement concrete repair methods. This technique is well documented in several studies conducted so far in

the same field (Fatma et al. 2016, Anitha et al. 2018, Kunamneni and Meena 2018)[38-40]. It is accomplished through the formation of calcium carbonate (CaCO_3) with the help of several ureolytic bacteria. These bacteria have been successfully applied to seal cracks in the recent past. Owing to their sufficient compatibility with concrete compositions, CaCO_3 is often regarded as the most appropriate filler for concrete cracks.

The bacterial degradation of urea increases the pH, facilitating the synthesis of calcium carbonate locally in a calcium-rich environment (Monishka and Nishanth 2017)[57].



Mechanism of Crack Healing

As water seeps inside the cracks formed naturally in cement concretes, it activates the dormant bacterial spores. Once these spores come into contact with water, their physiology changes from a dormant to an active state, leading to germination. Meanwhile, the bacteria synthesize crystals of CaCO_3 on their cell surfaces as well as on the surfaces in and around the cracks, thereby sealing them simultaneously (De Muynck et al. 2007, Zhu and Dittrich 2016, Kunamneni and Meena 2018, Mohammad Rizwan et al. 2018) [15, 30, 40, 41].

(b) Fungi

The following is a list of some fungi involved in the biomineralization of calcite (Table 2)

Table 2: Some of the fungi involved in the biomineralization of calcite

Fungi involved in the biomineralization of calcite	
Fungal genus	Species involved in calcite precipitation
Aspergillus	<i>Aspergillus niger</i>
Aspergillus	<i>Aspergillus nidulans</i>
Neurospora	<i>Neurospora crassa</i>
Cadophora	<i>Cadophora interclivum</i>
Beauveria	<i>Beauveria dimorpha</i>
Serpula	<i>Serpula himantoides</i>
Myrothecium	<i>Myrothecium gramineum</i>
Pseudophialophora	<i>Pseudophialophora magnispora</i>
Coprinus	<i>Coprinus</i> sp.
Morchella	<i>Morchella</i> sp.
Ustilago	<i>Ustilago</i> sp.

Trichoderma	<i>Trichoderma reesei</i>
Piloderma	<i>Piloderma fallax</i>
Acidomelania	<i>Acidomelania panivicola</i>
Cephalotrichum	<i>Cephalotrichum</i> sp.
Umbelopsis	<i>Umbelopsis dimorpha</i>
Colletotrichum	<i>Colletotrichum acutatum</i>

(Gharieb et al. 1998, Fomina et al. 2005, Burford et al. 2006, Masaphy et al. 2009, Tazoun and Arocena 2009, Li et al. 2014, 2015 & 2018, Zhu and Dittrich 2016, Ting Luo et al. 2018, Mohammad Rizwan et al. 2019) [15, 42–51].

Fungal Biomineralization of CaCO₃

While bacteria promoted mineral precipitation only via induced biomineralizations, the fungi can do it through both induced biomineralization and organomineralization processes. This is demonstrated that similar to bacteria, the fungi can also induce CaCO₃ biomineralizations mainly influenced by two factors as the carbonate alkalinity and the concentration of calcium ions (Dupraz et al. 2009, Hou et al. 2013, Li et al. 2014, 2015 & 2018) [4,47–49,52].

Similar experiments have demonstrated the fungal biomasses exposed to significant concentration of Ca²⁺ leads to CaCO₃ biomineralization and organomineralization processes. However, there is no in fact, in situ studies exist so far. Therefore, further studies only supported by the fact, what is presently known to us about the involvement of fungi in biomineralization of CaCO₃ so far (Ahmad et al. 2004, Mohammad Rizwan et al. 2019) [51,53].

(c) Photosynthetic Microorganisms (Microalgae)

The following microalgae have been documented in the biomineralization of calcite, and most of them are being used in the preparation of bioconcretes (Tabel : 3).

Table 3: Some of the microalgae involved in the biomineralization of calcite

Group	Genus	Species
Chlorophyceae (Green Algae)	<i>Chlorella</i>	<i>Chlorella vulgaris</i>
	<i>Dunaliella</i>	<i>Dunaliella salina</i>
	<i>Mychonastes</i>	<i>Mychonastes</i> sp.
	<i>Muriellopsis</i>	<i>Muriellopsis</i> sp.

	<i>Haematococcus</i>	<i>Haematococcus pluvialis</i>
Rhodophyceae (Red Algae)	<i>Porphyridium</i>	<i>Porphyridium cruentum</i>
Eustigmatophyceae	<i>Nannochloropsis</i>	<i>Nannochloropsis oculata</i>
Cyanophyceae (Cyanobacteria)	<i>Synechococcus</i>	<i>Synechococcus</i> sp.
	<i>Synechocystis</i>	<i>Synechocystis</i> sp.
	<i>Scytonema</i>	<i>Scytonema</i> sp.
	<i>Spirulina</i>	<i>Spirulina platensis</i>
		<i>Spirulina maxima</i>
	<i>Arthrospira</i>	<i>Arthrospira platensis</i>
	<i>Pinocyanobacteria</i>	<i>Pinocyanobacteria</i> sp.
	<i>Coccochloris</i>	<i>Coccochloris peniocyctis</i>
	<i>Nannochloris</i>	<i>Nannochloris atomus</i>
	<i>Anabaena</i>	<i>Anabaena cycadae</i>
		<i>Anabaena cylindrica</i>
		<i>Anabaena doliolum</i>
		<i>Anabaena variabilis</i>
	<i>Anacystis</i>	<i>Anacystis nidulans</i>
	<i>Nostoc</i>	<i>Nostoc calcicola</i>
		<i>Nostoc muscorum</i>
Actinobacteria	<i>Brevibacterium</i>	<i>Brevibacterium ammoniagenes</i>

(Miller and Colman 1980, Castanier et al. 1999, Hasan 2000, Dittrich et al. 2004, Giordano et al. 2005, Harun et al. 2010, De Muynck et al. 2010, Ramnan et al. 2010, Kumar et al.2011, Perez-Garcia et al. 2011, Ariyanti et al. 2012, Zhu and Dittrich 2016, Sweety and Marjadi 201, Zhu et al. 2018, Mohammad Salim et al. 2019) [15, 27, 54–66].

Microalgae in biocementation

While research conducted so far in the field of microalgae as biocementation is still in a juvenile stage, it has shown great potential. Microalgae naturally consume CO₂ and can metabolically

hydrolyze urea to produce calcium carbonates with the help of the urease enzyme. These organisms are renewable resources whose availability can be easily maintained. Further research is required to produce good-quality biocement (Ariyanti et al. 2011 & 2012, Mohammad Salim et al. 2019) [63, 66, 67].

Microbiologically Induced Calcite Precipitation (MICP) Applications

Further, it appears that MICP has not been fully exploited. Photosynthesis, ureolysis, ammonification, and denitrification-induced carbonate precipitation have commercially been utilized in a variety of applications, such as fillers, binding materials, restoration of construction materials, biogroups, restoration of stone monuments, and altering the fluorescence properties, as well as for the removal of heavy metals and radionuclides (Reeder et al., 1991; Yoshida et al., 2010; Sarayu et al., 2014; Erşan et al., 2015) [68–71].

Moreover, some of the denitrifying facultative anaerobic bacteria, such as *Micrococcus*, *Bacillus*, *Denitrobacillus*, *Pseudomonas*, *Spirillum*, and *Thiobacillus*, are able to reduce nitrate. This process increases the pH by consuming H^+ ions, thereby promoting the precipitation of $CaCO_3$. Unfortunately, very few studies have been conducted to establish a direct link between denitrification and calcium carbonate precipitation (Erşan et al., 2015b; Zhu and Dittrich, 2016) [15, 71].

Similarly, ureolytic microbial carbonate precipitation has recently been introduced to repair decaying monuments, statues, and buildings. Bioconcrete and biogroups are produced using bacteria such as *Bacillus cereus* and *Myxococcus xanthus*, which are popular for this purpose. In bioconcretes, cracks are filled naturally as bacterial spores germinate and produce calcite when water seeps inside. These bacterial spores are added to cement concrete in association with clay pellets (Castanier et al., 2000; Rodriguez et al., 2003; Ramakrishnan, 2007; Jonkers and Schlangen, 2009; Zhu and Dittrich, 2016) [6, 15, 37, 72, 73].

Several other bacteria, such as myxobacteria, have also been found to induce the formation of biominerals and are being explored in connection with bioconcrete technology. *Myxococcus xanthus* has the ability to induce the formation of a variety of minerals, including carbonates such as calcite and vaterite, phosphates such as struvite, schertelite, and newberyite, and sulphates such as taylorite and barite (Jimenez et al., 2007) [33].

Additionally, *Myxococcus xanthus*, through the ammonification of amino acids, produces CO_2 and NH_3 . The subsequent hydrolysis of NH_3 leads to the formation of calcium carbonates (González-Muñoz et al., 2010) [74]. A similar mechanism is observed in sulphate-reducing bacteria, where sulphate is reduced to sulphide while carbon is oxidized to bicarbonate (Baumgartner et al., 2006; Braissant et al., 2007) [75, 76]. Furthermore, the anaerobic oxidation of

methane also favours the precipitation of CaCO_3 (Kroos et al., 1986; Jimenez et al., 2007; Reeburgh, 2007) [73, 77, 78].

Advantages and Drawbacks of Bioconcrete

Biocementation is a process used to produce binding materials based on the mechanism of microbiologically induced carbonate precipitation (MICP) (Redoute et al. 2012). Bioconcrete has now been proven to be a better binding material. Some of the advantages of microbial concretes are described as follows:

Advantages:

- √ Bioconcretes are eco-friendly construction materials.
- √ This process is pollution-free and natural.
- √ It is a kind of self-healing (autonomous) mechanism without any external aid.
- √ It does not require extra construction work.
- √ The process is more efficient.
- √ It requires a much shorter time.
- √ The process takes place at a much faster rate.
- √ Significant increase in compressive strength.
- √ Significant increase in flexural strength.
- √ It reduces the permeability and increases the durability of concrete.
- √ To make the process more economical, microbes may be cultured using industrial by-products such as lactose mother liquor and corn steep liquor as nutrient sources.
- √ Scientists have also developed a liquid formulation to fix cracks in conventional concrete.

Drawbacks:

However, there are some drawbacks. The cost of microbial concrete is approximately double that of conventional concrete. The cost of bioconcrete technology is slightly higher because it has not yet been implemented on a large scale. Nevertheless, the cost could be reduced by using substitutes for the main ingredients, such as corn steep liquor and lactose mother liquor. Likewise, instead of pure microbial cultures, mixed or non-axenic microbial cultures should be developed (Achal et al. 2009 & 2010, Silva et al. 2015) [80–82]. Another concern associated with the use of bioconcrete is the potential for the uncontrolled growth of microorganisms, which may lead to several environmental problems (Dei and Chelazzi 2013, Perito et al. 2014, Zhu and Dittrich 2016) [39, 83, 84].

Environmental and Health Concerns of MICP

While the urease enzyme plays a key role in the precipitation of CaCO_3 in concrete, the process also produces ammonia, which has already been documented as a pollutant causing numerous

health problems in humans. Similarly, it also causes deterioration of monumental stones (Sutton et al. 2008, De Muynck et al. 2013) [85, 86]. Similarly, during the course of denitrification, if reactions occur incompletely, a powerful greenhouse gas (GHG) such as nitrous oxide (N₂O) is produced. N₂O has a global warming potential approximately 300 times greater than that of CO₂ (Alley et al. 2007, Change 2007) [87, 88].

Further, the emission of hydrogen sulphide (H₂S) gas during the process, due to the involvement of sulphur-reducing bacteria (SRB), has been found to be highly toxic to human health, causing various ailments and diseases such as nausea, vomiting, corneal ulceration, keratoconjunctivitis, olfactory nerve ending fatigue, rhinitis, bronchitis, and pulmonary edema. At concentrations between 50 ppm and 300 ppm, H₂S may also cause cardiopulmonary arrest, unconsciousness, and even death when these concentrations exceed 1000 ppm.

CONCLUSION

The present review deals with the study and scope of bioconcretes prepared with the applications of urease-producing microorganisms to produce rather more powerful and strengthened calcium carbonate-based bioconcrete in the future. The review discusses the overall methodology of bacterial, fungal, and algal cultures, the biotechnology of bioconcrete production, and their physicochemical and bioengineering properties in the light of recent research carried out in this field.

The concretes mixed with urease-producing microorganisms, just to improve the quality and strength of concretes, are called bioconcretes. Recently, it has been observed that this technology has some extra capabilities to change the scenario of concrete technology in the future. This is usually referred to as microbial biologically enhanced crack remediation (MECR) or microbiologically induced calcite precipitation (MICP) (Meldrum 2003, Dick et al. 2006, Vijay et al. 2007, Gavimath et al. 2012) [89–92].

Cement concrete is a strong, relatively cheap, and one of the most commonly used building materials; however, it deteriorates well before the end of its service life. In recent years, a novel technology has been proposed to restore cement concrete structures via the biomineralization of calcium carbonate with the help of ureolytic bacteria such as *Bacillus subtilis*. The results revealed that infrastructures made of bioconcrete enhance the durability of building materials (Pradeep et al. 2015, Alshalif et al. 2017, Kunamineni and Meena 2018, Mohammad Rizwan et al. 2018 & 2019) [40, 41, 93, 94].

Further, cement concrete can withstand compressive forces very well but not tensile forces. When it is subjected to tension, it starts to crack. These microcracks in concrete are sometimes formed naturally as a result of abiotic phenomena such as drying, shrinkage, and external forces. These

microcracks weaken the concrete mechanically, allowing water to percolate and deteriorate the structures slowly (Jonkers and Schlangen 2009, Jonkers 2011) [1, 73].

As the qualities of bioconcretes mainly depend upon the types of microorganisms they contain, more microbes perpetuate accordingly to fill the cracks. The specific chemical reactions of bioconcrete with bacteria, fungi and photosynthetic microbes strengthen the whole material in such a way that it fills and tighten the gap successfully. In addition, the added microbes into concrete products copious amounts of minerals required to plug the freshly formed cracks. Simultaneously, it autonomously decreases the permeability and increases the durability of concrete matrix. It would thus save not only the time and labour of manual inspection and repair but money in future also (Ramchandran et al. 2001, Bujord et al. 2006, Magaphy et al. 2009, Jonkers et al. 2010, Frigyanti et al. 2011 & 2012, Li et al. 2011 & 2015, Bindschdler et al. 2016, Ting and Luo et al. 2018, Krishnamoorthi and Meena 2018) [32,40,44,45,47,48,50,63,67,95,96]. Similarly, the scientists working in the same field have also discovered ecofriendly biogrouts. These are self-healing binding materials especially containing bacteria for self healing cracks and probe to improve the mechanical properties and impermeability of any particular system(Bang et al. 2001, Van Tittelboom et al. 2010, Pradeep et al. 2015) [93, 97, 98].

This is a kind of interdisciplinary research work discussed with the study of composition, types, qualities and the scope of bioconcrete and biogrouts incorporating living microorganisms with some abiotic materials to improve the quality of concrete technology and to fill the cracks successfully in future. This is quite a new technology to restore the concrete structures with the help of biomineralization of CaCO_3 using microbes in future.

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Conflict of interest

The authors have declared no conflicts of interest. Compliance with ethical standards:

Ethical clearance

The article does not contain any studies involving either human or animal subjects.