

Bibliographic survey and comprehensive review on mechanical and durability properties of microorganism based self-healing concrete

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ABSTRACT Concrete is the most widely utilized material for construction purposes, second only to water, in the ever-increasing need for construction globally. Concrete is a brittle material and possesses a high risk of crack formation and consequent deterioration. Cracking, which allows chemicals to enter and can cause concrete structures to lose their physico-mechanical and durability features. Repairing and rehabilitating concrete structures involves high costs and leads to various repair methods including coating, adhesives, polymers, supplementary cementitious materials (SCMs), and fibers. One of the latest technologies is the use of microorganisms in concrete. These added microorganisms lead to calcite precipitation and thereby heal the cracks effectively. This study presents a comprehensive literature survey on bacteria-included concrete, before which a bibliographic survey is performed using VOSViewer software. In addition to regular bacterial concrete, this study focuses on also using SCMs and fibers in bacterial concrete. A detailed literature review with data representation for various mechanical properties including compressive strength (CS), split tensile strength (SS), and flexure strength (FS), along with durability properties including carbonation, water absorption, resistance against chloride ion penetration, gas permeation, and resistance against cyclic freeze-and-thaw is presented. A study on the use of X-ray computed tomography (XCT) in bacterial concrete is highlighted, and the scope for future research, along with identification of the research gap, is presented.

KEYWORDS bacterial concrete, bibliographic survey, biomineralization, carbonation, fibers

1 Introduction

One of the main reasons for concrete deterioration is cracking, which allows the ingress of moisture and chemicals into the concrete, thereby reducing its durability and physico-mechanical strengths. The addition of polymeric resins (such as epoxy, polyester, and vinyl ester), adhesives [1], and autogenous healing of unreacted cement particles, usually in the ultra-high performance concrete (UHPC) structures [2], are just a few of the significant repair approaches used in recent decades. Alternative binding agents (such as alkali-activated compounds) [3–8] and waterproofing chemicals [1] have also been used. However, there are many situations where choosing the best repair method is difficult due to factors

such as high cost, high environmental impact, lack of coherent and homogeneous interfacial transition zone delamination problems (especially for polymer-based materials), and difficulties with actual on-site applications (such as handling a liquid activator for alkali-activated materials) [9].

Current investigations have yielded encouraging findings regarding the potential application of microbial calcification for resolving cracking and durability problems [10,11]. The primary mechanism due to embedded bacteria in concrete is based on the breakdown of urea and calcium to form calcium carbonate (CaCO_3), so that the material is frequently referred to as self-healing, bio- or bacterial concrete/microbial concrete/bio-cement/microbial-induced calcite precipitation cement [12]. The precipitation methods of bio- CaCO_3 vary according to different bacterial metabolic pathways.

Moreover, there are two main categories of autotrophic and heterotrophic CaCO_3 precipitation, both leading to the calcification and biomineralization of CaCO_3 [13,14]. Autotrophic bacterial action involves various processes such as non-methylotrophic methanogenesis, oxygenic, and anoxygenic photosynthesis. The nitrogen cycle, sulfur cycle and the utilization of organic carbon are the basis of pathways for heterotrophic precipitation. The nitrogen cycle involves numerous methods, like ammonification of amino acids, degradation of urea and uric acid and dissimilatory reduction of nitrates whereas sulfur cycle involves dissimilatory reduction of sulphates [15].

In accordance with studies [1,16], if bacteria are appropriately applied to the fractured area, they can seal cracks with a width section of 0.46 mm. Microorganism driven healing could be as much as four times more effective in filling micropores and repairing cracks than autogenous healing, utilizing unreacted cementitious materials, which can only repair cracks up to 0.1 mm wide [17,18]. Due to the filler effect of bacteria, bacterial concrete heals the microcracks and leads to a reduction in the porosity by an order of 28% to 50% [19,20] and an increase in the mechanical strengths (of the order of 42% and 72% for compressive strength (CS) and flexural strength (FS), respectively) [21,22].

Applying bacteria directly to the mixture, encapsulating bacteria in the mix to keep them latent until activation, and spraying or injecting bacteria onto the surface of the fractures are the three techniques used to apply microorganism-induced healing to concrete [23]. In each of these situations, a nearby supply of organic food, such as yeast extract [13] and a urea medium [24], is also necessary to provide the potential for the bacteria to develop and react.

The most common procedure for healing cracks in bacterial concrete involves the propagation of cracks, activation of bacteria, chemical reaction, precipitation of calcite, and finally sealing cracks. The bacteria can mainly be classified into three types, neutrophils (which exist and grow at a pH of approximately 7, e.g., *Escherichia coli*, *Staphylococci*, and *Salmonella* species), acidophiles (which optimally grow below pH of 5.5, e.g., *Lactobacillus*) and alkaliphiles (which grow best at a pH range of 8–10, e.g., *Bacillus subtilis*, *Bacillus firmus*, etc., and are suited to highly alkaline media such as concrete [25].

Though several papers were found showing exhaustive data on bacterial concrete, research on the property-based analysis of concrete is rare. There are few comprehensive examinations of the material qualities, mechanical properties, permeability properties, resistance against carbonation and cyclic freeze–thaw and addition of bacteria percentage, to determine which would make the best concrete. In this analysis, we seek to explore connections between the relevant parameters of the raw material's physical and chemical qualities or its mechanical and

durability properties as well as to focus on the effect of bacteria on the properties of blended concrete and fiber-reinforced concrete. The study examines the literature and analyzes the current trend of research on bacterial concrete, making use of VOSviewer software, and is intended to be a reference for future researchers. The current research gaps are highlighted in the conclusion of the study.

2 Bibliographic analysis

The bibliographic analysis is carried out using VOSViewer software, and the data are obtained from the Scopus search engine. The data are obtained from the Scopus search engine using the keywords “concrete” and “bacterial concrete” and the filter is limited to the subject area “engineering”. From this search, only 589 documents have been found to date. The types of the 589 documents are classified in Table 1.

To analyze the data based on countries, the setting in the VOSviewer software is carried out by selecting the type of analysis as “bibliographic coupling” and the unit of analysis as “countries.” The visual network obtained based on this analysis can be termed country mapping (Fig. 1). Along with country mapping, a network can be visualized concerning yearly publications. It is found that the contribution of developed countries in the last five years is less than that of developing countries.

Sources encouraging research on using bacterial concrete as supplementary cementitious materials (SCMs) are analyzed. The top 15 journals with the highest number of documents, citations, and total link strength. It is observed that Construction and building materials published 36.6% of the total documents, and the total proportion of citations is about 56.0% (Fig. 2).

To framework the research adequately to cover all the significant factors, the choice of keywords is very important. To study this, the type and unit of analysis

Table 1 Classification Scopus data obtained based on documents type

Sr. No.	Document type	No. of documents
1	article	369
2	conference paper	139
3	book chapter	28
4	review	26
5	conference review	19
6	short survey	3
7	erratum	2
8	report	2
9	book	1

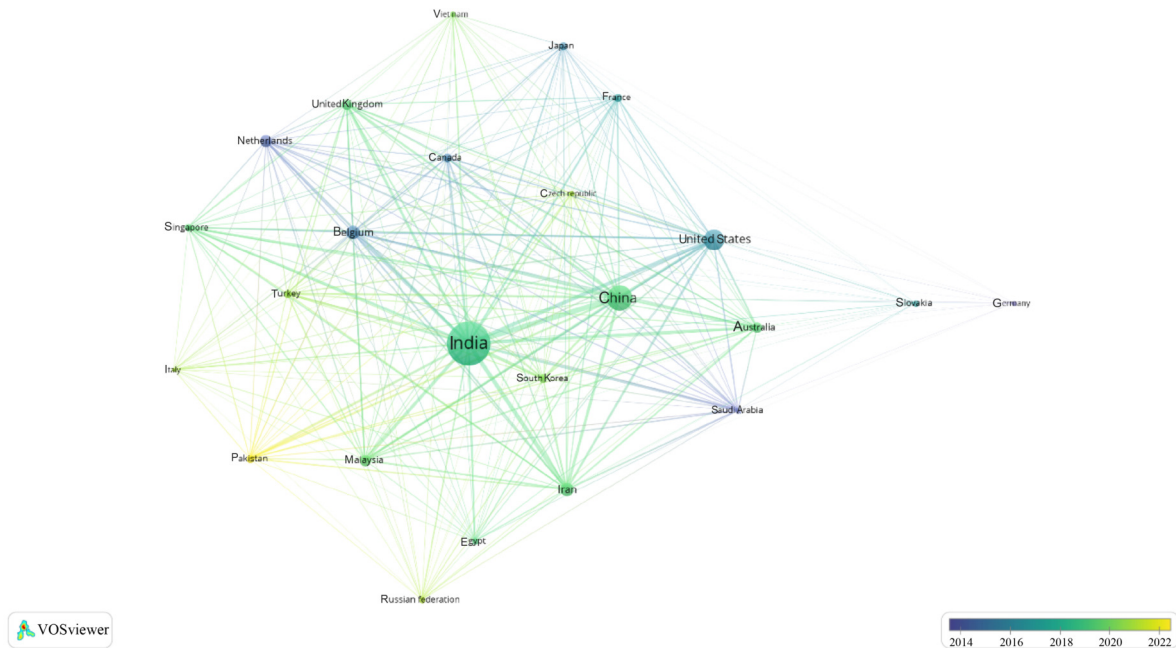


Fig. 1 Country mapping: based on Scopus search data.

Table 2 Documents and citations of the top 15 sources in the search field

Sr. No.	Journal	Documents	Citations	Total link strength	Documents (%)	Citations (%)
1	Construction and Building Materials	106	5547	31186	36.6	56.0
2	Lecture Notes in Civil Engineering	32	20	8385	11.0	0.2
3	Cement and Concrete Composites	26	2587	10817	9.0	26.1
4	IOP Conference Series: Materials Science and Engineering	19	59	1780	6.6	0.6
5	Journal of Building Engineering	18	128	10172	6.2	1.3
6	International Journal of Civil Engineering and Technology	14	131	1990	4.8	1.3
7	Journal of Materials in Civil Engineering	13	500	7142	4.5	5.0
8	Advanced Materials Research	11	4	602	3.8	0.0
9	ACI Materials Research	9	108	5615	3.1	1.1
10	Water Research	8	334	225	2.8	3.4
11	Indian Concrete Journal	8	10	647	2.8	0.1
12	Materials and Structures	7	158	4180	2.4	1.6
13	Journal of Advanced Concrete Technology	7	284	3478	2.4	2.9
14	Materials Science Forum	6	3	534	2.1	0.0
15	MATEC Web of Conferences	6	28	335	2.1	0.3

considered are “co-occurrence” and “all keywords”. To have the maximum repeated keywords, the minimum number of occurrences of a keyword is set to 25 times. Based on this, the top 43 keywords are shortlisted, and their visualization network is presented in Fig. 3. These keywords are classified under four major categories: self-healing mechanism of the bacterial concrete; process involved in bacterial concrete; effect on mechanical properties; effect on durability properties.

3 Cultivation of bacteria and their inclusion in concrete

Cultivating bacteria involves introducing them in a suitable medium comprising ingredients, such as peptone, beef extract, and urea, under sterile conditions [26]. After the cultivation stage, bacteria can be utilized in the concrete, most commonly in three ways: direct addition into the concrete, injection/spray of bacteria culture, or

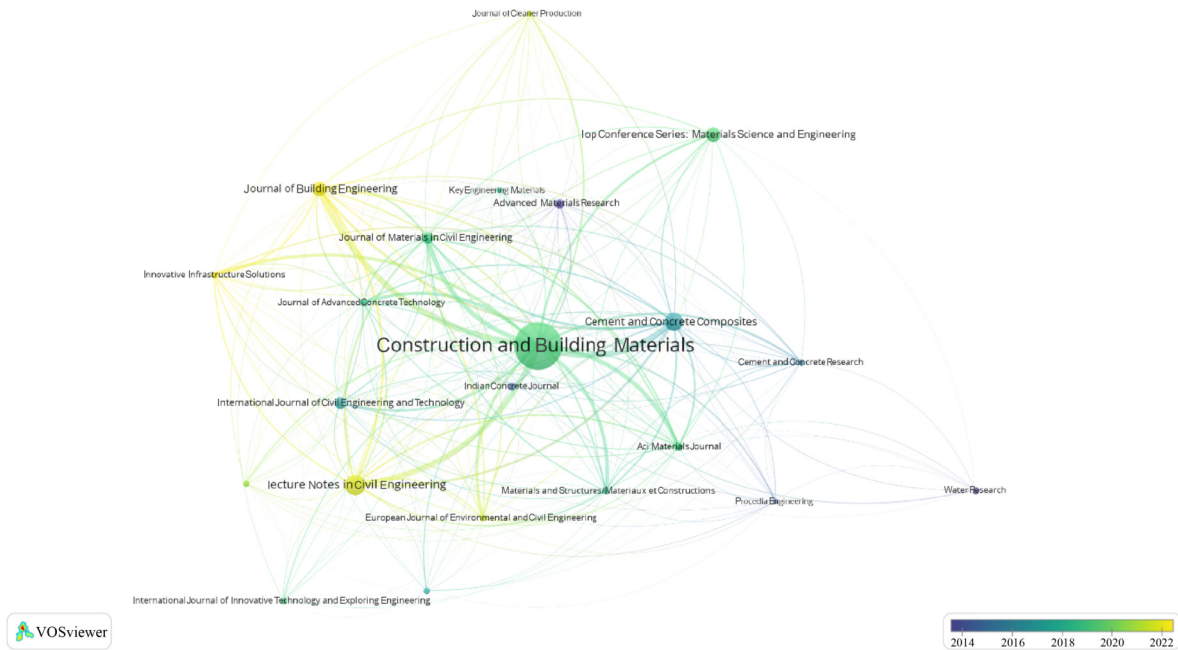


Fig. 2 Source mapping: based on Scopus search data.

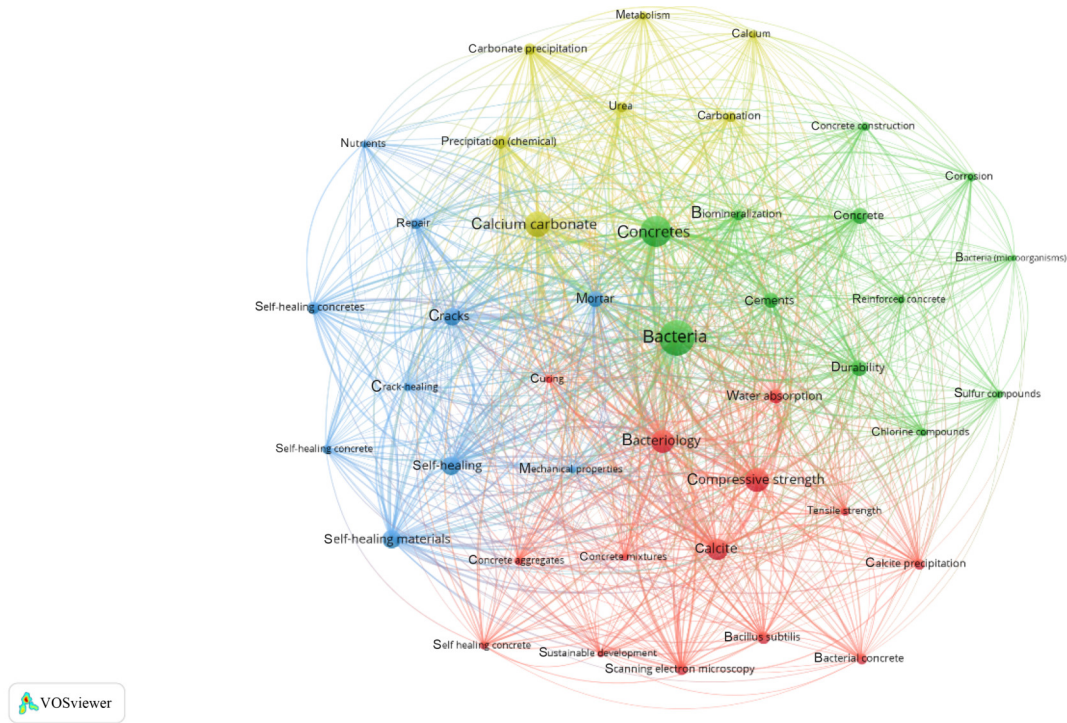


Fig. 3 Keywords mapping: based on Scopus search data.

the encapsulation of the bacteria in the concrete (Fig. 4) [23]. Calcium lactate and bacterial spores are directly added to the concrete after the concrete is mixed. The most commonly used carrier media that have been reported in the previous literature are water [27], aggregates (mostly coarse and light-weight aggregates) [28,29], expanded clay [30], micro and nanoparticles (e.g., nano iron oxide) [27,31] and others [32].

Surface treatment of concrete is a highly successful

method for improving the overall durability of the material, as the majority of degradation processes primarily initiate from the surface. de Muynck et al. [33,34] were among the early researchers who implemented bacterial treatments on the surface of cementitious materials by a two-step immersion process. The mortar specimens were initially submerged in a culture of *B. Sphaericus* that had been cultivated for a period of 24 h. The presence of CaCO_3 precipitation on the surface of the

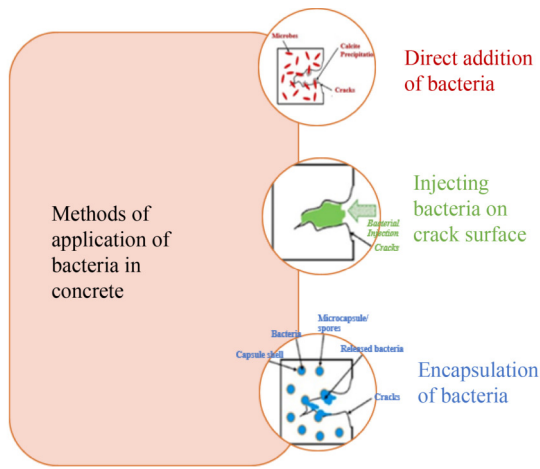


Fig. 4 Principles of three techniques for application of bacterial action in concrete.

mortar led to a reduction in the absorption of capillary water and in gas permeability. As a result, the mortar exhibited enhanced resistance to carbonation, chloride penetration, and the detrimental effects of freezing and thawing. Similar results were also reported by Nosouhian et al. [35]. In contrast, Qian et al. [36] employed a single-step bacterial treatment. The cement stones were immersed in a solution containing *S. Pasteurii* cells, urea, and Ca^{2+} . Concrete was mixed, and the precipitation of carbonate primarily occurred on its outer surfaces, resulting in the formation of a compact and cohesive layer with a thickness ranging from 150 to 290 μm and in enhanced durability properties. Another such application of bacteria on the surface of concrete is the biofilm formation. According to research by Soleimani et al. [37,38], bacterial biofilms have the potential to exhibit antagonistic properties against sulfur-oxidizing bacteria

(SOB), thereby serving as a protective barrier against SOB-induced deterioration of concrete structures.

Bacteria provide the basis for dealing with damage caused by cracks in general, involving 'self-healing'. During the casting process, dormant bacteria, typically in the form of spores, as well as other necessary agents such as nutrients and precipitation precursors, are integrated into the concrete matrix. The activation of latent bacteria in the crack zone and subsequent precipitation of CaCO_3 to facilitate crack healing in situ only occurs when cracking takes place, typically in response to factors such as presence of moisture and oxygen. The most common mechanisms for such crack healing are bacterial oxidation of organic compounds [13], bacterial ureolysis [39–41] and denitrification [42–44].

4 Biomineralisation

The inclusion of bacteria in concrete is very effective in crack healing, producing calcite crystals that help in blocking pores and cracks in concrete [45]. Precipitation of calcite, to block cavities in concrete, can be linked with biologically induced mineralization in the presence of a calcium source. Calcium carbonate is produced by microorganisms through various metabolic processes that can mainly be classified into two types. The first of these is autotrophic precipitation of CaCO_3 , which can be further classified into different methods like non-methylotrophic methanogenesis, oxygenic and anoxygenic photosynthesis [46]. The second is the heterotrophic precipitation of CaCO_3 which can further be categorized into various other processes like utilization of organic acid, reduction of calcium sulfate, ureolysis, and dissimilatory nitrate reduction, and ureolysis. Figure 5 presents the different

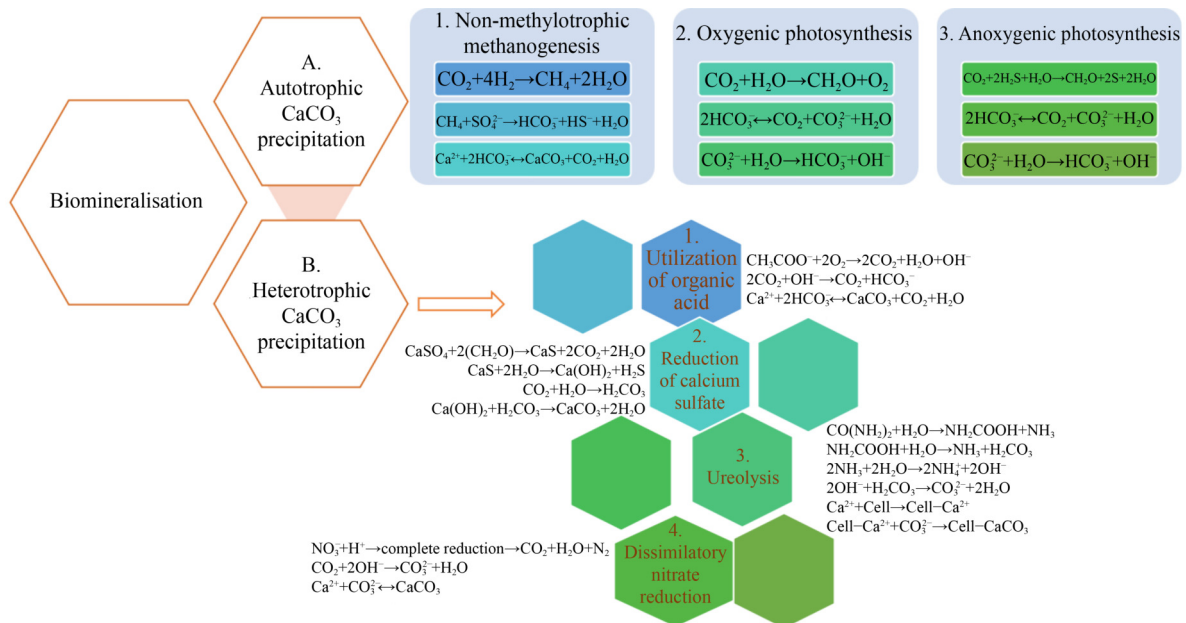


Fig. 5 Various methods of the process of biomineralization.

methods of biomineralization and the chemical reactions involved in those processes.

5 Mechanical properties

The general mechanism for strength development and healing of cracks in microbial concrete is the conversion of soluble organic compounds to inorganic crystals of calcite, which then seals the cracks [21,22]. Previous studies have demonstrated that strength restoration of cracks using CaCO_3 follows a multi-factor approach that can be classified into physical and chemical features. The most important variables for the development of strength, in terms of chemical properties, are the bacteria type and concentration [22], the medium's pH [14], and the nucleation site for bacterial immobilization [47].

Tables 3 and 4 provides the different types of bacteria used in previous literatures and detailed data analysis of mechanical properties reported in previous literatures,

respectively. In previous research, the most optimal concentration of bacteria for greater CS has been found to be in the range of 10^5 – 10^8 cfu/mL, where cfu is 'colony forming unit'. The type of bacteria also plays a vital role in the strength development of concrete. Rauf et al. [22] conducted research on application of different kinds of bacteria and reported that the higher strength was obtained with the inclusion of *Bacillus sphaericus*, rather than *B. cohnii* or *B. subtilis*, due to more significant calcium carbonate precipitation. Chen et al. [21] studied the influence of ceramsite sand, and Rauf et al. [22] used various natural fibers (coir, flax, and jute) as carriers for bacterial immobilization and their effect on the CaCO_3 precipitation. The latter study revealed that there can be as much as a 50%–70% rise in flexural strength.

Natural fiber inclusion can also result in concrete protection from an alkaline medium inside the concrete and around a 42% increase in CS values. An increase in strength can be linked with the formation of extra calcite from the carriers that already included nutrients [21,22].

Table 3 Different types of bacteria utilization in self-healing concrete/mortar and corresponding mix design reported in previous literatures

Ref.	Mix design cement/ NFA/CA (kg/m^3) + mineral precursors	Tests conducted	Curing (d)	SP (%)	W/B	Bacteria
Li et al. [19]	1/3 (ratio by mass) Nutrient Broth-Ureas	compressive strength	28	–	0.5	<i>B. cereus</i>
Khushnood et al. [52]	400/840/760* Calcium Lactate	compressive strength, split tensile strength	28	1	0.4	<i>B. subtilis</i>
Huynh et al. [53]	–	compressive strength, flexural strength	7, 28	–	0.5	<i>B. subtili</i>
Khushnood et al. [45]	930/1400 Calcium Lactate	compressive strength	7, 28	–	0.4	<i>B. subtilis</i>
Jena et al. [54]	432.55/628/1226	compressive strength, split tensile strength, flexural strength	7, 28	–	0.43	<i>B. subtilis</i>
Khaliq and Ehsan [55]	370/840/990 Calcium Lactate	compressive strength	7, 28	1	0.4	<i>B. subtilis</i>
Siddique et al. [56]	390/569/1164	compressive strength	7, 28	–	0.5	<i>B. aerius</i>
Saxena and Tembhurkar [57]	325/848/587	compressive strength	7, 28	0.7	0.5	<i>B. cereus</i>
Siddique et al. [58]	390/569/1164, SF is replaced at 0%, 5%, 10%, and 15%	compressive strength	28	–	0.5	Obtained from rhizospheric soil and marble sludge
Siddique et al. [59]	450/650/1343, CBFD is replaced at 10%, 20%, and 30%	compressive strength	28	–	0.5	AKKR5 strain
Sahoo et al. [60]	372/563.54/1136.64 (RCA) or 1232.75 (NCA)	compressive strength	7, 28	–	0.5	<i>B. subtili</i>
Achal et al. [20]	1/3 (ratio by weight) Nutrient Broth-Ureas	compressive strength	7, 28	–	0.47	<i>Bacillus sp.</i> CT-5
Rauf et al. [22]	385/850/937 Calcium Lactate and Urea	compressive strength	28	1.2	0.45	<i>B. subtilis</i> , <i>B. cohnii</i> , <i>B.</i> <i>sphaericus</i> (coir, flax, and jute fibers)
Pie et al. [61]	NFA/cement = 2 CaCl_2 -Urea	compressive strength	7, 28	–	0.68	<i>B. subtilis</i>
Salmasi and Mostofinejad [62]	450/575/393 Calcium Lactate and Urea	compressive strength	28- tap water (w), lactate (c), nutrient broth (n)	0.25 (no urea-calcium f)-0.3 (f)	0.35	<i>B. subtilis</i>
Afifudin et al. [63]	302/845/1033	compressive strength	7, 28	–	0.62	<i>B. subtilis</i>

Notes: CC: control concrete; IONPs: iron oxide nano-sized particles; LSP: micro sized limestone particles; CS: compressive strength; SS: split tensile strength; FS: flexure strength; LWA: light-weight aggregate (as carrier); GNP: graphite nano platelets (as carrier); f: steel fibres; SF: silica fume; BSF: bacterial silica fume; CBFD: cement baghouse filter dust; NCA: natural coarse aggregate; *: RCA, recycled coarse aggregate; MK: Metakaolin; #: fibers content in kg/m^3 .

Table 4 Mechanical properties of bacteria based self-healing concrete/mortar

Ref.	Mix design cement/ NFA/CA (kg/m ³) + mineral precursors	Bacteria concentration (cfu/mL)	Mix description	Mechanical property (MPa) CS/SS/FS (7–28 d)	Author inferences
Li et al. [19]	1/3 (ratio by mass) Nutrient broths-ureas	0	0% MK	NA-29.2/NA-NA/NA-NA	Use of bio cement enhanced the compressive strength by about 20%–28% at all replacements of MK.
		0	25% MK	NA-33.2/NA-NA/NA-NA	
		0	50% MK	NA-21.6/NA-NA/NA-NA	
		5×10^8	0% MK	NA-37.2/NA-NA/NA-NA	
		5×10^8	25% MK	NA-40.2/NA-NA/NA-NA	
		5×10^8	50% MK	NA-27.4/NA-NA/NA-NA	
Khushnood et al. [52]	400/840/760* Calcium Lactate	0	CC	NA-35/NA-1.75/NA-NA	An increase of about 4%–6% in compressive strength and 11%–38% in split tensile strength due to enhanced microstructure densification caused by biomineralization.
		1.9×10^7	direct	NA-36/NA-2/NA-NA	
		1.9×10^7	RCA	NA-34/NA-2.4/NA-NA	
		1.9×10^7	RCA & 50% NFA	NA-36.5/NA-2.55/NA-NA	
Huynh et al. [53]	–	0	–	39-49/NA-NA/5-7	Increased strength performance with the inclusion of <i>B. subtilis</i> bacteria.
		10^9	–	49-59/NA-NA/6-7	
Khushnood et al. [45]	930/1400 Calcium Lactate	0	CC	28-41/NA-NA/NA-NA	When used as an immobiliser, IONPs had a better self-healing effectiveness than LSP and siliceous sand grains.
		10^7	IONPs	35-53.07/NA-NA/NA-NA	
		10^7	LSP	30-42/NA-NA/NA-NA	
Jena et al. [54]	432.55/628/1226	0	–	35-44/2.65-3.7/2.9-3.45	Enhanced strength of bacterial concrete compared to CC. Strength increased upto 10^5 cfu/mL concentration of bacteria and decreased further.
		10^2	–	39-50/3-3.8/3.5-3.8	
		10^4	–	41.5-54.5/3.35-4.1/3.9-4.15	
		10^6	–	40-53/3.3-3.95/3.8-3.9	
Khaliq and Ehsan [55]	370/840/990 Calcium Lactate	0	-	18-26/NA-NA/NA-NA	Increased CS with inclusion of <i>B. subtilis</i> with notable improvement by LWA technique.
		10^8	direct	20-27/NA-NA/NA-NA	
		10^8	LWA	21-29.5/NA-NA/NA-NA	
		10^8	GNP	22-28/NA-NA/NA-NA	
Siddique et al. [56]	390/569/1164	0	–	22-33.5/NA-NA/NA-NA	Increment of about 10% in strength of bacterial concrete compared to CC.
		10^5	–	23-37/NA-NA/NA-NA	
Saxena and Tembhurkar [57]	325/848/587	0	CC	27-32.5/NA-NA/NA-NA	An increment of about 35% in CS is observed for bacterial concrete compared to CC.
		–	BC	36-45/NA-NA/NA-NA	
Siddique et al. [58]	390/569/1164, SF is replaced at 0%, 5%, 10%, and 15%	0	SF0	NA-32.9/NA-NA/NA-NA	Upto 10% replacement of SF, strength increased significantly both in normal as well as bacterial concrete reason being continuous hydration and pozzolanic reaction of SF. However increasing SF volume beyond 10% lead to decrease in strength due to reduction in hydration product.
		0	SF5	NA-34.8/NA-NA/NA-NA	
		0	SF10	NA-38.7/NA-NA/NA-NA	
		0	SF15	NA-36.6/NA-NA/NA-NA	
		10^5	BSF0	NA-36.5/NA-NA/NA-NA	
		10^5	BSF5	NA-38.4/NA-NA/NA-NA	
		10^5	BSF10	NA-43/NA-NA/NA-NA	
		10^5	BSF15	NA-40.2/NA-NA/NA-NA	
Siddique et al. [59]	450/650/1343, CBFD is replaced at 10%, 20%, and 30%	0	CBFD0	NA-32.89/NA-NA/NA-NA	Addition of CBFD does not lead to enhancement in strength however addition of bacteria enhanced the strength probably due to deposition of Calcite on the concrete.
		0	CBFD10	NA-27.23/NA-NA/NA-NA	
		0	CBFD20	NA-24.64/NA-NA/NA-NA	
		0	CBFD30	NA-20.53/NA-NA/NA-NA	

(Continued)

Ref.	Mix design cement/ NFA/CA (kg/m ³) + mineral precursors	Bacteria concentration (cfu/mL)	Mix description	Mechanical property (MPa) CS/SS/FS (7–28 d)	Author inferences
Siddique et al. [59]	450/650/1343, CBFDF is replaced at 10%, 20% and 30%	10 ⁵	BCBFD0	NA-36.33/NA-NA/NA-NA	Addition of CBFDF does not lead to enhancement in strength however addition of bacteria enhanced the strength probably due to deposition of Calcite on the concrete.
		10 ⁵	BCBFD10	NA-29.28/NA-NA/NA-NA	
		10 ⁵	BCBFD20	NA-25.02/NA-NA/NA-NA	
		10 ⁵	BCBFD30	NA-21.18/NA-NA/NA-NA	
Sahoo et al. [60]	372/563.54/1136.64 (RCA) or 1232.75 (NCA)	0	RCA	29.06-38/NA-NA/NA-NA	The CS of bacterial concrete has found increasing with increase in cell concentration up to 10 ⁶ cells/mL. Further increase in conc. lead to reduction in strength.
		10	–	31.27-41/NA-NA/NA-NA	
		10 ³	–	32.7-41.3/NA-NA/NA-NA	
		10 ⁶	–	34.1-46.2/NA-NA/NA-NA	
		10 ⁷	–	32.8-44.6/NA-NA/NA-NA	
		0	NCA	33.15-44/NA-NA/NA-NA	
Achal et al. [20]	1/3 (ratio by weight) Nutrient broths-ureas	0	13.4 [@]	12.5-22.5/NA-NA/NA-NA	5 × 10 ⁷ bacterial concentration induced maximum CS.
		0	18.8 [@]	12-21/NA-NA/NA-NA	
		0	27.2 [@]	11.5-20/NA-NA/NA-NA	
		5 × 10 ⁶	13.4 [@]	13-26.5/NA-NA/NA-NA	An increase of 37% in strength was observed for deepest crack with microbial inclusion.
		5 × 10 ⁶	18.8 [@]	12.5-23/NA-NA/NA-NA	
		5 × 10 ⁶	27.2 [@]	11.7-22/NA-NA/NA-NA	
		5 × 10 ⁷	13.4 [@]	14.2-31/NA-NA/NA-NA	
		5 × 10 ⁷	18.8 [@]	13-30/NA-NA/NA-NA	
		5 × 10 ⁷	27.2 [@]	12-28/NA-NA/NA-NA	
		5 × 10 ⁸	13.4 [@]	13.5-29/NA-NA/NA-NA	
		5 × 10 ⁸	18.8 [@]	12.8-28.5/NA-NA/NA-NA	
5 × 10 ⁸	27.2 [@]	11.8-27.5/NA-NA/NA-NA			
Rauf et al. [22]	385/850/937 Calcium Lactate and Urea	0	CC	NA-29.3/NA-NA/NA-NA	Compressive strength is greater in all other mixes compared to control concrete. Higher strength values are obtained in coir fiber based microbial concrete due to its greater diameter than other two fibers as well as the presence of amine compounds.
		0	CC (coir)	NA-37.5/NA-NA/NA-NA	
		0	CC (Flax)	NA-31/NA-NA/NA-NA	
		0	CC (Jute)	NA-34/NA-NA/NA-NA	
		6 × 10 ⁸	<i>B. subtilis</i> (coir)	NA-40/NA-NA/NA-NA	
		6 × 10 ⁸	<i>B. subtilis</i> (flax)	NA-37.5/NA-NA/NA-NA	
		6 × 10 ⁸	<i>B. subtilis</i> (jute)	NA-35/NA-NA/NA-NA	
		6 × 10 ⁸	<i>B. cohnii</i> (coir)	NA-39.5/NA-NA/NA-NA	
		6 × 10 ⁸	<i>B. cohnii</i> (flax)	NA-39.2/NA-NA/NA-NA	
		6 × 10 ⁸	<i>B. cohnii</i> (jute)	NA-39/NA-NA/NA-NA	
		6 × 10 ⁸	<i>B. sphaericus</i> (coir)	NA-42/NA-NA/NA-NA	
6 × 10 ⁸	<i>B. sphaericus</i> (flax)	NA-41/NA-NA/NA-NA			
6 × 10 ⁸	<i>B. sphaericus</i> (jute)	NA-39/NA-NA/NA-NA			
Pie et al. [61]	NFA/cement = 2 CaCl ₂ -Urea	0	–	29-30/NA-NA/NA-NA	Bacterial cell walls of <i>B. subtilis</i> lead to an improved compressive strength.
		10 ²	–	31-35/NA-NA/NA-NA	
		10 ³	–	25.5-35/NA-NA/NA-NA	
		10 ⁴	–	16-28/NA-NA/NA-NA	

(Continued)

Ref.	Mix design cement/ NFA/CA (kg/m ³) + mineral precursors	Bacteria concentration (cfu/mL)	Mix description	Mechanical property (MPa) CS/SS/FS (7–28 d)	Author inferences
Salmasi and Mostofinejad [62]	450/575/393 Calcium Lactate and Urea	0	cc-w	NA-44.31/NA-NA/NA-NA	Change in curing medium from water to urea-lactate or nutrient broth showed detrimental effect on compressive strength. Steel fibres inclusion compensated the strength loss caused by the bacterial presence.
		0	cc-w-f	NA-58.44/NA-NA/NA-NA	
		0	cc-c	NA-43.56/NA-NA/NA-NA	
		0	cc-c-f	NA-46.83/NA-NA/NA-NA	
		0	cc-n	NA-42.95/NA-NA/NA-NA	
		0	cc-n-f	NA-45.63/NA-NA/NA-NA	
		10 ⁷	bs-w	NA-41.92/NA-NA/NA-NA	
		10 ⁷	bs-w-f	NA-42.86/NA-NA/NA-NA	
		10 ⁷	bs-c	NA-40.25/NA-NA/NA-NA	
		10 ⁷	bs-c-f	NA-42.96/NA-NA/NA-NA	
		10 ⁷	bs-n	NA-40.96/NA-NA/NA-NA	
Afifudin et al. [63]	302/845/1033	0	–	24-37.5/NA-NA/NA-NA	<i>B. subtilis</i> inclusion showed positive results at higher concentration with an increment of about 28% is observed in CS of concrete with 10 ⁶ cfu/mL concentration.
		10 ³	–	34-39/NA-NA/NA-NA	
		10 ⁴	–	21.5-30/NA-NA/NA-NA	
		10 ⁵	–	24-34/NA-NA/NA-NA	
		10 ⁶	–	29.5-43.5/NA-NA/NA-NA	
		10 ⁷	–	20-27.5/NA-NA/NA-NA	

Notes: CC: control concrete; IONPs: iron oxide nano-sized particles; LSP: micro sized limestone particles; CS: compressive strength; SS: split tensile strength; FS: flexure strength; 7–28 d: 7 and 28 d result; LWA: light-weight aggregate (as carrier); GNP: graphite nano platelets (as carrier); f: steel fibres; SF: silica fume; BSF: bacterial silica fume; CBF: cement baghouse filter dust; NCA: natural coarse aggregate; *: RCA, recycled coarse aggregate; MK: Metakaolin; @: crack depth (mm); NA: not available.

Also, there are conflicting findings about how bio-based healing agents affect the strength of concrete. According to an investigation done by Wang et al. [48] adding encapsulated *B. Sphaericus* to mortar causes a 15%–34% reduction in CS. However, others have found that the CS was raised at 7 and 28 d when *B. Sphaericus* was used in a cube mortar [20]. The CS of the mortar decreased for greater cell concentration (5×10^8 cfu/mL) even though the bio-based agent had a beneficial impact on the cell concentration of 5×10^6 cfu/mL.

Bang et al. [49] investigated the impact of *S. Pasteurii* bacteria on CS at 7 and 28 d for mortar specimens. They discovered that the immobilized *S. Pasteurii* concentration at the most significant level on porous glass beads significantly increased the CS of the mortar samples by 24%. Additionally, the CS increased as the cell concentration rose from 6.1×10^7 to 3.1×10^9 cells/mL. Figures 6 and 7 represent the CS and tensile strength (split tensile strength (SS) and FS) of concrete, respectively, with various bacterial concentrations.

Apart from the above factors, one of the governing factors affecting the strength of microbial concrete is the usage of different nutrients, such as calcium lactate, calcium formate, calcium nitrate, yeast extract, and urea as food source for bacteria. A study was performed on the

behavior of various nutrient dosages (bacterial spore powder, calcium lactate, formate, and nitrate) on the CS of microbial mortar by Luo and Qian [50]. Results show decreased early strength gain by incorporating calcium lactate with bacterial spore powder. However, strength was found to be enhanced at later curing ages. Including calcium formate also produced an enhancement in strength; however, an increase in concentration resulted in a decrease in the enhancement effect. Contradictory to the effects of abovementioned nutrients, Calcium nitrate inclusion resulted in about a 17% decrease in CS after 28 d of testing. Another research performed by Schreiberová et al. [51], on the performance of nutrients on strength properties of microbial concrete demonstrated a drastic decrease in strength with the addition of yeast. Adding other nutrients like calcium formate, nitrate, lactate, and urea enhanced CS and FS.

Very few studies have been done on blended concrete. The inclusion of various waste materials, such as fly ash (FA), silica fume, and rice husk ash, in bacterial concrete has been reported in previous literature. Significant findings of earlier literature on blended microbial concrete are tabulated in Table 5. Figure 8 represents blended bacterial concrete's CS, SS, and FS with different SCMs at 28 d. The table data confirm that the

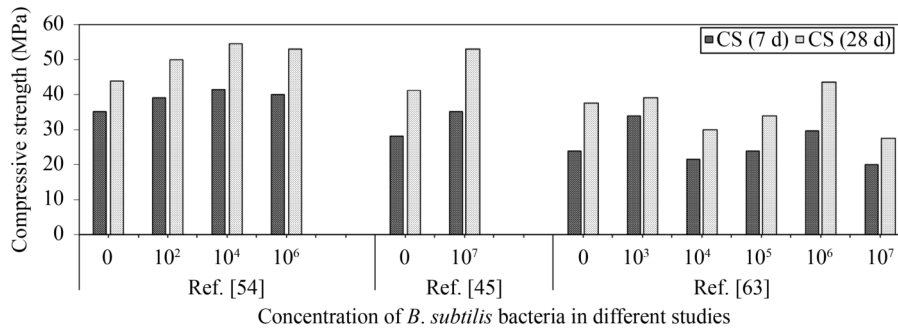


Fig. 6 CS of microbial concrete at various concentrations of bacteria.

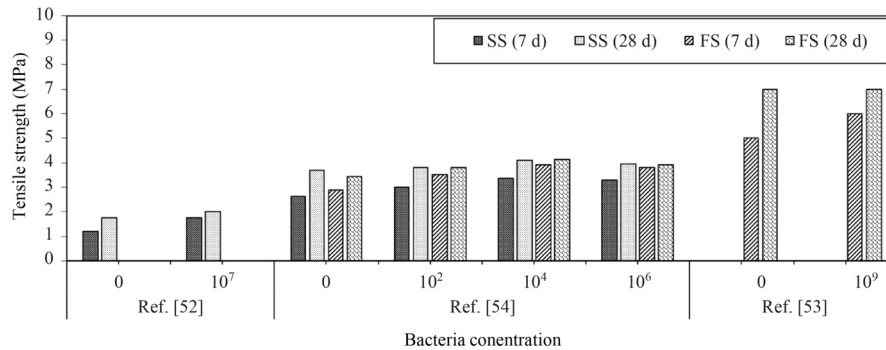


Fig. 7 Tensile strength of microbial concrete at various concentrations of bacteria.

effect of bacteria concentration is dependent on type and concentration, so it is necessary to optimize these factors, to help researchers to standardize the mix design process. Some studies exist in the literature on the efficiency of different supplementary materials like FA and silica fume [64–66] but no research has been found on the efficiency of bacteria to achieve equal 28 d strength, and there is scope for future research.

Studies on the inclusion of different fibers like coconut fibers [72], polyvinyl alcohol (PVA) [73], polypropylene (PP) [74,23], steel fibers [2], glass fibers [75], waste tire rubber fibers [76], natural fibers like coir, flax, jute [22], etc. in concrete have been done on the literature. However, in bacterial concrete, very few studies [22,62,75–81] have been done so far involving fibers. Including glass fibers (3% by volume of cement) [75] and steel fibers (2%) [77] in bacterial concrete resulted in a 12%–16% increase in CS. In addition, FS was enhanced by 50% in a study conducted by Zhang et al. [77]. In a study conducted by Rauf et al. [22], different natural fibers (coir (1.19%), flax (0.31%), and jute (0.38%)) were used as carriers for bacteria immobilization. Compared to other fibers, flax fibers have proved to protect bacteria more efficiently, whereas coir fibers imparted greater mechanical strength than the other fibers [22]. A summary of the effects of fibers on bacterial concrete is presented in Table 6. Figure 9 portrays CS, SS, and FS at 28 d in fiber-reinforced bacterial concrete, as reported in previous literature.

6 Durability properties

6.1 Carbonation

Carbonation is a process of reaction of CO₂ with the hydration products of concrete, leading to reduced reserve alkalinity [83]. One of the most critical factors affecting the carbonation is the porosity of concrete. Greater porosity leads to the increased diffusion of CO₂, resulting in a faster reaction of CO₂ with hydration products [84].

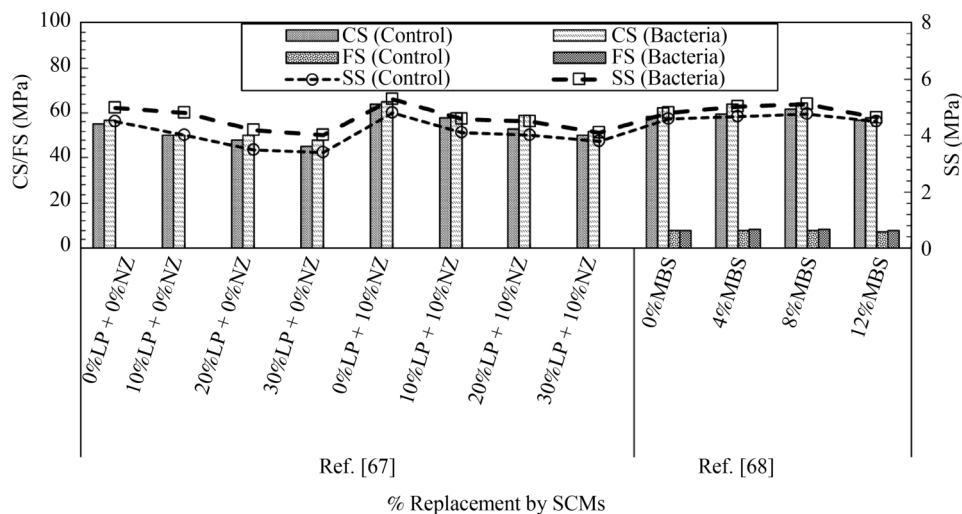
Out of the various methods, one method for reduction of the porosity of concrete is by adding bacteria [53,85]. Although very few studies have been performed on the impact of bacteria on concrete carbonation, some research has shown positive results regarding carbonation resistance. A recent study by Joshi et al. [86], has shown the positive impact of bacteria (admixed and sprayed) on the carbonation of concrete, with about a 50% reduction in carbonation depth compared to that in normal concrete. Adding fibers in bacterial concrete has also demonstrated a promising result against carbonation compared to behavior of non-bacterial concrete [87]. Table 7 presents the literatures on the carbonation performance of bacterial concrete.

Curing conditions have also impacted concrete carbonation [62,79]. Concrete cured in the urea-lactate medium has shown poor resistance against carbonation, leading to increased carbonation depth than in concrete cured in tap water. This might be because of the acidic

Table 5 Effect of Bacteria on the mechanical and durability properties of blended concrete/mortar

Reference	PB-SB (%)	Bacteria type and concentration	Major observations
Jafarnia et al. [67]	OPC-LP (0, 10, 20, 30)-NZ (0, 10)	<i>S. pasteurii</i> (10^7 cells/mL)	NZ inclusion improved the strength values of bacterial and non-bacterial concrete by about 23% and 20%, respectively. About 53% and 64% increases in electrical resistivity were reported for bacterial and non-bacterial concrete, respectively, compared to control concrete. Concrete cured in Urea-CaCl ₂ medium effectively healed cracks of about 0.58 mm. to the equivalent figure for standard water curing is 0.16 mm.
Priya et al. [68]	OPC-MBS (0, 4, 8, 12)	<i>B. sphaericus</i> (10, 20, 30 mL)	Optimal replacement of cement by MBS is found to be 8%. The inclusion of 20 mL bacteria at 8% MBS reported 13.5%, 16.38%, and 13.32% higher compressive, split tensile, and flexure strength, respectively. Reduced water absorption and sorptivity with the inclusion of bacteria and MBS due to increased CSH gel formation.
Siddique et al. [58]	OPC-SF (0, 5, 10, 15)	AKKR5 (10^5 cells/mL)	About a 12% increment in strength with the inclusion of bacteria at 10% substitution of cement with SF. About 50%–55% reduction in water absorption and porosity occurred with the inclusion of bacteria.
Li et al. [19]	OPC-MK (0, 25, 50)	<i>B. cereus</i> (5×10^5 cells/mL)	Use of bio cement enhanced the compressive strength by about 20%–28% at all replacements of MK.
Siddique et al. [56]	OPC-RHA (0, 5, 10, 15, 20)	<i>B. aerius</i> (10^5 cells/mL)	10% is the optimal replacement of cement by RHA with a 10%–15% increment in strength compared to CC. Bacteria inclusion in RHA concrete reported reduced chloride ion penetration, porosity, and water absorption due to calcite precipitation.
Siddique et al. [59]	OPC-CBFD (0, 10, 20, 30)	AKKR5 (10^5 cells/mL)	Improved strength by up to 11% with the inclusion of AKKR5 bacterial strain. Inclusion of CBFD resulted in a decrease of strength. Improved durability properties like water absorption, porosity, and permeability due to the densification of microstructure by the formation of calcite.
Kunal et al. [69]	OPC-CKD (0, 10, 20, 30)	KG1 Bacterial strain	Increase of about 26% in strength was observed with 10% inclusion of bacterial-treated CKD; however, further addition led to decreased strength due to reduced cement content, formation of ettringites leading to increase of pore numbers, and reduction of strength.
Chahal and Siddique [70]	OPC-FA (0, 10, 20, 30)-SF (0, 5, 10)	<i>S. pasteurii</i> (10^3 , 10^5 , 10^7 cells/mL)	Optimum replacement of SF and FA was found to be 10% with bacterial conc. Of 10^5 cells/mL with 11% and 13% increases in strength with that of 10^3 cells/mL and 10^7 cells/mL. Minimum water absorption, porosity, and RCPT values were observed for the concrete with 30% FA and 10% SF, at bacterial conc. of 10^5 cells/mL.
Chahal et al. [10]	OPC-FA (0, 10, 20, 30)	<i>S. pasteurii</i> (0 , 10^3 , 10^5 , 10^7 cells/mL)	20%–22% increase in strength by including bacteria in both CC and FA-concrete. Optimum dosage of bacteria was found to be 10^5 cells/mL, with maximum strength gain, maximum water absorption reduction, and maximum reduction in chloride ions.
Achal et al. [71]	OPC-FA (0, 10, 20, 40)	<i>B. megaterium</i> (5×10^7 cells/mL)	Around 21%, 19%, 14%, and 10% increase in strength was observed for bacterial mortar samples corresponding to non-bacterial samples with 0, 10%, 20%, and 40% FA replacements, respectively.

Notes: PB-SB: primary binder-secondary binder; CBFD: cement baghouse filter dust; MBS: micronized biomass silica; LP: limestone powder; NZ: natural zeolite; CKD: cement kiln dust; OPC: ordinary Portland cement; FA: fly ash; SF: silica fume; RHA: rice husk ash; MK: Metakaolin.

**Fig. 8** Mechanical strength values of blended bacterial concrete with different replacement by SCMs.

nature of lactate, which leads to reduced pH of concrete pore solutions [62].

Chen et al. [88] studied the carbonation behavior by using *Paenibacillus* bacteria (3.1×10^8 cells/mL) in different water-to-cement ratio (w/c) (0.3, 0.4, and 0.5)

concrete. The bacteria were added in four different amounts (0.4%, 0.6%, 0.8%, and 1.5% by weight of cement) in the concrete and tested for carbonation after curing age of 14, 28 and 60 d. The optimum bacteria content is 0.6% for 0.4 to 0.5 w/c , and 0.4% for 0.3 w/c .

Table 6 Effect of fiber inclusions on various mechanical and durability properties of bacterial concrete/mortar

Reference	Binder	Fibers	Bacteria type and concentration	Major observations
Manojkumar et al. [75]	OPC	Glass Fiber	<i>B. subtilis</i> (3×10^7 cells/mL)	About 12% increase in compressive strength of bacterial concrete reinforced with glass fiber is observed.
Zhang et al. [77]	OPC-SF	Steel fiber	<i>S. pasteurii</i>	Around 16% and 50% increase in compressive and flexure strength is observed with the addition of bacteria and steel fiber in UHPC. Enhanced bonding between fiber and concrete is found due to calcite precipitation.
Thakare et al. [76]	OPC	WTR	<i>E. coli</i> (10^6 – 10^8 cells/mL)	Enhanced mechanical and permeation properties with the inclusion of bacteria through WTR fibers compared to direct inclusion.
Su et al. [78]	OPC	PP	<i>B. alkagophilus</i> (2×10^9 cells/mL)	Better healing capacity of mortar when bacteria and fibers are combined compared to individual action. Improved water resistance and mechanical performance after repair.
Rauf et al. [22]	OPC	Coir, Flax and Jute	<i>B. subtilis</i> , <i>B. cohnii</i> , <i>B. sphaericus</i> (6×10^8 cells/mL)	As a carrier, flax fibers offered bacteria more excellent protection, as evidenced by their effective crack-healing and pore-filling capacity. Compared to flax and jute fibers, coir fibers had superior mechanical qualities, allowing them to achieve greater compressive strength. <i>B. Sphaericus</i> performed better in terms of crack healing due to its greater ability of calcite formation than is shown by the other two bacteria types.
Karimi and Mostofinejad [82]	OPC	Barchip, Polypropylene, steel fibers	<i>B. subtilis</i> (10^7 cells/mL)	Inclusion of steel fibres leads to an increase in charge passing. However, the inclusion of bacteria leads to an almost 40% reduction in charge passing. Bacterial surface treatment was also found to significantly improve water penetration in concrete, as shown by a reduction in water penetration of 60% compared to the corresponding control after being applied to bar chip-reinforced concrete specimens cured in calcium lactate-urea solution. The presence of fibers is beneficial for concrete samples as it leads to reduced diffusion of CO ₂ , thereby reducing carbonation depth.
Salmasi and Mostofinejad [62]	OPC	Steel fibers	<i>B. subtilis</i> (10^7 cells/mL)	About a 13% reduction in water absorption is observed by including bacteria and steel fibers in concrete cured in the urea-lactate medium.
Kua et al. [82]	OPC	PVA, Steel fibers	<i>B. sphaericus</i> (10^{10} cells/mL)	The addition of bacteria along with SAP and fiber shows greater permeability recovery than control concrete. Utilising biochar as bacterial spores carrier, compared to direct inclusion of spores, leads to improved sorptivity regain and low permeation levels.
Feng et al. [80]	OPC	PP (6mm, 9mm), PVA(6mm)	Carbonic Anydrase (10^9 cells/mL)	In contrast to samples that include fiber, those containing bacteria displayed a more excellent repair ratio of crack area. Significant reduction in sorptivity with the inclusion of bacteria and fibers due formation and deposition of calcite on fibres. The highest flexure strength is obtained by including 9 mm PP fibers along with bacteria.
Sierra-Beltran et al. [81]	OPC-FA-BFS	PVA	<i>B. cohnii</i> (1.2×10^7 cells/mL)	Compared to mortar without the bio-based ingredient, the bio mortar used as a repair material exhibits less delamination from the concrete substrate. In comparison to control mixtures without a bio-based healing agent, the mixtures containing bacteria showed more excellent recovery of both deflection capacity and flexural strength.

Notes: BFS: blast furnace slag; PVA: polyvinyl alcohol; PP: polypropylene; SAP: super absorbent polymer; SF: silica fume; UHPC: ultra-high-performance concrete; WTR: waste tire rubber.

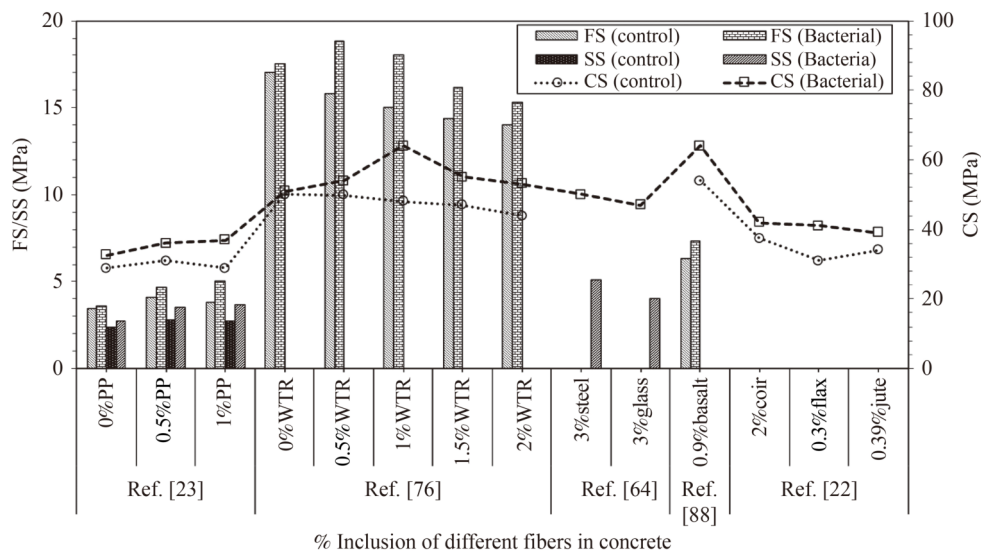


Fig. 9 28 d mechanical strength values of fiber-reinforced bacterial concretes.

Table 7 A brief literature survey on the carbonation behavior of bacterial concrete

Reference	Binders and fibers	Bacteria type and concentration (cells/mL)	Exposure conditions	Carbonation depth (min–max) (mm)	Major findings
Joshi et al. [86]	OPC	<i>B. sphaericus</i> CT5 (4×10^8)	Curing-28 d PC- (15 d, $T = 30^\circ\text{C}$, $RH = 60\%–70\%$) EC-(60/120 d, $\text{CO}_2 = 5\%$, $T = (30 \pm 2)^\circ\text{C}$, $RH = 60\%–70\%$)	5.41–10.95 (60 d) 9.28–18.27 (120 d)	Enhanced carbonation resistance of bacterial admixed concrete and bacterial sprayed concrete. About 40%–50% reduced carbonation depth than in control concrete. Decreased porosity due to calcite formation, leading to less diffusion of CO_2 .
Vijay and Murmu [87]	OPC, Basalt fibers	<i>B. subtilis</i> (10^5)	Curing-28 d EC-(28 d, $\text{CO}_2 = 5\%$, $T = (30 \pm 2)^\circ\text{C}$, $RH = 70\%$)	1.5–4.5 ($w/c = 0.35$) 2.5–6 ($w/c = 0.4$) 3.5–8 ($w/c = 0.45$)	Accumulation of basalt fibers resulting in a 55%–65% reduction in carbonation depth compared to control concrete for different w/c . Similarly, bacterial concrete also demonstrated reduced depth. Due to enhanced concrete densification, the decreased carbonation depth is observed in concrete containing bacteria and basalt fibers.
Karimi and Mostofinejad [79]	OPC, Barchip, PP, steel fibers	<i>B. subtilis</i> (10^7)	Curing-28 d EC- RILEM CPC-18 standard [91]	–	Specimens without bacteria cured in the urea-lactate medium showed enhanced carbonation depth; however, specimens with bacteria cured in the urea-lactate medium have shown less depth. Lowest carbonation depth is noted in bar chip-reinforced bacterial concrete.
Salmasi and Mostofinejad [62]	OPC, Steel fibers	<i>B. subtilis</i> (10^7)	Curing-28 d EC- RILEM CPC-18 standard [91]	9.86–28.49 (28 d) 7.13–25.86 (56 d)	Lowest carbonation depth is observed for concrete with bacteria and steel fiber cured in tap water. Concrete cured in urea lactate medium demonstrated the highest carbonation depth, possibly because of the acidic nature of lactate.

Notes: PC: pre conditioning; RH: relative humidity; T: temperature; EC: exposure conditions.

The optimum bacteria content contributes to an increase in carbonation depth, while a greater bacteria concentration prevents such an increase. This is due to the fact that the biomineralization rate is greater during the preliminary phase of hydration, and increased CaCO_3 synthesis causes the porosity of the surface layer to drastically decrease, making it more challenging for CO_2 to continue to diffuse in the sample, leading to prevention of further biomineralization [88].

A study on the efficient application of *B. Sphaericus* (10^7 cells/mL) has been conducted on mortar samples with the different w/c values (0.5, 0.6, and 0.7) by de Muynck et al. [89]. The samples were exposed to 10% CO_2 concentration and tested after 2, 4, and 6 weeks of exposure and resistance to carbonation were quantified in terms of carbonation rate constant (K) ($\text{mm} \cdot \text{s}^{-1/2}$) [90]. Results demonstrated that after two weeks of accelerated carbonation, there were already evident changes in the carbonation depth between treated and untreated specimens. When compared to untreated cubes, the carbonation rate was significantly reduced by the added bacterial biomass. With increasing w/c (0.5, 0.6, and 0.7), this addition had a better effect, resulting in a decrease in K of roughly 24%, 30%, and 37%, respectively, and with the combination of calcium source and bacteria, a further drop in carbonation rate is observed [89].

6.2 Permeation properties

Permeability is regarded as the critical characteristic for illustrating the durability of concrete since it determines how quickly aggressive substances can penetrate concrete and cause degradation under pressure gradients. It

depends on the characteristics of the materials' pore network, which can be measured in terms of pores, tortuosity, specific surface, distribution of size, connectedness, and microcracks. These features are influenced by various factors, including the age of hardened cementitious materials, their particle size distribution, the water/binder ratio, and the presence of aggressive compounds [92]. Precipitation of calcite due to biomineralization has proved beneficial in the resistance against water permeability and absorption. Deposition of CaCO_3 decreased water absorption and permeability, resulting in enhanced durability properties [93,94].

Chahal et al. [10] in their study on the behavior of microbial concrete blended with various percentages of FA, reported that the concrete showed 4-fold reduced water absorption at a concentration of 10^5 cells/mL *S. pasteurii*. In bacterial concrete, pores are filled with calcite resulting in decreased water absorption [95]. The addition of *Bacillus Megaterium* at a concentration of 5×10^7 cells/mL resulted in about threefold decrease in water absorption in FA-concrete. An overview of various water absorption tests conducted on concrete with the addition of different types of bacteria and their concentration is presented in Table 8.

The reduction in water absorption can also be influenced by the bacteria type and its concentration, presence of different nutrients, etc., as reported in various literatures [92,93,98]. In a long-term study of durability performance of concrete conducted by Bhaskar et al. [103], it was reported that the greatest reduction of 90% was obtained for the specimens containing *S. pasteurii* bacteria immobilized in Zeolite at a duration of 8 months. When it comes to bacterial concentration, it is seen that

Table 8 Beneficial findings on water absorption of bacterial concrete/mortar from the research literature

Reference	Binders	Bacteria type and concentration (cells/mL)	Water absorption (WA) test	Major observations and conclusions
Sadeghpour and Baradaran [93]	OPC-FA (0, 20%)	<i>B. megaterium</i> (10^4 – 10^7), <i>B. sporosarcina</i> (10^4 – 10^7), <i>B. subtilis</i> (2.8×10^6 – 1.0×10^8)	WA Test (ASTM C 642)	Greatest WA reduction (33%) is observed for 2.8×10^6 cells/mL conc. of <i>B. subtilis</i> for FA-concrete.
Tabalvandani et al. [94]	OPC	<i>B. licheniformis</i> (10^4 – 10^8)	Water Permeability (Bs-EN-12390-8)	Decrease in water permeability with an increase in bacteria concentration. About 46% reduction at 10^8 cells/mL conc.
Joshi et al. [86]	OPC	<i>B. sphaericus</i> CT5 (4×10^8)	Sorptivity (ASTM C185)	About 60% reduction of capillary suction is observed for bacterial concrete samples.
Smitha et al. [85]	OPC	<i>B. megaterium</i> (10^4 – 10^6)	Water Permeability (IS 3085-2002)	98.7% reduction in water permeability coefficient with the inclusion of 10^5 cells/mL bacteria in concrete compared to CC.
Wang et al. [96]	OPC	<i>S. pasteurii</i> (5.9×10^7)	Water Tightness test	A water tightness regains ratio of 99% is observed for bacterial specimens.
Mondal et al. [97]	OPC	<i>D. radiodurans</i> (10^3 , 10^5 , 10^7)	WA Test	Greatest reduction ($\approx 36\%$) in WA is observed for concrete samples with 10^5 cells/mL bacterial conc. at normal curing.
Mondal and Ghosh [98]	OPC	<i>B. subtilis</i> (10^3 , 10^5 , 10^7)	WA Test	WA is found to decrease with an increase in conc. of bacteria. 27% reduction in WA at 10^7 cells/mL conc. in bacterial concrete compared to CC.
Wang et al. [99]	OPC	<i>B. sphaericus</i> LMG22257 (3×10^8 , 10^9)	WA Test (NBN B15-215)	The greatest decrease in WA was found in samples for which aggregates were immersed for 24 hours in grown culture.
Siddique et al. [56]	OPC-RHA (0%, 5%, 10%, 15%, 20%)	<i>B. aerius</i> (10^5)	WA Test (ASTM C 642)	About 37% reduction in WA with the inclusion of bacteria in RHA-concrete.
Nugroho et al. [100]	OPC	<i>B. subtilis</i> (10^4 – 10^6)	Water Permeability	38% reduction in WA of concrete with 10^5 cells/mL bacteria compared to CC.
Xu et al. [101]	OPC	<i>B. cohnii</i> (ACC10230) (10^9)	Sorptivity	50% reduction in sorptivity of concretes treated with bacteria in the presence of Ca source compared to untreated samples.
Wang et al. [48]	OPC	<i>B. sphaericus</i> (LMG22557) (10^9)	Sorptivity (RILEM 25 PEM (II-6))	The specimens with microcapsules with or without bacteria demonstrated more than a 40% decrease in WA.
Kunal et al. [102]	OPC-CKD (0, 5, 10, 15%)	<i>B. halodurans</i> KG1 (10^8)	WA Test (ASTM C 642)	20% reduction in WA of 10% CKD-bacterial concrete compared to CKD-control concrete.
Chahal et al. [10]	OPC-FA (0%, 10%, 20%, 30%)	<i>S. pasteurii</i> (0 , 10^3 , 10^5 , 10^7)	WA Test (ASTM C 642)	Four times reduction in WA with the inclusion of bacteria (10^5 cells/mL).
Achal et al. [71]	OPC-FA (0%, 10%, 20%, 40%)	<i>B. megaterium</i> (ATCC 14581) (5×10^7)	Sorptivity (RILEM 25 PEM (II-6))	2 to 3.5 times less WA in bacterial concrete compared to CC.
de Muynck et al. [92]	OPC	<i>B. sphaericus</i> (10^7)	Sorptivity (RILEM 25 PEM (II-6))	Five times less WA with the inclusion of bacteria and CaCl_2 than CC.

Notes: WA: Water Absorption; CC: control concrete; CKD: Cement kiln dust; RHA: rice husk ash.

greater concentrations resulted in the most significant reduction in water absorption because higher precipitation slows down the water flow inside the matrix of concrete [94,98].

Steel reinforcement corrosion is caused by ion transport, and concrete's resistance to this process depends on the pore structure [104] and can be measured through Rapid Chloride Permeability Test (RCPT) [105]. The ASTM C1202 code provides a qualitative demonstration of chloride ion penetrability based on measured values (charge passed) from the test and is presented in Table 9.

The improvement of particle packing density of the material also results in a reduction of chloride permeability [106]. Additionally, bacterial precipitation enhances the aggregate-cement interface, which lowers the chloride permeability [20]. That research also showed that inclusion of bacteria in concrete can result in the

Table 9 Penetration of chloride ion based on charge passed [105]

Charge passed (C)	Chloride ion penetrability
> 4000	High
2000–4000	Moderate
1000–2000	Low
100–1000	Very low
< 100	Negligible

enhancement in the permeation resistance of chloride ions. Around 12% reduction in average charge passed is observed for the concrete containing bacteria compared to bacteria-less concrete. Addition of *S. pasteurii* and *B. subtilis* has reduced the penetration of chloride ion in concrete [107]. Inclusion of *B. aerius* bacteria in the concrete has reduced the charge passed in both control and rice husk ash (RHA)-concrete. Reductions of 55%, 50%, and 48% in the charge passed are observed for

bacterial concrete compared to that in normal concrete after 7, 28, and 56 d, respectively [56]. Another study conducted by Chahal et al. [95], on the inclusion of *S. pasteurii* bacteria on the silica fume (SF) based concrete, demonstrated excellent resistance against chloride penetration (380 coulombs passing level) at a concentration of 10^5 cells/mL for 10% SF concrete. Table 10 presents details from the literature survey, and Fig. 10 presents a

graphical representation of the RCPT behavior of bacterial concrete.

According to Rao et al. [106], bacterial concrete has shown an improvement in resistance to chloride migration of 85%–90% compared to conventional cementitious composites. Joshi et al. [108] and Achal et al. [20] reported similar findings in their studies. A study conducted by Bhaskar et al. [103] on the long-term

Table 10 Chloride ion permeability behavior of bacterial concrete

Reference	Binders	Bacteria type and concentration (cells/mL)	Charge passed (coulombs) (min-max)	Optimal mix and bacteria concentration	Conclusions
Tabalvandani et al. [94]	OPC	<i>B. licheniformis</i> (10^4 – 10^8)	1243–2856	Bacterial concrete (10^8)	All bacterial specimens fell in the “low” range, whereas control concrete fell in the “moderate” range. 57% reduction in charge passed of concrete with bacteria (10^8) than control concrete.
Smitha et al. [85]	OPC	<i>B. megaterium</i> (10^4 – 10^6)	986–1450	Bacterial concrete (10^5)	About 21.7%, 32.8%, and 11.3% reduction in charge passed for bacterial concrete (10^4 , 10^5 , 10^6) compared to control concrete. All concrete samples fall in the “very low” to “low” range.
Rao et al. [106]	OPC (M20, M40) OPC-MS (6%, 10%) (M60, M80)	<i>B. subtilis</i> JC3 (10^5)	85–327	M80 Bacterial concrete	Each grade of concrete containing bacteria falls in the range of “negligible” to “very low”, showing better resistance against chloride penetration compared to non-bacterial concrete, which fall in the “very low–low–moderate” range of the charge passed, showing chloride ingress.
Siddique et al. [56]	OPC-RHA (0%, 5%, 10%, 15%, 20%)	<i>B. aerius</i> (10^5)	799–1947	10% RHA (10^5)	Inclusion of RHA reduced the charge passed through concrete. “low to moderate” and “very low to moderate” for all RHA and bacterial RHA concrete, respectively.
Nosouhian et al. [107]	OPC	<i>S. pasteurii</i> , <i>B. subtilis</i> (10^6 , 10^7 , 10^9)	4536–5385	A mix containing both bacteria (2×10^9)	11.7% reduced charge passed in concrete with bacteria compared to that in non-bacterial concrete. Inclusion of <i>S. pasteurii</i> and <i>B. subtilis</i> can be potentially beneficial against chloride ion ingress.
Siddique et al. [59]	OPC-CBFD (0%, 10%, 20%, 30%)	AKKR5 (10^5)	1066–2982	0% CBFD bacterial concrete	10% CBFD and control concrete with and without bacteria fall in the “low” range of chloride permeability.
Achal et al. [20]	OPC, Biocement	<i>B. sphaericus</i> CT-5 (5×10^6 , 10^7 , 10^8)	818–3396	Bacterial concrete	The addition of bacteria increases the resistance against chloride ion penetration, thereby changing the permeability class from “moderate” to “very low”.
Chahal et al. [95]	OPC-SF (0%, 5%, 10%)	<i>S. pasteurii</i> (10^2 , 10^5 , 10^7)	380–1950	10% SF (10^5)	Inclusion of SF resulted in better resistance of chloride ion penetration. All SF-concrete samples fall in the “very low” range.
Chahal et al. [10]	OPC-FA (0%, 10%, 20%, 30%)	<i>S. pasteurii</i> (0 , 10^3 , 10^5 , 10^7)	762–1988	30% FA (10^5)	All concrete samples found to lie in the range of “very low to low” against resistance towards Cl^- ion penetration.

Notes: MS: micro silica; FA: fly ash; SF: silica fume; RHA: rice husk ash; CBFD: cement baghouse filter dust.

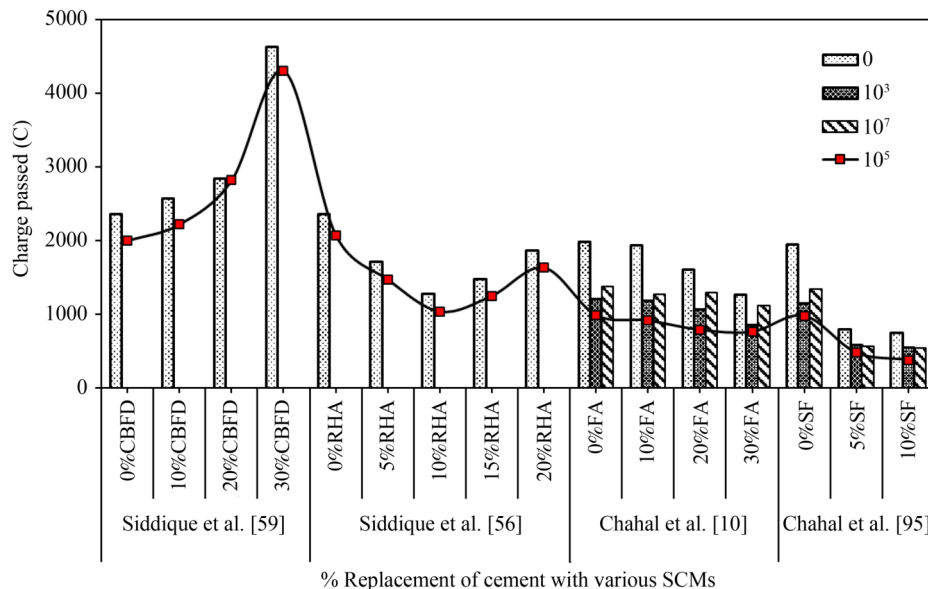


Fig. 10 28 d RCPT value of blended concrete at various bacterial concentrations.

durability properties of concrete has demonstrated reduction in chloride permeability of about 30% at 120 d, 44% at 180 d, and 54% at 240 d. Ling and Qian [109] studied the behavior of bacteria inclusion for reinforcement protection in concrete. Chloride concentration at various depths revealed that the presence of bacteria prevented chloride from penetrating the cracks, prevented corrosion products from spilling into the cracks, and reduced the weight-loss ratio of the reinforcements, thereby slowing the progression of reinforcement corrosion [109].

The evaluation of gas permeability in concrete is important in the assessment of the long-term durability and overall performance of concrete structures. Gas permeability quantifies the degree to which gases can traverse the porous and fractured structure of concrete and has implications for many concrete degradation mechanisms, such as reinforcement corrosion, concrete carbonation, and infiltration of deleterious compounds [110]. de Muynck et al. [34] in his study of bacterial carbonate precipitation using *B. Sphaericus*, found that gas permeability decreased with the addition of bacteria at all w/c due to the presence of precipitated calcite. In another study, greater gas permeability is reported in samples with greater w/c . The use of biodeposition treatment in the presence of calcium chloride led to reductions in permeability of approximately 31%, 47%, and 63% as the w/c increased from 0.5 to 0.6 and 0.7, respectively. The application of traditional surface treatments, such as acrylate-based surface coatings or the use of penetrating sealants like silanes or siloxane mixtures, led to more significant decreases in gas permeability than occurred with biodeposition treatment [92]. Nguyen et al. [111] had also reported the similar behavior of concrete treated with *B. subtilis* bacteria.

6.3 Freeze–thaw resistance

The experimental findings by de Muynck et al. [34], demonstrated that cementitious composites enhanced with bacterial technology, with a w/c of 0.7, and exhibited a notably greater SS following 21 freeze–thaw cycles than occurred in the composites with a lower w/c . This improvement can be attributed to the presence of a higher quantity of water capable of freezing within the pore system. Bang et al. [49] studied the performance of *S. pasteurii* bacteria cells (8.6×10^8 cells/cm³) in cement mortar beams, cured in urea–CaCl₂ medium for 7 d, prior to 14 d of air curing, and subjected to 300 cycles of freezing–thawing. Throughout the test cycles, there were notable decreases (ranging from 44% to 73%) in the average expansion of mortar beams containing bacteria, in comparison to the control specimens. The average expansion of control specimens was found to be 0.054% after being subjected to 300 cycles of freeze–thaw. In

contrast, the expansion of bacterial mortar beams was seen to be just 0.03%. The specimens containing bacteria exhibited a retention rate of 98% in terms of their initial weight after undergoing 300 cycles of freezing and thawing. In contrast, the specimens lacking bacteria displayed a significantly lower retention rate of just 69% of their original weight [49]. The better performance of the bacterial mortar specimens was because of the formation of an impermeable calcite layer on the surface of samples which resisted the samples to an adverse environmental condition.

7 Material characterization: X-ray computed tomography (XCT)

XCT is a non-destructive method used to examine the internal microstructure of materials. The emitter, which releases X-rays at a specific intensity, and the detector, which measures the X-ray reception intensity, make up the tomography apparatus. The outcome is a three-dimensional image in gray scale, where each area of gray corresponds to a different level of absorption of X-rays by the material, which is related to material density. Lighter greys represent higher densities of material, whereas lower densities are described by darker tones [112]. Although reports of XCT studies on concrete appear in the literature, only some were on bacterial concrete [35,88,113]. Wang et al. [113] conducted X-ray μ -CT to understand the microstructure and deposition of self-healing materials in concrete with bacteria encapsulated in hydrogel. The precipitation of calcite on the surface and inside the specimens could be seen clearly in the X-ray tomography images. The generated precipitates can be seen by subtracting the two-dimensional (2D) X-ray images taken before and after the healing process. Results show that no significant difference was found in ‘before’ and ‘after’ images for the sample with no nutrients (Reference sample, R). However, samples with hydrogel incorporation (m-H) and bacteria encapsulated in hydrogels (m-HS) show significant differences before and after the healing process. A larger amount of precipitation was formed in M-HS samples after the healing process than in the m-H sample.

To get a clearer idea about the precipitation of calcite, 2D reconstructed slices were rendered in three-dimensional (3D) (with the help of VG Studio) to learn more about the precipitation distribution in a 3D concrete matrix.

Even though there was little precipitation seen in the 2D images, there was some precipitation visible in sample R. There was a little concentration near the sample’s surface. The precipitation was dispersed randomly across the remainder of the sample, not confined to the cracks. A dense deposition was

discovered at the surface of sample (m-H). The quantity significantly decreased inside the matrix and in the subsurface layer. It appears that more precipitation was generated for the sample m-HS.

Likewise, in sample m-H, a substantial layer was seen, but it was more prominent on the sample's surface and less inside the body of material. Quantitative data of the produced precipitation were acquired using the software Morpho+. Results show 0.21%, 1.37%, and 2.2% precipitation by volume of the samples (R, m-H, and m-HS, respectively).

8 Conclusions

1) The most popular ways to incorporate bacteria into concrete are to spray it on the surface, add it to the mix, and encapsulate it there so it stays dormant until a crack forms; at that point the bacteria become active and begin the biomineralization process. Although most of the examined articles added the bacteria to the mixture, spraying the bacteria onto the mixture may be more appropriate because it keeps the bacteria out of the concrete mixture's heavily alkaline media.

2) Bacteria can produce calcium carbonate both autotrophically and heterotrophically as part of the biomineralization process. The heterotrophic method primarily depends on utilizing other nutrients and minerals for biomineralization, whereas the autotrophic pathway occurs when the bacteria use carbon dioxide or light to generate calcium carbonate.

3) Three criteria are determined to be the main determinants of the CS of microbial concrete: the type of bacteria, the application process, and the concentration of bacteria in the concrete matrix. Most researchers have also noted that the most significant improvement in CS is found at an intermediate bacterial cell concentration of 10^5 cells/mL.

4) Use of various SCMs such as FA, RHA, SF, have also enhanced mechanical and durability properties at an optimum replacement level.

5) As much as a 50% increase is observed in FS with the addition of fibers in bacteria-based self-healing concrete with significant enhancement in durability properties.

6) Although very few studies have been performed on the carbonation of bacterial concrete, as much as a 50%–60% reduction in carbonation depth is observed for bacterial concrete compared to the depth in control concrete because of the densification of concrete matrix and pore filling. Contradictorily, concrete cured in a nutrient medium like calcium lactate-urea is subject to increased risk of carbonation because of acidic nature of lactate media.

7) As much as 60% reduction in water absorption is

observed for bacterial concrete due to intense pore filling with the calcite precipitates, thereby leading to more durable concrete. Similar trends have also been observed in the case of gas permeability.

8) Bacterial concrete is also effective against the penetration of chloride ions in concrete. As much as 57% reduction is observed in charge passed (RCPT) for bacterial concrete compared to that in normal concrete. Most of the bacterial concrete samples were found falling in the region of “very low–low–moderate” range as defined for chloride ion permeability by ASTM C1202, making the bacterial concrete more durable against chloride ion penetration.

9) Bacteria induced mortar/concrete samples have shown better resistance against cyclic freezing and thawing. About 44%–73% reduction in expansion of bacterial concrete/mortar specimens is observed. A retention rate of about 98% in their initial weight is also reported in the bacterial concrete samples subjected to cycles of freezing and thawing.

10) XCT is a technique which gives us an idea about the microstructure of concrete. With the help of XCT technology in bacterial concrete, we learn about the amount of precipitates formed and its distribution along the concrete matrix.

9 Research gaps and recommendation for further studies

1) The majority of the research being done now is on a laboratory scale. As a result, more extensive outside experiments are required to be conducted.

2) Unlike concrete, mortar has been the subject of the majority of experimental work until now. The behavior of bacteria in concrete needs to be thoroughly investigated because mortar and concrete behave differently due to the influence of particles and different fracture patterns.

3) The behavior of bio concrete in actual environmental conditions, such as when the concrete gets old, has multiple cracks or faces a variety of prolonged loads, has to be studied further.

4) Carbonation of concrete is an essential aspect of the durability of concrete. Extensive research should be performed on the bacterial concrete regarding its behavior against carbonation.

5) Use of SCMs and fibers in bacterial concrete needs further research to encourage sustainable practices in construction industry.

6) It is crucial to consider ways to cut costs of producing bacterial concrete, including consideration of nutrients as well as bacteria.

Competing interests The authors declare that they have no competing interests.

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