Jurnal
Teknologi

Full Paper

RESEARCH PROGRESS ON MICROBIAL SELF-HEALING CONCRETE

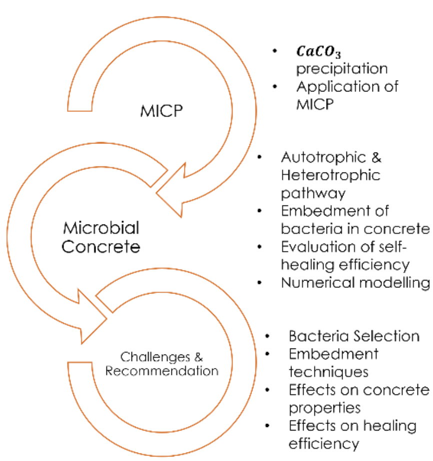
Bao Fang Yip, Mohd Ridza Mohd Haniffah, Erwan Hafizi Kasiman*, Ahmad Razin Zainal Abidin

School of Civil Engineering, Faculty of Engineering, Universiti Teknologi Malaysia, 81310 UTM Johor Bahru, Johor, Malaysia

Article history
Received
28 October 2021
Received in revised form
23 January 2022
Accepted
3 February 2022
Published Online
20 April 2022

*Corresponding author
bfyip2@graduate.utm.my

Graphical abstract



Abstract

Crack formation in concrete is inevitable. The cracks allow the penetration of harmful substances which may decrease the durability and the service life of concrete structures. Self-healing concrete is therefore emerging as an innovative construction material to tackle the cracking issues. In recent years, microbial self-healing concrete is garnering interest from many researchers due to its environmentally-friendly nature and the concrete compatibility of microbially-induced calcium carbonate precipitation. Various metabolic mechanisms have been used for microbial self-healing concrete production and urea hydrolysis is the most preferable metabolic pathway due to its fast and high precipitation of calcium carbonate. In this paper, a comprehensive review on the research progress on microbial self-healing concrete is presented together with the numerical modelling of microbial self-healing concrete. The challenges and limitations of microbial self-healing concrete are discussed along with the recommendations for its prospective uses in the construction industry. It is found that the survival of bacteria through direct addition technique is limited and needs further investigation. The immobilization technique gives a promising result in durability properties but doesn't reach the mechanical requirement. Moreover, a comprehensive assessment of self-healing efficiency is required, and more efforts are needed to improve from laboratory scale to large-scaled application.

Keywords: Microbial concrete, self-healing, microbial induced calcium carbonate precipitation, bacteria, crack

Abstrak

Pembentukan retakan dalam konkrit tidak dapat dielakkan. Keretakan tersebut mempercepatkan penembusan bahan berbahaya yang boleh mengurangkan ketahanan dan jangka hayat struktur konkrit. Konkrit penyembuhan diri telah muncul sebagai bahan pembinaan inovatif untuk mengatasi masalah retakan konkrit. Pelbagai cara dan teknik telah dikaji untuk menghasilkan konkrit penyembuhan diri yang lebih berkualiti. Kebelakangan ini, konkrit penyembuhan diri mikroba semakin banyak dikaji oleh para penyelidik disebabkan ciri-ciri mesra alam dan keserasian kalsium karbonat yang dihasilkan oleh mikrob dengan konkrit. Pelbagai mekanisme metabolik telah digunakan dalam konkrit penyembuhan diri mikroba dan ia telah didapati bahawa cara hidrolisis urea selalu digunapakai kerana pemendakan kalsium karbonatnya yang cepat dan tinggi. Dalam kertas kerja ini, kajian komprehensif mengenai konkrit penyembuhan diri mikroba dibentangkan. Cabaran dan had konkrit penyembuhan diri mikroba dibincangkan bersama dengan cadangan untuk prospek masa depan di industri pembinaan. Didapati bahawa kewujudan bakteria dalam konkrit melalui teknik campuran langsung terhad dan pemeriksaan lanjut diperlukan. Teknik imobilisasi memberikan keputusan baik dari segi ketahanan tetapi lemah dalam kekuatan mekanikal. Di samping itu, penilaian yang lebih komprehensif diperlukan untuk kecekapan penyembuhan diri serta memerlukan usaha yang berterusan untuk meningkatkan aplikasi dari skala makmal ke skala yang lebih besar.

Kata kunci: Konkrit microba, penyembuhan diri, *microbial induced calcium carbonate precipitation,* bakteria, retakan

1.0 INTRODUCTION

Concrete is the most widely and massively used material in the construction of buildings and infrastructures. High rise buildings, water dams, water / sewerage treatment plants, road pavements, bridges and tunnels are all constructed with concrete. Apart from its high availability, concrete is a popular material choice as it has a high compressive strength with the addition of embedded steel reinforcements to increase its tensile properties. However, the inherently low tensile strength of concrete makes it vulnerable to the formation of cracks, which are often caused by external loading, plastic shrinkage, drying shrinkage, thermal stress, creep and rebar corrosion. The presence of cracks in concrete allows the penetration of harmful chemical substances like chloride and sulphide, which may result in the corrosion of the steel reinforcements. The penetration of moisture will even speed up the formation and propagation of cracks through recurrent freezing and thawing processes in winter climates. The formation of cracks will consequently decrease the durability and the service life of concrete structures if left unchecked.

In order to mitigate the concrete cracking issues, researchers have applied the concept of self-healing into cementitious materials, giving rise to the emergence of self-healing concrete. Generally, self-healing concrete is classified into two major groups based on its healing approaches, which is either autogenous or autonomous. The autogenous healing approach relies on natural healing caused by chemical reaction(s) which involves the hydration process of un-hydrated cement particles in the concrete matrix. Now, more focus is aimed towards the autonomous-based self-healing approach instead, which involves the usage of engineered additives such as polymers, chemical compounds and superabsorbent polymers, along with several techniques such as micro-encapsulation, macro-encapsulation and vascular-network techniques to deliver the healing agents to damaged areas. Researchers are still working on strategies to scale up the production of these types of self-healing concrete from laboratory settings and implementing it in large-scale applications [1], [2]. Nonetheless, their works do give positive results which suggest that the aforementioned self-healing techniques are indeed feasible and warrant further research.

Typically for autonomous self-healing, chemical healing agents are applied to prolong the service life of concrete structures. However, the application of chemical healing agents such as polyurethane, epoxy resins and polymers are environmentally unfriendly and are hindered by their limited compatibility with concrete due to the different thermal expansion coefficients between the concrete and the agents themselves. With these issues in mind, researchers are looking for various environmentally friendly alternatives that are still ideal and would still give rise to concrete with good quality, long shelf life, high pervasiveness, and provide repeatable crack healing when the structure is subjected to multiple loading and damage [3].

Recently, a bio-based self-healing approach has emerged as a sustainable and a promising alternative, in which the healing process is initiated by the precipitation of calcium carbonate as a by-product of the metabolic process induced by specific bacteria in the concrete. To date, there are several published reviews on the application of such microbially induced calcium carbonate precipitation (MICP) in various fields. It has been reported that the application of MICP is environmentally friendly, long-lasting and has good compatibility with concrete. Most importantly, these bacteria-based self-healing treatments do provide promising results in crack healing, resulting in a good recovery in terms of mechanical strength and durability, thus resulting in a prolonged lifespan of concrete structures and a reduced need for repair and maintenance.

As of this point in writing, most of the works on microbial self-healing concrete have been focusing on laboratory and experimental investigations, with a limited amount of numerical-based studies that are yet to be reviewed. This paper aims to provide an encompassing review and assessment of microbial self-healing concrete by detailing its research progress thus far, along with a comprehensive review of the metabolic pathways, embedment methods and self-healing evaluation techniques used by various researchers. The challenges and future direction of microbial self-healing concrete is also discussed in subsequent sections.

2.0 MICROBIALLY INDUCED CALCIUM CARBONATE PRECIPITATION (MICP)

MICP is one of the biomineralization processes that involves the formation of minerals (calcium carbonate) by microorganisms or bacteria through their metabolic reaction with the microenvironment. In MICP, the precipitation of calcium carbonate requires sufficient concentrations of calcium ions (Ca^{2+}) and carbonate ions (CO_3^{2-}), as shown in Equations (1) and (2). In addition to the presence of these two primary ions, the precipitation process also depends on the concentration of dissolved inorganic carbon (DIC) and calcium ions, the pH value, as well as the existence of nucleation sites [4]. The concentration of DIC, on the other hand, can be affected by the pressure of carbon dioxide (CO_2), the surrounding temperature and other environmental factors [5].

$$\boldsymbol{Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3} \quad (1)$$
$$\boldsymbol{Ca^{2+} + 2HCO_3^{-} \rightarrow CaCO_3 + CO_2 + H_2O} \quad (2)$$

MICP has been studied extensively and applied in many fields in a worldwide scale. Several works have been published on various applications of MICP in Civil Engineering such as the remediation of groundwater with heavy metal and radio-nuclide contamination [6], soil improvement [7]–[10], restoration of stone monuments [11]–[13], concrete surface treatments [5], [14]–[16], shotcrete improvements [17] and enhancement of concrete structures [18]–[22].

In regard to concrete enhancement, the MICP biotechnology is originally intended for concrete surface treatments, which helps to reduce concrete permeability and the penetration of harmful substances as well as to increase the durability of concrete as a whole. It was not until recently that engineers in the concrete industry have made the innovative decision to apply the MICP techniques in the production of self-healing concrete.

3.0 MICROBIAL SELF-HEALING APPROACHES IN CONCRETE

3.1 Autotrophic and Heterotrophic Bacteria

3.1.1 Autotrophic Bacteria

Figure 1 shows the taxonomy on the metabolic pathways in microbial self-healing concrete. Generally, there are two major metabolic pathways in MICP, which includes the autotrophic pathway and the heterotrophic pathway. Autotrophic pathways such as photosynthesis and methane oxidation uses carbon dioxide (CO_2) as the carbon source. The autotrophic metabolic pathways include non-methylotrophic methanogenesis (methane oxidation) by methanogenic archaea, anoxygenic photosynthesis by purple bacteria and oxygenic photosynthesis by cyanobacteria [23]. Calcium carbonate precipitation from CO_2 is then induced by the autotrophic bacteria in the presence of calcium ions (Ca^{2+}) and water in its microenvironment (Equation 3 to 5). The difference between oxygenic and anoxygenic photosynthesis is the electron donor type. Oxygenic photosynthesis uses water as the electron donor, while anoxygenic photosynthesis uses inorganic compounds like H_2S instead.

$$\boldsymbol{CO_2 + H_2O \rightarrow H_2CO_3} \quad (3)$$
$$\boldsymbol{H_2CO_3 \leftrightarrow HCO_3^{-} + H^{+}} \quad (4)$$
$$\boldsymbol{Ca^{2+} + HCO_3^{-} \rightarrow CaCO_3 + H^{+}} \quad (5)$$

To date, only a few authors have reported the application of autotrophic bacteria in self-healing concrete. Zhu *et al.* [24] have studied the biomineralization of cyanobacteria *Synechococcus* PCC8806. Their experiments have shown positive results in which the cyanobacteria have formed a thick calcite-cell aggregate layer adhering to the concrete which can reduce its water absorption and increase its sonication resistance. Zhu extended the investigation of biomineralization of phototrophic cyanobacteria in mortars with live and UV-killed cyanobacteria *Gloeocapsa* PCC73106 [25]. It was subsequently observed that UV-killed cells performed better by contributing to a higher concrete compressive strength and reduction in water absorption and porosity, while the live cells have resulted in a higher amount of calcium carbonate precipitation. Kaur *et al.* [26] on the other hand, focuses on ureolytic bacteria, and have suggested the replacement of urea with a direct influx of CO_2 as the production of ammonia from urea hydrolysis by the bacteria can deteriorate the concrete structures. When Kaur *et al.* studied the carbonate precipitation by *Bacillus megaterium* SS3 with CO_2, the bacteria grew well and the amount of calcium carbonate precipitation through CO_2 was comparable to that when urea was used for precipitation. The treated specimens have shown a 117% improvement in compressive strength over the control specimens and 47% improvement over the urea-treated specimens. Water absorption was also reduced in the carbon dioxide-treated specimens.

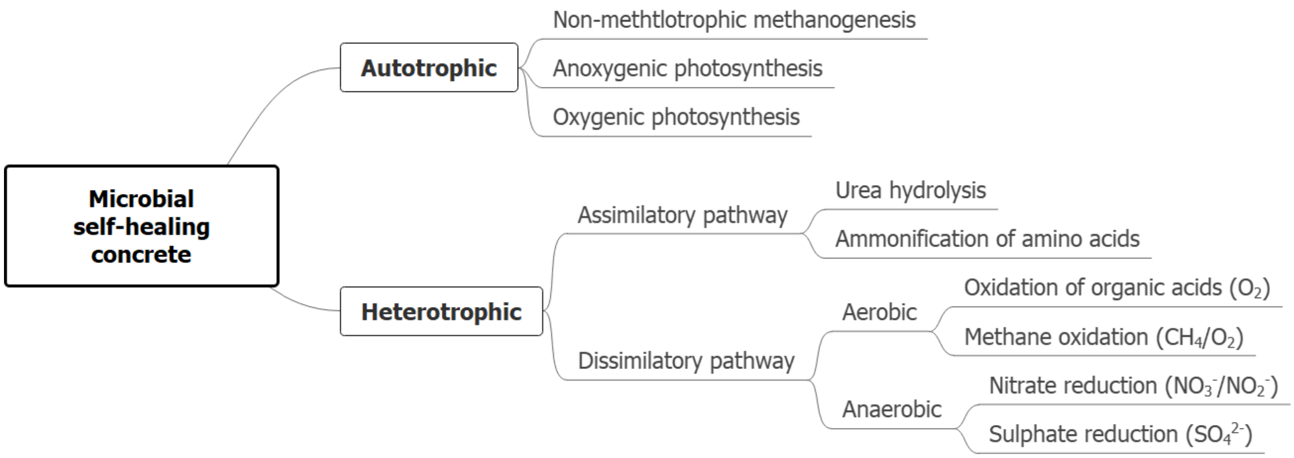


Figure 1 Taxonomy on the metabolic pathways in microbial self-healing concrete

Table 1 Summary on microbial self-healing concrete

Mechanisms	Microorganisms	Embedment method	Reported major findings	Ref.
Autotrophic Frequency = 3	*Bacillus megaterium* SS3	Direct	Improvement in compressive strength. Reduction in water absorption.	[26]
	Cyanobacteria *Gloeocapsa* PCC73106	Direct	Live cells increased the amount of precipitation. UV-killed cells increased the compressive strength. Reduced water absorption, with the lowest porosity.	[25]
	Cyanobacteria *Synechococcus* PCC8806	Direct	Reduced water absorption and increased resistance to sonication.	[24]
Denitrification Frequency = 6	*D. nitroreducens* *P. aeruginosa*	Immobilized ACDC, expanded clay	Denitrification can occur under minimum nutrient conditions. The results showed the capability of ACDC to inhibit steel corrosion.	[27]
	Diaphorobacter nitroreducens *Pseudomonas aeruginosa*	Immobilized expanded clay	Improvement in water tightness - absorbed 50% and 40% less water.	[28]
	ACDC	Direct	More than 90% of the cracks (500 μm) were closed and 68% less water was absorbed.	[29]
	ACDC	Direct and Immobilized	Immobilized in diatomaceous earth, expanded clay, granular activated carbon. ACDC performed better than bacteria, yielding positive effects in corrosion inhibition and crack healing.	[30]
	ACDC, CERUP	Direct	Protected rebar from corrosion, 300 μm cracks were healed.	[31]
	Pseudomonas aeruginosa, Diaphorobacter nitroreducens	Direct	Withstood alkaline environment and were concrete-compatible.	[32]
Fungi Frequency = 2		Direct	Comparison between *Trichoderma reesei, Aspergillus nidulans, Cadophora interclivum, Umbeliopsis dimorpha, Acidomelania panicicola, Pseudophialophora magnispora* was reported. *T. reesei* spores germinated into hyphal mycelia and grew equally well with or without concrete.	[33]
	Aspergillus nidulans	Direct	*A. nidulans* could grow on concrete plates.	[34]
Heterotrophic, iron-reducing bacteria Frequency = 2	Shewanella bacteria	Direct	Increased compressive strength.	[35]
	Shewanella bacteria	Direct	25% increase in compressive strength in 28 days.	[36]

Mechanisms	Microorganisms	Embedment method	Reported major findings	Ref.
Oxidation of organic acid Frequency = 19	*Bacillus sphaericus*	Direct	14.3% regain in mechanical strength.	[37]
	Bacillus pseudofirmus	Direct	Polyacrylic acid and citric acid affected the concrete strength. No strength was developed for gluconate- and ascorbic acid	[38]
	Bacillus cohnii	Immobilized expanded clay particles	Healed 0.46 mm cracks, but resulted in a decrease in compressive strength and decrease in permeability. Immobilization increased bacteria survival.	[39]
		Direct	Comparison between *Bacillus cohnii, Bacillus halodurans* and *Bacillus pseudofirmus* was reported. Decreased compressive strength.	[40]
	Bacillus pseudofirmus *Bacillus cohnii*	Direct	Strength reduction of about 10% at 3, 7 and 28 days and only gave positive effects in compressive strength with the addition of calcium lactate. Survival of bacteria spore decreased due to decreasing matrix pore size. Yeast extract and peptone addition reduced compressive strength; especially after peptone is added the late strength of concrete may even be lower than early strength.	[41]
	Bacillus subtilis	Immobilized Lightweight aggregates, graphite nano-platelets	Bacteria were distributed evenly in concrete matrix due to immobilization in GNP. Healing efficiency was higher in the early stages for GNP and the later stages for LWA. Increased compressive strength.	[42]
	Bacillus cohnii	Direct	59% increase in compressive strength, absorption rate and drying shrinkage of cement mortar decreased.	[43]
	Spore-forming bacteria	Direct	Improve rheology. Calcium lactate delayed hydration kinetics and decreased the compressive strength in early stages but increased in later stages. Calcium nitrate gave a negative result while calcium formate gave a positive result.	[44]
	Spore-forming bacteria	Direct	The effects and influences of crack width, curing ways and cracking age were determined. Water curing is the best. Healing efficiency decreased as crack age increased.	[45]
	Bacillus pseudofirmus	Direct	Comparison between Bac*illus pseudofirmus, Bacillus halodurans* and *Bacillus cohnii* was reported. *B. pseudofirmus* is the most effective.	[46]
	Bacillus cohnii	Immobilized LWA	Increased compressive strength.	[47]
	Bacillus genus	Immobilized LWA, expanded clay	Recovery of water tightness increased. 54% and 63% reductions in compressive and flexural strength respectively	[48]
	Bacillus	Immobilized LWA, expanded clay	Numerical model overestimated the volume of filling product as all healing agent in LWA was being converted to calcium carbonate.	[49]
	Bacillus alkalinitrilicus	Immobilized expanded clay particles	Crack-healing of up to 0.46 mm.	[50]
	Bacillus cohnii	Direct	Loss in compressive and flexural strength by about 8–10%. Calcium source affected the concrete properties. Increased flexural strength when calcium glutamate was used.	[51]
	Bacillus cohnii *B. pseudofirmus*	Immobilized expanded clay capsule	The influence of different parameters on the rate and quality of the crack healing was estimated.	[52]
	Bacillus cohnii	Immobilized Expanded perlite	Healing of up to 0.79 mm in EP. Has a higher bacteria content, lower incorporated amount and a high cost-effectiveness to make EP particles.	[53]
	Bacillus H4	Direct	ORT contained calcium peroxide CaO_2 and lactic acid (9:1) to provide a stable oxygen supply and a maintained pH level (9.5–11.0) for effective metabolic activities.	[54]
	Bacillus H4	Direct	Excessive Ca^{2+} inhibited $CaCO_3$ precipitation.	[55]
Oxidation of organic acid & Denitrification	Alkaliphilic bacteria	Direct	Increased crack-sealing efficiency and gave an improvement in frost salt scaling.	[22]

Mechanisms	Microorganisms	Embedment method	Reported major findings	Ref.
Urea hydrolysis Frequency = 44	*B. megaterium*	Direct	Average number of viable bacteria decreased. Improved strength and permeability properties.	[56]
	Bacillus sp. CT-5	Direct	Increased compressive strength and pullout strength, reduction in corrosion rate and in mass loss.	[57]
	Bacillus sphaericus	Direct	Reduced water absorption and improved compressive strength.	[58]
	Bacillus sphaericus CT-5	Direct	Reduced water permeability and improved compressive strength.	[59]
	Sporosarcina pasteurii	Direct	35% improvement in compressive strength and higher activity in CSL-urea medium.	[60]
	Sporosarcina pasteurii	Direct	Increased compressive strength.	[61]
	Sporosarcina pasteurii	Direct	UV-induced mutant Bp M-3 has the best performance.	[62]
	Bacillus megaterium	Direct	24% increase in compressive strength and increased flexural strength.	[63]
	S. pasteurii	Immobilized PU foam	Polyurethane matrix provided protection to bacterial cells from the extreme alkaline nature of concrete.	[64]
	Exiguobacterium mexicanum	Direct	Increase in compressive strength of up to 23.5%, reduction in water absorption.	[65]
	Sporosarcina pasteurii	Direct	Increased compressive strength with reduced porosity.	[66]
	Sporosarcina pasteurii	Direct	Nutrient medium and bacteria retarded the hydration kinetics. Increased compressive strength	[67]
	Sporoscarcina pasteurii	Direct	Improved compressive strength and reduced water absorption.	[68]
	Sporoscarcina pasteurii	Direct	20% improvement in strength and reduced water absorption and chloride penetration.	[69]
	Bacillus subtilis	Direct	Improvements in compressive strength, ultrasonic pulse velocity and a decrease in water absorption.	[70]
	Bacillus sphaericus *Bacillus cohnii* CERUP	Direct	Lower cost for CERUP.	[71]
	CERUP	Direct	Crack healing up to 0.45 mm. Addition of 0.5% and 1% by weight of cement did not adversely affect the compressive strength but higher dosages of 3% and 5% had significant adverse effects on strength.	[72]
	B. sphaericus	Direct	Decrease in uptake of water and gas permeability.	[15]
	Bacillus sphaericus	Direct	Increased resistance of mortar specimens towards carbonation, chloride penetration and freezing and thawing.	
	Bacillus sphaericus	Direct	Comparison between *Bacillus sphaericus, Sporosarcina psychrophile, Sporosarcina ureae* and *Sporosarcina pasteurii* was reported. *B. sphaericus* precipitate more carbonate than *S. psychrophila* at cold temperatures. 46 % decreased sorptivity and 64 % lower weight loss upon sonication.	[16]
	B. megaterium SS3	Direct	40% decrease in water absorption. 31% decrease in porosity and 18% increase in compressive strength	[5]
	Bacillus sphaericus	Direct	Increased compressive and tensile strength.	[73]
	Lysinibacillus sphaericus	Direct	Healing of 0.4mm cracks in 70 days.	[74]
	Bacillus subtilis	Direct	Increased compressive strength with enhanced tensile strength and decreased water absorption and porosity of shotcrete.	[17]
	Bacillus sphaericus *S. pasteurii*	Direct	*B. sphaericus* is more effective between the two.	[75]
	S. pasteurii *Bacillus cereus*	Direct	Reduction in rapid chloride permeability. Increase in compressive strength. *B. cereus* performed better.	[76]
	Bacillus massiliensis	Direct	Comparison between *Sporosarcina soli, Bacillus massiliensis* and *Arthrobacter crystallopoietes* was reported. Increased compressive strength.	[77]
	Bacillus subtilis	Direct	Cell wall improved compressive strength - 14.8% regain of compressive strength with decreased porosity.	[78]

Mechanisms	Microorganisms	Embedment method	Reported major findings	Ref.
	Bacillus sphaericus	Immobilized sodium alginate	Used extrusion, spray-drying, and freeze-drying techniques for immobilization. Pore size reduced bacteria survival.	[79]
	S. pasteurii *Pseudomonas aeruginosa*	Direct	Increased compressive strength.	[80]
	Bacillus aerius	Direct	Increased strength properties and reduced water absorption, permeability and concrete porosity.	[81]
	Bacterial strain AKKR5	Direct	CBFD displayed negative effects on compressive strength. Increased concrete permeability. Bacteria increased strength and reduces water absorption and chloride penetration.	[82]
	Bacillus sphaericus	Immobilized Silica gel	Reduced water permeability.	[10]
	Bacillus subtilis strain JC3 *Salinicoccus* sp.	Direct	JC3 has better improvement with 19.2% strength regain.	[83]
	Bacillus sphaericus	Immobilized Diatomaceous earth	Reduced water absorption. Healed 0.15 - 0.17 mm cracks.	[84]
	Bacillus sphaericus	Immobilized hydrogel	Healed 0.5 mm cracks under wet-dry cycles and reduced water permeability to 68%. Moisture absorption and retention properties of hydrogel also helped in higher bacterial action. Decrease of compressive strength by 15 to 34%.	[85]
	Bacillus sphaericus	Immobilized hydrogel	Improved durability of concrete via crack closure and calcite precipitation.	[86]
	Bacillus sphaericus	Immobilized modified sodium alginate-based hydrogel	Bacterial activity was observed only for encapsulated samples at crack face. Reduced tensile strength and compressive strength.	[87]
	Bacillus sphaericus	Immobilized silica gel and polyurethane (PU) in glass tubes	PU-immobilized bacteria showed lowest permeability and high strength recovery. Higher bacteria activity in silica sol.	[88]
	Bacillus sphaericus	Immobilized melamine based microcapsules	Healed 0.97mm crack and reduced permeability. The best healing performance was observed during wet and dry curing cycles. Addition of nutrients and capsules significantly affected the hydration degree, compressive and tensile strength. Addition of 5% microcapsules by weight of cement reduced the compressive strength by up to 34%. Tensile strength was significantly affected with capsule addition above 3%.	[89]
	S. pasteurii	Direct	Yeast extract was replaced with meat extract. Reduced retardation by up to 75%.	[90]
	Bacillus cereus	Direct	Reduced water absorption and chloride permeability up to 12% and 10.9% respectively. Wet-dry cycle curing is the most preferable.	[91]
	Sporosarcina pasteurii	Direct	Higher rate of $CaCO_3$ precipitation with calcium lactate than with calcium nitrate.	[92]
	Sporosarcina pasteurii	Immobilized calcium sulphoaluminate cement	Regained mechanical properties, permeability and durability. Regain of the ratio of compressive strength and increase of water tightness up to 130% and 50% respectively.	[93]
	Sporosarcina pasteurii	Immobilized Porous ceramsite	Compressive strength recovery increased by 24% and the water absorption coefficient decreased by 27%.	[94]
Urealysis & Denitrification	*Diaphorobacter nitroreducens* *Bacillus sphaericus*	Immobilized	Comparison between diatomaceous earth, expanded clay, granular activated carbon, metakaolin, zeolite, air entrainment, CERUP and ACDC was reported. Reduced compressive strength.	[95]

3.1.2 Heterotrophic Bacteria

A. Sulphur cycle

Apart from autotrophic pathways, MICP can take place via heterotrophic pathways in the form of sulphur and nitrogen cycles [96]. In the sulphur cycle, $CaCO_3$ precipitation is induced by sulphate reducing bacteria (SRB) through the dissimilatory reduction of sulphate (Equation 6). *Desulfovibrio* sp. can precipitate $CaCO_3$ by reducing the sulphate released from gypsum ($CaSO_4.2H_2O$). The calcium ions released from the dissolution of gypsum due to sulphide removal can also react with carbonate ions to form $CaCO_3$ under alkaline conditions (Equation 7).

$$\mathbf{6CaSO_4 + 4H_2O + 6CO_2 \rightarrow CaCO_3 + 4H_2S + 2S + 11O_2} \quad (6)$$

$$\mathbf{CaSO_4.2H_2O \rightarrow Ca^{2+} + SO_4^{2-} + 2H_2O} \quad (7)$$

Alshalif *et al*. [97] have reported the utilization of SRB in self-healing concrete. The compressive strength of concrete was improved by 13% and the water permeability was reduced by 8.5%. Another study on SRB was conducted by Tambunan *et al.* [98]. Their results showed improvements in compressive and flexural strengths by 60.87% and 52.30% respectively, following the addition of SRB in concrete.

B. Nitrogen cycle

In the nitrogen cycle, $CaCO_3$ precipitation can take place through three different mechanisms, which include the ammonification of amino acids (in the presence of organic matter and calcium under aerobic conditions), the dissimilatory reduction of nitrate (in the presence of organic matter, calcium and nitrate under anaerobic conditions) and urea degradation (in the presence of organic matter, calcium and urea under aerobic conditions).

i. Urea hydrolysis

Urea hydrolysis is the most preferable MICP pathway due to its faster and higher precipitation of calcium carbonate in comparison to other metabolic pathways [99]–[101]. In urea hydrolysis, ureolytic bacteria produce the urease enzyme, which catalyses urea ($CO(NH_2)_2$) to carbamate (NH_2COOH) and ammonia (NH_3) (Equation 8). NH_2COOH can be further hydrolyzed to ammonia (NH_3) and carbonic acid (H_2CO_3) (Equation 9). The formation of NH_3 through urea hydrolysis will increase the pH levels and create an alkaline environment in concrete which favours $CaCO_3$ precipitation. NH_2COOH is converted into bicarbonate and hydrogen ions (Equation 10) whereas NH_3 reacts with moisture to form ammonium (NH_4^+)and hydroxide ions (OH^-) (Equation 11). The hydroxide ions will then react with the bicarbonate ions to form carbonate ions (CO_3^{2-}) (Equation 12). The calcium cations (Ca^{2+})will attach to the negatively charged bacterial cell wall and deposit (Equation 13), where CO_3^{2-} will react with Ca^{2+} and form calcium carbonate ($CaCO_3$) on the cell surface (Equation 14). The bacteria cell serves as a nucleation site for $CaCO_3$ precipitation.

$$\mathbf{CO(NH_2)_2 + H_2O \rightarrow NH_2COOH + NH_3} \quad (8)$$

$$\mathbf{NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3} \quad (9)$$

$$\mathbf{H_2CO_3 \leftrightarrow HCO_3^- + H^+} \quad (10)$$

$$\mathbf{2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^-} \quad (11)$$

$$\mathbf{2NH_4^+ + 2OH^- + HCO_3^- + H^+ \rightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O} \quad (12)$$

$$\mathbf{Ca^{2+} + Cell \rightarrow CellCa^{2+}} \quad (13)$$

$$\mathbf{CellCa^{2+} + CO_3^{2-} \rightarrow CellCaCO_3} \quad (14)$$

Various researchers have shown interest to the application of urea hydrolysis in self-healing concrete. Achal and his fellows [56]–[62] have studied intensively on the effects of ureolytic bacteria on concrete. They have used *Bacillus megaterium*, *Bacillus sphaericus* CT-5, *Sporosarcina pasteurii* and even UV-induced mutant bacteria in their experiments. Based on their results, the treated samples have shown an improvement in compressive strength and reduction in water permeability. Wang and his fellows [84]–[89], [102] have studied the application of ureolytic bacteria (*Bacillus sphaericus*) with different capsule materials such as diatomaceous earth, hydrogel and polymer. Their works will be further discussed in Section 3.2.2.

ii. Aerobic oxidation of organic compounds

Although urea hydrolysis provides a faster and a higher precipitation of $CaCO_3$, the production of ammonium ions (NH_4^+) might cause environmental concerns. Ammonium ions can react with other substances to form harmful compounds such as nitrogen oxide, ammonium salts and nitric acid, which are detrimental to both the environment and the concrete structure itself. Due to these concerns, researchers have come up with a new alternative in the form of aerobic oxidation of organic compounds in the presence of a calcium source. The oxidation of organic acids produces calcium carbonate and carbon dioxide (Equation 15). The CO_2 reacts with calcium hydroxide (by-product of hydration of cement) to form $CaCO_3$, thus resulting in autogenous self-healing (Equation 16).

$$\mathbf{CaC_6H_{10}O_6 + 6O_2 \rightarrow CaCO_3 + 5CO_2 + 5H_2O} \quad (15)$$

$$\mathbf{5CO_2 + Ca(OH)_2 \rightarrow 5CaCO_3 + 5H_2O} \quad (16)$$

Several authors have published various works on bacterial self-healing concrete through the oxidation of organic acids. The common bacteria used are *Bacillus sphaericus*, *Bacillus cohnii*, *Bacillus halodurans, Bacillus pseudofirmus, Bacillus subtilis* and *Bacillus alkalinitrilicus*. Gandhimathi and Suji [37] used *B. sphaericus* with lactose broth as the nutrient medium. Calcium lactate, calcium glutamate, calcium formate, calcium acetate and calcium nitrate were the organic acids used, that also doubled as a calcium source. Based on the reported findings, the treated samples regained 14.92% of compressive strength at the 28-day mark. Sharma *et al*. [46] have suggested that *B. pseudofirmus* has a better performance in comparison to *B. halodurans* and *B. cohnii*. Sierra-Beltran *et al*. [47] used B. cohnii (immobilized in lightweight concrete), managed to record an improvement in compressive strength for the treated samples as well. Tziviloglou et al. [48] used the *Bacillus* genus too, where the spores were immobilized in expanded clay particles beforehand. Their samples have shown a good recovery of water tightness, but the concrete mechanical strength was adversely affected.

iii. Nitrate reduction

Due to limited oxygen in the deeper parts of concrete, aerobic oxidation of organic acids is very slow and may hinder the precipitation of $CaCO_3$. Researchers have thus used nitrate-reducing bacteria as an alternative to replace the aerobic oxidation of organic acids. In the nitrate reduction pathway, organic matter was oxidized by using nitrate (NO_3^-), nitrite (NO_2^-), nitric oxide (NO) and nitrous oxide (N_2O) as the electron acceptor, instead of O_2, which is used in aerobic oxidations (Equations 17-20). Calcium carbonate precipitation from CO_2 is then induced by the bacteria in the presence of calcium ions (Ca^{2+}) and water in its microenvironment (Equation 21).

$$\mathbf{2HCOO^- + 2NO_3^- + 2H^+ \rightarrow 2CO_2 + 2H_2O + 2NO_2^-} \quad (17)$$

$$\mathbf{HCOO^- + 2NO_2^- + 3H^+ \rightarrow CO_2 + 2NO + 2H_2O} \quad (18)$$

$$\mathbf{HCOO^- + 2NO + H^+ \rightarrow CO_2 + 2N_2O + 2H_2O} \quad (19)$$

$$\mathbf{HCOO^- + 2N_2O + H^+ \rightarrow CO_2 + N_2 + 2H_2O} \quad (20)$$

$$\mathbf{Ca^{2+} + CO_2 + H_2O \rightarrow CaCO_3 + 2H^+} \quad (21)$$

It was reported that $CaCO_3$ precipitation via nitrate reduction can take place in a minimum-nutrient environment. The production of nitrite ions (NO_2^-) can inhibit the corrosion of steel reinforcements. Ersan *et al*. [27]–[32], [95] have studied the usage of nitrate reducing bacteria such as *Pseudomonas aeruginosa* and *Diaphorobacter nitroreducens*. In their works, they recommended the selection of these two nitrate-reducing bacteria species due to their ability to withstand an alkaline environment and are able to perform under minimum-nutrient conditions. Apart from these strains, a thermophilic, iron-reducing anaerobic bacteria strain belonging to the *Shewanella* genus was also studied [35], [36]. The treated samples have shown an enhanced cement mortar compressive strength by 25% in 28 days, but displayed no significant improvements over that treated with *Escherichia coli*.

iv. Fungi

Despite its promising uses, bacteria do have a prominent weakness when it comes to surviving under extreme environmental conditions, such as high alkalinity, extreme temperatures and dry conditions within the concrete. In recent years, researchers have made attempts to use fungi as a replacement for bacteria in self-healing concrete [33], [34]. These fungi can precipitate $CaCO_3$ in the presence of nutrients, water and oxygen. In comparison to bacterial strains, filamentous fungi have a particular advantage in which they have a higher surface-to-volume ratio and thus provide a larger surface area of organic substrate transfer for biomineralization. This has resulted in a wide usage of filamentous fungi in many biotechnological fields. However, the fungi-driven $CaCO_3$ precipitation mechanism in self-healing concrete is still not well-established and requires further investigations. Luo *et al.* [33] have experimented on a few types of fungi such as *Trichoderma reesei, Aspergillus nidulans, Cadophora interclivum, Umbeliopsis dimorpha, Acidomelania panicicol* and *Pseudophialophora magnispora*. Their results have shown that *T. reesei* grew well on concrete. Later, Menon *et al.* [34] chose *A. nidulans* because this fungal strain is the best characterized member for gene regulation by ambient pH. They have also added that *A. nidulans* (MAD1445) grew well on concrete too and is harmless to human health.

3.2 Embedment of Bacteria in Concrete

3.2.1 *Direct Addition*

There are two different ways to introduce bacteria into concrete, and this includes direct addition and encapsulation techniques. For the direct addition method, an optimum concentration of bacteria is added during concrete mixing, along with its nutrients and calcium source. Andalib *et al.* [63] have studied the optimum concentration of *Bacillus megaterium* to improve the concrete mechanical strength properties. Based on their reports, the compressive strength was increased by 24% for high strength concrete. Chahal and his fellows [68], [69] have studied the effect of ureolytic bacteria (*Sporosarcina pasteurii*) on concrete, with silica fume and fly ash as concrete admixtures. Different concentrations of bacteria cells were added directly to the concrete specimens containing silica fume and fly ash. Their results have indicated that the direct addition of *S. pasteurii* have increased the concrete compressive strength and reduced the water absorption and chloride permeability, thus implying that *S. pasteurii* performed well in concrete made with silica fume and fly ash. Kim *et al.* [75] have studied the direct addition of two different bacteria (*Bacillus sphaericus* and *S. pasteurii*) into normal and lightweight concrete. *B. sphaericus* was recommended due to its higher $CaCO_3$ precipitation.

3.2.2 *Encapsulation or Immobilization*

A. Lightweight aggregate

Aside from direct addition, encapsulation or immobilization techniques are also used to embed bacteria within the concrete matrix [103], [104].

Various encapsulation techniques have been used to protect the bacteria in the concrete, such as lightweight aggregates, expanded clay, diatomaceous earth, melamine-based microcapsules, hydrogels, silica gels and polyurethane (PU). Khaliq and Ersan [42] immobilized *Bacillus subtilis* by using lightweight aggregates (LWA) and graphite nano platelets (GNP). Sierra-Beltran *et al.* [47] used LWA to immobilize *Bacillus cohnii* and the treated concrete samples have shown an improvement in compressive strength. Tziviloglou *et al.* [48], [49] incorporated the *Bacillus* genus with LWA to increase the water tightness of concrete. However, their specimens have shown a reduction in mechanical strength due to the low strength of LWA.

B. Expanded clay and perlite

A few researchers have studied the immobilization of bacteria with expanded clay and perlite [28], [39], [50], [53]. Expanded clay and perlite exhibits expansion characteristics and can be distributed evenly in concrete. Similar to expanded clay, expanded perlite has a high porosity and water absorption, which is suitable for bacterial activities. Jonkers [39] studied the feasibility of bacteria-based self-healing concrete with *Bacillus cohnii* immobilized in expanded clay particles. He recommended the use of the immobilization technique as it can increase the survival rate of bacteria in concrete. Wiktor and Jonker [50] have also experimented with the introduction of *Bacillus alkalinitrilicus,* encapsulated in expanded clay particles, into concrete, and they have observed the healing of cracks up to 0.46 mm in width. Zhang *et al.* [53] experimented with immobilized *Bacillus cohnii* in expanded perlite for self-healing concrete and they observed that crack widths up to 0.79 mm were healed after 28 days of healing. They have suggested the usage of expanded perlite over expanded clay due to the former material allowing a higher bacteria content, requiring a lower incorporated amount and its cost-effectiveness in general.

Xu and his fellows [93], [94] have studied the carbonate precipitation of ureolytic bacteria *Sporosarcina pasteurii* in self-healing concrete, and in the experiment, *S. pasteurii* was incorporated into concrete, with porous ceramsite and calcium sulphoaluminate cement serving as a protective carrier for the bacteria spores. Porous ceramsite is a type of expanded clay and calcium sulphoaluminate cement is a type of weakly alkaline cementitious material. Their treated concrete samples have shown enhancements in compressive strength and durability as well as reduced water absorption. Another team of researchers, Wang *et al.* [84], utilized diatomaceous earth to immobilize the ureolytic bacteria *Bacillus sphaericus* before incorporating it into the concrete. Diatomaceous earth is a silica-rich mineral compound and consists of diatomic skeletons which are highly porous, lightweight and inert to chemical substances. Based on their results, 0.15 – 0.17 mm cracks in mortar were almost healed depending on the immersion media.

C. Polyurethane and silica gel

In a study by Wang *et al.* [88], two components – polyurethane and silica gel – were used to immobilize *Bacillus sphaericus* before being placed inside glass tubes. The glass tubes were glued together to ensure that the tubes would rupture at the same time and would allow the healing agents, i.e., the bacteria immobilized in polyurethane and silica gel as well as the nutrient medium to fill the concrete cracks. The specimen treated with polyurethane-immobilized bacteria has shown a lower permeability and a higher strength recovery whereas the bacterial activity was higher in that of silica sol. The low permeability of the specimen treated with polyurethane-immobilized bacteria could be attributed to the water-proof nature of polyurethane, which can double as a protective barrier for bacteria cells against the extreme alkaline environments in concrete [64]. Van Tittelboom *et al.* [10], who studied concrete crack healing by using *B. sphaericus* immobilized in silica gel, have similarly observed low water permeability in the treated specimens.

D. Hydrogel and sodium alginate

Hydrogel can also be used to immobilize bacteria before being incorporated into concrete [85]–[87]. It is speculated that hydrogel can sufficiently protect the bacteria spores in concrete and serve as a water reservoir to promote spore germination and bacterial activity. Hydrogel, with its high water absorbing capacity, is able to retain a large amount of water or aqueous solution within concrete without dissolving, and at the same time is capable of releasing water slowly to the environment. Hydrogel additives can absorb moisture from the surrounding air and provide better concrete curing without requiring much external manual water curing. Wang *et al.* [85] have studied the healing efficiency of bacteria-based self-healing concrete with *B. sphaericus* immobilized in hydrogel. Crack widths up to 0.5 mm were successfully healed and the water permeability was reduced by 68%. In addition to hydrogel usage, Wang [89] has experimented on the microencapsulation technique as well by using melamine-based microcapsules to immobilize *B. sphaericus* and unsurprisingly, it was found that the bacteria-treated specimens had higher healing ratios (48 – 80%) than specimens without bacteria (18 – 50%). Cracks of up to 0.97 mm in width were healed and water permeability was reduced to 10 times lower than the specimens that were not treated with bacteria. Wang [87] soon extended his study further by using modified-alginate based hydrogel to immobilize *B. sphaericus*, and the results showed a good compatibility between the bacteria and concrete. However, the mechanical strength results were deemed unsatisfactory.

Another research team, Pungrasmi *et al.* [79] have studied the carbonate precipitation of *B. sphaericus,* immobilized in sodium alginate gel via three different immobilization techniques, namely the extrusion, spray drying and freeze-drying techniques. It was observed that the freeze-drying technique yielded the highest bacteria spore survival rate and the highest healing efficiency when compared to the two other techniques.

E. CERUP and ACDC

Due to the high production cost of axenic cultures, researchers have developed low-cost, self-protected non-axenic mixed cultures such as Cyclic EnRiched Ureolytic Powder (CERUP) [71], [72], [105] and activated compact denitrifying core (ACDC) [27], [29]–[31], [95]. CERUP is a ureolytic culture protected by its high salt content and obtained from the further processing of sub-streams in vegetable treatment plants. ACDC is a non-axenic granulated nitrate-reducing microbial community protected by various bacterial companions and is produced via a sequential batch reactor by applying selective stress conditions. It is estimated that the use of CERUP can decrease the overall costs by about 40 times when compared to the use of axenic cultures [72], [105]. Table 1 summaries the research progress of microbial self-healing concrete with the details on the mechanisms involved, microorganisms and its embedment in the concrete matrix as well as the major findings from the studies.

3.3 Evaluation of Microbial Self-Healing Efficiency

To evaluate the healing efficiency of bacteria-based self-healing concrete, researchers have focused on the recovery of mechanical and durability properties as the key evaluation parameters. Different types of testing have been conducted to evaluate the self-healing efficiency of microbial self-healing concrete. Table 2 presents a summary of the evaluation methods used in various studies. It is evident that durability, mechanical properties and microstructure visualization are the main criteria that were mostly focused on microbial self-healing concrete studies. The table shows that 53 authors have studied the mechanical properties of microbial self-healing concrete and 43 authors have studied its durability properties. Interestingly, the majority of the authors (up to 59 authors) have also studied the microstructure in healed concrete through visualization testing to verify the presence of calcium carbonate precipitation in concrete cracks.

Table 2 Summary on the evaluation methods of self-healing efficiency in microbial self-healing concrete.

Dependent variables	Evaluation method	Remarks	Reference
Mechanical properties	Compression test Flexural test Tensile strength test Ultrasonic velocity pulses (UVP) Rebound hammer	To determine the recovery of mechanical strength after healing. Both positive and negative effects on mechanical strength were reported in previous studies. The results might be affected by curing time, curing conditions, temperature, microcapsule size and dosage, as well as the concentration of bacteria and nutrients added.	[5], [10], [15], [17], [19], [25]–[27], [30], [35]–[37], [39], [41]–[44], [47], [51], [56]–[61], [63], [65]–[73], [76]–[78], [80]–[85], [87]–[89], [93]–[95], [106]–[108] Frequency = 53
Durability properties	Sorptivity test Water permeability Gas permeability pH test Corrosion test Rapid chloride permeability test Mercury intrusion porosimetry Freeze and thaw resistance	To determine the crack healing efficiency by measuring the water tightness and volume of permeable voids and the flowrate of air through it. To determine the resistance against the penetration of harmful substances like chloride. Mostly reported on reduction in permeability and absorption of concrete because of calcium carbonate precipitation by bacteria. The results might be affected by the size of pre-cracking in the specimen.	[5], [10], [15]–[17], [19], [22], [25]–[30], [32], [33], [39], [41], [43], [44], [46], [48], [49], [56], [58], [59], [65], [66], [68]–[70], [75], [76], [78], [81], [82], [84], [85], [88], [89], [91], [93], [94], [109] Frequency = 43
Microstructure visualization	Scanning electron microscopy (SEM) Infrared analysis Environmental scanning electron microscopy Optical microscopy and image analysis X-ray computed tomography X-ray diffraction (XRD) Energy dispersive X-ray spectroscopy Raman spectroscopy Compact ion chromatography Fourier-transform infrared spectroscopy	To determine the formation of healing products by visualizing the crystal deposits. To visualize the microcapsule, crack filling and morphology of healing products. Most studies reported the presence of $CaCO_3$ precipitation with the observation via microstructure visual analysis. The accuracy of the results highly depends on the resolution of microscopy. The tests are costly when compared to other testing methods.	[5], [15], [17], [19], [24]–[36], [39], [41], [43], [45]–[48], [50], [51], [53]–[58], [60], [62], [63], [65], [67]–[69], [74], [75], [78]–[84], [86], [89], [90], [92]–[94], [106]–[110] Frequency = 59

Mechanical strength recovery is widely regarded as the primary evaluation measurement in concrete research because enhanced concrete strength plays a key role in providing an overall support to the entire structure. There are several tests to measure the mechanical strength performance of bacteria-based self-healing concrete, and this includes compression, flexural, split tensile and non-destructive tests (NDT). Compression test is the most used testing method to evaluate the strength recovery of microbial self-healing concrete. In literature, several authors reported on the positive effects of microbial self-healing in terms of improvement in concrete strength [35], [36], [43], [47], [56], [59], [63], [66]–[70], [73], [76]–[78], [80], [83], [93], [94], whereas several others observed a negative effect on mechanical strength in some cases [39]–[41], [51], [72], [85], [87], [89], [95]. For cases with hardened concrete, a few researchers have used ultrasonic velocity pulses to determine the concrete strength and its dynamic modulus of elasticity [37], [50], [51], [70].

Another parameter that is hugely considered when measuring self-healing efficiency is the durability recovery of concrete. Durability is often connected to the permeability and the porosity of the concrete matrix. An increased permeability of concrete, which are often caused by the presence of interconnected crack networks, will allow the penetration of harmful substances and in turn, results in reinforcement corrosion and the degradation of concrete strength and durability. In literature, researchers have measured the durability recovery of self-healing concrete in terms of porosity, tortuosity, specific surface, size distribution, connectivity and micro-cracks in the concrete matrix. Several tests such as water permeability, water absorption, gas permeability and rapid chloride permeability tests were used during the evaluation process. Most of the aforementioned studies on bacteria-based self-healing concrete have reported on an overall reduction in permeability and absorption for bacteria-treated concrete specimens. It is inferred that the reduced permeability and absorption of concrete is caused by the bacteria-induced precipitation of the highly-insoluble calcium carbonate, which fills up the voids and interconnected pores in the concrete matrix and thusly prevents the entry of air and water.

In addition to measuring strength and durability recovery, researchers have also evaluated the healing efficiency of self-healing concrete via visual and optical analyses. Light microscopes, optical microscopes and high-resolution digital imaging (computed tomography) were mainly used to observe the healed cracks. Tests which involve high-cost instruments such as scanning electron microscopy (SEM), energy dispersive spectrum (EDS), X-ray diffraction (XRD) and energy dispersive X-ray analyses have been occasionally used as well to verify the presence of calcium carbonate precipitation in microstructures.

3.4 Numerical Modelling of Microbial Self-Healing Concrete

To date, most of the previous works have been focusing on laboratory and experimental investigations. There are a limited number of pre-existing studies that highlight the numerical modelling of bacteria-based self-healing concrete. According to these studies, to determine the self-healing efficiency, the rate of calcium carbonate precipitation needs to be calculated by measuring the healed crack width and crack volume over time.

Tziviloglou *et al*. [49] have studied the healing performance of bacteria-based self-healing in mortar. In their study, the healing agent (organic acids such as calcium lactate) was introduced into lightweight concrete together with alkaliphilic bacteria spores of the *Bacillus* genus. A model was developed to simulate the healing efficiency and to optimize the required amount of healing agent and expanded clay particles. To evaluate the healing efficiency, three different parameters were used, namely the sealing percentage based on microscopic observations (image processing), a_m, the rate of recovery of water tightness based on the crack permeability test, r, and the sealing percentage based on computer simulations, α_s.

$$\boldsymbol{a_m = \frac{V_i - V_t}{\alpha_s}} \tag{22}$$

$$\boldsymbol{r = \frac{f_{n-h} - f_h}{f_{n-h}}} \tag{23}$$

$$\boldsymbol{\alpha_s = \frac{V_{sp}}{V_{cr}} = \frac{V_{cp} \cdot \beta}{0.5 \cdot d_{cr} \cdot w_{cr} \cdot l_y}} \tag{24}$$

where, V_i is the initial crack volume and V_t is the crack volume at time t (Equation 22). f_{n-h} is the average crack flow of the specimen without healing and f_h is the average flow of the healed specimen (Equation 23). V_{sp} is the volume of sealing product, V_{cp} is the volume of cracked particles, V_{cr} is the crack volume, d_{cr} is the depth of crack, w_{cr} is the crack width, l_y is the model length and β is a constant (Equation 24). The experimental results have shown a greater crack closure percentage, indicating that the simulation had underestimated the crack sealing.

Zemskov *et al*. [52] have developed a 2D numerical model to simulate the healing process of cracks driven by the bacteria-induced precipitation of calcium carbonate via the oxidation of organic acids. When developing the model, the team has considered various factors such as crack width and capsule size, and has studied their effects on the rate and efficiency of crack healing. The model was used to find the optimum conditions for crack healing. In the model, the diffusion equation was used to describe the diffusive transport of healing agents along the cracks and was numerically solved by the Galerkin finite element method. The authors have also tracked the moving boundary of calcium carbonate precipitation in the crack by using the level set method. The simulation results indicated that the crack was healed completely with 60% of calcium lactate. The excessive calcium lactate may be used for further healing events.

Another team of researchers, Xu *et al.* [94], have conducted a numerical analysis on the kinetics of urea decomposition and $CaCO_3$ precipitation to determine the influence of various factors (pH, temperature, and dosage of urea) on the bacterial activity and its precipitation rate. The biochemical rate was modelled according to a fitted logistic curve:

$$y = \frac{a}{1 + e^{-k(t-t_0)}} \tag{25}$$

where a is the range of y variation, t is time (d), t_0 is the time at the maximum dy/dt and k is the rate constant (d^{-1}), which is a reflection on the kinetics of the biochemical process. k values were calculated from regression analyses. This equation was later used by Hassan *et al.* [74] to calculate the concentration of insoluble calcium as a key measure to monitor the productivity rate.

In recent years, Hassan *et al.* [74] have developed another model to simulate the calcium carbonate precipitation through urea hydrolysis in bacteria-based self-healing concrete. The model has taken various factors into account, which include urea transport and urea hydrolysis rate depending on bacteria cell concentrations and calcium carbonate precipitation. The diffusive transport of urea was described by Fick's law, and first-order kinetics were used to model the reactions. The numerical simulation was validated against the experimental results obtained from a self-healing beam specimen. It was observed that the simulation had overestimated the crack healing performance, i.e., 60 days of complete healing, instead of the actual 70 days that were taken during the experiment. The discrepancy could be caused by the assumption of a uniform continuous supply of urea in each discrete crack. In a more realistic situation, once the cracking activity ruptures the microcapsule, the urea embedded within will be released and will diffuse throughout the water in the crack. The diffusion of the limited concentration of urea along the cracks is non-uniform and is instead similar to the scenario of an instantaneous pollutant release into a stagnant narrow channel.

It should be noted that up to this point, the existing numerical models were only developed for MICP pathways via aerobic oxidation of organic acids and urea hydrolysis. Modelling and simulation studies for other metabolic pathways such as nitrate dissimilatory reduction, sulphate dissimilatory reduction and autotrophic pathways have yet to be reported in literatures.

4.0 CHALLENGES AND FUTURE DIRECTION

4.1 Bacteria and Metabolic Process Selection

In the previous sections, several types of bacterial metabolic pathways were introduced, and despite their promising potential, there are some drawbacks depending on the type of bacteria selected for the production of self-healing concrete. In the case of the precipitation of calcium carbonate through photosynthesis, which occurs in the presence of carbon dioxide and light [111], the bacteria can only conduct photosynthesis if they are located within concrete areas which are exposed to sunlight. Thus, autotrophic bacteria are only suitable for shotcrete and plasters with a large area of exposure to sunlight. Other autotrophic pathways, such as the oxidation of methane, is yet to be studied and applied in bacteria-based self-healing concrete.

For some other metabolic processes, the by-products may be a detriment to the overall integrity of the concrete structure. The production of H_2S and O_2 from the sulphate reduction process for instance, can cause reinforcement corrosion within the concrete structure. The formation of sulphur when H_2S reacts with oxygen can be damaging to concrete as it can form a biofilm layer on the concrete surface. The oxygen in the concrete matrix can also catalyze the rusting of iron. Although positive healing results have been observed [97], [98], the production of H_2S is still a significant concern which hinders the widespread application of said pathway in the production of self-healing concrete. The combination of SRB and cyanobacteria is suggested [112] in response to these issues, but further investigation on the oxygen-producing cyanobacteria and anaerobic SRB is still required. Another example would be the precipitation of calcium carbonate via urea hydrolysis, which is typically the most preferable metabolic pathway due to its fastest precipitation and its relative ease to maintain the bacterial culture. However, the formation of ammonium ions as a by-product can cause some issues to the environment and to the concrete structure itself. It is possible for ammonium to react with other substances to form nitrogen oxide, ammonium salts and nitric acid, which can deteriorate the concrete structure as a whole and lead to environmental issues especially when released to the atmosphere.

Several other metabolic pathways contain a list of caveats such as being hard to sustain and may get easily impeded by other common additives in the concrete matrix. The aerobic oxidation of organic acids for example, requires a continuous carbon source for the precipitation of calcium carbonate. It has also been reported that the addition of nutrient solutions could affect its hydration kinetics and the mechanical properties of concrete as a whole. Both urea hydrolysis and aerobic oxidation of organic acids require oxygen for their metabolic processes. Although this may limit the corrosion of concrete reinforcements as the oxygen is continuously used up by the heterotrophic bacteria, the bacterial activity may get disrupted due to the limited concentrations of oxygen within the deeper parts of concrete. In addition to these issues, the precipitation of calcium carbonate around the bacteria cell, which serves as a nucleation site, could entrap the bacteria itself and starve the cell of nutrients and oxygen, thus further hindering the aerobic-based precipitation process.

Among the available metabolic processes, the denitrifying process, induced by nitrate-reducing bacteria, can be a generally promising candidate. The process itself will produce carbon dioxide (as the carbon source), water and nitrogen gas. The emission of nitrogen gas from this process is very low as compared to the conventional sources of nitrogen pollution. The consumption of hydrogen ions during the nitrate reduction process increases the pH in the concrete microenvironment, and is conducive to the precipitation of calcium carbonate. As a potential solution for the aforementioned oxygen starvation caveats, a combination of bacterial strains can be used, such as the nitrate-reducing bacteria being used in deeper parts of concrete and autotrophic bacteria for the outer parts, to maximize self-healing.

4.2 Embedment of Bacteria in Concrete

The main challenges of directly incorporating bacteria spores into concrete are the high pH environment and the small pore size in concrete, which could affect the bacteria survival rate in the long run. A few studies have reported that the viability of bacteria spores have decreased due to the highly-alkaline environment, high temperature during the hydration of cement and reduced pore size, which sometimes can get even smaller than the size of bacteria spores [41], [45].

To improve the survival odds of bacteria in concrete, immobilization techniques have been proposed to protect the bacteria spores from the extreme concrete microenvironment [39]. The use of immobilization techniques when producing bacteria-based self-healing concrete has resulted in a better healing efficiency due to the uniform distribution of the immobilized bacteria spores, which are also protected against the extreme environment in concrete [42]. In literature, different types of capsule materials have been used to immobilize bacteria, such as lightweight aggregates, expanded clay and perlite with porous media which promotes bacterial activity. Microcapsules made with polymers such as silica gel, polyurethane and hydrogel have been used as well.

Despite its benefits, several studies have reported that the immobilization technique can cause a negative effect on the mechanical strength of concrete. This is due to the weak bonding between the microcapsules and the concrete matrix. After rupturing and releasing the healing agents into the concrete matrix, the empty microcapsules left will form voids within the concrete, which in turn reduces the mechanical strength of the entire concrete structure. Thus, the microcapsule dosage needs to be controlled within an optimum concentration range without adversely affecting the mechanical strength of concrete. In a study where CERUP was applied in self-healing concrete, the results showed that the concrete strength was significantly altered between scenarios when dosage of 3% and 5% by weight of cement were added into concrete [72]. Wang *et al.* [89] have observed that the compressive strength and tensile strength of concrete were reduced when microcapsules with 5% by weight of cement were used, when compared to the case where 3% by weight of cement were added. In addition to strength reduction, the microcapsules may affect the rheology of fresh concrete. Spherical microcapsules can reduce the interlocking in the aggregate and thus may enhance the concrete flow despite adversely affecting the concrete strength [104].

Of all the capsule materials, hydrogel might serve as a better choice due to its good water absorptivity and retention ability, which can improve the water curing process in concrete. However once again, the dosage of hydrogel microcapsules must be optimized to ensure that there is enough water for cement hydration at the early stages of concrete. Excessive capsule dosage may affect the cement-to-water ratio and negatively affect the strength development of concrete. Apart from hydrogel, porous materials such as lightweight aggregates, expanded clay and perlite are decent candidates to immobilize bacteria spores and the germinated bacteria. Once a crack penetrates the protective carrier, the bacteria will induce the calcium carbonate precipitation process in the presence of oxygen and water. Since the main contributor of concrete strength is the aggregates, the usage of soft and brittle lightweight aggregates in self-healing concrete will inevitably cause a reduction in concrete strength. Jonkers [39] has found out that the strength of the concrete specimen made with lightweight aggregates was reduced by 50% at the 28-day mark. Such low strength is generally deemed unsatisfactory for structural applications.

The survival of capsules is also important especially during the concrete-mixing stage. Wang *et al.* [89] have examined the survival of capsules after gentle concrete mixing, by using light microscopy and they have observed that most of the microcapsules remained unbroken. This meant that the bacteria could have a longer shelf life in the concrete thanks to microcapsule immobilization.

4.3 Effects on Concrete Properties and Healing Efficiency

The healing efficiency of self-healing concrete caused by bacterial-induced calcium carbonate precipitation depends on various factors, such as bacteria species, bacteria concentration, urea concentration, nutrients, organic acids, nitrate and calcium concentrations, temperatures, curing methods and capsule materials. The pore structures, which determines the mechanical and durability properties of concrete, depends on the mix design, curing conditions, degree of hydration, as well as the addition of supplementary cementitious materials, additives and construction practices [99].

A. Effect of embedment methods on concrete strength

In literature, the precipitation of calcium carbonate can be induced by different types of bacteria and metabolic pathways, and yet, even if the same bacteria species were used, different researchers have obtained different results. Wang *et al.* [85] studied the usage of *Bacillus sphaericus*, immobilized with hydrogel, and its application in mortar. The treated specimens have shown a reduction in compression strength by 15 to 34%. In contrast, the experiment conducted by Achal *et al.* [59] (by using the same bacteria genus with a different immobilization technique applied in cube mortars) has yielded specimens with enhanced compressive strength. This indicates that the healing efficiency will be influenced by other factors such as the embedment method of bacteria in concrete. As seen above, the embedment of hydrogel might decrease the strength of mortar because the hydrogel capsules have formed hollow pores in the mortar matrix.

B. Effect of bacteria and nutrient concentrations on concrete properties

The bacteria concentration could affect the concrete mechanical properties, and by extension, the healing efficiency of concrete. In the same experiment conducted by Achal *et al.* [59], the maximum compressive strength was obtained with a bacteria concentration of $5 \times 10^7 \mathrm{cells/mm^3}$. A reduction of compressive strength was observed when a concentration of $5 \times 10^8 \mathrm{cells/mm^3}$ was added to the mortar. Andalib *et al.* [63] have studied the incorporation of *Bacillus megaterium* into concrete and has recorded an optimum concentration of $30 \times 10^5 \mathrm{cfu/ml}$ for maximum compressive and flexural strength recovery.

In addition to the bacteria themselves, the nutrient solutions as well as the organic carbon and calcium sources, which are required to necessitate or accelerate the precipitation of calcium carbonate [102], can affect the mechanical strength of concrete as well by changing the microstructure in the concrete matrix, as reported by several studies. The addition of organic acids for instance, can affect the hydration kinetics and the setting time of fresh concrete. Bundur *et al.* [67], who have studied the influence of inoculated vegetative cells on the concrete properties, have concluded that the nutrient media can retard the hydration kinetics but increased the compressive strength of the specimens. In a crack repairing study using *Bacillus pseudofirmus*, different types of organic acids were experimented along with the bacteria, such as citric acid, polyacrylic acid, aspartic acid, glutamic acid, gluconate acid and ascorbic acid [38]. The results showed that polyacrylic and citric acids will affect the concrete strength while no strength is developed in cases where gluconate and ascorbic acids were added. Another study conducted on self-healing concrete with *B. pseudofirmus* and *B. cohnii* revealed that the addition of calcium lactate can improve the compressive strength of concrete whereas the addition of yeast extract and peptone yielded negative effects. These results were supported by the experiment conducted by Luo and Qian [44] as they found that calcium lactate can delay the hydration kinetics while calcium formate and calcium nitrate can accelerate the hydration kinetics. According to their findings, the addition of calcium lactate can negatively affect the early stages of compressive strength development but result in a strength increase in later stages, whereas the addition of calcium formate can improve the compressive strength while a negative result was obtained for calcium nitrate. In response to the original studies that have used yeast extract which in turn yielded a reduced compressive strength, the yeast extract was replaced with meat extract to decrease the delay of hydration kinetics [90]. It was observed that the meat extract has reduced 75% of retardation in comparison to the yeast counterpart.

Apart from nutrient solutions, organic calcium sources may affect the concrete properties, and sometimes the healing process itself. In the aerobic oxidation process of organic acids, a calcium source which is capable of supplying a continuously high amount of calcium is required for the precipitation of calcium carbonate. However, an overly high concentration of calcium ions in the concrete matrix can cause problems to the concrete structures. This is due to the fact that the accumulation of calcium salts in the concrete matrix can adversely affect the properties of concrete [51]. Moreover, excessive calcium ions in the concrete might inhibit calcium carbonate precipitation in the long run [55].

C. Effect of temperature and curing conditions on healing efficiency

Surrounding temperatures can affect the bacterial metabolic rate, which in turn may influence the rate of calcium carbonate precipitation. An ideal bacteria strain is expected to adapt to varying temperatures without experiencing significant effects on bacterial activity, and by extension, the precipitation of calcium carbonate. De Muynck *et al.* [16] studied the influence of temperature on the healing efficiency of surface treatment used for limestone conservation by using *Bacillus sphaericus*, *Sporosarcina psychrophila*, *Sporosarcina ureae* and *Sporosarcina pasteurii.* The authors have concluded that *B. sphaericus* gave a higher carbonate precipitate yield than *S. psychrophile* at cold temperatures. This indicated that *B. sphaericus* is more resilient and can be used for self-healing concrete production in regions with cold climates.

The healing efficiency of self-healing concrete might also be affected by the curing conditions, namely wet curing, wet-and-dry curing cycle and water curing [44], [113]. Luo *et al.* [45], a team of researchers who studied the effects of various factors such as crack widths, curing methods and crack ages on the crack healing efficiency in bacteria-based self-healing concrete, have stated that water curing is the most suitable and practical curing method in self-healing concrete production, but the wet-and-dry curing cycle has provided a better healing efficiency [89]. These findings were also supported by Wu *et al.* [91].

D. High cost of cultivated media

The usage of high-cost cultivated media makes the microbial-based self-healing approach rather difficult to be upscaled and applied commercially in the construction industry. Due to cost concerns, several solutions have emerged to decrease the overall costs of bacteria-based self-healing system [105]. As aforementioned, self-protected non-axenic mixed cultures such as CERUP [71], [72] and ACDC [27], [29]–[31], [95] have been proposed, as the cost of these non-axenic cultures is much lower than that of the combo of axenic cultures and encapsulations for culture embedment. The application of these non-axenic cultures needs to be further studied in a larger scale in the construction industry. Additionally, several low-cost nutrient media such as corn steep liquor (CSL) [57], [60] and lactose mother liquor (LML) [61], [90] have been proposed as well. Apart from using cheaper alternatives of bacterial cultures and nutrient media, supplementary cementitious materials can be added to replace some amount of cement in concrete, thus further reducing the production costs. Fly ash [56], [69], silica fume [68], rice husk ash [81] and cement baghouse filter dust [82] have all been tested as low-cost cementitious additives to bacteria-based self-healing concrete and coincidentally, improvements in the concrete properties were observed.

E. Lack of standard assessment and measurement of healing efficiency

To evaluate the healing efficiency of concrete (which is in turn governed by different factors and circumstances), it is imperative to have a clear and standard healing assessment with a set of standard parameters and testing methods. As different combinations of various factors such as bacteria cell concentration, nutrient concentration, calcium concentration, curing conditions and immobilization methods may significantly influence the healing efficiency, without a set of quantitative measurements from standard testing, it is very difficult to make any meaningful comparisons in terms of healing efficiency for different scenarios. According to the available studies on microbial self-healing concrete, different evaluation parameters, methods and techniques have been used to evaluate the efficiency of self-healing. Most of the researchers from the published studies have performed self-healing evaluations by measuring and comparing the mechanical and durability properties of the concrete specimens before and after the healing process. While these properties are widely regarded as the only primary measures in self-healing assessment, new evaluation parameters and testing methods including indices, optical and visual analyses, and analytical judgments can and should be further studied and implemented to form a more comprehensive and representative assessment framework to better evaluate the healing efficiency of self-healing concrete as a whole.

F. Lack of numerical simulations

To date, only a handful of published studies have focused on the numerical modelling of bacteria-based self-healing concrete [49], [52], [74], [94]. Numerical modelling and simulations are required alongside experimental works as they help the researchers to have a better understanding on the mechanics of the bacteria-based self-healing processes and hopefully this allows them to design better and more optimized self-healing systems in the future.

5.0 CONCLUSION

This paper reviewed and discussed several metabolic pathways of microbially-induced calcium carbonate precipitation, bacteria embedment techniques in concrete and the various testing methods conducted to evaluate self-healing efficiency in microbial self-healing concrete. It was found that there are a few types of metabolic pathways involved in microbially-induced calcium carbonate precipitation namely, the autotrophic pathway, the denitrification pathway, the iron-reducing (heterotrophic) pathway, oxidation of organic acids as well as fungi and urea hydrolysis. It should be noted that urea hydrolysis is the most popular and widely used metabolic pathway in microbial self-healing concrete production.

In this paper, several conclusions can be made. Firstly, direct addition of bacteria into self-healing concrete has provided satisfactory results in terms of mechanical and durability properties. The chances of bacteria survival under extreme concrete environmental conditions (high pH, temperature and small pore size) is debatable and requires further discussion. It was found that the immobilization techniques of bacteria in concrete can improve the durability of self-healing concrete by reducing the water permeability and water absorption of the healed specimens. However, a few authors have reported a reduction in mechanical strength for specimens with the embedment of lightweight aggregates, diatomaceous earth, expanded clay and hydrogel capsules.

Moreover, it has been proved that the bacteria used in self-healing concrete are typically harmless to humans and can be easily cultured and handled in laboratory settings. However, the commercial production of bacteria-based self-healing concrete is still impeded by its high initial cost and the low survival rate of bacteria under extreme concrete environmental conditions such as high alkalinity, fluctuating temperatures, dry conditions and small pore size in the concrete matrix.

Furthermore, the bacteria-induced calcium carbonate precipitation process in self-healing concrete has provided promising results in the laboratory scale. More evaluation parameters and testing methods need to be studied and added to the list of existing ones to form a more comprehensive assessment to better evaluate the self-healing efficiency of the treated specimens. Further efforts are required to increase the healing efficiency of available methods to allow the application and implementation of microbial self-healing systems in large-scaled actual site environments.

The findings in this paper were reported based on the current research progress in microbial self-healing concrete. More research is required to address the aforementioned gaps and to improve the performance of self-healing systems as a whole. Suffice to say, there are still big challenges ahead in order to improve the self-healing efficiency before the technology gets commercially introduced for widespread industrial use.

Acknowledgement

The authors would like to acknowledge the financial supports by the Ministry of Higher Education Malaysia and Universiti Teknologi Malaysia under the Skim Latihan Akademik IPTA (SLAI) scholarship.

References

[1] A. Al-Tabbaa, C. Litina, P. Giannaros, A. Kanellopoulos, and L. Souza. 2019. First UK Field Application and Performance of Microcapsule-based Self-healing Concrete. *Construction and Building Materials*. 208: 669-685. Doi: 10.1016/j.conbuildmat.2019.02.178.

[2] R. Davies *et al.* 2018. Large Scale Application of Self-Healing Concrete: Design, Construction, and Testing. *Front. Mater.* Doi: 10.3389/fmats.2018.00051.

[3] V. C. Li and E. Herbert. 2012. Robust Self-Healing Concrete for Sustainable Infrastructure. *Journal of Advanced Concrete Technology*. 10(6): 207-218. Doi: 10.3151/jact.10.207.

[4] F. Hammes and W. Verstraete. 2002. Key Roles of pH and Calcium Metabolism in Microbial Carbonate Precipitation. *Reviews in Environmental Science and Biotechnology*. 1(1): 3-7. Doi: 10.1023/A:1015135629155.

[5] N. K. Dhami, M. S. Reddy, and A. Mukherjee. 2013. Bacillus Megaterium Mediated Mineralization of Calcium Carbonate as Biogenic Surface Treatment of Green Building Materials. *World Journal of Microbiology and Biotechnology*. 29(12): 2397-2406. Doi: 10.1007/s11274-013-1408-z.

[6] P. A. M. Lesley A. Warren Nagina Parmar, F. Grant Ferris. 2001. 'Microbially Mediated Calcium Carbonate Precipitation: Implications for Interpreting Calcite Precipitation and for Solid-Phase Capture of Inorganic Contaminants', *Geomicrobiology Journal*. 18(1): 93-115. Doi: 10.1080/01490450151079833.

[7] B. C. Martinez *et al.* 2013. Experimental Optimization of Microbial-induced Carbonate Precipitation for Soil Improvement. *Journal of Geotechnical and Geoenvironmental Engineering*. 139(4): 587-598. Doi: 10.1061/(ASCE)GT.1943-5606.0000787.

[8] R. Micallef, D. Vella, E. Sinagra, and G. Zammit. 2016. Biocalcifying Bacillus Subtilis Cells Effectively Consolidate Deteriorated Globigerina Limestone. *J Ind Microbiol Biotechnol*. 43(7): 941-952. Doi: 10.1007/s10295-016-1768-0.

[9] S. Stocks-Fischer, J. K. Galinat, and S. S. Bang. 1999. Microbiological Precipitation of CaCO3'. *Soil Biology and Biochemistry*. 31(11): 1563-1571. Doi: 10.1016/S0038-0717(99)00082-6.

[10] K. Van Tittelboom, N. De Belie, W. De Muynck, and W. Verstraete. 2010. Use of Bacteria to Repair Cracks in Concrete. *Cement and Concrete Research*. 40(1): 157-166. Doi: https://doi.org/10.1016/j.cemconres.2009.08.025.

[11] G. Le Métayer-Levrel, S. Castanier, G. Orial, J.-F. Loubière, and J.-P. Perthuisot. 1999. Applications of Bacterial Carbonatogenesis to the Protection and Regeneration of Limestones in Buildings and Historic Patrimony. *Sedimentary Geology*. 126(1): 25-34. Doi: 10.1016/S0037-0738(99)00029-9.

[12] C. Rodriguez-Navarro, M. Rodriguez-Gallego, K. Ben Chekroun, and M. T. Gonzalez-Muñoz. 2003. Conservation of Ornamental Stone by Myxococcus xanthus-Induced Carbonate Biomineralization. *Applied and Environmental Microbiology*. 69(4): 2182-2193. Doi: 10.1128/AEM.69.4.2182-2193.2003.

[13] P. Tiano, L. Biagiotti, and G. Mastromei. 1999. Bacterial Bio-mediated Calcite Precipitation for Monumental Stones Conservation: Methods of Evaluation. *Journal of Microbiological Methods*. 36(1): 139-145. Doi: 10.1016/S0167-7012(99)00019-6.

[14] Q. Chunxiang, W. Jianyun, W. Ruixing, and C. Liang. 2009. Corrosion Protection of Cement-based Building Materials by Surface Deposition of CaCO3 by Bacillus Pasteurii. *Materials Science and Engineering: C*. 29(4): 1273-1280. Doi: https://doi.org/10.1016/j.msec.2008.10.025.

[15] N. W. V. De Muynck Willem, Kathelijn Cox, De Belie. 2008. Bacterial Carbonate Precipitation as an Alternative Surface Treatment for Concrete. *Construction and Building Materials*. 22(5): 875-885. Doi: https://doi.org/10.1016/j.conbuildmat.2006.12.011.

[16] W. De Muynck, K. Verbeken, N. De Belie, and W. Verstraete. 2013. Influence of Temperature on the Effectiveness of a Biogenic Carbonate Surface Treatment for Limestone Conservation. *Applied Microbiology and Biotechnology*. 97(3): 1335-1347. Doi: 10.1007/s00253-012-3997-0.

[17] H. Kalhori and R. Bagherpour. 2017. Application of Carbonate Precipitating Bacteria for improving Properties and Repairing Cracks of Shotcrete. *Construction and Building Materials*. 148: 249-260. Doi: https://doi.org/10.1016/j.conbuildmat.2017.05.074.

[18] W. De Muynck, N. De Belie, and W. Verstraete. 2010. Microbial Carbonate Precipitation in Construction Materials: A Review. *Ecological Engineering*. 36(2): 118-136. Doi: https://doi.org/10.1016/j.ecoleng.2009.02.006.

[19] C. Qian, R. Wang, L. Cheng, and J. Wang. 2010. Theory of Microbial Carbonate Precipitation and Its Application in Restoration of Cement-based Materials Defects. *Chinese Journal of Chemistry*. 28(5): 847-857. Doi: 10.1002/cjoc.201090156.

[20] E. Tziviloglou *et al.* 2016. Bio-Based Self-Healing Concrete: From Research to Field Application. *Self-healing Materials*. M. D. Hager, S. van der Zwaag, and U. S. Schubert, Eds. Cham: Springer International Publishing. 345-385. Doi: 10.1007/12_2015_332.

[21] V. Wiktor and H. Jonkers. 2011. Determination of the Crack Self-Healing Capacity of Bacterial Concrete. *Proc. Concrete Solutions*.

[22] V. Wiktor and H. M. Jonkers. 2015, Field Performance of Bacteria-based Repair System: Pilot Study in a Parking Garage. *Case Studies in Construction Materials*. 2: 11-17. Doi: https://doi.org/10.1016/j.cscm.2014.12.004.

[23] S. Castanier, G. L. Métayer-Levrel, and J.-P. Perthuisot. 1999. Ca-carbonates Precipitation and Limestone Genesis — The Microbiogeologist Point of View. *Sedimentary Geology*. 126(1): 9-23. Doi: https://doi.org/10.1016/S0037-0738(99)00028-7.

[24] T. Zhu, C. Paulo, M. L. Merroun, and M. Dittrich. 2015. Potential Application of Biomineralization by Synechococcus PCC8806 for Concrete Restoration. *Ecological Engineering*. 82: 459-468. Doi: https://doi.org/10.1016/j.ecoleng.2015.05.017.

[25] T. Zhu, X. Lu, and M. Dittrich. 2017. Calcification on Mortar by Live and UV-killed Biofilm-forming Cyanobacterial Gloeocapsa PCC73106. *Construction and Building Materials*. 146: 43-53. Doi: https://doi.org/10.1016/j.conbuildmat.2017.04.026.

[26] G. Kaur, N. K. Dhami, S. Goyal, A. Mukherjee, and M. S. Reddy. 2016. Utilization of Carbon Dioxide as an Alternative to Urea in Biocementation. *Construction and Building Materials*. 123: 527-533. Doi: https://doi.org/10.1016/j.conbuildmat.2016.07.036.

[27] Y. C. Ersan. 2016. Microbial Nitrate Reduction Induced Autonomous Self-healing in Concrete. PhD Thesis. Ghent University. Faculty of Bioscience Engineering.

[28] Y. Ç. Erşan, E. Hernandez-Sanabria, N. Boon, and N. de Belie. 2016. Enhanced Crack Closure Performance of Microbial Mortar through Nitrate Reduction. *Cement and Concrete Composites*. 70: 159-170. Doi: https://doi.org/10.1016/j.cemconcomp.2016.04.001.

[29] Y. Ç. Erşan, E. Gruyaert, G. Louis, C. Lors, N. De Belie, and N. Boon. 2015. Self-protected Nitrate Reducing Culture for Intrinsic Repair of Concrete Cracks. *Frontiers in Microbiology*. 6: 1228. Doi: 10.3389/fmicb.2015.01228.

[30] Y. Ç. Erşan, H. Verbruggen, I. D. Graeve, W. Verstraete, N. D. Belie, and N. Boon. 2016. Nitrate Reducing CaCO3 Precipitating Bacteria Survive in Mortar and Inhibit Steel Corrosion. *Cement and Concrete Research*. 83: 19-30. Doi: https://doi.org/10.1016/j.cemconres.2016.01.009.

[31] Y. Ç. Erşan, K. Van Tittelboom, N. Boon, and N. De Belie. 2018. Nitrite Producing Bacteria Inhibit Reinforcement Bar Corrosion in Cementitious Materials. *Scientific Reports*. 8(1): 14092. [Online]. Available: https://doi.org/10.1038/s41598-018-32463-6.

[32] Y. Ç. Erşan, N. de Belie, and N. Boon. 2015. Microbially Induced CaCO3 Precipitation through Denitrification: An Optimization Study in Minimal Nutrient Environment. *Biochemical Engineering Journal*. 101: 108-118. Doi: https://doi.org/10.1016/j.bej.2015.05.006.

[33] J. Luo *et al.* 2018. Interactions of Fungi with Concrete: Significant Importance for Bio-based Self-healing Concrete. *Construction and Building Materials*. 164: 275-285. Doi: https://doi.org/10.1016/j.conbuildmat.2017.12.233.

[34] R. R. Menon *et al.* 2019. Screening of Fungi for Potential Application of Self-Healing Concrete. *Scientific Reports*. 9(1): 2075. [Online]. Available: https://doi.org/10.1038/s41598-019-39156-8.

[35] P. Ghosh, S. Mandal, S. Pal, G. Bandyopadhyaya, and B. Chattopadhyay. 2006. Development of Bioconcrete Material using an Enrichment Culture of Novel Thermophilic Anaerobic Bacteria.

[36] P. Ghosh, S. Mandal, B. D. Chattopadhyay, and S. Pal. 2005. Use of Microorganism to Improve the Strength of Cement Mortar. *Cement and Concrete Research*. 35(10): 1980-1983. , Doi: https://doi.org/10.1016/j.cemconres.2005.03.005.

[37] A. Gandhimathi and D. Suji. 2015. Studies on the Development of Eco-friendly Self-healing Concrete - A Green Building Concept. *Nature Environment and Pollution Technology*. 14(3): 639.

[38] H. M. Jonkers and E. Schlangen. 2007. Crack Repair by Concrete-immobilized Bacteria. *Proceedings of the First International Conference on Self Healing Materials*. 18: 20.

[39] H. M. Jonkers. 2011. Bacteria-based Self-healing Concrete. *Heron.* 56(1/2).

[40] H. M. Jonkers and E. Schlangen. 2008. Development of a Bacteria-based Self Healing Concrete. *Proc. int. FIB Symposium*. 1: 425-430.

[41] H. M. Jonkers, A. Thijssen, G. Muyzer, O. Copuroglu, and E. Schlangen. 2010. Application of Bacteria as Self-healing Agent for the Development of Sustainable concrete. *Ecological Engineering*. 36(2): 230-235. Doi: https://doi.org/10.1016/j.ecoleng.2008.12.036.

[42] W. Khaliq and M. B. Ehsan. 2016. Crack Healing in Concrete using Various Bio Influenced Self-healing Techniques. *Construction and Building Materials*. 102: 349-357. Doi: https://doi.org/10.1016/j.conbuildmat.2015.11.006.

[43] C. Kumari, B. Das, R. Jayabalan, R. Davis, and P. Sarkar. 2016. Effect of Nonureolytic Bacteria on Engineering Properties of Cement Mortar. *Journal of Materials in Civil Engineering*. 29: 06016024.

Doi: 10.1061/(ASCE)MT.1943-5533.0001828.

[44] M. Luo and C. Qian. 2016. Influences of Bacteria-based Self-healing Agents on Cementitious Materials Hydration Kinetics and Compressive Strength. *Construction and Building Materials*. 121: 659-663. Doi: https://doi.org/10.1016/j.conbuildmat.2016.06.075.

[45] M. Luo, C. Qian, and R. Li. 2015. Factors Affecting Crack Repairing Capacity of Bacteria-based Self-healing Concrete. *Construction and Building Materials*. 87: 1-7. Doi: https://doi.org/10.1016/j.conbuildmat.2015.03.117.

[46] T. K. Sharma, M. Alazhari, A. Heath, K. Paine, and R. M. Cooper. 2017. Alkaliphilic Bacillus Species Show Potential Application in Concrete Crack Repair by Virtue of Rapid Spore Production and Germination Then Extracellular Calcite Formation. *Journal of Applied Microbiology*. 122(5): 1233-1244. Doi: 10.1111/jam.13421.

[47] M. G. Sierra-Beltran, H. M. Jonkers, and E. Schlangen. 2014. Characterization of Sustainable Bio-based Mortar for Concrete Repair. *Construction and Building Materials*. 67: 344-352. Doi: https://doi.org/10.1016/j.conbuildmat.2014.01.012.

[48] E. Tziviloglou, V. Wiktor, H. M. Jonkers, and E. Schlangen. 2016. Bacteria-based Self-healing Concrete to Increase Liquid Tightness of Cracks. *Construction and Building Materials*. 122: 118-125. Doi: https://doi.org/10.1016/j.conbuildmat.2016.06.080.

[49] E. Tziviloglou, Z. Pan, H. M. Jonkers, and E. Schlangen. 2017. Bio-based Self-healing Mortar: An Experimental and Numerical Study. *Journal of Advanced Concrete Technology*. 15(9): 536–543. Doi: 10.3151/jact.15.536.

[50] V. Wiktor and H. M. Jonkers. 2011. Quantification of Crack-healing in Novel Bacteria-based Self-healing Concrete. *Cement and Concrete Composites*. 33(7): 763-770. Doi: https://doi.org/10.1016/j.cemconcomp.2011.03.012.

[51] J. Xu and W. Yao. 2014. Multiscale Mechanical Quantification of Self-healing Concrete Incorporating Non-ureolytic Bacteria-based Healing Agent. *Cement and Concrete Research*. 64: 1-10. Doi: https://doi.org/10.1016/j.cemconres.2014.06.003.

[52] S. V. Zemskov, H. M. Jonkers, and F. J. Vermolen. 2014. A Mathematical Model for Bacterial Self-healing of Cracks in Concrete. *Journal of Intelligent Material Systems and Structures*. 25(1): 4-12. Doi: 10.1177/1045389X12437887.

[53] J. Zhang *et al.* 2017. Immobilizing Bacteria in Expanded Perlite for the Crack Self-healing in Concrete. *Construction and Building Materials*. 148: 610-617. Doi: https://doi.org/10.1016/j.conbuildmat.2017.05.021.

[54] J. L. Zhang *et al.* 2016. A Binary Concrete Crack Self-healing System Containing Oxygen-releasing Tablet and Bacteria and its Ca2+-precipitation Performance. *Applied Microbiology and Biotechnology*. 100(24): 10295-10306. Doi: 10.1007/s00253-016-7741-z.

[55] J. L. Zhang *et al.* 2016. Screening of Bacteria for Self-healing of Concrete Cracks and Optimization of the Microbial Calcium Precipitation Process. *Applied Microbiology and Biotechnology*. 100(15): 6661-6670. Doi: 10.1007/s00253-016-7382-2.

[56] V. Achal, X. Pan, and N. Özyurt. 2011. Improved Strength and Durability of Fly Ash-amended Concrete by Microbial Calcite Precipitation. *Ecological Engineering*. 37(4): 554-559. Doi: https://doi.org/10.1016/j.ecoleng.2010.11.009.

[57] V. Achal, A. Mukherjee, S. Goyal, and Ms. Reddy. 2012. Corrosion Prevention of Reinforced Concrete with Microbial Calcite Precipitation. *ACI Materials Journal*. 109(2): 157-164. Doi: 10.14359/51683702.

[58] V. Achal, A. Mukherjee, and M. S. Reddy. 2011. Microbial Concrete: Way to Enhance the Durability of Building Structures. *Journal of Materials in Civil Engineering*. 23(6): 30-734.

[59] V. Achal, A. Mukerjee, and M. S. Reddy. 2013. Biogenic treatment Improves the Durability and Remediates the Cracks of Concrete Structures. *Construction and Building Materials*. 48: 1-5. Doi: https://doi.org/10.1016/j.conbuildmat.2013.06.061.

[60] V. Achal, A. Mukherjee, and M. S. Reddy. 2010 Biocalcification by Sporosarcina Pasteurii using Corn Steep Liquor as the Nutrient Source. *Industrial Biotechnology*. 6(3): 170-174. Doi: 10.1089/ind.2010.6.170.

[61] V. Achal, A. Mukherjee, P. C. Basu, and M. S. Reddy. 2009. Lactose Mother Liquor as an Alternative Nutrient Source for Microbial Concrete Production by Sporosarcina Pasteurii. *Journal of Industrial Microbiology & Biotechnology*. 36(3): 433-438. Doi: 10.1007/s10295-008-0514-7.

[62] V. Achal, A. Mukherjee, P. C. Basu, and M. S. Reddy. 2009. Strain Improvement of Sporosarcina pasteurii for Enhanced Urease and Calcite Production. *Journal of Industrial Microbiology & Biotechnology*. 36(7): 981-988. Doi: 10.1007/s10295-009-0578-z.

[63] R. Andalib *et al.* 2016. Optimum Concentration of Bacillus Megaterium for Strengthening Structural Concrete. *Construction and Building Materials*. 118: 180-193. Doi: https://doi.org/10.1016/j.conbuildmat.2016.04.142.

[64] S. S. Bang, J. K. Galinat, and V. Ramakrishnan. 2001. Calcite Precipitation Induced by Polyurethane-Immobilized Bacillus pasteurii'. *Enzyme and Microbial Technology*. 28(4): 404-409. Doi: https://doi.org/10.1016/S0141-0229(00)00348-3.

[65] R. Bansal, N. K. Dhami, A. Mukherjee, and M. S. Reddy. 2016. Biocalcification by Halophilic Bacteria for Remediation of Concrete Structures in Marine Environment. *Journal of Industrial Microbiology & Biotechnology*. 43(11): 1497-1505. Doi: 10.1007/s10295-016-1835-6.

[66] Z. Basaran. 2013. Biomineralization in Cement based Materials: Inoculation of Vegetative Cells. PhD Thesis.

[67] Z. B. Bundur, M. J. Kirisits, and R. D. Ferron. 2015. Biomineralized Cement-based Materials: Impact of Inoculating Vegetative Bacterial Cells on Hydration And Strength. *Cement and Concrete Research*. 67: 237-245. Doi: https://doi.org/10.1016/j.cemconres.2014.10.002.

[68] N. Chahal, R. Siddique, and A. Rajor. 2012. Influence of Bacteria on the Compressive Strength, Water Absorption and Rapid Chloride Permeability of Concrete Incorporating Silica Fume. *Construction and Building Materials*. 37: 645-651. Doi: https://doi.org/10.1016/j.conbuildmat.2012.07.029.

[69] N. Chahal, R. Siddique, and A. Rajor. 2012. Influence of Bacteria on the Compressive Strength, Water Absorption and Rapid Chloride Permeability of Fly Ash Concrete. *Construction and Building Materials*. 28(1): 351-356. , doi: https://doi.org/10.1016/j.conbuildmat.2011.07.042.

[70] B. Chattopadhyay and M. Sarkar. 2016. Genetically Modified Bacillus subtilis Bacterial Strain for Self-healing and Sustainable Green Bioconcrete Material. Organic *Chem Curr Res 2016*. 5(46). Doi: http://dx.doi.org/10.4172/2161-0401.C1.013.

[71] F. B. da Silva. 2015. Up-scaling the Production of Bacteria for Self-healing Concrete Application. PhD Thesis. Ghent University. Faculty of Bioscience Engineering.

[72] F. B. da Silva, N. De Belie, N. Boon, and W. Verstraete. 2015. Production of Non-axenic Ureolytic Spores for Self-healing Concrete Applications. *Construction and Building Materials*. 93: 1034-1041. Doi: https://doi.org/10.1016/j.conbuildmat.2015.05.049.

[73] C. Gavimath *et al.* 2012. Potential Application of Bacteria to Improve the Strength of Cement Concrete.

International Journal of Advanced Biotechnology and Research. 3(1): 541-544.

[74] A. A. Hassan, A. B. Suhaimi, Mohd. S. Abdul Rahman, Z. A. Ahmad Razin, S. Shafinaz, and H. A.-T. Wahid Ali. 2018. Numerical Modeling for Crack Self-healing Concrete by Microbial Calcium Carbonate. *Construction and Building Materials*. 189: 816-824. Doi: https://doi.org/10.1016/j.conbuildmat.2018.08.218.

[75] H. K. Kim, S. J. Park, J. I. Han, and H. K. Lee. 2013. Microbially Mediated Calcium Carbonate Precipitation on Normal and Lightweight Concrete. *Construction and Building Materials*. 38: 1073-1082. Doi: https://doi.org/10.1016/j.conbuildmat.2012.07.040.

[76] S. Maheswaran *et al.* 2014. Strength Improvement Studies Using New Type Wild Strain Bacillus Cereus on Cement Mortar. *Current Science*. 106(1): 50-57. [Online]. Available: http://www.jstor.org/stable/24099862.

[77] S.-J. Park, Y.-M. Park, W.-Y. Chun, W.-J. Kim, and S.-Y. Ghim. 2010. Calcite-forming Bacteria for Compressive Strength Improvement in Mortar. *Journal of Microbiology and Biotechnology*. 20(4): 782-788. [Online]. Available: http://europepmc.org/abstract/MED/20467254.

[78] R. Pei, J. Liu, S. Wang, and M. Yang. 2013. Use of Bacterial Cell Walls to Improve the Mechanical Performance of Concrete. *Cement and Concrete Composites*. 39: 122-130. Doi: https://doi.org/10.1016/j.cemconcomp.2013.03.024.

[79] W. Pungrasmi, J. Intarasoontron, P. Jongvivatsakul, and S. Likitlersuang. 2019. Evaluation of Microencapsulation Techniques for MICP Bacterial Spores Applied in Self-Healing Concrete. *Scientific Reports*. 9(1): 12484. [Online]. Available: https://doi.org/10.1038/s41598-019-49002-6.

[80] S. K. Ramachandran, V. Ramakrishnan, and S. S. Bang. 2001. Remediation of Concrete using Micro-organisms. *ACI Materials Journal-American Concrete Institute*. 98(1): 3-9.

[81] R. Siddique, K. Singh, Kunal, M. Singh, V. Corinaldesi, and A. Rajor. 2016. Properties of Bacterial Rice Husk Ash Concrete. *Construction and Building Materials*. 121: 112-119. Doi: https://doi.org/10.1016/j.conbuildmat.2016.05.146.

[82] R. Siddique *et al.* 2016. Influence of Bacteria on Compressive Strength and Permeation Properties of Concrete Made with Cement Baghouse Filter Dust. *Construction and Building Materials*. 106: 461-469. Doi: https://doi.org/10.1016/j.conbuildmat.2015.12.112.

[83] S. R. Vempada, S. S. P. Reddy, M. S. Rao, and C. Sasikala. 2011. Strength Enhancement of Cement Mortar Using Microorganisms - An Experimental Study. *Int J Earth Sci Eng*. 4: 933-936.

[84] J. Y. Wang, N. De Belie, and W. Verstraete. 2012. Diatomaceous Earth as a Protective Vehicle for Bacteria Applied for Self-healing Concrete. *Journal of Industrial Microbiology & Biotechnology*. 39(4): 567-577. Doi: 10.1007/s10295-011-1037-1.

[85] J. Y. Wang, D. Snoeck, S. V. Vlierberghe, W. Verstraete, and N. D. Belie. 2014. Application of Hydrogel Encapsulated Carbonate Precipitating Bacteria for Approaching a Realistic Self-healing in Concrete. *Construction and Building Materials*. 68: 110-119. Doi: https://doi.org/10.1016/j.conbuildmat.2014.06.018.

[86] J. Wang, J. Dewanckele, V. Cnudde, S. V. Vlierberghe, W. Verstraete, and N. D. Belie. 2014. X-ray Computed Tomography Proof of Bacterial-based Self-healing in Concrete. *Cement and Concrete Composites*. 53: 289-304. Doi: https://doi.org/10.1016/j.cemconcomp.2014.07.014.

[87] J. Wang *et al.* 2015. Application of Modified-alginate Encapsulated Carbonate Producing Bacteria in Concrete: A Promising Strategy for Crack Self-healing. *Frontiers in Microbiology*. 6: 1088. Doi: 10.3389/fmicb.2015.01088.

[88] J. Wang, K. V. Tittelboom, N. D. Belie, and W. Verstraete. 2012. Use of Silica Gel or Polyurethane Immobilized Bacteria for Self-healing Concrete. *Construction and Building Materials*. 26(1): 532-540. Doi: https://doi.org/10.1016/j.conbuildmat.2011.06.054.

[89] J. Y. Wang, H. Soens, W. Verstraete, and N. D. Belie. 2014. Self-healing Concrete by Use of Microencapsulated Bacterial Spores. *Cement and Concrete Research*. 56: 139-152. Doi: https://doi.org/10.1016/j.cemconres.2013.11.009.

[90] S. L. Williams, M. J. Kirisits, and R. D. Ferron. 2016. Optimization of Growth Medium for Sporosarcina pasteurii in Bio-based Cement Pastes to Mitigate Delay in Hydration Kinetics. *Journal of Industrial Microbiology & Biotechnology*. 43(4): 567-575. Doi: 10.1007/s10295-015-1726-2.

[91] M. Wu, X. Hu, Q. Zhang, D. Xue, and Y. Zhao. 2019. Growth Environment Optimization for Inducing Bacterial Mineralization and Its Application in Concrete Healing. *Construction and Building Materials*. 209: 631-643. Doi: https://doi.org/10.1016/j.conbuildmat.2019.03.181.

[92] J. Xu, Y. Du, Z. Jiang, and A. She. 2015. Effects of Calcium Source on Biochemical Properties of Microbial CaCO3 Precipitation. *Frontiers in Microbiology*. 6: 1366. Doi: 10.3389/fmicb.2015.01366.

[93] J. Xu and X. Wang. 2018. Self-healing of concrete Cracks by Use of Bacteria-containing Low Alkali Cementitious Material. *Construction and Building Materials*. 167: 1-14. Doi: https://doi.org/10.1016/j.conbuildmat.2018.02.020.

[94] J. Xu, X. Wang, and B. Wang. 2018. Biochemical Process of Ureolysis-based Microbial CaCO3 Precipitation and Its Application in Self-healing Concrete. *Applied Microbiology and Biotechnology*. 102(7): 3121-3132. Doi: 10.1007/s00253-018-8779-x.

[95] Y. Ç. Erşan, F. B. D. Silva, N. Boon, W. Verstraete, and N. D. Belie. 2015. Screening of Bacteria and Concrete Compatible Protection Materials. *Construction and Building Materials*. 88: 196-203. Doi: https://doi.org/10.1016/j.conbuildmat.2015.04.027.

[96] S. Castanier, G. L. Métayer-Levrel, and J.-P. Perthuisot. 2000. Bacterial Roles in the Precipitation of Carbonate Minerals. *Microbial Sediments*, R. E. Riding and S. M. Awramik, Eds. Berlin, Heidelberg: Springer Berlin Heidelberg. 32-39. Doi: 10.1007/978-3-662-04036-2_5.

[97] A. F. Alshalif, J. M. Irwan, N. Othman, and L. H. Anneza. 2016. Isolation of Sulphate Reduction Bacteria (SRB) to Improve Compress Strength and Water Penetration of Bio-Concrete. *MATEC Web of Conferences*. 47: 01016. Doi: 10.1051/matecconf/20164701016.

[98] T. Tambunan, M. I. Juki, and N. Othman. 2019. Mechanical Properties of Sulphate Reduction Bacteria on the Durability of Concrete in Chloride Condition. *MATEC Web Conf.* 258: 01024. Doi: 10.1051/matecconf/201925801024.

[99] K. Vijay, M. Murmu, and S. V. Deo. 2017. Bacteria based Self Healing Concrete – A Review, *Construction and Building Materials*. 152: 1008-1014. Doi: https://doi.org/10.1016/j.conbuildmat.2017.07.040.

[100] S. Joshi, S. Goyal, A. Mukherjee, and M. S. Reddy. 2017. Microbial Healing of Cracks in Concrete: A Review. *Journal of Industrial Microbiology & Biotechnology*. 44(11): 1511-1525. Doi: 10.1007/s10295-017-1978-0.

[101] A. Sidiq, R. Gravina, and F. Giustozzi. 2019. Is Concrete Healing Really Efficient? A Review. *Construction and Building Materials*. 205: 257-273. Doi: https://doi.org/10.1016/j.conbuildmat.2019.02.002.

[102] J. Wang. 2013. Self-healing Concrete by Means of Immobilized Carbonate Precipitating Bacteria. PhD Thesis. Ghent University. Faculty of Engineering and Architecture.

[103] S. Gupta and H. Kua. 2016. Encapsulation Technology and Techniques in Self-Healing Concrete. *Journal of Materials in Civil Engineering*. 28: 04016165. Doi: 10.1061/(ASCE)MT.1943-5533.0001687.

[104] S. Gupta, S. D. Pang, and H. W. Kua. 2017. Autonomous Healing in Concrete by Bio-based Healing Agents – A Review. *Construction and Building Materials*. 146: 419-428.
Doi: https://doi.org/10.1016/j.conbuildmat.2017.04.111.

[105] F. B. da Silva, N. Boon, N. Belie, and W. Verstraete. 2015. Industrial Application of Biological Self-healing Concrete: Challenges and Economical Feasibility. *Journal of Commercial Biotechnology*. 21: 31-38. Doi: 10.5912/jcb662.

[106] H. M. Jonkers and E. Schlangen. 2007. Self-healing of Cracked Concrete: A Bacterial Approach. *IA-FraMCoS [internet].[Acesso em: 2017 out 04]*.

[107] H. M. Jonkers and H. Schlangen. 2021. Crack Repair by Concrete Immobilized Bacteria. *Proceedings of the First International Conference on Self Healing Materials*. 1-7. Accessed: Jul. 25. [Online]. Available: https://research.tudelft.nl/en/publications/crack-repair-by-concrete-immobilized-bacteria.

[108] H. Jonkers and E. Schlangen. 2008. Development of a Bacteria-based Self Healing Concrete. *Tailor Made Concrete Structures*. Doi: 10.1201/9781439828410.ch72.

[109] W. De Muynck, D. Debrouwer, N. De Belie, and W. Verstraete. 2008. Bacterial Carbonate Precipitation Improves the Durability of Cementitious Materials. *Cement and Concrete Research*. 38(7): 1005-1014. Doi: https://doi.org/10.1016/j.cemconres.2008.03.005.

[110] S. Dupraz, M. Parmentier, B. Ménez, and F. Guyot. 2009. Experimental and Numerical Modeling of Bacterially Induced pH Increase and Calcite Precipitation in Saline Aquifers. *Chemical Geology*. 265(1): 44-53. Doi: https://doi.org/10.1016/j.chemgeo.2009.05.003.

[111] M. Seifan, A. K. Samani, and A. Berenjian. 2016. Bioconcrete: Next Generation of Self-Healing Concrete. *Applied Microbiology and Biotechnology*. 100(6): 2591-2602. Doi: 10.1007/s00253-016-7316-z.

[112] M. J. Castro-Alonso, L. E. Montañez-Hernandez, M. A. Sanchez-Muñoz, M. R. Macias Franco, R. Narayanasamy, and N. Balagurusamy. 2019. Microbially Induced Calcium Carbonate Precipitation (MICP) and Its Potential in Bioconcrete: Microbiological an. d Molecular Concepts. *Frontiers in Materials*6: 126. Doi: 10.3389/fmats.2019.00126.

[113] S. Z. Qian, J. Zhou, and E. Schlangen. 2010. Influence of Curing Condition and Precracking Time on the Self-Healing Behavior of Engineered Cementitious Composites. *Cement and Concrete Composites*. 32(9): 686-693.
Doi:https://doi.org/10.1016/j.cemconcomp.2010.07.015.