

Bio-Engineered Concrete: A Critical Review on The Next Generation of Durable Concrete

Md. Fahad Shahriar Zawad, Md. Asifur Rahman*, Sudipto Nath Priyom

Department of Civil Engineering, Chittagong University of Engineering & Technology (CUET), Chattogram-4349, BANGLADESH
Kaptai, Highway Raozan Pahartali Rd, Chattogram-4349

*Corresponding authors: u1501011@student.cuet.ac.bd

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ABSTRACT Concrete is a prerequisite material for infrastructural development, which is required to be sufficiently strong and durable. It consists of fine, coarse, and aggregate particles bonded with a fluid cement that hardens over time. However, micro cracks development in concrete is a significant threat to its durability. To overcome this issue, several treatments and maintenance methods are adopted after construction, to ensure the durability of the structure. These include the use of bio-engineered concrete, which involved the biochemical reaction of non-reacted limestone and a calcium-based nutrient with the help of bacteria. These bio-cultures (bacteria) act as spores, which have the ability to survive up to 200 years, as they are also found to start the mineralization process and the filling of cracks or pores when in contact with moisture. Previous research proved that bio-engineered concrete is a self-healing technology, which developed the mechanical strength properties of the composite materials. The mechanism and healing process of the concrete is also natural and eco-friendly. Therefore, this study aims to critically analyze bio-engineered concrete and its future potentials in the Structural Engineering field, through the use of literature review. The data analysis was conducted in order to provide gradual and informative ideas on the historical background, present situation, and main mechanism process of the materials. According to the literature review, bio-engineered concrete has a promising outcome in the case of strength increment and crack healing. However, the only disadvantage was its less application in the practical fields. The results concluded that bio-engineered concrete is a new method for ensuring sustainable infrastructural development. And also, it indicated that more practical outcome-based analysis with extensive application in various aspects should be conducted, in order to assess the overall durability.

KEYWORDS Bio-Engineered Concrete; Mineral Precipitation; Bio-Culture; Self-Healing; Mechanical Strength.

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1 INTRODUCTION

Concrete's weakness against tension force is always an issue of great concern as it generates cracks, which reduces the performance and durability of composite materials. Although, cracks generation is not a new topic, the remedies have always been significant issues of concern. Furthermore, they act as pathways for moisture, causing the corrosion of the embedded rebar inside the concrete. The generation of both micro and macro cracks also severely damage the properties of the material, which ultimately causes serious long-term problems (Zulfikar *et al.*, 2021). This extremely leads to a decrease in the strength and durability of the concrete, as well as the decay of the structure (Thakur and Singh, 2017). After the construction, remedies to

fill up the cracks are often carried out, using a costly and non-eco-friendly method.

Based on the construction industry, a durable and cost-efficient structure is always preferable, as various attempts are often conducted to create high-performance concretes. In this regard, the use of natural resources, e.g., Bio-engineered concrete, is becoming popular, due to having the ability to help eradicate the threat to the environment. Furthermore, Sustainable Development Goals (SDGs) are being globally implemented, as the use of bio-resources is becoming a preferable topic for experts, based on the development of less energy-consuming bio-concrete. These materials mainly focus on the

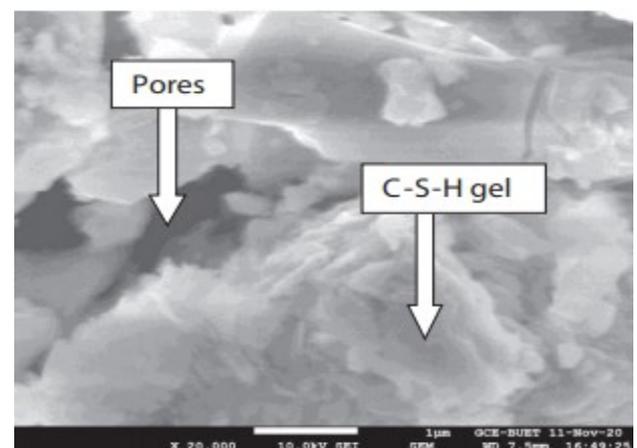
pre-construction measures, in order to make durable concretes. Therefore, the process of using bio-culture (bacteria) to heal cracks and improve mechanical properties is widely known as Bio-engineered concrete. This technique incorporates calcite precipitating bacteria within the material in certain concentrations. These bio-cultures precipitate calcium carbonate when in contact with water, and eventually solidifies the cracks (Wiktor and Jonkers, 2015). This bio-mineralization process is known as Microbiologically Induced Calcite Precipitation (MICP), which involves intracellular hydrolyzation of ammonia and Carbon dioxide. The ammonia present in this process causes an increase in the pH of the surroundings, which eventually leads to the mixture of the Calcium and carbonate cations that are deposited along the cell surface (Reinhardt and Joss, 2003).

Based on performance evaluation, several studies have been globally conducted on bio-engineered concrete, as materials having different bio-culture provided good results than the conventional types (Raina *et al.*, 2018). Furthermore, the properties of concrete were observed to effectively improve when bio-culture is used, based on the eco-friendly features of the material (De Belie, 2016). From Figure 1, the use of bacteria in concrete enriches the microstructural properties, which initiates a new and developed genus of durable material. This study aims to critically analyze the bio-engineered concrete, as well as determine the key aspects and applications of its future potentials, through the use of the literature review method.

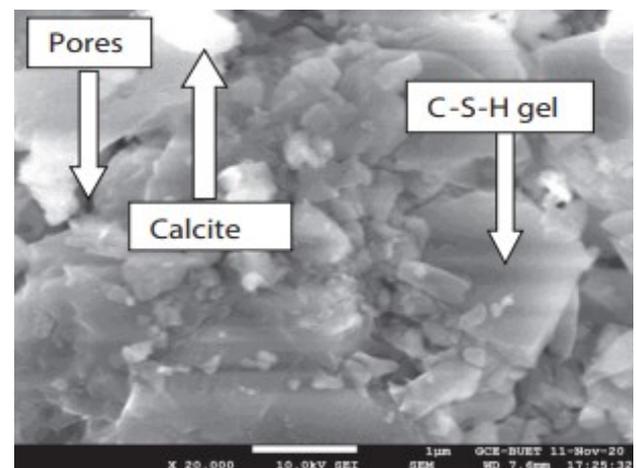
2 METHODOLOGY

This study was conducted by analyzing various published articles, based on the method adopted in using bio-cultures (bacteria) within concrete. The performance of this study was also based on significant results, obtained through key

information analyses of the publications. Approximately 39 laboratory-based research articles were reviewed, such as scientific journals, dissertations, and conference proceedings on bio-agent utilization in concrete. Three. potential publications focusing on practical results related to the application of bio-engineered materials in structural elements were also analyzed in section 8.2. These research articles were selected based on the titles, keywords and abstracts. In addition, Figure 2 represents the process flow being utilized in conducting this study.



(a)



(b)

Figure 1. (a). SEM image showing the less dense microstructure of conventional concrete, (b). dense microstructure of bio-engineered concrete (Priyom *et al.*, 2020).



Figure 2. The basic methodology followed for this research article.

3 HISTORICAL BACKGROUND AND CURRENT PROSPECTS OF BIO-ENGINEERED CONCRETE

Although the term bio-engineered concrete was new in the field of Structural Engineering, the concept of using bio-cultures (bacteria) was very familiar in increasing microstructural properties. In 1877, Ferdinand Cohn, a German biologist, claimed that the use of bacteria in the field of construction was an effective solution to the development of self-healing material genera. However, the study did not occur during this period, due to a lack of conception.

The use of bio-cultures to efficiently use the bio-mineralization process was first proposed by Me'tayer-Levrel *et al.* (1999). This study experimented on the use of bacterial carbonatogenesis, for the protection of architectural monuments. It also stated that the bio-mineralization process was a large field for future research, due to being a useful option to restore broken architectural monuments. In 2006, bio-engineered concrete was invented by Henk Jonkers, a professor at Delft University of Technology. Furthermore, H. Jonkers [7 publications], N. De Belie [7 publications], N.K. Dhami [4 publications], and S. P. Sivkova [5 publications], were prominent experts whose works in bio-agent (bacteria) concretes had generated a new possibility within the construction industry. Major studies based on this material had also been carried out in the last decade (2010-2020). In addition, the first bio-engineered concrete sample developed by Henk Jonkers through the encapsulation method, is shown in Figure 3.

Several laboratory and practical experiments had also been carried out on bio-engineered concrete, as various studies indicated that

efficient use of bio-cultures developed and enriched self-healing and mechanical properties, respectively. The flow dynamics of publication in this study within the last decade is shown in Figure 4.

Based on Figure 4, a major increment of interest in the study of the bio-engineered concrete occurred between 2014 to 2020, as practical application related to the research became a major focus for future experts.



Figure 3. Bio-engineered concrete developed by the encapsulation method (Jonkers, 2011)

4 SELECTION OF BIO-AGENT (BACTERIA) IN CONCRETE

The precipitation of CaCO₃ and other inorganic minerals by bacteria was strongly dependent on environmental conditions, which increased due to employing specific metabolic pathways. The designation of the ‘Limestone producing bacteria’ was always due to a tangible combination of the metabolic pathways, activities, and physicochemical environmental conditions. Therefore, a specific bacterium was ‘limestone producing’ in one environment, and different in another. The selection of bacteria was also highly dependent on their survivability within the concrete ambience. In addition, the pH value influenced the limestone precipitation, due to the urease enzyme being only activated for the specific acidic and basic level of Urea hydrolysis. This showed that the bacterial spores activated from the dormant stage had pH levels of 10-11.5, although they differed from the alkaliphile types and exposure conditions (Abo-El-Enein *et al.*, 2013). Carbonate yield also

played a vital role during the selection of bio-agents, as Table 1 represents the different metabolic pathways of calcium carbonate precipitation (De Belie and Wang, 2015).

5 METHODS OF APPLICATION OF BIO-AGENT (BACTERIA) IN CONCRETE

Based on various studies, the following methods are found more feasible,

(1) Direct application method: Bio-cultures containing spores and calcium lactate are added directly into the concrete mix in this method. When cracks occur on the structure, the spores germinate and feed on the calcium lactate, to carry out healing.

(2) Encapsulation method: In this method, treated clay pellets containing bacterial spores and calcium lactate are used in concretes. These pellets are degraded when cracks occur on the structures, as spores are observed to start germinating.

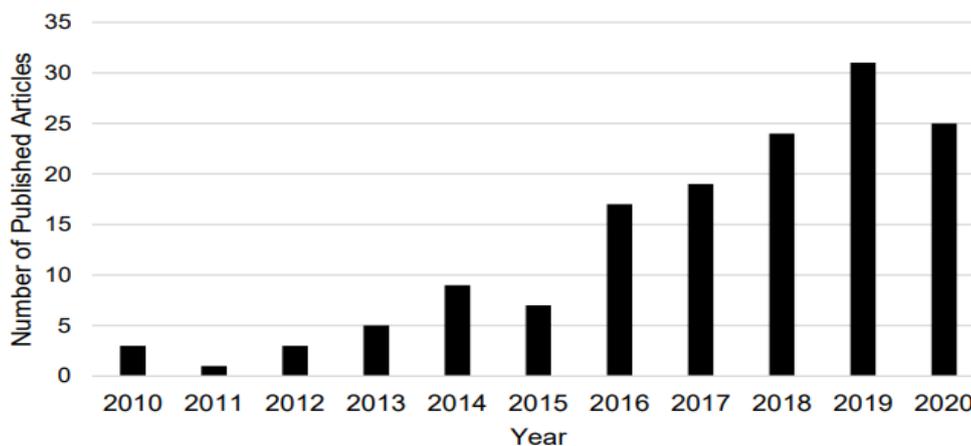


Figure 4. Frequency of published articles on bio-engineered concrete in the last decade.

Table 1. Different metabolic pathways of bacterial calcium carbonate (CaCO₃) precipitation (Belie *et al.*, 2015)

Autotrophic bacteria	Heterotrophic bacteria				
Non-methylotrophic methanogenesis	Assimilatory pathways		Dissimilatory pathways		
	Oxidation of organic carbon				
Anoxygenic photosynthesis	Urea decomposition	Aerobic		Anaerobic	
	Ammonification of amino-acids	Process	e ⁻ acceptor	Process	e ⁻ acceptor
Oxygenic photosynthesis		CH ₄ oxidation	Respiration	O ₂	NOx reduction
			CH ₄ /O ₂	SO ₄ ²⁻ reduction	SO ₄ ²⁻

(3) Vascular network method: This is a new method for using the self-healing mechanism, where temporary glass tubes are generally embedded in the concrete during casting. After this, these tubes are removed from the structures and tunnels produced within the structure. In addition, bio-cultures containing spores are then injected through the tunnel, when cracks are formed.

(4) Bio-agent as curing medium: One of the new and most promising techniques to produce bio-concrete is the curation of structural specimens within a medium containing bacterial spores. However, these bio-cultures are not directly applied to the concrete mix.

(5) Spraying bio-culture during curing: When the concrete specimens are cured for a desired limit, the spraying of bacteria-induced liquid is conducted, in order to produce a self-healing nature.

(6) Surface treatment agent: Bio-cultures are used as surface treatment cultures than conventional approaches, to protect the concrete from the ingress of water and other deleterious substances.

(7) Microcapsules enriched encapsulation method: In this method, microcapsule spores are applied into the concrete mix. These capsules are found to participate in self-healing processes, after the generation of cracks.

(8) Bacterial spores enriched powder method: In this method, bacterial spores with calcite ingredients are dry-sprayed within the tank by a peristaltic pump, with the dry powder directly added to the concrete mix

6 MICROBIAL INDUCED CALCITE PRECIPITATION (MICP)

The autogenous healing process is a remarkable concrete capacity to naturally repair cracks. This mainly depends on the presence and absence of moisture and tensile stress, respectively. However, it is limited to crack widths below 0.2 mm (Beltran and Jonkers, 2015). Therefore, the development of a self-healing system filling the

cracks of higher widths is becoming a popular topic for present studies. Furthermore, various studies had shown that microbial-induced calcite precipitation (MICP) was a promising technique for developing self-healing genera (Joshi *et al.*, 2017; Silva, 2015). This method mainly focused on the mechanism of calcium carbonate (CaCO_3) precipitation of bio-cultures, when cracks occurred on the concrete surface. In addition, the bacteria-induced CaCO_3 sealed the cracks in the presence of moisture (Wang *et al.*, 2015).

Bio-mineralization in the form of MICP is a process where CaCO_3 minerals are formed from a supersaturated solution, in the presence of micro-organisms. Figure 5 represents the basic mechanism of this process. Furthermore, bio-agent (bacteria) cells had the abilities to emit carbonate ions (CO_3^{2-}), which reacted with calcium (Ca)-rich solution and precipitated insoluble CaCO_3 . Various species of bacteria also produced different phases of calcium carbonate (CaCO_3). Therefore, calcite is the primary and most thermodynamically stable polymorph of calcium carbonate (CaCO_3), mostly formed in the MICP reactions (Anbu *et al.*, 2016).

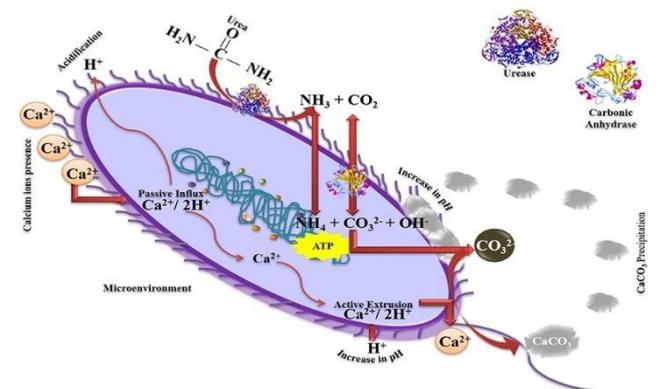


Figure 5. Basic mechanism of calcite precipitation at microbial cell walls (Castro-Alonso *et al.*, 2019)

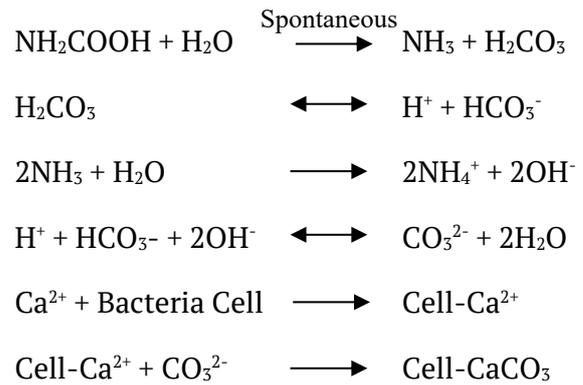
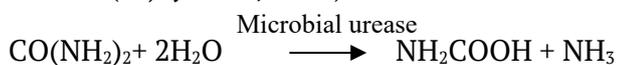
7 MECHANISM OF BIO-ENGINEERED CONCRETE

As previously mentioned, cement mortar has high alkaline and dry ambience, which creates an inhospitable atmosphere for specified lifeforms. However, alkaliphilic bacteria have the viable capabilities to survive in severe and harsh conditions. Different activities such as the CaCO_3

precipitation, is known as a typical metabolic pathway, leading to the increment of carbonate ion concentration and related saturation.

Based on several studies, the introduction of MICP in concrete was carried out through the enzymatic hydrolysis of urea, which was converted into NH_3 and CO_2 by bacteria. Therefore, the pH level increased from neutral to alkaline condition, causing the formation of carbonate. Although the process provided much effective self-healing capability in the concrete, a minor disadvantage was still observed, i.e., non-eco-friendly formation of ammonia. Furthermore, the long-term stability became limited in the alkaline environment within the material, due to the influence of urea. This indicated that the technique accurately performed as an externally applied repair mechanism than a self-healing agent.

Based on the process of MICP, a higher concentration of calcium carbonate was achievable within a short time. Urease also influenced the formation of minerals by four factors, namely concentration of Ca^{2+} , dissolved inorganic carbon ratio, pH and presence of nucleation sites, and the latter of great importance for continuous and stable calcite crystals formation. However, it was generally carried out by the bacteria lying on the cell surface in Bio-mineralization. This charged with negative groups, as the divalent cations were anchored at a neutral pH level, which created the ideal nucleation sites for necessary calcite deposition. Meanwhile, magnesium ions made the bond more frequent than the calcium ions, due to having strong ionic selectivity. The bound cations (metal ions) also reacted with anions (carbonates) to form insoluble calcium carbonate. Bacterial cells further affected the type of minerals to be created, due to being nucleation sites. The enzymatic urea hydrolysis procedures are shown in the following chemical reaction (Vijay *et al.*, 2017).



In this urease-mediated process, the reaction of urea ($\text{CO}(\text{NH}_2)_2$) and water yielded Carbon dioxide (CO_2), and ammonia (NH_3). Based on the high pK value (acid dissociation constant- a quantitative measure of acidic strength in a solution) of the $\text{NH}_3/\text{NH}_4^+$ system (about 9.2), the reaction produced a pH increase and concomitantly shifted in the carbonate equilibrium (CO_2 to HCO_3^- and CO_3^{2-}). This caused the precipitation of CaCO_3 , when a sufficient amount of calcium ions (Ca^{2+}) was present.

Based on Figure 6, the generation of calcium carbonate (CaCO_3) in the mortar sample surface (inner and outer) was observed, showed due to the addition of bio-cultures. The generation of this compound did not only act as a crack healer, it also developed the mechanical properties of concretes. The use of these carbonate-producing bio-cultures filled the pores of the cement-sand matrix (Stooks-Fischer *et al.*, 1999), causing the development of concretes with low permeability and higher strength.

8 LITERATURE ANALYSIS

8.1 Review of laboratory-based research study focusing on strength properties, microstructure and self-healing capability

This aspect focused on the laboratory-based articles that mainly investigated the healing capacity and ability of bio-genus as a strength increaser. Table 2 shows the analysis of the selected studies.

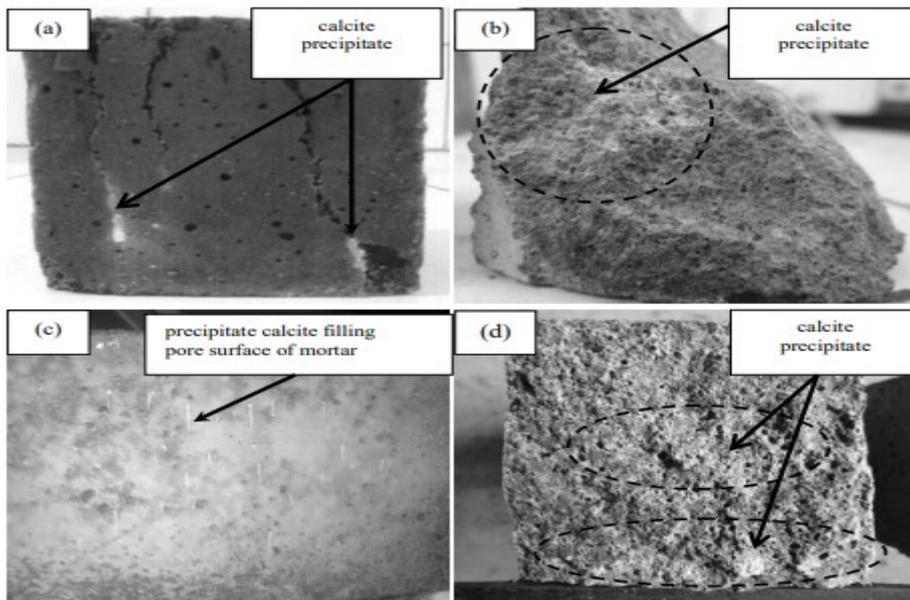


Figure 6. Precipitation of calcium carbonate in the outer and inner surface of mortar samples having bacterial spores in the mix (Nugroho *et al.*, 2015)

Table 2. Literature analysis of Bio-agent enriched laboratory-based research

Bio-agent used	Methodology	Major outcomes	References
<i>Bacillus pseudofirmus</i> and <i>Diaphorobacter nitroreducens</i>	a. Encapsulation method b. Bacterial strains were added as 0, 0.5, 1.5, and 3.0% of cement weight	a. Lower concentration of alginate beads within the capsule had very little significance on strength properties. b. Specimens with higher calcium alginate concentrations provided greater stiffness recovery. c. Samples having calcium alginate had superior self-healing ability over controlled concrete. d. Lower concentrations of alginate beads did not yield significant differences in compressive strength (at 0.5% by wt. of cement) and elasticity modulus (at 0.5% and 1.5% by wt. of cement), compared to the control specimens.	Hassan <i>et al.</i> , (2019)
<i>Bacillus sphaericus</i>	a. Direct application b. Bacterial spores with and without immobilized silica gel were directly added to the cracks	a. Culture protected with silica gel showed better performance. b. Crack sealing by bacterial approach reduced the water permeability of concrete. c. Bacteria, immobilized with silica gel accurately sealed the cracks than others.	Tittelboom <i>et al.</i> , (2010)
<i>Sporosarcina pasteurii</i>	a. Ceramsite particles were used as a carrier of bio-agent. b. Porous ceramsite particles with nutrients and bacterial spores were applied to the concrete mix	a. The viability of the bacterial spores within the concrete Ceramsite particles played an important role. b. Bacterial concrete showed a 20% increment in compressive strength than the control specimens. c. Bacteria concrete had 30% less water absorption ratio than the control specimens. d. Based on the mechanism of the self-healing process, the maximum width filled with precipitated calcite was 0.3 mm. e. Nutrients were frequently and easily accessed to the cells, when the bacteria and nutrients were incorporated within the Ceramsite particles.	Xu <i>et al.</i> , (2018)

Table 2. Literature analysis of Bio-agent enriched laboratory-based research (Cont.)

Bio-agent used	Methodology	Major outcomes	References
<i>Bacillus cereus</i>	a. Direct application b. 35% of cement replaced by fly ash was also added	a. At 28 days, 9.93% compressive strength was increased for the bacterial concrete, compared to the conventional specimens. b. Water absorption capacity for bacterial concrete was 1.98%, which was less than half of the conventional type.	Selvan and Dharani, (2016)
<i>Paenibacillus muscilagenosus</i>	a. Direct application of bacteria powder in concrete mortar b. Bacterial powder of 1.2 kg/m ³ was added into the concrete mix	After healing, a. Bio-concrete: 0 to 135 μ A (corrosion current) and -150 to -550 mV (corrosion potential), Normal concrete: 50 to 150 μ A (corrosion current) and -250 to -600 mV (corrosion potential) b. Bacterial concrete by healing cracks decreased the process of reinforced corrosion. c. The bio-concrete chloride contents at 5 and 10 mm depth were 0.02% lower than the conventional type.	Ling and Qian, (2017)
<i>S. pasteurii</i> and <i>Bacillus subtilis</i>	a. Direct application b. Two different bacterial concrete specimens were made and cured in both plain water and urea-CaCl ₂ solution	a. Bacterial concrete cured in urea-CaCl ₂ was found to prolong cement hydration. b. Specimens cured in urea-CaCl ₂ showed less mass increment than those in water. c. Increment of bulk density and reduction of voids were found in bacterial concrete. d. Compressive strength of bacterial concrete approximately increased by 20%, which was more than the conventional type, as reduction of chloride penetration was also observed. e. <i>S. pasteurii</i> concrete showed better strength performance.	Nosouhian et al., (2015)
<i>Bacillus pseudofirmus</i>	a. Encapsulation method b) Coated perlite with nutrient was applied to the mix	a. A reinforced concrete wall was made with bacterial concrete for further investigations after trial. b. Healing of cracks was initiated in bio-concrete. c. Encapsulation method did not modify the basic properties of concrete.	Paine et al., (2016)
<i>Bacillus flexus</i> , <i>Bacillus pasteurii</i> and <i>Bacillus sphaericus</i>	Microbial culture used as the medium of curing specimens	a. A maximum of 18% compressive strength was increased by using bacterial approach, as <i>Bacillus flexus</i> containing specimens showed great result. b. <i>B. pasteurii</i> and <i>B. sphaericus</i> showed marginally less strength than <i>B. flexus</i> . c. All the bacteria were urease positive. d. XRD analysis showed the phenomenon of bio-mineralization.	Jagadeesha et al., (2013)
<i>Bacillus subtilis</i>	Direct application of four different OD ₆₀₀ spores	a. UPV test indicated that bacterial concrete possessed higher density and compactness than the conventional type. b. Specimens having OD ₆₀₀ =0.637 showed better performance.	Priyom et al., (2018)
<i>Sporosarcina pasteurii</i> , <i>Bacillus cohnii</i> , <i>Bacillus halodurans</i> and <i>Bacillus pseudofirmus</i>	a. Direct application b. Re-suspension bacterial culture was added as a medium of the water required for the concrete mix	a. Except for <i>S. pasteurii</i> , three strains showed abundant production of spores in the mineral medium. b. <i>B. pseudofirmus</i> had the highest viability rate of 7.0%. c. From the ESEM analysis, high amount of calcite-like crystals were formed in the bacterial concrete. d. Incorporation of high bacteria amount (10 ⁹ /cm ³) did not affect the strength of concrete. e. Bacterial concrete had the ability of self-healing.	Jonkers & Schlangen, (2007)

Table 2. Literature analysis of Bio-agent enriched laboratory-based research (Cont.)

Bio-agent used	Methodology	Major outcomes	References
<i>Bacillus sphaericus</i> and <i>Bacillus pasteurii</i>	<ul style="list-style-type: none"> a. Direct application b. Bacterial culture was added as 10, 20 and 30% of cement weight b. Fly ash was added as 10, 20 and 30% replacement of cement 	<ul style="list-style-type: none"> a. Concrete specimens containing fly ash and <i>Bacillus sphaericus</i> showed better performance. b. Concrete specimens containing fly ash and <i>Bacillus pasteurii</i> showed marginally less performance than those with <i>B. sphaericus</i>. c. Concrete specimens containing bio-cultures showed better performance than conventional types. d. A maximum of compressive, split tensile, and flexural strengths at 10.8, 29.87, and 5.1%, was increased by using <i>Bacillus sphaericus</i>. 	Jagannathan <i>et al.</i> , (2018)
<i>E. coli</i>	<ul style="list-style-type: none"> a. Continuous growth setup was designed to grow bio-film on mortar cubes b. <i>E. coli</i> culture was injected into the medium 	<ul style="list-style-type: none"> a. The results from the ATP measurement and member filtration showed intensive continuous biomass concentration. b. SEM micrographs showed that the biofilm on the mortar surface was fairly distributed after 8 days. c. The result from the elemental mapping (EDS analysis) showed that there was an increase of 2.4% atomic fraction of the constituent Phosphorous, which was mainly liable for the formation of bio-film. d. Formation of biofilm on mortar surface was eco-friendly, cost-effective, and self-plenishing. 	Soleimani <i>et al.</i> , (2013)
<i>Bacillus megaterium</i>	<ul style="list-style-type: none"> a. Direct application b. A 2-phased diagram was adopted for bio-agent selection. c. After XRD analysis, the bacteria identification step was initiated 	<ul style="list-style-type: none"> a. Strength development rate for higher-grade concrete (50 MPa) was 24% (maximum), which was greater than the lower level structure, due to increased intensity of calcite precipitation. b. Bacterial concentration of 30×10^5 cfu/ml was optimal, due to obtaining positive characteristics from the concrete samples. c. For bacterial concentration of 30×10^5 cfu/ml, concrete samples had more calcite precipitation rate, due to its maximum compressive & flexural strength. d. In the construction industry, <i>B. megaterium</i> was part of green building material. 	Andalib <i>et al.</i> , (2016)
<i>Bacillus subtilis</i>	<ul style="list-style-type: none"> a. Direct application b. Depending on the cell concentration, 8 different bacterial agents were added to the concrete mix 	<ul style="list-style-type: none"> a. Bacterial specimens showed better strength performance than conventional concrete. b. The concentrations of 6.39×10^8 cells/ml were optimal for strength increment. c. 60% bacterial water provided better result. d. Bacterial concrete strength increment for 40MPa was 2-10% higher than the 20MPa specimens. e. The concentration of 6.39×10^8 cells/ml was highly optimal for strength increment. 	Islam <i>et al.</i> , (2018)
<i>Bacillus sphaericus</i>	<ul style="list-style-type: none"> a. Surface treatment agent b. Specimens were immersed in the bacterial culture of 0.3 and 0.6 L, before submersion in the nutrient solution. 	<ul style="list-style-type: none"> a. Concrete specimens were immersed in <i>Bacillus sphaericus</i> culture for 24 h, before being submerged in 0.3 and 0.6 L of nutrient solutions. b. SEM and XRD analysis indicated the presence of a newly formed calcite layer on the surfaces of bacterial specimens. c. Decrements in capillary suction and gas permeability were recorded for bacterial concrete. d. CaCO_3 crystal morphology was observed. e. Bacterial specimens performed better in all aspects than the controlled concrete. 	De Muyneck (2008)

Table 2. Literature analysis of Bio-agent enriched laboratory-based research (Cont.)

Bio-agent used	Methodology	Major outcomes	References
	c. Centrifuged ureolytic sludge were added		
<i>Bacillus megaterium</i>	a. Biogenic surface treatment agent b. Surface treatment agent was added as 10% of the weight	a. During 28 days of curing, the specimens' surfaces were regularly treated with 30 ml solution and precursors. b. Bacterial specimens showed 40% less water absorption capacity than the conventional concrete. c. Bacterial concrete showed a 31% decrement in porosity than the conventional specimen. d. Bacterial specimen showed a 22% increment in compressive strength, which was further improved to 18% more than the conventional concrete during freeze thaw test. e. SEM and XRD analysis established the presence of calcite crystals on the bacterial concrete.	Dhami <i>et al.</i> , (2013)
<i>Bacillus sphaericus</i>	a. Microcapsules encapsulation process b. Microcapsules were added as 1, 2, 3, 4 and 5% to weight of cement	a. Microcapsule dosage higher than 3% showed a significant (At a confidence level of 0.05) and negative effect on tensile strength. b. Increasing addition of microcapsules at 1-5% showed a decrement in compressive strength by 15-34%, based on the 28 days curing age. c. For 90 days of curing, the decrement in compressive strength was 22-47%. d. Healing ratio in the bio-specimens and controlled concretes were 48-80% and 18-50%. e. Maximum crack width healed by bacterial specimens was 970µm, which was 4 times lower in non-bacterial concretes at 250 µm. f. Bacterial specimens showed that water permeability was 10 times lower than the control concretes. g. At 95% RH, no self-healing was observed for all specimens, which strongly indicated the importance of the moisture presence.	Wang <i>et al.</i> , (2014)
<i>Bacillus sphaericus</i>	a. Direct application b. 10 and 20 ml bio-cultures were directly added to the concrete mix	a. For 10 and 20 ml addition, the compressive strength of bacterial specimens were 30.84 and 31.11%, respectively, which were higher than the conventional concrete. b. Increment in tensile strength was approximately 1.80 and 5.82% for 10 and 20 ml culture addition. c. Higher amount of culture addition showed better performance.	Gandhimathi <i>et al.</i> , (2015)
<i>Sporosarcina pasteurii</i>	a. Direct application b. Normal and light weight coarse aggregates were added into the concrete mix.	a. The coarse aggregates were left to soak in a bacterial inoculum with precursors for 6 days, and applied to the concrete mix. b. An average of 10% reduction in water absorption was observed for bacterial concrete. c. Bacterial concrete showed an average of 20% increment in compressive strength. d. The RCPT test showed a 20% reduction in chloride penetration than the controlled concrete. e. The SEM analysis showed denser and lower porosity of LWCA bacterial specimens.	Balam <i>et al.</i> , (2017)
<i>Bacillus pumilus</i>	a. Direct application b. As a curing agent having 1.5×10^8 , b. 12×10^8 and 24×10^8 cells/ml	a. In the case of direct application, 1.5×10^8 cells/ml showed better performance in compressive strength test. Also, a 6.3% increment in compressive strength was observed than the conventional specimen at 28 days. b. For bacterial specimens 24×10^8 cells/ml concentration showed better performance in compressive strength test.	Oriola <i>et al.</i> , (2018)

Table 2. Literature analysis of Bio-agent enriched laboratory-based research (Cont.)

Bio-agent used	Methodology	Major outcomes	References
		<ul style="list-style-type: none"> c. At 28 days, the weight losses for bacterial specimens were 11.8, 17.8, and 14.2% for 1.5×10^8, 12×10^8, and 24×10^8 cells/ml, respectively. However, it was 16.5% for conventional concrete. d. At 90 days, the weight losses for bacterial specimens were 15.5, 15.7, and 15% for 1.5×10^8, 12×10^8, and 24×10^8 cells/ml, respectively. However, it was 16.5% for conventional concrete. e. Specimens containing 1.5×10^8 and 24×10^8 cells/ml showed better performances in the compressive strength increment and durability test within all aspects. 	
<i>Bacillus sp.</i> CT-5	<ul style="list-style-type: none"> a. Direct application b. Bio-culture to w/c ratio was 0.47 	<ul style="list-style-type: none"> a. The result of compressive strength test showed a 30% increment for bacterial specimens than that of the controlled concrete. b. Bacterial concrete absorbed 6% less water than the controlled cubes. 	Achal <i>et al.</i> , (2010)
<i>Rhodobacter capsulatus</i>	<ul style="list-style-type: none"> a. Direct application b. Bacterial spores of 4.6×10^6 cell/ml were added to the mix c. Super absorbent polymer were used at 30% volume of fine aggregate replacement in the concrete mix 	<ul style="list-style-type: none"> a. The bacterial strain as an isolated and super absorbent polymer, was employed for immobilization, and was also 30% volume for replacements of fine aggregates in coating mortars. b. At 28 days, compressive strength of the bacterial and conventional concretes were 40.7 and 38.9 MPa, respectively. c. Approximately 38% increment in compressive strength was observed in the bacterial concrete. d. The compressive strength coefficient after exposure of the samples in a 5% H_2SO_4 solution, was 1.02 and 0.97 for bacterial and conventional concretes, respectively. e. Due to H_2SO_4 solution reaction, gypsum production was 17% lower for bacterial concrete than the conventional specimens. 	Yoon <i>et al.</i> , (2019)
<i>Bacillus pseudofirmus</i>	Encapsulation process (1.4×10^9 , 3.2×10^9 , 5.5×10^9 , 8.6×10^9 and 13×10^9 cells/ml)	<ul style="list-style-type: none"> a. Crack closure was observed for specimens containing 5.5×10^9 and 8.6×10^9 cells/ml, after 165 days. b. Sufficient healing compounds were not enough, as minimal bacterial spores were also required. 	Alazhari <i>et al.</i> , (2018)
<i>Bacillus subtilis</i>	<ul style="list-style-type: none"> a. Direct application b. Yeast Extract of 0.91 kg/m^3 and bacterial spores of 1.82×10^{10} cells/m^3 were added to the concrete mix 	<ul style="list-style-type: none"> a. The permeability and porosity for all tested concrete decreased with the increase in curing period. b. Bacterial concrete with $400 \mu\text{m}$ cracks were completely closed after 44 days. c. Bacterial concrete culture manifested better resistance against capillary suction. d. Bacterial specimens containing yeast extract and peptone reduced chloride ion diffusion e. A substantial decrease in gas permeability was also observed 	Nguyen <i>et al.</i> , (2019)
<i>B. subtilis</i> , <i>Brevi B. sp.</i> , <i>P. dendritiformis</i> , <i>B. methylotrophicus</i> , <i>B. licheniformis</i> and <i>S. maltophilia</i>	<ul style="list-style-type: none"> a. Direct application b. Six different alkaliphilic bacteria of 10^4 and 10^6 cells/ml concentrations were added to 	<ul style="list-style-type: none"> a. Specimens containing 10^4 cells/ml showed better performances. b. Compared to conventional concrete strength, the increment for bacteria specimens are, <i>B. subtilis</i> – 28.61%, <i>Brevibacillus sp.</i> – 22.1%, <i>P. dendritiformis</i> – 19.9%, <i>B. methylotrophicus</i> – 16%, <i>B. licheniformis</i> – 12.7%, 	Sreenivasulu <i>et al.</i> , (2018)

Table 2. Literature analysis of Bio-agent enriched laboratory-based research (Cont.)

Bio-agent used	Methodology	Major outcomes	References
	the concrete mix	<i>S. maltophilia</i> – 9.6%. c. SEM analysis showed calcite precipitation for all type of bio-groups.	
<i>Bacillus coagulans</i>	a. Direct Application b. Bacterial spores pf 1.5×10^8 , 12×10^8 and 24×10^8 cell/ml concentrations were added to the concrete mix	a. <i>B. coagulans</i> concrete showed the property of early strength than the conventional specimens. A maximum of 6.25% increment in compressive strength was observed for 24×10^8 cells/ml. b. The SEM images also showed that the increase in strength was the result of the calcite precipitation c. The highest compressive strength was found at a bacterial concentration of 1.5×10^8 cells/ml However, the strength and the durability reduced as the concentration increased d. Bacterial specimens exhibited better performances than the conventional concrete, in terms of durability and strength	Oriola <i>et al.</i> , (2018)
<i>Enterococcus faecalis</i> and <i>Bacillus cereus</i>	a. Direct application b. Three different bacterial concentrations of 2.1, 6.3, and 10.5 l/m^3 were added to the concrete mix	a. <i>E. faecalis</i> had high efficiency in increasing the compressive and tensile strengths of concrete than <i>B. cereus</i> (23% vs. 14.2%, and 13% vs. 8.5%, respectively). b. Both bacteria produced higher amount of calcite till their curing periods. c. The SEM images exhibited distinct amount of calcite crystals and decrease in pore sizes. d. From the EDX analysis, the highest percentage of calcium constituent was determined. e. Significant water penetration reduction was possible, by using both of the bacterial sample. f. Compared to conventional concrete, water absorption reduced at 3 and 5% for <i>E. faecalis</i> and <i>B. cereus</i> , at 28 days age.	Alshalif <i>et al.</i> , (2019)
<i>Sporosarcina pasteurii</i>	a. Direct application b. Bacterial concentrations of 10^8 and 10^9 per ml in different solutions were added to the mortar mix	a. The effect of varying bacterial concentrations on cement-sand mortar was significant b. The addition of <i>S. pasteurii</i> had positive impact on compressive and tensile strengths, due to the calcite precipitation. c. From SEM analysis, the growth of fibrous filler were observed within the pores, due to the precipitation of calcite. d. The mortar created with the 10^8 cells/ml was filled with narrow filler strand. e. The increment in cells provided better results, i.e., 4.9 and 4% compressive strength increment in NB and NH ₄ -YE media, respectively. This clearly indicated that the NH ₄ -YE medium did not have any influence.	Al-Salloum <i>et al.</i> , (2016)
<i>Bacillus subtilis</i>	a. Direct application b. Bacterial spores of 10^4 , 10^5 , and 10^6 cells/ml were directly added into pulverized fly ash medium	a. The mortars having bacterial spores, calcium lactate, and urea in pulverized fly ash medium, performed better in all aspects. b. For water submersion, samples with 10^6 cells/ml showed higher compressive strength increment at 10.66 and 16.11% for 3 and 7 days, compared to the control mortar. c. For submersion in 5% urea and 1% calcium lactate solutions, samples with 10^5 cells/ml showed higher compressive strength improvement at 25.38 and 21.40% for 3 and 7 days, compared to the conventional mortar. d. From flexural strength test, the stiffness recovery of the original strength was achieved for the bacterial specimens, at 34.85%. e. Based on water permeability test, bacterial spores were able to heal the cracks to 0.22 mm width	Nugroho <i>et al.</i> , (2015)

Table 2. Literature analysis of Bio-agent enriched laboratory-based research (Cont.)

Bio-agent used	Methodology	Major outcomes	References
		f. From XRD analysis, bacterial and control mortar had 39.70/25.20% calcite and 62.36/42.30% crystallinity, respectively.	
<i>Bacillus subtilis</i>	a. Direct application b. Bacterial culture having OD ₆₀₀ = 0.107, 0.2, 0.637, and 1.221 were directly added to the concrete mix, as 50% replacement of water	a. Concrete specimens containing OD ₆₀₀ = 0.637 showed better performance. b. Concrete Mix 4 with OD ₆₀₀ = 0.637 showed 17.91 and 11.95% increment in compressive strength for 20 and 30 MPa at 28 days, compared to the conventional specimens. c. For OD ₆₀₀ = 0.637, 12% increment in split tensile strength was also observed. d. Approximately 6-9% increment in pulse velocity was recorded for concrete specimens with 0.637 optical density, at the age of 28 days. e. OD ₆₀₀ = 0.5±0.1 was a good option for developing bacterial concrete genera.	Priyom <i>et al.</i> , (2021)
<i>Sporosarcina pasteurii</i>	a. Encapsulation method b. A protective carrier was developed for bacteria, by using calcium sulphoaluminate cement c. Microsilica of 0, 20 and 40%, were added to the concrete mix	a. The carrier acted as a support for the bacteria, ensuring preservation for long duration. b. The crack widths ranging from 20-450 μm were generated by applying compressive load. c. For visual observation of the cracks, a processing software known as the "Image-Pro plus" was used. d. Specimens having 20% silica fume with protective bacterial spores showed better performance. e. Self-healing of cracks with 0.42 mm width was obtained in 28 days. f. The regain ratio of compressive strength and water tightness increased at 130 and 50% for bacterial specimens, compared to plain mortar.	Xu and Wang, (2018)
<i>Bacillus</i>	a. Encapsulation method b. Mixture of viable and dormant bacteria in porous clay particles	a. Cracks were formed in concrete specimen slabs with 10 cm diameter and 2.5 cm thickness, by the controlled application of compressive and tensile stresses. A crack width of 0.15 mm was also generated. b. Cracks with 0.15 mm width and 8 cm length were totally sealed. c. All specimens containing bacterial spores and controlled concretes showed 100 and 33% healing of cracks.	Jonkers (2011)
<i>Bacillus sphaericus</i>	a. Encapsulation method b. Melamine-based capsule was added to the concrete mix	a. Bacterial spores were able to grow and germinate in high pH range. b. Optimal pH range for growth was 7-9. Although the growth rate decreased at pH of 10-11, it did not stop. c. Crack width of 0.97 mm was healed by bacterial spores. d. Crack healing ratio for bio samples was 48 to 80%.	Wang <i>et al.</i> , (2017)
<i>Shewanella and E. coli</i>	a. Direct application b. Seven different cell concentration ranging from 10 ⁵ to 10 ⁷ per ml were directly mixed with water.	a. Cell concentration was determined by developing OD ₆₂₀ vs. bacterial cell numbers, standard curve. b. Mortar specimens containing <i>Shewanella</i> spores of 10 ⁵ cells/ml showed 25% increment in compressive strength, than the conventional concrete. c. For <i>E. coli</i> , the increment in the compressive strength were less than 1%, compared to the conventional concrete.	Ghosh <i>et al.</i> , (2005)

Table 2. Literature analysis of Bio-agent enriched laboratory-based research (Cont.)

Bio-agent used	Methodology	Major outcomes	References
<i>Sporosarcina Pasteurii</i>	<ul style="list-style-type: none"> a. Direct application b. Microbial mortar was treated with three different calcium sources, namely CaCl_2, $\text{Ca}(\text{CH}_3\text{COO})_2$, and $\text{Ca}(\text{NO}_3)_2$ 	<ul style="list-style-type: none"> a. Samples treated by calcium acetate showed better result in compressive strength increment, which was 2.45, 2.58, and 1.32 times higher than other samples. b. Samples of bacterial mortar with calcium acetate showed better tensile strength, which was 2.4 and 3.0 times than chloride and nitrate samples respectively. c. SEM and XRD analysis showed that the use of $\text{Ca}(\text{CH}_3\text{COO})_2$ as calcium source for MICP, improved the mechanical properties and durability of the microbial mortar. 	Zhang <i>et al.</i> , (2015)
<i>Bacillus sphaericus</i>	<ul style="list-style-type: none"> a. Encapsulation method b. Bacterial spores were encapsulated into hydrogels, and incorporated into the specimens 	<ul style="list-style-type: none"> a. Prism specimens were subjected to multiple cracking by tensile load, with an average crack width of 150 μm. b. Maximum healing efficiency was observed in the specimens with bio-hydrogels, as 0.5 mm crack width was successfully healed. Approximately 40-90% healing ratio was also observed under wet-dry cycle. c. For non-bio hydrogel specimens, the healing width was 0-0.3 mm. d. Based on bacterial and control specimens, water permeability decreased by 68 and 15-55% in average. 	Wang <i>et al.</i> , (2014)
<i>Bacillus</i>	<ul style="list-style-type: none"> a. Direct application b. Bacteria-based healing agent was directly incorporated into lightweight aggregates, and mixed with fresh mortar. 	<ul style="list-style-type: none"> a. The liquid-tightness of mortar matrix with and without bacterial spores were evaluated through water permeability test, in both water immersion and wet-dry cycles. b. Water tightness of samples with and without bacterial spores were not different when immersed in water. c. During wet-dry cycles, samples with bacterial spores showed better performance than the control concrete. d. For bacterial samples, 96% of water tightness was achieved at day 56. 	Tziviloglou <i>et al.</i> , (2016)
<i>Bacillus mucilaginous and Brewers yeast</i>	<ul style="list-style-type: none"> a. Direct application b. Bacterial spores with and without nutrient were added to cement and water, mixed with prism and cylinder specimens 	<ul style="list-style-type: none"> a. Cracks were formed by bending test. b. Approximately 0.4-0.5mm with cracks were made by embedded method. c. For nutrient enriched bacterial samples, cracks with width of 0.4-0.5 mm were repaired after recurring for 28 days. d. For nutrient enriched bacterial samples, crack area repair ratio was 87.5%. e. The combination of ceramsite, brewer yeast, and <i>Bacillus Mucilaginous</i>, were able to reduce water permeability coefficient from $7.9-8.3 \times 10^{-5} \text{m/s}$ to $0.8 \times 10^{-7} \text{m/s}$, after 49 days of healing period. 	Chen <i>et al.</i> , (2016)
<i>Bacillus subtilis</i>	<ul style="list-style-type: none"> a. Direct application b. Bacterial spores were added to the mix, through the LWA and Graphite platelets 	<ul style="list-style-type: none"> a. Bacteria immobilized in graphite nano platelets provided better result in pre-cracked specimens, at 3 and 7 days old. b. Specimens immobilized in LWA were more effective in samples pre-cracked at 14 and 28 days old. c. Higher crack width was healed by bacterial samples with LWA, at 0.53 mm. d. Approximately 12% increment in compressive strength was observed for LWA immobilized specimens. 	Khaliq <i>et al.</i> , (2016)

8.2 Application of bio-engineered mortar and concrete in structural elements

Although most of these studies were laboratory-based, a few conducted in the last five years were still related to the application of bio-cultures as healers and strength increasers in practical structural elements.

Mors and Jonkers (2019), conducted several practical implementations on bio-engineered mortar and concrete. This was the largest practical study that mainly focused on the ability of bio-cultures, towards crack healing within a realistic environment. It also included two repair mortar and concrete construction demonstration projects, where a representative from the *Bacillus* genus was used as a healing agent.

Self-healing mortar: This was applied into a damaged reinforced concrete column (Figure 7), and cracked with active leaking garage basement walls, which were located at 20m below ground (Figure 8). To evaluate the performance of the self-healing mortar, visual determination of water tightness and hammer-knocking test was conducted with desired intervals. In addition, biennially monitoring for two subsequent years showed that the repaired patches were watertight, with the observation of sound bonding in both conditions.

Self-healing concrete: The research team carried out two full-scale demonstrator projects, by using self-healing concrete. The first project was conducted on the construction of a wastewater purification tank of 7 X 2.5 X 0.15 m (Figure 9), while the second was carried out on a rectangular water reservoir of 47 m long X 5 m high dimension, where the south and east-facing walls were fully constructed by using self-healing concrete (Figure 10).

Based on the wastewater purification tank, a 10 kg healing agent per m³ concrete mix was applied. The result showed that the tank

completed three years of successful operation till September 2019, with no cracks or degradation on the surface. For the water reservoir, a 5 kg/m³ self-healing agent was added to the concrete mix. Although the south-facing wall was more critical for cracking, the implementation of this method showed that no breaches were observed. However, minor cracks were found in the north-facing wall, where self-healing agent was not applied.



Figure 7. Application of bacteria-based self-healing repair mortar on steel reinforced concrete column (Mors & Jonkers, 2019).



Figure 8. Application of bacteria-based self-healing repair mortar on actively leaking cracked concrete basement walls (Mors & Jonkers, 2019).



Figure 9. Prefabricated wastewater treatment tank by using self-healing concrete (under construction) (Mors & Jonkers, 2019).



Figure 10. Full scale demonstrator in situ cast self-healing concrete water reservoir (Mors & Jonkers, 2019).

Zhang and Qian (2020) conducted the engineering application of self-healing concrete on ship lock walls, for practical outcomes. This study used *Bacillus mucilaginosus* and calcium nitrate powder for the purpose of the process, as a dry-spray method was utilized for making microbial particle. Also, both laboratory-based and practical analysis were also conducted, respectively. In the laboratory-based analysis, there was no significant difference between the workability levels of the control and microbial structures, as the compressive strength of self-healing concrete was slightly lower than the conventional type at the age of 28 days. During this period, 0.543 mm artificial crack was entirely healed by the self-healing material (Figure 11), as $2\theta = 29.49^\circ$ and 29.41° also indicated the production of minerals.



Figure 11. Sealing of 0.543 mm crack of laboratory specimen after 28 days of curing (Zhang and Qian, 2020)

Figure 12 shows the whole process of generating and applying self-healing concrete on the ship lock chamber. Based on temperature stress and shrinkage, cracks were generated on both the normal and self-healing concrete gates of the side wall.

At the age of 65 days, the cracks on the surface of the normal concrete were not healed. However, the generated breaches on the self-healing material were fully healed by calcite precipitation (Figure 13). In addition, the connectivity of the cracks were completely blocked on the bacterial enriched wall, as the leakages of water were not observed.

Mullem *et al.* (2020), conducted a large scale application of self-healing concrete by directly mixing MUC⁺ with the material (Figure 14). This compound (MUC⁺) was obtained from the combination of ureolytic culture with anaerobic granular bacteria.

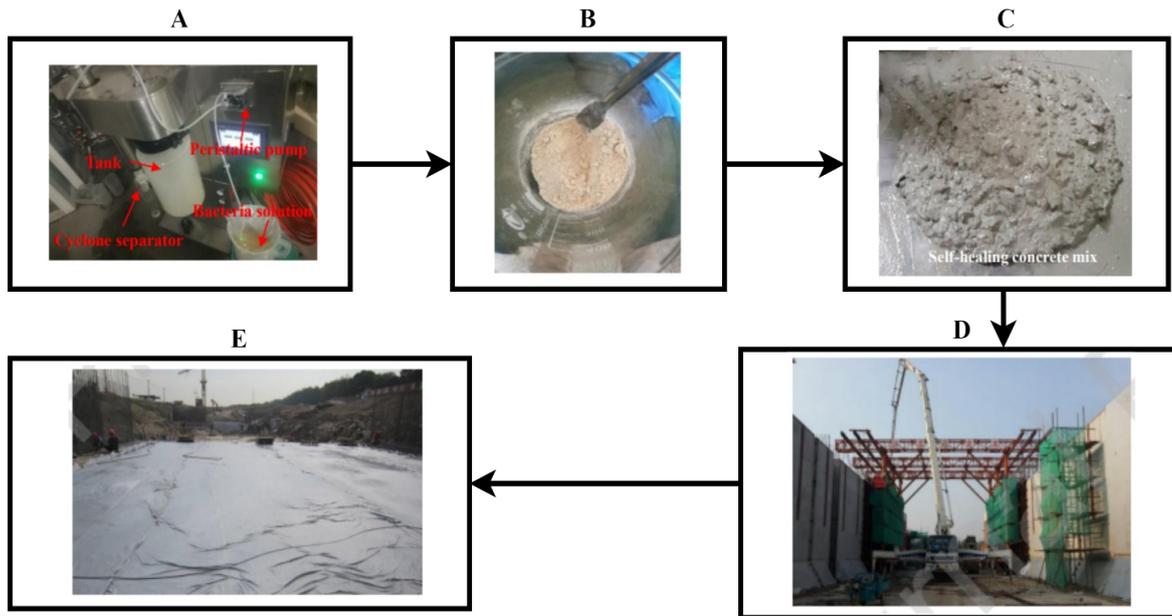


Figure 12. Whole process of the construction of bacteria-based self-healing ship lock wall: (A) Spray drying of bacterial sample, (B) Spore powder, (C) Self-healing concrete mix, (D) Pouring of self-healing concrete, (E) Curing of self-healing concrete (Zhang and Qian, 2020).



Figure 13. Comparison of self-healing effects of ship lock wall made by normal concrete (left) and made by bacterial concrete (right) (Zhang and Qian, 2020).

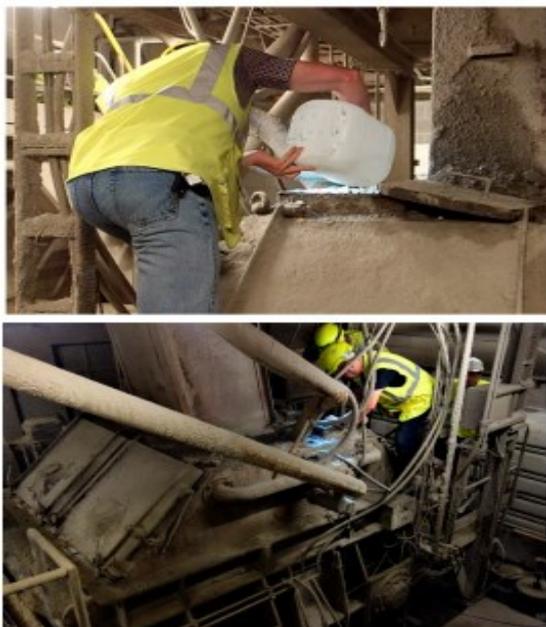


Figure 14. Addition of bacterial agent, urea and calcium nitrate tetrahydrate through inspection hatch above industrial concrete mixer (Mullem *et al.*, 2020).

Furthermore, the roof slab of a drainage pipe inspection pit (Figure 15) and laboratory prism specimens were casted by the same bacterial concrete mix (Figure 16). In the self-healing concrete mix, two different water-reducing plasticizers and one mass (%) of bio-agent were added. From the laboratory analysis, a significant amount of strength was increased at 93 days, through the addition of the self-healing agent, as increment was also observed to be 9.7 MPa.

Based on the laboratory specimens, cracks were created through the application of the tensile stress, as widths varying between 0.52-0.58 mm were perfectly sealed during the healing analysis. For bottom cracks with 245 μm width, 86.3% closure was observed, as the formation of stalactites was also found during the wet-dry cycle analysis (Figure 17).

The roof slab was also installed in the inspection room after five weeks of casting. After this, an onsite inspection was conducted after one year of casting, as no cracking sign was observed at the bottom of the slab. Approximately 30% of the slab top was covered by large condensation drops, which indicated that it was in a favourable condition to heal the cracks (Figure 18).



Figure 15. Roof slab made by bio-engineered concrete (Mulleem *et al.*, 2020).



Figure 16. Laboratory prism specimens made by bio-engineered concrete (Mulleem *et al.*, 2020).



Figure 17. Formation of stalactites on a crack location of a prism specimen subjected to W/D cycles (Mulleem *et al.*, 2020).



Figure 18. Condensation on the bottom side of the roof slab showing favorable condition for self-healing (Mulleem *et al.*, 2020).

9 HEALING CAPABILITY OF BIO-ENGINEERED CONCRETE AND TYPES OF CRACK HEALED

Based on the literature review, the maximum crack width completely sealed by the bio-engineered concrete is 0.97 mm. The healing of crack widths ranging from 0.3-0.6 mm was also observed in several pieces of the study, which had been reviewed in Table 2. In a practical situation, the healing capacity depended on the surrounding environment (Mulleem *et al.*, 2020). When this environment supply sufficient moisture and oxygen, the healing capability of bio-engineered concrete increases. This material is capable of healing cracks to a width of 1 cm in a practical situation (Zhang and Qian, 2020). In this case, the selection of bio-cultures played a vital role, as the use of calcite reagent also increased healing capability. Most of the studies focused on the amount of crack width, healed with complete crack closure by the bio-engineered concrete. However, Jonkers (2011), tried to focus on the length of crack that was efficiently sealed by the material. The study also showed that approximately 8 cm crack length was completely healed by the bio-engineered concrete, as the healing capability of bio-agent generally varied from 0.5 to 0.8 mm in practical situations (De Belie and Wang, 2015).

Concrete structures are also susceptible to various cracks, with most of them occurring before and after hardening. From the literature analysis, bio-engineered concrete mainly focused on the cracks generated after the hardening of the structural specimens. Several

pre-hardening cracks, such as construction movement and plastic breaches, were also healed by bio-engineered concrete.

The crack types healed by this method were often a topic of great interest among experts, as the implementation and further study in this field were more straightforward when summarized. Based on the literature reviewed, a diagram showing the crack type healed by bio-engineered concrete is shown in Figure 19.

10 PRIORITY OF BIO-GENUS USED IN THE RESEARCH STUDY

According to the reviewed literatures, more than 80% of the studies were carried out by adopting a representative from the genus-Bacillus. This was due to its tremendous ability to precipitate carbonate during harsh environment. In addition, the usage of various bio-genus is shown in Figure 20, through the representation of a bar chart.

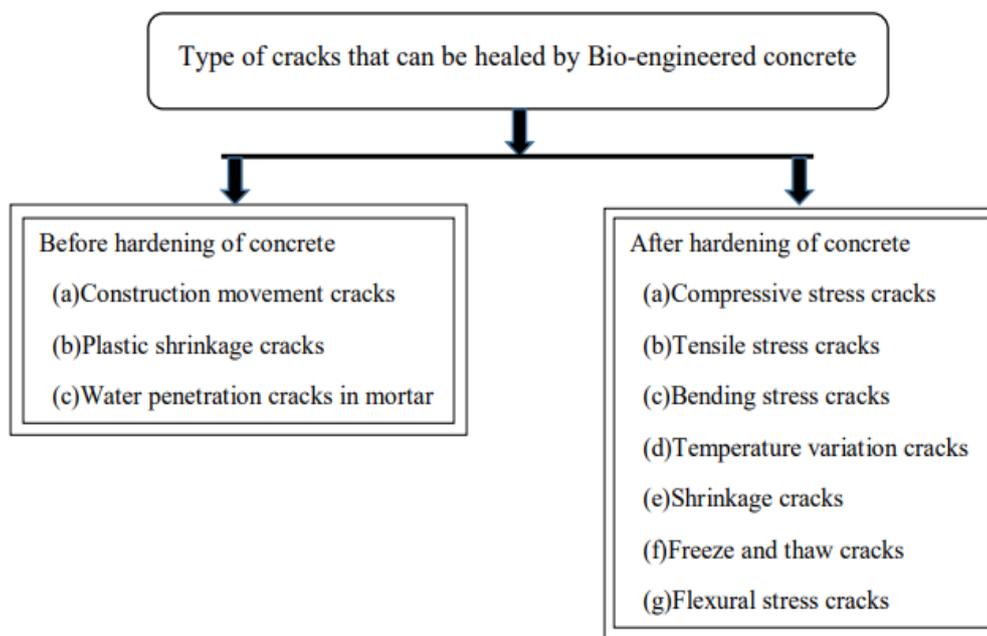


Figure 19. Type of crack healed by bio-engineered concrete.

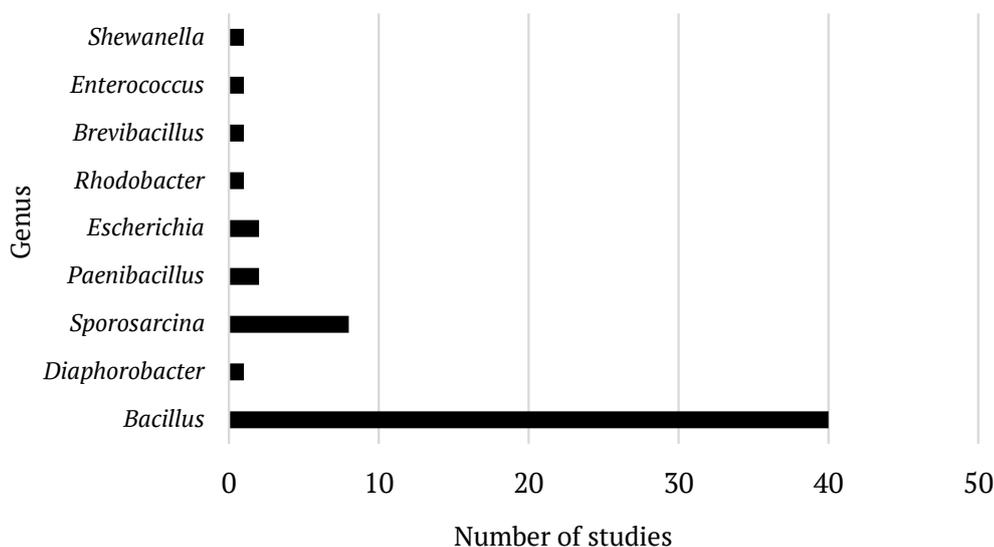


Figure 20. Usage of different bio-genus in reviewed literatures.

11 APPLICATION OF BIO-ENGINEERED CONCRETE

Sustainable urban amenities are very important for the better economy of a country, as most of the industrial works are infrastructurally related. This is because a small improvement on the durability of infrastructure has the ability to provide a larger result in the economy. Therefore, the use of bio-engineered concrete is becoming more popular in Structural Engineering. The roles and functions of this method are as follow,

- Sealing of cracks in concrete structure: Bio-engineered concrete are an excellent option for making durable structures, due to their bio-mineralization abilities.
- Mechanical strength increaser by conducting reaction with cement-sand matrix.
- Preparation of less permeable concrete: Bacteria within the concrete reduce void volumes, leading to more compaction and less permeability.
- Enhancement of resistance towards freeze-thaw action.
- Construction of low-cost durable housing.
- Durable rigid pavement preparation.
- Effect on sea-shore structures: It protects the structure from corrosion and prevents the deformation of rebar.

12 EFFECT OF BIO-AGENT ON THE PROPERTIES OF CONCRETE

- **Compressive strength:** The use of bio-cultures help in filling the pores between cement-sand microstructures, in order to acquire more compressive strength and become denser. According to Table 2, approximately 40% increase in this strength was observed through the use of bacteria.
- **Reduction of permeability:** Calcium carbonate helps in filling and reducing the pores and permeability of concrete specimens, respectively. Also, the permeability of bio-concrete is less than

half, compared to the conventional structure.

- **Setting time:** Based on the different bio-agent solutions, the set time of the concrete mix is accelerated or delayed. In addition, calcium lactate delayed this time, while $\text{Ca}(\text{HCOO})_2$ and $\text{Ca}(\text{NO}_3)_2$ (Calcium Formate and Calcium Nitrate) accelerated it (Jonkers *et al.*, 2010).
- **Development of the microstructure:** The SEM and XRD analysis indicated that the application of bio-agent caused calcite precipitation on the concrete microstructure, which eventually enriched the property of the structural specimens (Jagadeesha *et al.*, 2013; Jonkers and Schlangen, 2007).
- **Self-healing:** The use of bio agent enriches the ability of concrete, in order to heal cracks. From the literature analysis, the utilization of bacteria healed approximately 1 cm concrete crack width.

13 RECCOMENDATION FOR FURTHER STUDY

- Any new invention or improvement of the existing methodology in the construction industry needs a lot of practical research. Bio-concrete as a recent phenomenon is the most promising solution for durable structures. However, the long-term effect of this method is not well recognized. Previously, several studies were carried out on this topic, with most of them being laboratory-based. Only a few demonstration project was conducted to assess the suitability of this new method. Therefore, large scale practical implementation and the regular inspection to sort out any problem should be the next step.
- The selection of bio-agent is also a matter of great concern, due to the difference in the climatic and environmental situation suitable for the microorganisms. Therefore, separation and categorization

on various exposure conditions should be an important issue in the future.

- Based on practical implementation, bio-concrete should also be cost effective, with the budget mainly depending on the selection of bio-cultures. Therefore, the cost of the project should be focused on various exposure conditions.

CONCLUSION

Bio-engineered concrete is becoming a popular topic for research purposes. In this study, several publications focusing on the performance of bio-engineered concrete were reviewed. Based on the literature reviewed, it was found out that such type of concrete are useful for harsh environment where density and compactness of the concrete microstructure is needed. Bio-engineered concrete significantly improved the lifetime of construction, by reducing the repair and replacement costs. Therefore, the additional investment in the method was accurately worth the risk. In addition, future research on bio-engineered concrete should understand the cost-effectiveness and long-term effects.

DISCLAIMER

The authors declared no conflict of interest

AVAILABILITY OF DATA AND MATERIALS

All data are available from the author.

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