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Review

A Biomineralization, Mechanical and Durability Features of Bacteria-Based Self-Healing Concrete—A State of the Art Review

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Abstract: Cracking is one of the main ways that concrete ages, allowing pollutants to seep within and potentially lowering the physical and mechanical strength and endurance of concrete structures. One of the healing procedures that merits research is the use of bacterially generated calcium carbonate precipitation in concrete mixtures to mend concrete cracks. The impact of different variables, including the nucleation location, bacterial type, concentration, uratolytic activities, pH, nutrition, and temperature on the bio-mineralization of calcium carbonate are discussed in this review article. ATR-IR (Attenuated Internal Reflectance Fourier Transform Infrared Spectroscopy)/FTIR (Fourier Transform Infrared Spectroscopy)/NMR (Nuclear Magnetic Resonance) and FESEM (Field Emission Scanning Electron Microscope) are among the micro test techniques reviewed along with the biosynthetic pathway of bio mineralized calcium carbonate. The sealing ability and recovery of mechanical and durability properties of bio-mineralized concrete specimen is discussed. Moreover, we discussed the corrosion, damages, and challenges and their detection methods. Also, in-depth knowledge on the use, advancements, and drawbacks of bio-mineralized calcium carbonate is presented. Future potential for bio-mineralized (MICP) self-healing concrete are discussed in the final section.

Keywords: MICP; factors affecting MICP; assessing the MICP; self-healing concrete



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1. Introduction

Concrete has become one of the world's utmost widely utilized substances due to its unique properties, which include its good compressive strength, availability, and adaptability, its economical nature, its suitability with reinforced steel bar, its being flame retardant, its superior caloric weight (Highly resistant to weight loss against rise in temperature [1], and the ability to cast in specific forms and dimensions [2–4]. Concrete, however, is prone to cracking and corrosion, and these fissures and corrosion, which are caused by mechanical and environmental forces, considerably degrade the structure's performance, serviceability, and longevity [5–7]. Despite the fact that passive crack treatments (such as chemical and polymer sealants) are available on the market, they are typically time-consuming and non-sustainable and don't repair the inside of crack [8,9]. Currently, microbiologically influenced corrosion (MIC), a technique that describes the involvement of microorganisms in the corrosion process, has received the greatest attention in the scientific literature. If concrete

Crystals **2022**, 12, 1222 2 of 30

cracks are not promptly and properly treated, reinforced concrete structures are susceptible to collapsing. Concrete should not crack because, if it does, water and oxygen [10] will enter the concrete, accessing the steel bars, corroding it in the early stages of construction. Corrosion is a process that is significantly influenced by temperature, which enhances the pace of reaction [11]. The most prevalent cause of RC constructions failing early is rusting of the reinforcements [12]. Chlorides, which are created by chemical manufacturers and are present in seawater, antifreeze, and other goods, are primarily responsible for rusting [13]. Steel reinforcement in concrete commonly corrodes due to concrete carbonation [14]. The most frequent stages of RC construction deterioration are as follows: [15]: It will eventually collapse due to loss of passivity, cracking, corrosion, flaking of the protective coating, decreased adhesion between the reinforcements and the concrete, and other factors.

Spalling of concrete and rusting of reinforcing bars accelerate the deterioration of concrete structures as a result of ongoing crack growth [6,16,17]. High replacement costs and environmental problems are linked to these phenomena [18–20]. As a result, a realistic solution to the concrete cracking problem that does not compromise mechanical qualities is required. The inherent capability of microorganisms to produce calcium carbonate precipitation has inspired biotechnological methods for the development of a novel concrete auto-healing system [21]. When a split forms, bacteria within it get triggered, causing calcium carbonate minerals to form, which close the split (Whenever a crack forms, the encapsulated material becomes shattered and the bacteria enter an environment within the concrete. After that, the bacterium can access oxygen and water, and it starts to cause calcium carbonate to fill the concrete's fractures [22]). The microbially induced calcium carbonate (MICP)-based bio self-healing approach offers a long-term and ecologically benign solution to cracking compared to conventional chemical self-healing concrete [23–25]. The MICP production is done in the existence of CO₂ which is produced from the precursor used in bio-concrete. Different types of precursors are used by scientists to obtain CaCO₃. The most common precursors include amino acid salt [26,27] and urea [28,29]. The autotrophic microbes turn CO₂ to CaCO₃ which is produced when water comes in contact with urea or the salt of amino acid, given in Equation (1), when there is Ca²⁺ in the surrounding atmosphere. Detailed chemical reaction for forming CaCO₃ precipitation is given in Equation (1)–(6). The negatively charged bacterial cell membrane will pull positive charges from the surrounding area after the Ca²⁺ is in the process of demineralization. Ca²⁺ will ultimately be collected on a surface of the cell, which is portrayed in Figure 1. After that, Ca^{2+} the ions integrate with CO_3^{2-} ions, resulting in $CaCO_3$ precipitation as portrayed in Equation (6).

$$CO(NH2)2 + H2O \rightarrow NH2COOH + NH3$$
 (1)

$$NH_2COOH + H_2O \longleftrightarrow NH_3 + H_2CO_3$$
 (2)

Ammonia then forms ammonium and hydroxide ions in water (Equation (3)).

$$NH_3 + H_2O \longleftrightarrow NH_4^+ + OH^- \tag{3}$$

Carbonic acid also forms bicarbonate and hydrogen ions in water (Equation (4)).

$$H_2CO_3 \leftarrow \rightarrow HCO_3^- + H^+$$
 (4)

The bicarbonate equilibrium is altered by the formation of hydroxide ions, which also raises pH. Consequently, carbonate ions are produced. Equation (5), which is the total equation, is shown below.

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \longleftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O$$
 (5)

Crystals **2022**, 12, 1222 3 of 30

Calcium carbonate crystals can form when there are calcium ions present, as shown in Equation (6).

 $Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 \tag{6}$

Calcium carbonate is made outside of the cell membrane of bacteria via autotrophic and heterotrophic metabolic processes. The autotrophic microbes convert the CO_2 to $CaCO_3$ by non-methylotrophic methanogenesis, aerobic photosynthesis, and anaerobic photosynthesis by different bacteria in a variety of environments.

In a number of geological settings, such as soils, limestone caves, oceans, and soda lakes, (MICP) has been found to be involved in the creation of calcium carbonate [30]. Biotic mechanism can produce calcium carbonate minerals in two ways: autotrophic and heterotrophic [31]. Using MICP-based self-healing technology to address engineering, geotechnical, and environmental concerns has evolved as a realistic, sustainable, and repeatable solution over the last decade [26,27,29,32,33]. The use of MICP-based techniques to adapt and improve construction substances like concrete and mortar has caught the interest of researchers [26,27,29] while concern upon the quality and production of induced minerals has been noted.

The effectiveness of the MICP method is significantly influenced by the quantity and quality of generated minerals [25,34]. For example, the maximum calcium carbonate synthesis put up to more fracture and hole space filling, which improves the performance of bio-healing concrete. Calcium carbonate comes in a variety of polymorphs, each with its own set of physical and mechanical properties that have an influence on the characteristics of deposition [35,36].

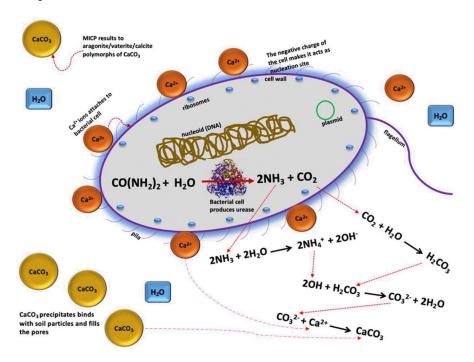


Figure 1. A diagram depicting the ureolytic bacterium Sporosarcina pasteur-role in CaCO₃ synthesis via the ureallysis-driven microbially induced carbonate precipitation route for soil improvement. Reproduce with permission from publisher [37].

Researchers have summarized the experimental findings [38,39] about the MICP pathway based on urease bacteria and largely concur that under the catalysis of urease, urea $(CO(NH_2)_2)$ is first used as a carbon source and converted to carbamate (NH_2COOH) ; next, NH_2COOH produces carbonic acid (H_2CO_3) through a hydrolysis reaction; next, H_2CO_3 produces carbonate ions (CO_3^{2-}) in water solution; and finally, Ca^{2+} and CO_3^{2-} combine to form $CaCO_3$ on the surface of microorganisms [40]. Figure 1 depicts the entire MICP route diagram. Clearly, the central process in the entire MICP pathway is the production

Crystals **2022**, 12, 1222 4 of 30

of CaCO₃. While the CO_3^{2-} comes from the initial carbon source, the Ca^{2+} comes from both external artificial replenishment and internal MSHC. The original carbon source is $CO(NH_2)_2$, and after urease catalysis and NH_2COOH hydrolysis, the source of the carbon is identified and transferred to H_2CO_3 . Our group has previously conducted in-depth research on the mechanisms of urease catalysis and NH_2COOH hydrolysis, therefore the current investigation is focused on the mechanism of H_2CO_3 transformation into CO_3^{2-} in MSHC. Equations (1)–(3), (6) and (7) contain the biosynthetic routes of microbially generated calcium carbonate depicted in Figure 1.

$$2OH^{-} + H_{2}CO_{3} \rightarrow CO_{3}^{2-} + 2H_{2}O$$
 (7)

A previous study [41] reviewed MICP and its potential in bio concrete. However, their study remained focused only on the metabolic pathways of bacteria. The many processes, such as urea hydrolysis, amino acid ammonification, dissimilatory sulphate reduction, photosynthesis, and methane oxidation are discussed in this article. The review study examines the ideas of microbially induced calcium carbonate precipitation (MICP) and its function in bio-concrete from a microbiological and molecular perspective. Additionally, urea hydrolysis, de-nitrification, dissimilatory sulphate reduction, and photosynthesis are a few of the significant metabolic activities engaged in MICP at various stages. In another previous study, Chuo [42] only the different bacteria and their efficiency in calcium carbonate precipitation are discussed. Seifan and Berenjian [43] has carried out a thorough analysis of MICP, however the focus of their work is mostly on the various factors influencing calcium carbonate precipitation. A tabular comparison has been provided in Table 1 to identify the shortcomings and highlighting research gap.

The purpose of this review is to determine the consequences subsequently reduced on MICP's uses in improving construction materials, in addition to the existing hurdles of implementing this breakthrough technology in situ. To determine the consequences on MICP, different factors are considered and discussed, such as type of bacteria, pH, temperature, concentration of microbes, nutrients, and ureolytic activity. To determine the quality of produced MICP (CaCO₃) different test techniques are discussed, their application and few new test techniques used in the recent literature appear in the coming section. We also present the reinforcement damages and challenges due to corrosion and the technological breakthroughs to detect corrosion in the infrastructure. The authors in this paper reviewed the different bacteria inducing calcium carbonate, factors affecting calcium carbonate precipitation, and application of MICP in field, in addition to the microstructure test and corrosion analysis for durability of concrete which was not yet studied.

Table 1. Different research on MICP and gap identification.

Reference	Studied Area	Gap Identified	Remarks
[41]	The author reviewed MICP and its potential in bio-concrete. However, their study remained focused only on the metabolic pathways of bacteria.	Future research on these issues will support the creation of novel bacterial strains through genetic and protein engineering to enhance their ability to survive and function in the harsh environment of concrete.	The author did not study or provide detail on durability of structure regarding corrosion, or whether these bacteria can prove potential in reducing or controlling the corrosion.
[42]	Discussed the different bacteria and their efficiency in calcium carbonate precipitation	Future research is anticipated to boost sustainability, lower application costs, and improve MICP performance.	Lacks the study of characterization of MICP and study of corrosion and how these different types of bacteria respond to the corrosion.

Crystals **2022**, 12, 1222 5 of 30

Table 1. Cont.

Reference	Studied Area	Gap Identified	Remarks
[43]	Conducted a detailed reviewed work on MICP but their work mainly limits the different parameters affecting the precipitation of calcium carbonate. The authors in this paper reviewed the different bacteria inducing calcium carbonate as well.	The adhesion behavior of calcium carbonate to the concrete matrix needs to be thoroughly investigated.	Lacks the study of adhesion behavior and the role of calcium carbonate in a corrosive environment.
[44]	This review covered the current development of MICP technology and the prospects of different application approaches.	The widespread use of bio-mineralization engineering still depends on lowering technical costs. There are many different application scenarios that are available, which prevents the development of a coordinated and efficient implementation approach. Domesticating local bacteria is regarded as a ground-breaking remedy. The problem of how to handle by-products (such ammonia) during the bio mineralization process has not been adequately resolved. It requires the steadfast assistance of experts in biological processes. Future inter-disciplinary collaboration should be more intense.	The author did not review the application of MICP technology for corrosive environment and microstructure analysis.
[45]	Overall, this article demonstrates that microbial induced precipitation via denitrification has great potential to resolve a wide range of building material problems, such as ground modification, mitigating the liquefaction and soil pollution, improving the durability and engineering properties of concrete, historic buildings, monuments, etc. under aerobic and anaerobic conditions; also studied the use of different chemical, environmental and biomedical-related science and applications.	It is an interdisciplinary study that calls for the participation of numerous academics and businesspeople with backgrounds in microbiology, biochemistry, geology, and geotechnical engineering.	The author did not study the application of MICP for corrosion and microstructure analysis.

2. Factor Affecting the MICP

To ensure the successful implementation of the MICP process, several criteria (there are different parameters to be considered for successful implementation of MICP process such as temperature, pH, water availability, oxygen availability for aerobic bacteria, carrier of bacteria, and nutrients) must be considered. The effects of those criteria on the production of calcium carbonate are explored in this section.

2.1. Nucleation Site

Bacteria's importance as crystal nucleation sites [44] has long been recognized [45]. Because of their large surface-to-volume ratio, bacteria are said to behave as crystal nucleating agents, according to geological data [46,47]. The first stage in crystal formation is the molar ratio contact of metallic ions with chemically reactive compounds created by microorganisms, which are found largely in the murein [22]. These locations nucleate the accumulation of additional mineral as chemical expedite after complexation. Only tiny-grain precipitates can be produced in this location due to the narrow intervals between wall polymers, whereas the outside surface sites have no such restriction. As a result,

Crystals **2022**, 12, 1222 6 of 30

large-grain precipitates can form if enough metal ions are available [48]. In order to promote mineral precipitation, the hydrophilic power between the bacterium and the mineral must be lower than the interaction tension between the mixture and the mineral [49].

With his staining technique, Christian Gram established the essential systematic distinction between gram-positive and gram-negative bacterial kinds. Unaware of it, he had also distinguished between the various eu-bacterial wall types and their chemical and structural composition [50,51].

2.1.1. Gram-Positive Bacteria

In terms of their chemical and structural makeup, gram-positive bacteria are less complex than gram-negative bacteria. Bacillus subtilis gram-positive walls have been well studied [52]. When this bacterium is cultivated in the presence of phosphate, peptidoglycan and teichoic acid comprise most of its wall. The true supporting structure of the wall is peptidoglycan. It protects the bacteria from the protoplast's turgor pressure and determines its shape and form [52]. The recurring f3 (1–4) linked N-acetylglucosaminyl-N-acetylmuramyl dimers that comprise the peptidoglycan's chemotype Al l' produce linear strands of 50 linkage units or longer. Each N-acetylmuramyl residue has a short amino acid sequence of four to five (some strains have shorter stems due to carboxypeptidase action). In order to create a network of intramolecularly linked peptidoglycan, or murein, that surrounds the protoplast and can hold secondary polymers like teichoic acid, tetramers frequently make covalent bonds with the peptide stems of adjacent strands. Depending on the B. subtilis strain and stage of the cell's growth, the peptidoglycan can account for 30–50% of the wall's dry weight, and its interstrand cross-linking can consume up to 20–30% of the muramyl residues.

2.1.2. Bacterial Metal Accumulation

Two characteristics make bacterial cell walls incredibly strong and resilient. Because the carboxylate groups of the peptide stems of the peptidoglycan dominate its charge density, this scaffolding is anionic [53]. Teichoic acid is a covalently bonded flexible, linear polymer of glycerol that is found in a variety of muramyl peptidoglycan residues in strain 168. The polymer has a net negative electrical charge because of the phosphates positioned between the glycerol residues. It is thought that the teichoic acid's linear strands penetrate the peptidoglycan framework by doing so that Mg²⁺ salt-bridging function stabilizes the strands, and that a variable percentage of strands are exposed at the wall surface [54–56]. Because they also contain electronegative teichuronic acids as additional secondary polymers in addition to the other two components, other bacilli, including Bacillus licheniformis NCTC 6324, are three-polymer systems. The majority of the wall's bulk and anionic nature are accounted for by the two secondary polymers in NCTC 6324 [57]. The interdigitation of the peptidoglycan with the secondary polymers, whether they be teichoic or teichuronic acids, results in an amorphous cell wall. The walls of pathogenic streptococci, for instance, include M-protein and group-specific carbohydrates, whereas mycobacteria contain mycolic acids [58]. Undoubtedly, gram-positive walls are also more chemically complicated. The general design of wall construction is rarely altered, despite the increased complexity in wall chemistry [59].

2.1.3. Gram Negative Bacteria

Gram-negative bacteria have more complex chemical and structural barriers than gram-positive bacteria. The peptidoglycan is not directly exposed at the surface of the wall since it is only found in the periplasm, which is located in the space between the plasma and outer membranes. It was previously believed that this Al 'Y peptidoglycan (murein), the typical paradigm for the gram-negative Al 'Y chemotype, was chemically homogeneous. However, analysis of solubilized E. coli murein using high-performance liquid chromatography has revealed approximately 80 different muropeptides. The intricacy resulted from the unrestricted combination of two different cross-bridge types and

Crystals **2022**, 12, 1222 7 of 30

seven different peptide stems. The composition of the growth media can also have an impact on murein chemistry, which makes the issue worse [60]. Only a murein sacculus one to three molecules thick can be formed by bacteria like E. coli and Pseudomonas aeruginosa using the available peptidoglycan [58], Despite the fact that the periplasmic space is filled with what is thought to be a gel-like substance, the new preservative method of freeze-substitution has revealed [59]. The three-layer notion is supported by the most recent mass estimates for *E. coli* murein sacculi [60]. The layer of peptidoglycan is followed by the outer membrane. In an aquatic environment, the amphipathic components of this lipopolysaccharide-phospholipid-protein mosaic are forced to create a bi-layer structure.

At least in E. coli and closely similar organisms, all of the constituents' molecular interactions limit the fluidity of the membrane and segregate the bulk of the lipopolysaccharide and phospholipid to opposing membrane faces, with the lipopolysaccharide going to the outer surface [58]. Porins generate aqueous channels in some bacterial species, and low-molecular weight lipoproteins covalently connect the membrane to the murein. The outcomes of the numerous research that have been done over the years on the outer membranes of various gram-negative bacteria are impossible to describe. In order for lipopolysaccharides with various chain lengths to emerge from a single membrane's surface and for all membrane components to be affected by the developing environment, various primary and secondary polypeptides must flow through and cross the membrane. Consult the references [61–63] for more details. The cell wall is composed of the gramnegative surface's outer membrane and murein [58]. Because of the coordination and exposure of the various polymers and macromolecules, gram-negative walls produce a net electronegative charge that allows them to interact with cations in their immediate surroundings [64–66].

The capacity of bacteria to interact with metallic ionic species in the culture can be influenced by a number of variables. extracellular polymeric molecules being present [67,68] and the existence of functional groups that can be changed by cell membrane configuration is the much more significant component in ionic sorption. It is well known that the net negative charge on bacterial cell membranes varies from species to species and is depending on the species. Gram-positive bacteria have a negative charge primarily due to the presence of functional groups in the cellular membrane, such as carboxyl or phosphate groups in teichoic acids [69]. Gram-negative bacteria, however, have a negative charge owing to the existence of phospholipids and lipopolysaccharides in the cell surface [70,71] Teichoic and teichuronic acid polymers are stable under normal conditions when divalent cations are present [49]. As illustrated in Figure 2, this results in a strong electrostatic affinity for cations, as well as crystallization, when bivalent positive ions bind to the anionic cellular membrane depends on bacteria surface to volume ratio.

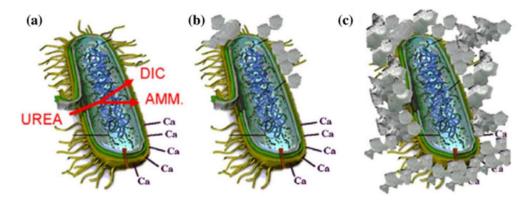


Figure 2. Schematic diagram (a) precursor intake through cell membrane for growth of bacteria (b) indicates the nucleation site and attachment of cationic ions and (c) suggests that urease-producing bacteria caused the calcium carbonate to precipitate. Reproduce with permission from [42].

Crystals **2022**, 12, 1222 8 of 30

The summary of the above discussion is that the precipitation of $CaCO_3$ depends upon the bacterial type whether gram positive or gram negative as the bacterial cell surface play a role in precipitation. The most important is the hydrophilic power of bacteria with mineral, which should be greater than the mixture and mineral interaction power for calcite precipitation.

2.2. Bacterial Type

Based on the many metabolic pathways used by microorganisms, the MICP process can be divided into four categories: ureaolysis, denitrification, sulphate reduction, and CO2 hydrolysis mechanism [72]. Due to its effectiveness in the deposition reaction of calcium carbonate by urease bacteria, ureaolysis has been studied in considerable detail in MICP. To increase CaCO₃ deposition efficiency, different urease bacteria, like *Bacillus sphaericus*, have their enzyme activity and production studied [73,74], including Bacillus subtilis [75,76], Bacillus megaterium [77,78], Bacillus aerius [79], Sporosarcina pasteurii [80,81], Bacillus cereus [82], and Bacillus cohnii [83,84]. Because of its efficient enzymatic activity and biosynthesis, Sporosarcina pasteurii has emerged as one of the most popular urease bacteria research respondents among them. For calcium carbonate precipitation, several genera of bacteria discussed above can be chosen based on abiotic conditions. Urease-positive bacteria, for example, are needed to produce urease, which stimulates urea decomposition in the ureaolysis biochemical process [85]. The urease enzyme, found in bacteria like Bacillus, initiates the conversion of urea to ammonia to CO2 [86]. Urease-positive bacteria can exist in a variety of places, including the human body and soil. Besides determining whether the bacteria is suitable for the metabolic route, its feasibility and efficacy in various scenarios must also be examined. The viability of microorganisms plays a crucial role in the process efficacy when MICP is used. Discovered under anoxic conditions, the activity of Sporosarcina pasteurii (NCIMB 8841) was suppressed [87]. Bacillus species, however, exhibit increased activity in a variety of environments [88]. The results show that B. sphaericus is the bacterium most frequently used in MICP operations that use a urea-fortified medium. B. mega-terium is one of the other species in this genus [89] and B. lentus [90], have also been identified as MICP-causing microorganisms.

To summarize the above discussion, the genera *sporosarcina* cannot exhibit calcium carbonate precipitation in various environments. Among the species the genera *Bacillus* exhibit activity in various environments that is beneficial to use.

2.3. Bacterial Concentrations and Ureolytic Activity

The efficiency of the MICP process and the crystals produced are influenced by bacterial concentration and ureolytic activity. The hydrolysis of urea is a very slow process $(3 \times 10^{-10}~\rm s^{-1})$, despite the fact that the urease enzyme can greatly speed up the process $(3 \times 10^{-4}~\rm s^{-1})$ [91]. As a result, the precursor with enhanced ureolytic activity should be chosen for increased calcium carbonate generation. Aside from that, the amount of bacteria present can influence calcium carbonate formation which seal the crack result in the strength recovery provided in Table 2 below. The number of bacterial cells participating in the fermentation process affects how quickly urea decomposes; a greater numeral of bacterial cells in the zymolysis activity leads to increased urease enzyme synthesis, which adds to MICP's efficiency [92]. Because urease-positive bacteria are predominantly aerobic, oxygen-limiting circumstances hinder bacterial growth and calcium carbonate production [43]. Denitrifying bacteria, however, can start MICP anaerobically by utilizing nitrate as an electrophile to oxidize organic materials to produce calcium carbonate [23]. Denitrifying strains have a reduced ability to cause calcium carbonate precipitation than ureolytic bacteria, which may influence the functionality of MICP activities [43].

Crystals **2022**, 12, 1222 9 of 30

Table 2. Demonstrates the effects of different factor on microbially induced calcium carbonate production which is depicted from the crack width sealed.

2	S. No	Microbes Type	Cell Concentrations (Cells/mL)	РН	Optimum PH	Temperature (°C)	Healing Substrate	Crack Width Sealed (mm)	Reference
2 Bacillus Pasteurii, Bacillus Subtilis - 8-14 13 28 Carbonate (CaCO ₃) 0.81 [2] 3 Bacillus Sphaericus 10° 9-12.5 12.5 28 Calcium (CaCO ₃) precipitation 4 Lysinibacillus sphaericus 10° 9 9 9 30 Microbial carbonate 5 Bacillus subtilis 2.8 × 10 ⁸ Highly alkaline 28 Microbial carbonate 6 Bacillus subtilis 10° Highly alkaline 20 ± 2 Microbial carbonate 7 Bacillus mucilaginous 10° Highly alkaline 20 ± 2 Microbial carbonate 8 Bacillus subtilis 10 ⁵ 8.5-10 10 - Bacterial carbonate 8 Bacillus subtilis 10 ⁸ 7 7 37 Bacterial calcium carbonate 9 Bacillus subtilis 10 ⁸ 7 7 37 Bacterial calcium carbonate 9 Bacillus subtilis 10 ⁵ 10-13.5 13-13.5 37 Bacterial calcium carbonate 10 Bacillus Pasteurii 5 × 10° 10-11 11 -3-90 - 0.80 190 11 Bacillus subtilis 2.8 × 10 ⁸ 11-13 - 27 - 0.80 190 12 Bacillus subtilis 2.8 × 10 ⁸ 11-13 - 27 - Calcium alginate hydrogel beads 120-174 μm 190	1	and native Lysinibacillus	2 × 10 ⁸	7–13	9	28	calcium	0.4	[23]
3 Bacillus sphaericus 10° 9–12.5 12.5 28 Calcium carbonate carbonate 4 Lysinibacillus sphaericus 10° 9 9 9 30 Microbial calcium carbonate 5 Bacillus subtilis 2.8 × 10° Highly alkaline Highly alkaline 28 Calcium carbonate 6 Bacillus mucilaginous 10° Highly alkaline Highly alkaline 20 ± 2 Calcium carbonate 7 Bacillus subtilis 10° 8.5–10 10 - Bacterial Calcium carbonate 8 Bacillus subtilis 10° 8.5–10 10 - Bacterial Calcium carbonate 8 Bacillus subtilis 10° 7 7 37 37 Bacterial calcium carbonate 9 Bacillus subtilis 10° 10–13.5 13–13.5 37 Bacterial calcium carbonate 10 Bacillus Pasteurii 5 × 10° 10–11 11 -3–90 - 0.80 [9° 11 Bacillus subtilis 2.8 × 10° 11–13 - 27 - - [9° 12–14 μm 10° 12–14 11° 12–14 12–14 12–14 12–14 12–14 13–14 13–14 14–14	2		-	8–14	13	28	Carbonate (CaCO ₃)	0.81	[24]
4 Lysinibacillus sphaericus 10^7 9 9 9 30 calcium carbonate 0.4 [34] 5 Bacillus subtilis 2.8×10^8 Highly alkaline Highly alkaline 28 Microbial calcium carbonate 0.37 [93] 6 Bacillus mucilaginous 10^9 Highly alkaline Highly alkaline 20 ± 2 Microbial calcium carbonate 0.4–0.6 [94] 7 Bacillus subtilis 10^5 $8.5-10$ 10 - Bacterial carbonate 0.5 [32] 8 Bacillus subtilis 10^8 7 7 37 Bacterial calcium carbonate - [98] 9 Bacillus sphaericus and Bacillus subtilis 10^5 $10-13.5$ $13-13.5$ 37 Non-reacted limestone and calcium-carbonate - [22] 10 Bacillus subtilis 5×10^9 $10-11$ 11 $-3-90$ - 0.80 [96] 11 Bacillus pseudofirmus and Diaphorobacter nitroreducens 10^7 - - - -<	3	Bacillus sphaericus	10 ⁹	9–12.5	12.5	28	Calcium	0.15-0.17	[33]
Second Process Sec	4		10 ⁷	9	9	30	calcium	0.4	[34]
6 Bacillus mucilaginous 10^9 Highly alkaline Highly alkaline 20 ± 2 calcium carbonate 0.4–0.6 [94] 7 Bacillus subtilis 10^5 8.5–10 10 - Bacterial Calcium Carbonate 0.5 [32] 8 Bacillus subtilis 10^8 7 7 37 Bacterial calcium carbonate - [95] 9 Bacillus subtilis 10^5 10 –13.5 13 –13.5 37 Non-reacted limestone and calcium-carbonate - [25] 10 Bacillus Pasteurii 5×10^9 10 –11 11 -3 –90 - 0.80 [96] 11 Bacillus pseudofirmus and Diaphorobacter nitroreducens 10^7 - - - $-$ [96] 13 Sporosarcina pasteurii 10^9 - - $ -$ <td>5</td> <td>Bacillus subtilis</td> <td>2.8×10^{8}</td> <td></td> <td></td> <td>28</td> <td>calcium</td> <td>0.37</td> <td>[93]</td>	5	Bacillus subtilis	2.8×10^{8}			28	calcium	0.37	[93]
7 Bacillus subtilis 10^5 8.5–10 10 - Calcium Carbonate 0.5 [32] 8 Bacillus subtilis 10^8 7 7 37 Bacterial calcium carbonate - [98] 9 Bacillus sphaericus and Bacillus subtilis 10^5 10 –13.5 13 –13.5 37 Non-reacted limestone and calcium-carbonate - [28] 10 Bacillus Pasteurii 5×10^9 10 –11 11 -3 –90 $ 0.80$ [96] 11 Bacillus pactuofirmus and Diaphorobacter nitroreducens 10^7 $ -$ </td <td>6</td> <td>Bacillus mucilaginous</td> <td>10⁹</td> <td></td> <td></td> <td>20 ± 2</td> <td>calcium</td> <td>0.4-0.6</td> <td>[94].</td>	6	Bacillus mucilaginous	10 ⁹			20 ± 2	calcium	0.4-0.6	[94].
8 Bacillus subtilis 10^8 7 7 37 calcium carbonate - [98] 9 Bacillus sphaericus and Bacillus subtilis 10^5 $10-13.5$ $13-13.5$ 37 Non-reacted limestone and calcium-carbonate - 25 10 Bacillus Pasteurii 5×10^9 $10-11$ 11 $-3-90$ - 0.80 196 11 Bacillus subtilis 2.8×10^8 $11-13$ - 27 - - 197 12 Bacillus pseudofirmus and Diaphorobacter nitroreducens 10^7 - - - Calcium alginate hydrogel beads $120-174 \mu m$ 198 13 Sporosarcina pasteurii 10^9 - - $27-100$ 0.3 198	7	Bacillus subtilis	10 ⁵	8.5–10	10	-	Calcium	0.5	[32]
9 Bacillus sphaericus and Bacillus subtilis 10^5 $10-13.5$ $13-13.5$ 37 limestone and calcium-carbonate - [28] 10 Bacillus Pasteurii 5×10^9 $10-11$ 11 $-3-90$ - 0.80 [96] 11 Bacillus subtilis 2.8×10^8 $11-13$ - 27 - - [97] 12 Bacillus pseudofirmus and Diaphorobacter nitroreducens 10^7 - - - - Calcium alginate hydrogel beads $120-174 \mu m$ [98] 13 Sporosarcina pasteurii 10^9 - - $27-100$ 0.3 [96]	8	Bacillus subtilis	10 ⁸	7	7	37	calcium	-	[95]
11Bacillus subtilis 2.8×10^8 $11-13$ - 27 [97]12Bacillus pseudofirmus and Diaphorobacter nitroreducens 10^7 Calcium alginate hydrogel beads $120-174 \mu m$ [98]13Sporosarcina pasteurii 10^9 $27-100$ 0.3[99]	9		10 ⁵	10–13.5	13–13.5	37	limestone and calcium-	-	[25]
Bacillus pseudofirmus 12 and Diaphorobacter 10 ⁷ Significant alginate hydrogel beads 13 Sporosarcina pasteurii 10 ⁹ 27–100 Calcium alginate hydrogel beads 120–174 μm [98]	10	Bacillus Pasteurii	5×10^9	10–11	11	-3-90	-	0.80	[96]
Bacillus pseudofirmus 12 and Diaphorobacter 10^7 alginate hydrogel beads 13 Sporosarcina pasteurii 10^9 27–100 0.3 [99]	11	Bacillus subtilis	2.8×10^{8}	11–13	-	27	-	-	[97]
	12	and Diaphorobacter	10 ⁷	-	-	-	alginate hydrogel	120–174 μm	[98]
14 Bacillus mucilaginous $1.0 \times 10^{10} \text{CFU/g}$ 27 - 0.3 -0.5 [10	13	Sporosarcina pasteurii	10 ⁹			27–100		0.3	[99]
	14	Bacillus mucilaginous	$1.0 \times 10^{10} \text{CFU/g}$	-	-	27	-	0.3-0.5	[100]

The summary of the above section is that denitrifier bacteria can be utilized where there is a limited oxygen and is used for cracks with very limited width and the ureolytic bacteria is used for cracks wider than that for denitrifying bacteria and in the environment with plenty of oxygen. Also, when employed, a greater concentration of bacteria lead to more MICP production.

2.4. PH

Despite the fact that microbes affect microbial decomposition by changing almost any deposition factor [49], The ability of bacteria to create an alkaline environment through a variety of biological activities has been credited as the primary function in the formation of calcium carbonate [29]. Calcium carbonate precipitation and dissolution are mediated by microbial activity in response to environmental circumstances [101]. Generally, precipitation is influenced by the saturation index (SI) [102]; Using the formula in Equation (8), which is based on the interactions of temperature, calcium hardness, total alkalinity, and

Crystals **2022**, 12, 1222 10 of 30

pH, which is connected to PH medium, one may calculate the saturation index, which is a number.

$$SI = \log (IAP/K_{sp})$$
 (8)

The ion activity product is known as IAP, while Ksp denotes the solubility product. Calcium carbonate tends to be produced if SI surpasses 0.8 in the absence of particular inhibitors, according to one study [103]. In the MICP procedure, the importance of bacteria in creating an alkaline condition that favors crystal development was outlined by [104]. Microbial metabolism increases alkalinity when a solution contains a calcium supplier, shifting the balance toward crystal formation [91].

Different factors can influence pH fluctuations depending on the biological pathways. For example, the formation of ammonia is linked to pH variation in the ureaolysis and ammonification routes, which are the most commonly employed techniques for creating bioconcrete [105]. According to a recent study, in the presence of Bacillus species, the calcium carbonate deposition rises at a higher pH compared to an uncontrolled pH solution [88]. This is because, at high pH levels, carbonate maintains deposition in the medium rather than being dissolved [106]. [86] In a different study, Stock et al. found that S. pasteurii's urease activity increased significantly in the PH range of 6.0 to 8.0. Similarly, [107] tested the influence of different pH on S. pasteurii urease activity, and the results indicated that pH 7–9 for a 5-hour incubation time resulted in a high specific urease activity (the effect of PH on the calcium carbonate synthesis is listed in Table 2). As a result, the MICP process requires the use of food that might raise the pH and cause a lavish environment. Apart from its importance in calcium carbonate bio precipitation, pH also has an important influence in bacterial proliferation. Because the number of cells has a correlation with the synthesis of urease enzyme, maintaining an appropriate pH is crucial for controlling the microorganism's survivability and metabolic activity without jeopardizing MICP efficacy.

The entire summary is that bacteria can proliferate and deposit calcite at high pH values; some studies indicate the pH values in the range of 7–9 for other strains discussed above, but the *Bacillus* may induce calcite in a highly alkaline pH of 9–14 as well.

2.5. Nutrients

Since nutrients provide the energy needed for bacterial production and biochemical activity, their availability has a significant impact on calcium carbonate bio mineralization [43,108]. Bacteria have a wide range of sources and amounts of critical components that are required for their functioning [109]. Carbon and nitrogen supplies are two of the most significant dietary needs listed in Table 3 below, serving as energy pockets and a means of life for microbes, respectively [110]. Depending on the metabolic route, multiple minerals should be added to the reaction media [111]. In the non-methylotrophic methanogenesis pathway, for example, microorganisms employ CO_2 for energy production and carbonate bio-manufacture [31]. Acetate, citrate, oxalate, glyoxylate, and other organic acids are used by some common soil bacteria, including Pseudomonas fluorescens, Ralstonia eutropha, Xanthobacter autotrophicus, Flavobacter sp., Acinetobacter sp., Vibrio spp., Nesterenkonia halobia, and Halomonas eurihalina [112,113], Jonkers et al. advocated using calcium lactate is the only cause of carbon and energy for bacterial synthesis and crystal deposition to overcome the challenges connected with construction materials (2010, 2011). Urea is employed as a carbon source in the ureaolysis metabolic pathway to produce carbonate minerals.

Calcium carbonate bio mineralization is dependent on the presence of free Ca²⁺ in the nearby habitat, in addition to the carbon source [114]. The disintegration of 1 mole of urea develops the creation of 1 mole of calcite, as demonstrated in Equations (1)–(3) and(7) [115]. It is obvious that a higher urea content enhances mineral precipitation. In addition, it has been discovered that inclusion a large amount of calcium salt reduces enzyme activity and, as a result, causes calcium carbonate precipitation [116–121]. Employing *Bacillus* genera in the existence of urea, [2] studied the influence of varying Ca²⁺ causes on the deposition of calcium carbonate. According to the authors, CaCl₂ is the best calcium supply for biogenesis of calcium carbonate. The presence of soluble Ca²⁺ may cause a

Crystals **2022**, 12, 1222 11 of 30

calcite production and urease functioning, which could be explained by adding ions species to the suspension media [116]. It's also important to remember that, in order to obtain the maximum amount of calcium carbonate, reagent concentrations must be kept within acceptable ranges to prevent microbial growth inhibition [102]. Table 3 shows the various nutrients used by bacteria for self-healing of cementitious material.

Table 3. Displaying nutrients used by different bacteria for self-healing of cementitious material.

S. No	Application	Microorganism	Nutrients	Analysis of the Specimens	References
1	Bio-mortar	Bacillus cereus	Nutrient broth + $CaCl_2 \cdot 2H_2O$, Actical, Natamycine ($C_{33}H_{47}NO_{13}$)	-	[26]
2	Concrete crack remediation	Bacillus subtilis	Nutrient broth, urea $CO(NH_2)_2$, Calcium dichloride dihydrate, Ammonium chloride (NH_4Cl), Sodium hydrogen carbonate	Compressive strength, water absorption test and SEM analysis	[28]
3	Concrete crack remediation	Bacillus sphaericus	Extract yeast, urea, Calcium dichloride dihydrate	Compressive strength, sorptivity test, SEM, gas permeability, XRD, chromatic analysis of specimens	[29]
4	Bio- concrete	Bacillus subtilis	Peptone: 5 gram/liter, Sodium chloride: 5 gram/liter, Yeast extract: 3 gram/liter.	Compressive strength, water absorption test, and SEM-EDX analysis	[27]
5	Monumental limestone conservation	Micrococcus sp. Bacillus subtilis.	B_4 nutrient medium (calcium acetate, yeast extract, dextrose)	Water absorption, colorimetric analysis, stone cohesion, SEM, XRD, and FTIR of crystals	[122]
6	Porous ornamental limestone	Myxococcus xanthus	Pancreatic digest of casein, calcium acetate, potassium carbonate	Weight increase, MIP, XRD, SEM analysis, sonication analysis.	[123]
7	Archaeological gypsum plasters	M. xanthus	M-3P nutrient solution (pancreatic digest of casein, calcium acetate, potassium carbonate)	Drilling resistance analysis, TGA, XRD, SEM, MIP, TEM, and colorimetric analysis	[124]
8	Marble substrate	B. pumilus	Primary growth medium (bacteriolog-ical peptone, calcium acetate)	Chromatic analysis, weight loss with ultrasonic treatment, XRD, SEM, and FTIR	[125]
9	Porous sand column	Sporosarcina pasteurii	Calcium chloride with urea medium	XRD, SEM, CaCO ₃ estimation	[126]
10	Cement mortar	S. pasteurii Pseudomonas aeruginosa	Urea and calcium chloride medium	Compressive strength, XRD, and SEM analysis	[127]

From the above discussion, carbon and nitrogen are the two most important dietary needs of bacteria for calcite deposition. Some bacteria also consume organic acids (acetate, citrate etc.) and result in calcium carbonate production. Some use calcium lactate as a carbon and energy source for bacteria. Some use urea as a carbon source. It is also evident from the study that using more calcium salt results in greater deposition of CaCO₃. But much higher concentration may limit the microbial growth.

Crystals 2022, 12, 1222 12 of 30

2.6. Temperature

The temperature of the incubation chamber is one of the most important (the incubation chamber temperature is the laboratory static and shaking incubator temperature for the bacteria growth that is kept 37 °C for effective growth of bacteria; Higher temperature may affect the growth of bacteria) operating parameters that might influence bacterial growth and, as a result, the bio-mineralization process [2]. The catalysis of urea hydrolysis, like other enzymatic reactions, is a temperature-dependent process [128]. The ideal temperature, however, lies between 20 and 37 °C, depending on the surrounding conditions and the concentrations of chemical reagents in the fermentative media [129] [130,131]. The rate of urea disintegration is increased by 5 and 10 times, respectively, when the reaction temperature is raised from 10 to 20 °C and between 10-15 °C, according to previous research [132]. In a solution medium with 0.02 gram/Liter of catalyst, we discovered that increasing the temperature from 20 to 50 °C enhances calcium carbonate production [133]. However, a relatively high temperature had a negative impact on bacterial metabolism and enzyme function [91]. Despite a consistent urease activity at comfortable temperature (35 °C), we discovered that at a high temperature (55 °C), the enzyme activity significantly declines [134].

The effect of different temperatures on the performance of bacteria are displayed in Table 2. We adjusted the operating conditions for calcium carbonate biosynthesis and discovered that temperature ranges of 33–45 °C have no effect on the *Bacillus* species' calcium carbonate bio-precipitation [2]. Increased temperature, however, hindered bacterial production and enzymatic activity [135]. Mechanisms of deposition and hydrolysis controlled by biology are the solubility constant guides balance reactions [49]. Equation (9) defines the solubility constant for calcium carbonate, which is temperature-dependent [136]. A larger solubility product constant (Ksp) is associated with a higher reaction temperature, and as a result, the reaction favor deposition.

Ksp =
$$[Ca^{2+}]$$
 + $[CO_3^{2-}]$ = 4.83 × 10⁻⁹ (calcite, 25 °C),
= 7.24 × 10⁻⁹ (aragonite, 25 °C) (9)

The conclusion of the entire discussion was that the temperature has no effect on Bacillus species until it reaches 45 $^{\circ}$ C, and that for nearly all other types of bacterial species, temperatures above 35 $^{\circ}$ C inhibit bacterial precipitation, while increases in temperature between 10 and 20 $^{\circ}$ C result in greater calcium carbonate precipitation.

3. Tests for Assessing the Bio-Mineralized Calcium Carbonate Based SELF-Healing Concrete

Several methods for evaluating the crack self-healing effect were put forth in order to evaluate the potential of microbial self-healing concrete (MSHC) to close cracks. For instance, X-ray diffraction, scanning electron microscopy, and energy dispersive spectroscopy (XRD) [137,138] were employed to examine the shape of crystals that precipitated at the break. The ability of the bio-mineralized self-healing precipitate was further evaluated using water permeability, porosity, and chloride ion permeation resistance [139,140] and nondestructive detection methods including ultrasonic and acoustic emission were used to assess the effectiveness of bio-self-healing. concrete [141–144]. TEM analysis was used to assess the sealing ability or deposition of MICP by [124] on archeological gypsum plasters employed *M. xanthus* bacteria. However, characterization techniques like SEM, XRD, and water permeability are only suitable for testing in the lab and not real engineering since they cannot concurrently satisfy the demands of operability and nondestructive testing. Acoustic emission and ultrasonic technology are expected to be heavily used in practical engineering due to the advantages of simple detecting equipment, practical operation, and economical detection. The majority of MSHC research is based on previously published experiments. The research on the self-healing process, particularly the quantitative studies, hasn't received a lot of attention, though.

Crystals **2022**, 12, 1222

However, several scientists have chosen to use measurements of the deposited minerals to confirm the encapsulation of bacteria in self-healing concrete [91,145–148]. In the realm of encapsulating materials in self-healing concrete, techniques of testing based on the restoration of mechanical characteristics by the transmission of ultrasonic waves are still unexamined. Quantification of precipitated crystals in cementitious and biochar materials has been investigated using TGA for concrete or mortar samples. Some researchers [149–155] focused solely on durability, whereas others [93,156,157] focused solely on mechanical qualities. The bulk of the authors [158–167] mechanical and durability characteristics were seen. Because experimental research on the dependability of bacterial transporters in various materials has been constrained, there are specific assessment techniques employed to gauge the effectiveness of self-healing in nanomaterials, cementitious materials, unique minerals, and charcoal [3,168–170]. Seifan [70,171] is the only researcher who has examined the evaluation of the self-healing performance of nanomaterial bacterial vehicles with respect to durability and mechanical qualities. More experimental procedures and investigation on self-healing bacterial vehicles are strongly recommended to improve reliability [172]. Testing of mechanical properties and durability in cemented materials is limited to compression and water penetration, respectively. There are several study methodologies used to quantify deposited minerals. Only compression and flexural qualities for mechanical characteristics and TGA (microstructure test) for measurement of deposited minerals are examined to determine self-repairing, suggesting that a wide range of durability tests have been conducted for the distinctive crystals shown in Figure 3 below.

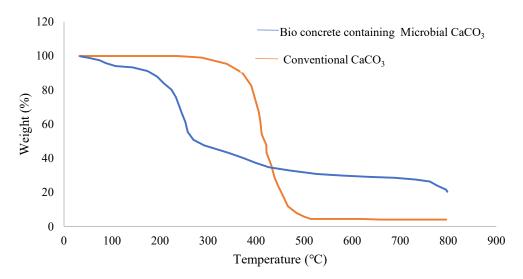


Figure 3. Demonstrates the TGA Analysis of bio-concrete [97].

Microstructure tests are carried out using tools including scanning electron microscopes (SEMs), field-emission scanning electron microscopes (FESEMs), and X-ray diffraction to identify and describe embedded materials after self-healing (XRD) [173] used bio-cement with a variable urea-CaCl2 and bacterial cell density concentration. Based on its 40% greater compressive strength than conventional concrete, improved material finish, aesthetic qualities, and environmental effect, the authors came to the conclusion that bio-cement has potential as a sustainable design material. The XRD spectra of the calcium carbonate content in bio cement are shown in Figure 4. [174] used jute fibers with different lengths and content in bio-cement. Figure 5 demonstrates the SEM images of MICP treated bio-cement specimens.

Crystals **2022**, 12, 1222

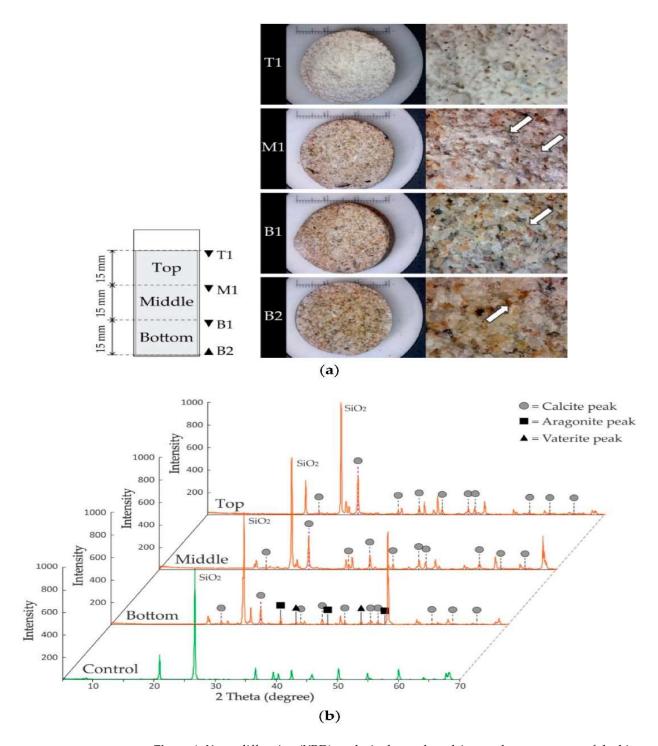


Figure 4. X-ray diffraction (XRD) analysis shows the calcium carbonate content of the bio cement sample as follows: (a) The bio-cement sample section was divided into three equal pieces and examined. There is more calcium carbonate precipitation in the upper section (T1) than the bottom (B2). (b) In each portion of the sample, XRD was used to determine the mineral composition of the bio cement sample (top, middle, and bottom). Control refers to sand that is free of microorganisms. Triangle, vaterite; square, aragonite; and circle, calcite. Reproduce with permission from [173].

Crystals **2022**, 12, 1222 15 of 30

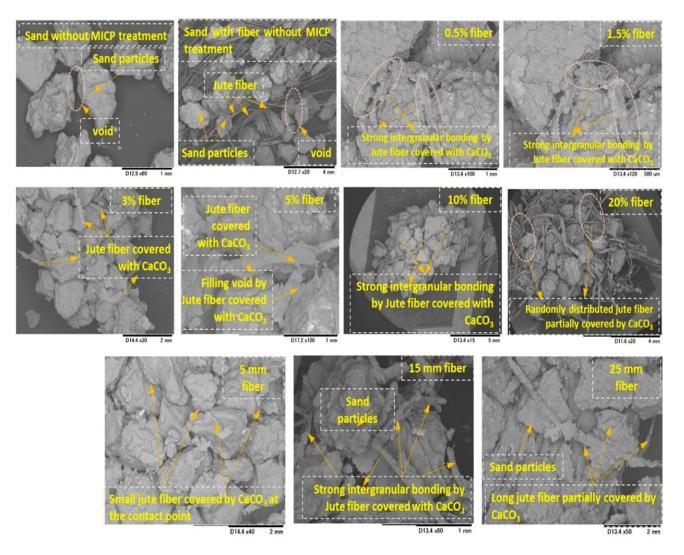


Figure 5. SEM images of MICP-treated bio-cemented samples with the addition of jute fibers (considering different lengths and contents) and distribution of CaCO₃ within the sand matrix, reproduce with permission from [174].

ATRIR [149,152] revealed the chemical composition of the deposit, which consisted of a calcite and aragonite combination in addition to two CaCO₃ polymorphs. For more confirmation of the characterization results, the XRD is frequently employed, along with SEM-EDX by various researchers provided in Table 2. This method was utilized to search for verified healing agents in the precipitates based on macrostructural and SEM inspection. The most prevalent microstructural test used by investigators to trace deposition products in crack specimens was SEM analysis [175]. In addition, for qualitative and quantitative elemental analysis, several researchers have combined EDS with SEM [163,164,176,177]. X-ray tomography, Raman spectroscopy, and Nuclear Magnet Resonance (NMR) can be utilized to monitor and study crack healing qualitatively and quantitatively besides the existing microstructure level research [178,179]. The nanotechnology level study of self-restoration effectiveness by bacteria encased materials has yet to be completed. These tests are performed to ensure that the results of the microstructural testing are as reliable as possible. Nanoscale tests should be performed to determine the bonding strength within the fractures at the interface between the deposited minerals and the cement substrate [180]. Despite this, there remains a dilemma because it is improbable that CaCO₃ will form in the MSHC if H₂CO₃ only produces CO₂ and H₂O. The evidence for CaCO₃ production has, however, been explicitly reported by multiple experiments. In order to study the self-healing ability

Crystals **2022**, 12, 1222 16 of 30

of MSHC cracks, [181] used hydrogel-coated bacterial spores and detects 10–50 nm cubic or spherical particles, which were identified as CaCO₃ by EDS analysis. In order to explore the self-healing of cement paste, [137] encapsulates bacteria with synthetic polyurea; the attribution of the precipitation to polymorph CaCO₃ was confirmed by FTIR and XRD. To encapsulate microorganisms, [182] used carbonized bagasse, and the author studied how it might be used to repair microcracks and fracture characteristics in concrete. CaCO₃ is identified as a micro- and nano-spherical precipitation using SEM, EDS, and XRD. Ref [183] uses XRD, FTIR, and thermogravimetric (TGA) analysis to examine the impacts of biomineralization on the localized phase and microstructure evolutions of bacteria-based self-healing cementitious composites, and finds that polymorph CaCO₃ would form to close the crack area. Additionally, a number of researchers, including [184–189], validated the existence of CaCO₃ using a variety of test techniques. Figure 6 portrays the FTIR spectral analysis of MICP.

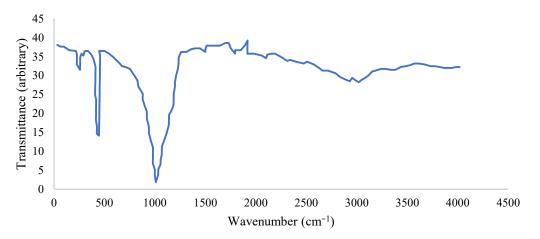


Figure 6. Portrays the FTIR spectral analysis of microbially induced CaCO₃ modified and adaptive from [127].

4. Sealing Ability and Recovery of Mechanical and Durability Properties

The crack width healed, the precipitate's bonding with the mixture and structure, and the precipitate's strength would all influence the restoration of original concrete properties. For efficient self-healing, properties that are as close to those of the original concrete as possible are desirable. The capability of bio-based self-healing concrete to heal is dependent on a variety of parameters, such as the curing condition, concentrations of doable spores and nutrients, the age of the concrete, and the amount of time it takes for the concrete to heal. Healing can occur in two different formats: calcium carbonate precipitation to close the fractures, and carbon dioxide produced by bacteria metabolism reacting with unreacted portlandite at the crack region to make more deposits. The use of a bio-based restoring chemical was found to cure a wide variety of fracture widths. Although it is dependent on a variety of conditions, healing efficiency is optimal when crack width is kept between 100 and 200 µm.

4.1. Recovery of Mechanical Properties

Wang [190] used glass tubes to encapsulate bacteria cells in PU and silica gel. The crack width used to measure mechanical strength recovery was around 0.35 mm. In the case of silica gel, there was more calcium carbonate precipitation, but only around 5% strength recovery. PU-containing specimens recovered between 50 and 80 percent of their potency. Conversely, the bacterial role in regaining mechanical strength was questioned because the strength recovery for live and dead bacteria cells was not significantly different. The precipitate quantity was larger in silica gel than in PU, although PU had a higher strength recovery. It's reasonable to assume that PU, as an excellent sealing agent, played a major role in the mechanical strength recovery.

Crystals **2022**, 12, 1222 17 of 30

Pei [191] discovered that adding bacterial cell walls to concrete can boost its strength in three ways. When encapsulated bacterium spores become metabolically active, the same may be true. The unreacted portlandite surrounding the fracture face reacts with carbon dioxide produced by bacterial activity to form stronger calcium carbonate. Direct deposition or conversion of portlandite produces insoluble calcium carbonate, increases granular stacking capacity, and reduces permeability, leading to increased mechanical strength. The particle packing model [192] explains this. Finally, negatively charged bacterial cells may serve as cement hydration nucleation sites. However, this is more appropriate with newer concrete. Even though calcite is a powerful constituent that is suitable with cement, the bonding of calcium carbonate to concrete may limit the strength recovery.

4.2. Recovery of Durability Properties

In concrete, effective self-healing means that the durability and mechanical strength are totally or almost restored to the original specimen. Water permeability and water absorption tests are frequently used to assess durability. Healing cracks also entails sealing any voids or linked pores through which foreign chemicals from the air or water could enter. As a result, water permeability and absorption are reduced. Pore blockage by calcium carbonate, which has a relatively low solubility, causes absolute permeability reduction through bacterial action [193–195].

According to [196], the healing ratio declined from 83% for 0.1–0.3 mm crack width to 30% for 0.8 mm crack width. Moisture or other entering fluids tend to wash healing materials away from the fracture face when the crack width is larger than 30–50 μm. The drop in restorative chemical from the crack surface or a lack of restoring substance to cover larger cracks could be causing the decrease in repairing proportion. Xu and Yao [145] also agree with this. Such observations may be correct because the quantity of bacteria, nutrients, and precursor compounds at the fracture region may limit bacterial metabolism and precipitation. If the crack width is significant, deposits may be rinsed away by water or other substances before the crack is shut because of the slow rate of precipitation. The healing ratio of cementitious materials specimens immersed in water was more than that of specimens treated to damp curing [197], that could be related to enhanced restorative chemical transfer due to the content differential between the mixture as well as the surface in the moist environment. The maximum restoration ratio for moisture cure was demonstrated in early age, implying that plenty of moisture could enter inside and become accessible for bacterial activity. The repair rate for wet curing was modest at first, but eventually matched that of water curing.

Wang [162] used microencapsulated Bacillus Sphaericus spores in melamine-based capsules to make a similar observation. Immersion in water cured cracks with a maximum width of 850-970 mm, although wet-dry cycles of water resulted in the largest healed crack area. Moisture was able to infiltrate the matrix during the wet cycle, while the spores had enough oxygen during the dry cycle. In the event of incubation via continuous immersion in water, the spores do not have access to oxygen, and in most practical instances, continual exposure to water is not practicable. Crack region is a more useful statistic of recovery than crack size since it counts healing in both directions. In a water permeability test, concretes containing 5% pellets had the lowest final permeability coefficient after being liable to wet and dry cycles. Although the result was like those with 3 percent capsules, the permeability values in specimens with 5 percent demonstrated substantially less variance. It is worth noting that the study found that concrete with 5% pellets was weaker in terms of strength. As a result, lesser permeability might be explained by a reduction in porosity due to the presence of more capsules. When the capsules are intact, this is true. Decreased porosity for 5% pellets after rupture could be due to the waterproofing effect of the included chemical, resulting in somewhat lower permeability. Conversely, 3% pellets were indicated to be the best with respect to mechanical strength and water permeability coefficient reduction.

The capsule material or immobilization media utilized may have a considerable impact on water permeability reduction. Wang [190] used silica gel and polyurethane

Crystals **2022**, 12, 1222

to encapsulate bacterium spores. When silica gel was used to immobilize the cells, the permeability was reduced by two orders of magnitude. Bacterial activity was higher in silica gel, which resulted in the formation of a precipitate that clogged pores and reduced permeability. However, minimal precipitation was found in the case of polyurethane immobilization, and a larger reduction in permeability was reported when compared to silica gel, which is attributable to the waterproofing effect of polyurethane [190]. Because PU foam was employed to encapsulate spores in glass capsules, this impact was noticeable. After the foam has ruptured, it may pour out and obstruct the concrete pores. Bacterial activity resulted in the formation of calcite, which only reduced the permeability of the PU foam and had no effect in directly blocking concrete pores.

Wang [151] used hydrogel as an encapsulation for bacteria spores and bio-reagents and reported a significant reduction in permeability of about 68 percent. Even for 0.3–0.4 mm cracks, the maximum crack size of 0.5 mm was repaired, albeit there was a wide range of healing ratios (40–90%). However, when compared to when only hydrogel was used, there is an improvement. Due to the proportionate dispersion of spores and bio reagents when encased along in hydrogel, better healing may be expected. Because of this encapsulation approach, bacteria would have fast reach to nutrients and precursor compounds in the case of cracking. Furthermore, in addition to bacterial precipitation, some autogenous healing helped by internal hydrogel curing may improve permeability reduction.

When bacteria entrapped in diatomaceous earth was utilized, the water-holding data obtained by Wang [190] indicated a significant improvement. When fractured specimens were incubated in deposition medium (including yeast extract, calcium nitrate, and urea) and water, water retention was inhibited by one-third and 50%, respectively. Calcium ion and urea from the deposition medium could permeate within during the healing process, boosting calcium carbonate precipitation. This effect was more pronounced since the cementitious samples were just 14 days old before cracks were created, allowing nutrients and calcium from the deposition media to easily enter within the cementitious mixture. However, Wang [190] claim that a high calcium ion quantity limits the quantity of hydroxyl ion released during calcium hydroxide dissolution, resulting in a pH drop. Because of the decline in optimum alkalinity for bacterial action, precipitation may be slowed. Calcium ions cannot easily infiltrate inside older concrete due to more complete hydration, hence calcium ions cannot easily ingress within the concrete when completely soaked in deposition media. As a result, calcium contents at the face would be sufficient, preventing bacteria spores from gaining access to an external calcium supply and deposition source.

5. Field Application of Bio-Mineralized Self-Healing Concrete

Without well-planned field experiments, it is impossible to determine whether MICP is effective as a simple engineering fix for fixing concrete fractures. To test the capacity of Sporosarcina pasteurii cells to self-heal, broken rock was repaired with the cells [198]. In 17 h of handling, a sizable amount of calcite precipitated (around 750 gramme), and a water permeability test of a single fracture over a large area revealed a significant reduction. These results suggested that MICP can be utilised to reduce the porosity of cracked rock in practise, suggesting that a MICP-based strategy would be a suitable choice for reducing unwelcome groundwater flow through fractured channels. In another investigation, investigators used bacteria to treat the faces of limestone at various temperatures in attempt to discover the optimal microorganism for practical use [199]. The limestone was reinforced to depths of 30 mm after a surface modification with bacteria twice in 12 h. The treatment cost was reduced to within the reach of common consolidates by optimizing urease dosages and carbonate precursor solutions. Subsurface drilled well fluid leaking in typical oil and gas exploitation or carbon capture technologies can be stopped by using the MICP a novel way by sealing gaps and porosity and lowering the system's liquid penetration. So because activation solutions used in MICP-based sealants are water-soluble and have lower viscosities than those used in cement-based sealants, they are simpler to move into the consolidation deposit and are therefore more appropriate for usage with cracked structures. Crystals **2022**, 12, 1222 19 of 30

This study used conventional fluid conveyance methods to treat sandstone strata fractures 340.8 m below ground level with a S. pasteurii culture and a urea-calcium mixture (packer and bailer). Leading to decreased in insertion rate of flow from 1.9 to 0.47 L/min and a reduction in well pressure gradient from more than 30 percent to 7 percent. Moreover, following MICP therapy, the crack extension stress while re-fracturing improved in comparison to before MICP administration. The applications for which bio-concrete might be especially advantageous are shown in Figure 7. These applications' main goals were to cut maintenance costs and prevent water infiltration. Additional uses included those for hard-to-reach locations, the nuclear sector, water-retaining structures, and airports [200].

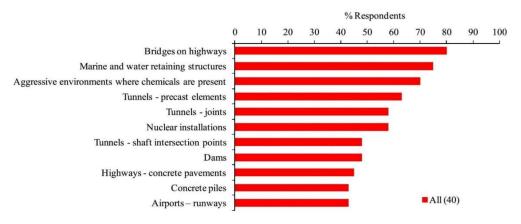


Figure 7. Proposed application areas of self healing bioconcrete [201].

5.1. Damages and Challenges Due to Corrosion

Steel corrosion is a global issue. Over half of structures have corroded, and billions of dollars are needed to rehabilitate other structures. In 2002, corrosion issues cost the globe 276 billion USD. In 2016, that cost is anticipated to be around 2.2 trillion USD [202,203]. The estimated loss from corrosion prepared globally is listed in Table 4 below.

Event	Damage	Economic Loss	Reference
Estimate in USA	Damage due to corrosion of Highway bridges	\$90–150 billion	[204] Federal highway Administration (1991)
Estimate in USA	Repair cost of bridge deck, substructures, car park	\$200–450 billion	[205] Transportation Research Board (1991)
Estimate in UK	Damage caused by corrosion of trunk and motorway bridges in Wales (England)	GBP 616.5 million	[206] Wallband (1989)
Estimate in UK	Annual repair cost to concrete structures	GBP 500 million	[207] Rosenberg (1989)

Table 4. The estimated loss due to corrosion.

5.2. Corrosion Monitoring Techniques

In concrete, corrosion of the reinforcing steel occurs electrochemically [208]. Electrochemical techniques are thus the most frequently utilized to determine the reinforcement's status (passivity or corrosion) or the rate of corrosion. Physical approaches are also becoming more and more common, since they may be used to evaluate the progression of the corrosion process based on indirect criteria including permeability change, decreased adhesion at the steel/concrete interface, and cracking brought on by corrosion products buildup.

Numerous reviews are currently being written about sensors for determining the corrosion status of concrete reinforcement. Some of them, such as electrochemical ones, are focused on a detailed examination of certain methodologies [209], chloride content regulation [210], fiber-optic [211], piezoelectric [12], and others. In other investigations, which are often conducted in lab settings, types of techniques are covered in detail, along

Crystals **2022**, 12, 1222 20 of 30

with certain specific applications of sensor designs [212]. Researchers frequently underestimate the significance of pre-made commercial solutions and system applications to actual structures. The main goal of our research was to examine the literature and identify the most prevalent sensors and non-destructive tracking systems for RC constructions.

5.3. Corrosion Challenges

5.3.1. Societal and Economic Challenges

The effective and stable operation of the economy and of everyday life depends on many the engineering structures that have been constructed. Maintaining and developing an economy's infrastructure is crucial for maintaining its competitiveness. This is one of the primary constraints limiting economic growth and international competitiveness in the United States of America (U.S.) [213]. The majority of bridges have been in use for 40 or more years despite being built with a 50-year service life in mind [214]. As a result, the quantity of bridges that require maintenance after their design lifespan will significantly grow. According to Polder et al. [215] the number of Dutch bridges in need of repair will rise by a factor of 2–4 over the next 20 years and even by a factor of 3–6 over the next 40 years. Their projections were based on information such as the dates of construction of Dutch highway bridges, experience with corrosion-induced deterioration, and average maintenance lifespans. As a result, it is expected that the infrastructure age profile in industrialized countries would cause corrosion costs to increase further during the coming decades.

5.3.2. Technological Challenges

The high societal costs of corrosion directly reflect a severe lack of fundamental knowledge of corrosion-related degradation of materials exposed to the environment, according to a committee recently established by the National Research Council of the United States to identify challenges in corrosion research and engineering [216]. This is due to a variety of factors, such as the multidisciplinary and multiscale character, the absence of pure and well-defined materials, the vast variety of exposure settings, the prolonged time periods, and the striking difference between laboratory and real-world surroundings. Despite the problem's technical commonality, national policies for dealing with corroding RC structures differ substantially from one another. The high societal costs of corrosion directly reflect a severe lack of fundamental knowledge of corrosion-related degradation of materials exposed to the environment, according to a committee recently established by the National Research Council of the United States to identify challenges in corrosion research and engineering [217] and engineering strategies continue to be heavily empirical and based on prior experience, which highlights the lack of a fundamental understanding [215–217]. At the international RILEM training event on upcoming and existing durability challenges for reinforced concrete structures, held in Zurich, Switzerland, in 2012, experts provided briefings and participated in a discussion panel [217], there is a vital requirement for an experience and understanding solution to the issue of forecasting the durability of RC.

5.3.3. Educational Challenges

This can be attributed, among many other things, to the restricted options for RC corrosion-specific education, which immediately arises from the decline in the number of university professors who are able to provide research posts in the field and who can also supervise and educate PhD candidates. This is illustrated, for instance, by the fact that the number of technical university professors conducting scientific research and teaching the subject of reinforcing steel corrosion in concrete at their institutions in the German-speaking region of Europe (Germany, Austria, and Switzerland) has decreased from 7 in the early 2000s to 3 as a result of retirements, followed by replacements of academic staff with knowledge and experience in other fields. Therefore, in the author's opinion, there is a need for more faculty positions in the fields of corrosion and concrete durability. To improve information transfer from materials science to engineering, this should ideally take place

Crystals **2022**, 12, 1222 21 of 30

in engineering departments instead of those that study materials. Furthermore, since the subject of concrete durability and corrosion might be effectively connected into curriculum at the B.Sc. and M.Sc. level, which is presently rarely the case [218], it would ensure that future generations of civil engineers receive a minimal degree of corrosion education.

In conclusion, the writers agree that there are not currently enough qualified specialists to handle the major issues connected to the resilience of RC systems. This can be due to the fact that, despite the subject's tremendous societal and economic significance, there are currently fewer academic positions available at institutions that are capable of supervising research projects and educating specialists in the field of corrosion in RC. These positions are necessary in order to expand the opportunities for the training of RC corrosion specialists and to include the topic of concrete durability and corrosion in the education of civil engineers at the B.Sc. and M.Sc. levels. They are also necessary to provide the scientific foundation necessary to overcome technological obstacles, such as questions relating to novel cementitious materials.

Forecasting the repair-free service life based on the degree of environmental corrosivity and the state of the RC requires the use of methods for evaluating the efficiency of primary and secondary prevention. Systems for managing and maintaining assets, like RC structures, rely heavily on non-destructive monitoring techniques (and continuous monitoring systems built on them).

The most effective monitoring systems use electrochemical, physical, and sensor technology. The structure's complexity and applicability, the operational conditions' difficulties, and the techniques' economic viability all have an impact on the decision of which combination to choose.

6. Outlooks and Drawbacks

Calcium carbonate generated within cracks in self-healing systems mostly closes cracks but scarcely recovers loss in strength. Furthermore, because calcium carbonate is resolved in acidic environments, it will not provide long-term fracture sealing when exposed to low pH levels. Another disadvantage of calcium carbonate is that it is brittle, making the bacterial calcium carbonate creation unsuitable in long-term closure of fractures in cementitious materials under various loading circumstances, as cracks will open and close again. To address these shortcomings, a more acid tolerant, potent, and/or more flexible (low modulus of elasticity) substance will be necessary. Minerals such as hydroxyapatite, which are stronger and more acidic tolerant than calcium carbonate-based minerals such as nacre, are both stronger and more flexible [219]. Another factor to consider is the expense of bio self-healing. The nutrition, bacterial preparation, and immobilizing agents are the principal sources of expense. Alternative nutrition compounds derived from waste streams could be used to achieve this. In this scenario, a screening investigation is needed to determine how different nutrients affect MICP and the polymorphism that results. The adhesion of the calcium carbonate that has been deposited to the fracture surface is one of the most important factors in determining the efficacy of the bio self-healing mechanism. As a result, a thorough examination into the calcium carbonate's adhesion behaviour to the concrete matrix is required.

7. Conclusions

According to previous studies, some bacteria can be dangerous to human health, others, including Bacillus Sphaericus, Bacillus pasteurii, Bacillus subtilis, and Bacillus flexus, don't. and have a considerable capability to precipitate calcite. As a result of this feature, these bacteria are perfect for the creation of bacterial concrete.

1. It is necessary to investigate several metabolic products and foods that are used in the development of ossifying bacteria. Moreover, calcium carbonate generated within cracks in (stimulated) autogenous and bacterial self-healing systems mostly closes cracks but scarcely recovers loss in strength. The nutrition, bacterial preparation, and immobilizing agents are the principal sources of expense.

Crystals **2022**, 12, 1222 22 of 30

2. Test methods most widely used for bacterial quantification approach of self-healing, including SEM, followed by XRD, were the most prominent technologies used by researchers to verify the precipitated crystals by therapeutic agents. No one has tested encapsulated bacteria at the nanostructure level, according to the authors. The results of the literature review have been used to select appropriate test techniques for assessing the self-healing efficacy of novel bacteria-based healing agents. To verify the accuracy of the results, microstructural tests like SEM, XRD, or FT-IR can be performed to recognize and describe the existence of precipitated products in crack specimens. In addition, the nanotechnology level study of self-restoration effectiveness by bacteria encased materials has not yet been completed.

The fracture repair period for microbial restoration under proper curative settings, according to the findings so far, is often a lengthy time, often 2 to 3 weeks. To develop genetically manipulated bacteria cultures that can survive longer and precipitate more quickly, further transdisciplinary study may be required.

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Crystals **2022**, 12, 1222 25 of 30

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