

Review

Advancements in Exploiting *Sporosarcina pasteurii* as Sustainable Construction Material: A Review

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Abstract: With the development of bioinspired green solutions for sustainable construction over the past two decades, bio-cementation, which exploits the naturally occurring phenomenon of calcium carbonate precipitation in different environments, has drawn a lot of attention in both building construction and soil stabilization. Various types of microorganisms, along with specific enzymes derived from these microorganisms, have been utilized to harness the benefits of bio-cementation. Different application methods for incorporating this mechanism into the production process of the construction material, as well as a variety of experimental techniques for characterizing the outcomes of bio-cementation, have been developed and tested. Despite the fact that the success of bio-cementation as a sustainable method for construction has been demonstrated in a significant body of scientific literature at the laboratory scale, the expansion of this strategy to construction sites and field application remains a pending subject. The issue may be attributed to two primary challenges. Firstly, the complexity of the bio-cementation phenomenon is influenced by a variety of factors. Secondly, the extensive body of scientific literature examines various types of microorganisms under different conditions, leading to a wide range of outcomes. Hence, this study aims to examine the recent advancements in utilizing the most commonly employed microorganism, *Sporosarcina pasteurii*, to emphasize the significance of influential factors identified in the literature, discuss the findings that have been brought to light, and outline future research directions toward scaling up the process.

Keywords: bio-cementation; sustainable construction materials; building construction; soil stabilization; *Sporosarcina pasteurii*



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1. Introduction

Calcium carbonate (CaCO_3) precipitation is a naturally occurring phenomenon in different environments, including marine water, freshwater, and soils. Microbially induced calcium carbonate precipitation Induced Calcium Carbonate Precipitation (MICP), which exploits the microbial metabolic processes for bio-cementation to enhance the durability of construction materials, has drawn attention not only in soil stabilization [1–11] and building construction [12–22] but also in wind-induced desertification [23–26], stone artwork conservation [27], and even subsurface-related applications [28,29]. Microorganisms engaged in the nitrogen cycle, sulphate-reducing bacteria, and photosynthetic microorganisms have all been reported to induce calcium carbonate [30,31]. Although the bio-cementation process produces ammonia gas that is undesirable [32], in comparison to the traditional methods, which make use of Portland cement, it offers a variety of benefits, including the following:

- Bio-cementation can be produced at room temperature; thus, the amount of energy needed for its manufacturing is significantly less than that required for conventional cement, resulting in a 43–95% decrease in embodied energy [33];
- The carbon footprint from the bio-cementation process is about 18–49.6% lower than that from traditional cement [33];

- Due to the relatively low viscosity of the cementation solution and bacterial suspension, the bacterium can flow like water during the bio-cementation process and move through the pores of the concrete [34];
- Bacterial sizes are less than 10 μm , which is considerably smaller than the sizes of cement ($<40 \mu\text{m}$); therefore, the pore openings can be as small as 6 mm [35];
- The cost-effectiveness of the MICP treatment compared to conventional treatments demonstrated a lifecycle cost reduction of about 98% [36,37].

1.1. Bio-Cementing Agents

Over the last two decades, researchers have investigated various techniques to exploit bio-cementation. These strategies are founded on three primary approaches, as follows:

- (i) Using allochthonous or autochthonous alive cells to exploit their metabolism [7,11,12,21,38,39];
- (ii) The cell-free approach, which uses bacterial fraction components in the absence of viable cells [40];
- (iii) Isolating specific enzymes from microorganisms or plants for Enzymatically Induced Calcium Carbonate Precipitation (EICP) [7,9,41–46].

A wide range of bacteria [38], including *Sporosarcina pasteurii* [2,46–55], *Bacillus Subtilis* [14,16–18,40,56–59], *Bacillus Cereus* [20,60,61], and *Bacillus Megaterium* [23,24,62,63], as well as enzymes isolated from plants, such as Jack bean [42,45,64] and soybean [36,41,44], have been investigated in the literature. Navigating through the literature, however, it can be observed that *Sporosarcina pasteurii* (*S. pasteurii*) is the most widely employed bacteria, as it demonstrates a higher rate and quality of calcium carbonate precipitation due to its species characteristics [65,66]. Parallel comparison investigations comparing the results of native bacteria present in soil with *S. pasteurii* cementation proved that this bacterium is highly efficient for bio-cementation and outperforms indigenous bacteria [47,67,68].

1.2. Application Methods of Bio-Cementation

Various application strategies, including mixing, injection, spraying, immersion [20,69–71], and even 3D printing [72–74], have been explored in the literature for introducing the bio-cementing agent into cementitious materials for MICP or EICP. However, the majority of the literature primarily uses three methods: injection or grouting, surface percolation or spraying, and mixing [11].

Injection is the most popular method to introduce bio-cementing agents into the system, in particular for soil stabilization applications [11]. Using a peristaltic pump, bacterial suspension and cementation solution are injected into the sand in either a vertical [10,75] or horizontal [76] trajectory. The advantage of this approach is that testing conditions, such as injection flow, pressure, and hydraulic gradient, may be easily modified for optimal treatment [10,77]. The main disadvantages of this method are unequal bacterial dispersion and inhomogeneity of CaCO_3 concentration, which result in non-uniform treatment along the injection path. This problem derives from the fact that when bacteria are injected via the pore space, they are likely to be filtered by sand, resulting in a linear drop in bacterial concentration along the injection direction [78].

Surface percolation and spraying are superficial treatments in which the bacterial suspension and cementation solution are alternately dripped or sprayed over the soil surface, followed by solution penetration into the soil by gravity [37,79–82]. Due to the similarity of these methods to the injection technique, they share the drawbacks of uneven precipitation and surface clogging, which are mostly influenced by the sand's particle size. A comparative study on the effect of sand dimension in 2 m columns of one-dimensional trials conducted by Cheng and Cord-Ruwisch [83] revealed that, after repeated treatments, the column of fine sand (size 0.3 mm) showed blockage at the injection end, resulting in a limited cementation depth of less than 1 m, but this issue was not observed in the column of coarse sand (size $> 0.5 \text{ mm}$).

In the mixing method, which is relatively new, the bacteria are first cultured in a nutrient broth medium, and the broth is mechanically mixed with cementitious material until the

desired homogeneity is attained. This method provides the opportunity to control the growth of bacteria and optimize the bacteria culture prior to the mixing procedure in order to achieve the highest possible concentration of bacteria. However, this approach has the potential downside of causing disturbance to the soil, which is significant since it may cause false stress to form in the soil sample due to the vigorous mixing of the soil and the cementing agent [11]. Although this method can open the doors to the development of “smart-living concrete”, both the protection or immobilization of bacteria and the source of nutrients for establishing the long-term and repetitive self-healing effect are still open questions [17].

1.3. Parameters That Influence Bio-Cementation

Bio-cementation treatment is a complex phenomenon influenced by several factors, including bacteria type (e.g., high, medium, or low urease activity), bacteria condition (e.g., live, bacterial fraction, or enzyme extracted from bacteria or plant) [27], application method (e.g., injection, mixture, spraying) [3,19], as well as environmental factors, such as pH [84], temperature [20], nutrition media [9,85,86], and grain size and nucleation sites [46,49,87], all of which affect the precipitation quantity and quality of calcium carbonate crystals and, consequently, alter the permeability and mechanical properties of the treated construction material. Figure 1 depicts a summary of factors that affect the bio-cementation process. For instance, a study on the morphology and evolution of crystals by two *Escherichia Coli* (*E. Coli*) strains altered to have either high or low urease activity over the course of seven days revealed that the strain with low urease activity produced nanocrystalline sheets during the first day, which after seven days of precipitation resulted in well-crystallized and extraordinarily large crystals that were 1924% larger than those with high urease activity due to the difference in kinetics of mineralization between the two strains [88].

Like any other chemical process, temperature significantly impacts the kinetics of the enzymatic activity of the bacteria in bio-cementation, and it can have a direct impact on crystal dissolution behavior following the Ostwald law [89]. A modest adjustment of 1 or 2 °C in the reaction temperature might cause changes in the outcomes of 10% to 20%, and most enzymes' activity increases by 50% to 100% when the temperature rises by 10 °C [90]. However, this rise only lasts as long as the elevated temperature does not denature the enzyme and change its structure. Similar to temperature, the pH of the environment (construction material in this case) substantially impacts bacterial enzymatic activity. At the microlevel, pH variation affects microbial cells by two different mechanisms: it alters the enzymatic reaction rate and the transport of nutrients into the cell [91]. Changing the pH level outside of its optimum range may slow down or even stop the bacteria's enzymatic activity and CaCO₃ precipitation, as the bacteria will not survive [43].

One additional factor that impacts the bio-cementation process and contributes to its complexity is the nutrition medium on which the bacteria rely for food [20,85]. Calcium carbonate crystal types include calcite, vaterite, and aragonite, with calcite being the most common and stable [92,93]. The composition of the nutritional medium has a significant effect on the CaCO₃ crystal morphology [93]. Even though yeast extract slows down the rate of hydration by a large amount, it is often used as a carbon source in bio-cementation applications as a nutritional medium. As a result, ongoing research is being conducted to explore alternative solutions [64,85,86].

Grain size has a significant impact on the outcomes of bio-cementation, as it can lead to non-homogeneity in large-scale applications. The particle's granularity directly affects the spacing between particles, which in turn acts as nucleation sites for the precipitation of CaCO₃ by the bio-cementing agent. In the case of a mixture containing predominantly fine grains, when the particles are excessively small, the bio-cementing agent will fill the narrow gaps between the particles and become restricted within these confined spaces. In the case of a mixture containing predominantly coarse grains with excessive distance between the particles, on the other hand, bacteria will have unrestricted movement between the pores. However, over time, gravity will cause the bacteria to migrate towards the lower level

of the specimen, resulting again in non-homogeneity [46]. The urease activity of bacteria or enzymes and the kinetics of CaCO_3 precipitation also play an additional role in this matter. In the case of a low urease activity bacteria that exhibits a slower precipitation rate of CaCO_3 , the reduced kinetic activity may allow for the potential migration of the bacteria to other pores, resulting in a more uniform outcome.

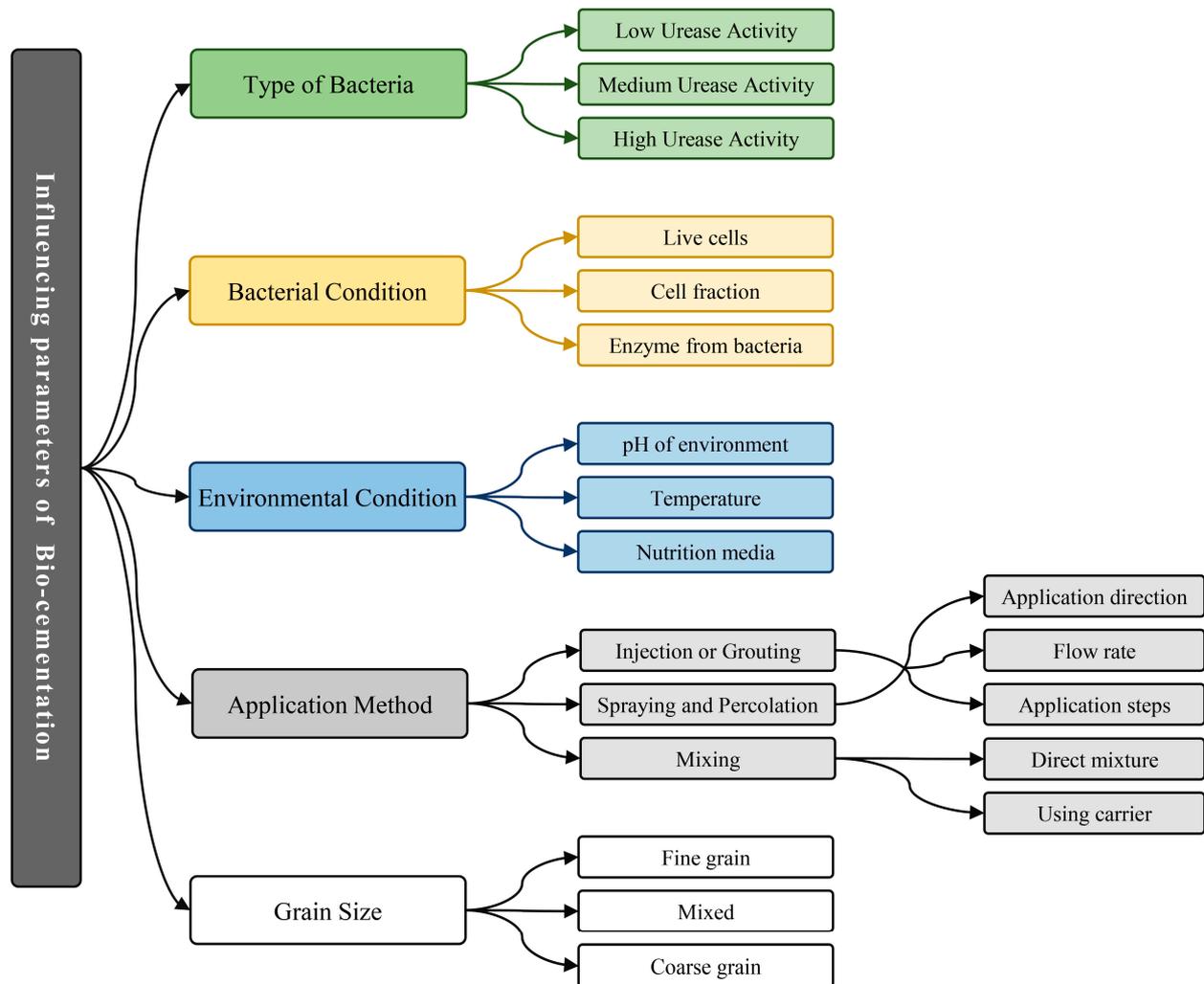


Figure 1. Schematic representation of influencing parameters on bio-cementation.

1.4. Characterization Techniques

Various experimental methodologies for qualitative and quantitative characterization of treated material and bio-cementation efficiency were employed. Scanning electron microscopy (SEM) has been employed for morphological analysis, which offers a wide range of information, including the efficiency of bio-cementation in filling nucleation sites [46], as well as the effect of the bacterial strain and its urease activity rate [21,39,47,68,88], bacterial condition [40,67], nutritional solution [37,64], and the difference between MICP and EICP [46,84] on the shape and quantity of precipitated CaCO_3 crystals. For instance, Phua and Røyne [64] used SEM to highlight the influence of nutritional solution on the CaCO_3 crystal shape precipitated by Jack bean (*Canavalia Ensiformis*) urease, revealing that calcium chloride solution resulted in rhombohedral CaCO_3 crystals with dimensions ranging from 20 to 80 μm , while calcium lactate and dissolved chalk solution precipitated spherical CaCO_3 crystals with a diameter between 100 and 250 μm (Figure 2).

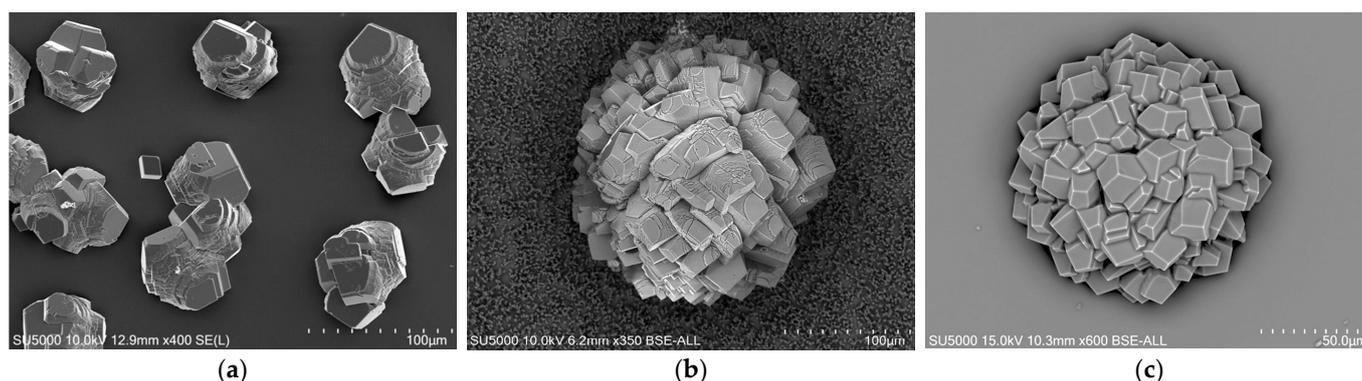


Figure 2. SEM images of CaCO_3 crystals formed in (a) calcium chloride, (b) calcium lactate, and (c) dissolved chalk solution, adapted with permission from Ref. [64] (2023, Elsevier).

Several studies took advantage of the elemental mapping capability of energy-dispersive X-ray spectroscopy (EDS or EDX) to study the surface composition and polymorphs of calcium carbonate (calcite, vaterite, and aragonite) in treated samples [46,55,64,68]. X-ray diffraction analysis (XRD) has been used to determine the crystallographic structure of the newly formed precipitated CaCO_3 as well as the intensity of each polymorph compared to the other [84,94].

One of the most important outcomes of bio-cementation is the CaCO_3 concentration, which has a significant impact on the mechanical properties of the treated construction material. The distribution of CaCO_3 content after the completion of the curing time or treatment is used to determine whether sand columns are adequately solidified. In a comparative study conducted by Choi et al. [95], six different methods, namely titration, inductively coupled plasma (ICP), X-ray diffraction (XRD) TOPAS, thermogravimetric analysis (TGA), ASTM, and washing methods, were adopted to measure the calcium carbonate content, which highlighted that titration and ICP techniques gave the lowest value and the washing method the highest value for CaCO_3 content.

Regarding the mechanical properties characterization, experimental techniques, such as unconfined compressive strength tests (UCSs), California bearing ratio (CBR), and cone penetration testing (CPT), based on ASTM D2166/D2166M-16 [96], ASTM D1883-21 [97], and ASTM D3441-16 [98], respectively, as well as direct shear tests (DSTs), have been employed. Permeability is another important property that indicates the efficiency of bio-cementation treatment. The significance of this property may vary across different application fields; however, it is evident that achieving low permeability is the objective across all application fields. In the context of building construction applications, high permeability leads to the rapid penetration of water containing harmful substances, thereby compromising durability and causing gradual deterioration. In soil stabilization, on the other hand, the elevated permeability characteristic of certain soils can facilitate the infiltration of liquids, such as water, thereby potentially causing the erosion and removal of particles. Depending on the application field and sand properties, the permeability properties of treated samples have been characterized using a variety of methods, including the rapid chloride permeability test (RCPT) [20,61], water absorption, constant head test method [8,10], falling head test [94], and standard protocols, such as ASTM D2434-22 [99], ASTM D2435/ D2435-11 (2020) [100], ASTM C 1585-20 [101], and ASTM 5856-15 [102].

1.5. Focus of This Review and Bibliometric Analysis

As a result of technological advancement and actions towards multidisciplinary with the involvement of microbiology and material engineering, the window of opportunity to exploit this phenomenon has widened and provided an infinite number of possibilities, which in turn makes it more difficult to reach the optimized solution. Although a large body of scientific literature has been devoted to this topic, the viability of scaling up this technology to the construction site- and field-scale remains to be extensively explored.

Therefore, an overview of the most recent advances in this context will be required to emphasize the advantages, drawbacks, and knowledge gaps, and to shed light on the road towards scaling up this process. Although the mechanism of bio-cementation has been described in many studies devoted to exploiting bio-cementation, it is important to elaborate on the chemical reactions occurring during this phenomenon. In light of this, the first topic that will be covered in this review is the process by which bio-cementing agents precipitate calcium carbonate. Herein, the biochemical reactions for CaCO_3 precipitation by microorganisms involved in the nitrogen cycle are described as an example. For the sake of brevity and in order to have a more concise view on the influencing factors and different application methods, this review concentrates on the bacteria that has been referenced most frequently across the literature, namely *Sporosarcina pasteurii*. Thus, a section of this review article is devoted to the properties of *S. pasteurii* from a microbiological standpoint, as well as how various parameters influence the outcomes of bio-cementation processes that are assisted by this bacterium in particular. Following that, recent advances in employing *Sporosarcina pasteurii* as a sustainable construction material, specifically in soil stabilization and the building construction sector, will be presented. This section will examine studies that presented systematic comparative analysis in these application sectors, and the most significant results will be highlighted. Last but not least, concluding remarks, knowledge gaps, and a future perspective will be presented.

The process of selecting the cited literature in this review article has been conducted according to the following steps:

1. The four keywords, namely “bio-cementation”, “*Sporosarcina pasteurii*”, “Microbially Induced Calcium Carbonate Precipitation (MICP)”, and “Enzymatic Induced Calcium Carbonate Precipitation (EICP)”, were searched on Google Scholar and Scopus.
2. The pre-screening process was conducted to determine the relevance of the search results. A total of 194 articles were identified as primarily relevant within the scope of this review article.
3. The articles that were chosen were classified into two categories: “review articles” and “original research”.
4. The original research articles were classified according to two criteria: “the type of bacteria or enzyme studied”, and the specific “application field” in which the research was conducted.
5. For the selected original articles, the “application method”, the primary “experimental methodology” used for characterization, and the “key outcomes” were highlighted. Following this secondary screening, 140 publications were chosen to be included in this manuscript.
6. The review articles and original research articles that were not specifically related to *Sporosarcina pasteurii* but presented results that highlighted specific outcomes for bio-cementation, such as the effect of influencing parameters or application methods or specific outcomes for experimental methods used in a creative or critical manner, were used in the introduction section to depict a clear background of bio-cementation and governing parameters for the reader.
7. Articles that focus on the specific characteristics of *S. pasteurii* and its behavior in various environments, as well as the results obtained from its application in soil stabilization and building construction, are used in Sections 3 and 4.

2. Bio-Cementation Mechanism

Bio-cementation is a complex multi-step process that starts with the production of urease, a multi-subunit, nickel-containing enzyme that is synthesized by ureolytic bacteria. Figure 3 depicts a schematic representation of the chemical reaction for microbially induced calcium carbonate precipitation by a microorganism involved in the nitrogen cycle as an example. The stepwise chemical reactions of MICP are as follows:

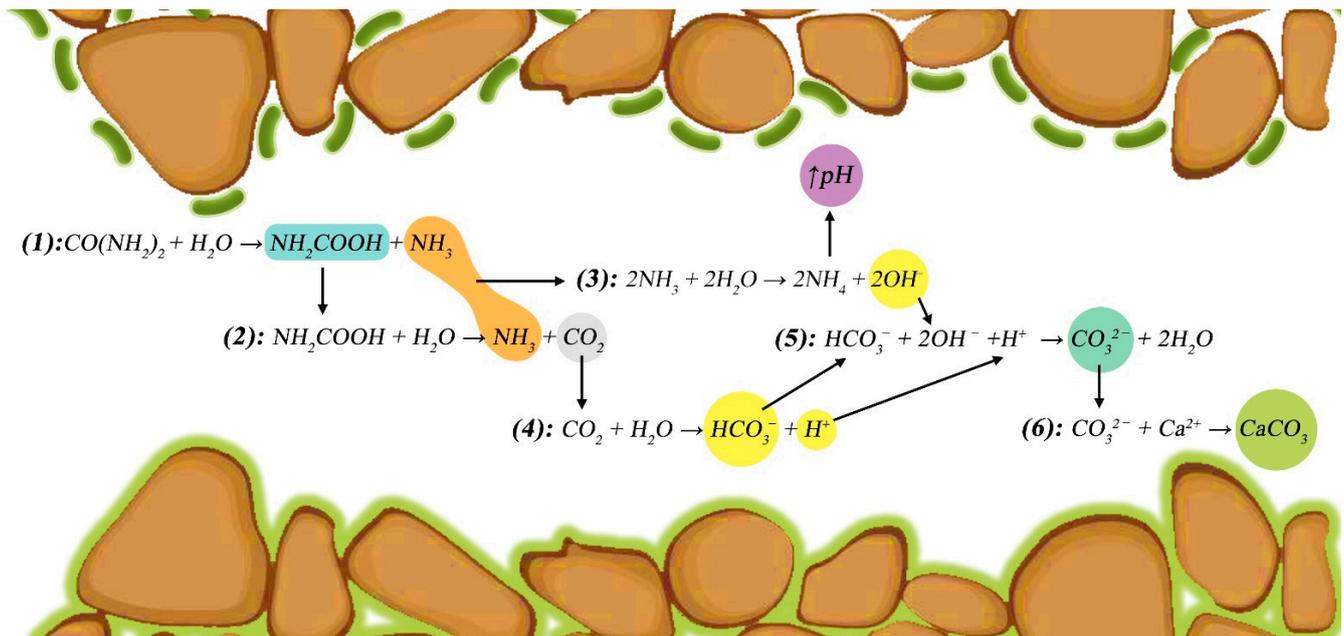


Figure 3. Chemical reactions of microbially induced calcium carbonate precipitation by nitrogen cycle bacteria.

1. The hydrolysis of urea ($\text{CO}(\text{NH}_2)_2$) under the catalysis of the bacteria's urease produces ammonia (NH_3) and carbamic acid (NH_2COOH);
2. Carbamic acid hydrolysis leads to ammonia and carbon dioxide;
3. Ammonia interacts with water and generates hydroxide (OH^-), and ammonium (NH_4) leading to a pH increase of about 1–2 pH;
4. Meanwhile, carbon dioxide in the system reacts with water, resulting in bicarbonate (HCO_3^-) and hydrogen ions (H^+);
5. The system reaches equilibrium and generates carbonate ions (CO_3^{2-}) and water;
6. Eventually, the reaction of carbonate with calcium ions (Ca^{2+}) in the environment results in the precipitation of CaCO_3 crystals.

It is important to keep in mind that Ca^{2+} and other cations in this system are bonded with water dipoles in an aqueous environment (nutrient media). As a result, these ions need to be dehydrated before they can attract CO_3^{2-} and precipitate CaCO_3 [103].

Although the EICP method employs urease obtained directly from plants or bacterial cells rather than live urease-producing bacteria (MICP) for the purpose of urea hydrolysis, it relies on identical biochemical reactions. The alterations seen in EICP treatment pertain primarily to the reaction rate and, as a result, the characteristics of the precipitated calcium carbonate [46]. In addition, it should be noted that the cost of EICP is higher compared to MICP. This is primarily attributed to the significant expenses involved in acquiring commercially purified urease enzymes derived from plants or bacteria, even for small-scale laboratory experiments. According to previous research findings, this expense can account for up to 60% of the overall operating costs, which is almost twice the cost of the growth medium ingredients themselves [10,104].

3. *Sporosarcina pasteurii* (*S. pasteurii*)

Sporosarcina pasteurii, previously referred to as *Bacillus Pasteurii* in older classifications, is an aerobic, mesophilic, rod-shaped (0.5–1.2 μm in width and 1.3–4.0 μm in length), gram-positive bacterium that is the most dominant microorganism used in MICP and EICP. *S. pasteurii* is able to form endospores, which are non-reproductive structures generated by bacteria, or the dormant form to which the bacterium can reduce itself [65]. Endospore development is frequently driven by a lack of nutrition. Endospores allow the bacterium to remain dormant for extended periods of time in the absence of nutrition, surviving

ultraviolet radiation, desiccation, high temperatures, freezing temperatures, and chemical disinfectants until the environment improves and the endospore can revive itself [66]. This feature improves *S. pasteurii*'s ability to survive in hostile environments and gives it the opportunity to have its metabolic system enabled or disabled depending on the requirements of the engineers. Various strains of *S. pasteurii* have been utilized in the literature, including ATCC 11859 [2,46–55], ATCC 1376 [105,106], PTCC 1645 [107,108], DSM 33 [8,76,81,109], and NB28 (SUTS) [1,4]. Being an alkaliphile, this bacterium grows best in an alkaline environment with a pH between 9 and 10 [110]; however, it can survive in moderately harsh circumstances up to a pH of 11.2 [111], making it also a suitable admixture component for building construction applications.

S. pasteurii, like all other *Sporosarcina* species, is heterotrophic, which means it cannot make its own food and must rely on other sources for nutrition. *S. pasteurii* needs, in particular, urea and ammonium as nutrients for growth [112]. Depending on the type of nutrient provided for bacteria, its metabolism and, as a consequence, its urease activity and the rate at which calcium carbonate precipitates will differ, which emphasizes the significance of nutrition media in bio-cementation [38]. An investigation into the optimum concentrations of urea, calcium, and nickel to enhance calcium carbonate precipitation and *S. pasteurii* growth rate revealed that high urea and nickel concentrations and low calcium concentrations are required [113].

As mentioned before (Section 1.3), yeast extract is a commonly employed nutritional medium in bio-cementation applications, despite its significant impact on hydration rate reduction. However, the study on the effect of nutrient medium for optimizing *S. pasteurii* growth and cement hydration using six different nutrition media, namely yeast extract, lactose mother liquor, corn steep liquor, meat extract, glucose, and sodium acetate, revealed that the combination of meat extract and sodium acetate is a suitable replacement for yeast extract, as it reduced retardation by 75% (as compared to yeast extract) without compromising bacterial growth, urea hydrolysis, cell zeta potential, or the ability to promote calcium carbonate formation [86]. However, additional research is required to investigate the impact of these different growth media on other characteristics of concrete, such as its cost, strength, shrinkage, resistance to corrosion, and workability. Another investigation on the viability of *S. pasteurii* under extreme conditions (30, 45, and 55 °C) and pH (12.5–13.6) conditions demonstrated that exposing the bacteria to extreme temperatures (specifically 55 °C for 4 h) and extreme pH (specifically 13.6 for 4 h) led to the most significant decrease in both the concentration of viable cells and the initial rate of ammonia production through urea hydrolysis [114]. However, to the best of the author's knowledge, a comprehensive parametric study in the literature providing insights on the effect of temperature within a larger range (−5–50 °C) and extreme pH (1–14) on variation of CaCO₃ precipitation or denaturation of bacteria or enzymes, which could help the simulation of this process for optimization, does not exist.

As mentioned before, for the same microorganism, the agent condition (live, enzyme extracted, or cell fraction) affects the result of precipitated CaCO₃ and, consequently, the properties of the treated material. Hoang et al. [46] compared the crystallography, mechanical, and permeability properties of bio-cementation via MICP and EICP by *S. pasteurii* after 12 cycles of treatment. The results of this comprehensive investigation concluded the following:

- The MICP strategy creates large crystal clusters with a thickness ranging from 50 to 100 µm, whereas the size of crystals via EICP are significantly smaller, ranging from 5 to 20 µm.
- The same number of treatments resulted in a higher CaCO₃ content for MICP, and at a constant percentage of CaCO₃, the mechanical properties of EICP-treated samples were superior to those treated by MICP.
- While CaCO₃ precipitation levels differed (2.5–16%) and (1.5–8%), the peak stress for UCS tests of treated samples in both cases ranged from 200–2400 kPa.
- Permeability levels for EICP-treated samples were slightly lower in the range of 1.5–4% CaCO₃ precipitation compared to MICP-treated, while the MICP-treated permeability decreased by 3 to 4 orders of magnitude between 13–16% CaCO₃ content.

The comparison of MICP and EICP treatment by *S. pasteurii* in relation to pH fluctuation by Lai et al. [84] highlighted three key aspects. First, in acid environments (pH = 4–7), MICP treatment appears to be faster than EICP treatment since the delay time for visible flocculation—an indication of precipitation kinetics—is shorter in MICP treatment, and MICP appears to be less influenced by pH variation as it begins to precipitate CaCO_3 , even at a pH of about 4.5 when the EICP process does not even start (Figure 4a). Second, EICP treatment appears to outperform MICP treatment in terms of average calcium carbonate content (CCC) at a pH above 5.5; by decreasing the pH below 5.5, CCC decreases and declines dramatically at pH = 4.5, while MICP treatment appears to have an almost consistent precipitation up to pH = 4.5 and a decrease at pH = 4 (Figure 4b). The third outcome of this investigation concerns the morphology of calcium carbonate crystals. For the same initial pH, EICP treatment seems to predominantly precipitate calcite, which is the most stable polymorph of CaCO_3 , while MICP treatment produces both calcite and vaterite. This aspect results in significantly higher mechanical properties for EICP treatment (Figure 4c,d). Despite the numerous advantages of EICP over MICP, the MICP treatment continues to receive more attention due to its significantly lower cost in comparison to EICP.

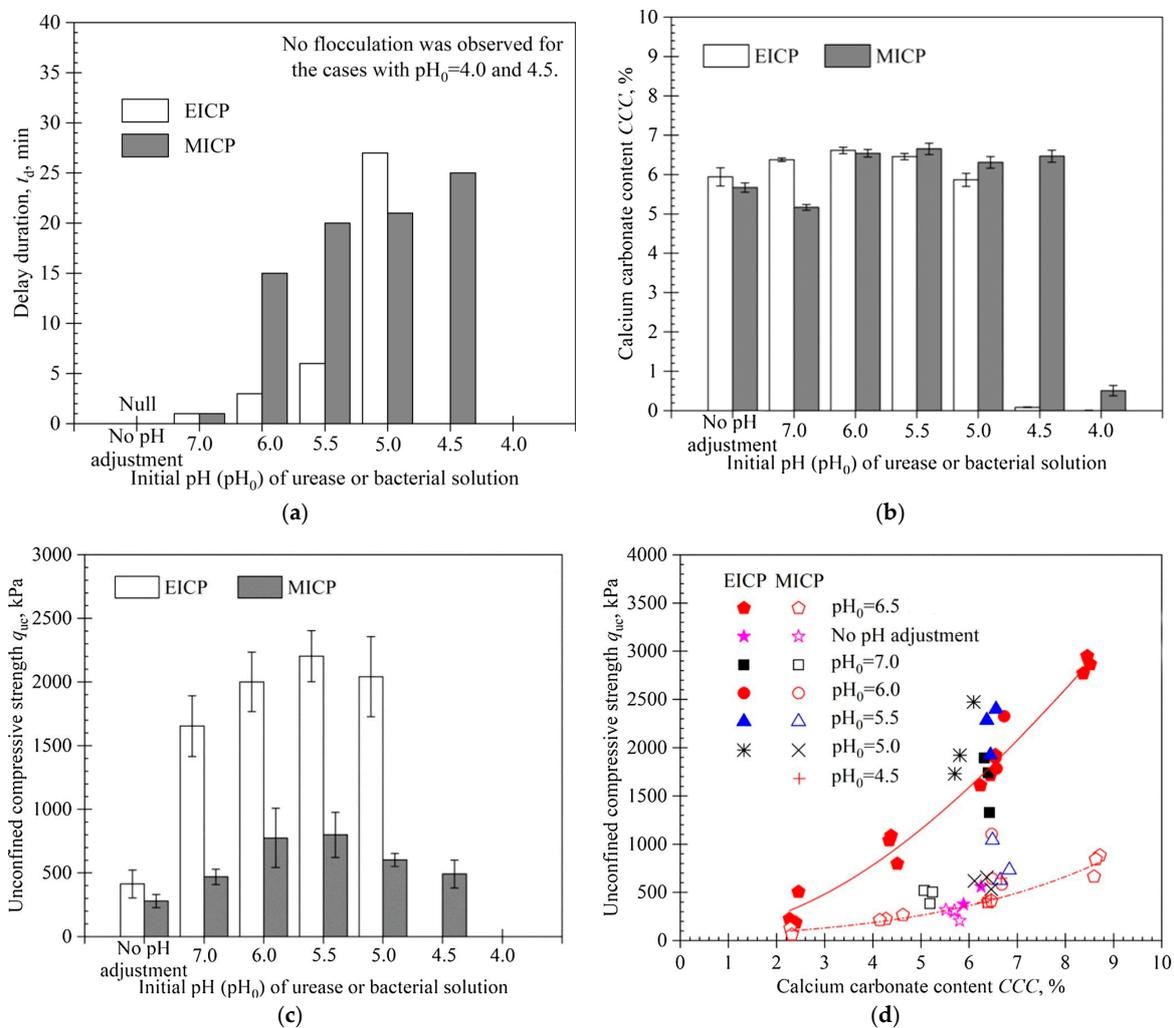


Figure 4. Effect of pH variation on EICP- and MICP-treated soil by *S. pasteurii*: (a) delay in flocculation at various initial pH conditions; (b) average calcium carbonate content in the whole sample; (c) unconfined compressive strength; (d) relation between strength and calcium carbonate content. Adapted with permission from Ref. [84] (2023, Springer Nature).

4. Recent Advancements in Exploiting *S. pasteurii*

As mentioned before, microbially induced calcium carbonate precipitation is already a complex phenomenon that can be influenced by a variety of parameters. On the other hand, a large body of literature has been devoted to the study of *S. pasteurii* as a sustainable construction material, investigating one or two parameters at a time. Hence, this section focuses only on the literature that presents parallel comparative methodologies and systematic characterizations and assessments. The preceding section (Section 3) has covered the latest developments concerning the factors that impact the distinct characteristics of *S. pasteurii*, as well as its behavior and viability in different environments. This section is dedicated to discussing the in situ application of bio-cementation. It will cover relevant literature that explores the effects of different application methods and the challenges encountered in various application sectors. Among the application domains of bio-cementation, the soil stabilization and building construction sector has investigated this strategy the most.

Although researchers were able to employ bio-cementation to develop commercially available building construction materials, such as bio-based bricks that received a patent [115] and reached mass production level (e.g., Biolith[®] tiles by Biomason, Inc. (Durham, NC, USA) [116]), the viability of scaling up this technology to the construction site and field scale remains to be extensively explored [1]. Since application methods of bio-cementation (Section 1.2), in particular injection or grouting, are already widely used in soil stabilization, exploiting MICP in field-scale applications in this sector is much more advanced than building construction. Hence, a closer look at the latest developments in soil stabilization can shed light on the most recent findings and areas of knowledge that require further exploration for scaling up this technology in this field as well as in building construction applications.

4.1. Soil Stabilization

Traditional soil improvement strategies, such as adding natural and synthetic materials (e.g., recycled glass fibers, tires, fruit brunches, polypropylene, and polyester), injection of chemical grouting or deep mixing using cement and/or lime, and application of sand or stone columns, rely on synthetic substances that require significant energy for production and application and raise the pH of groundwater, causing serious environmental problems and contributing to ecosystem disturbance [11]. With the widespread implementation of bio-cementation in soil stabilization applications, researchers have investigated the effects of different parameters, such as application method, nutrition media, and sand granularity, on the properties of *S. pasteurii*-treated soil at both laboratory and in situ field scales. Regarding the application method, injection is the most common method for MICP-based soil stabilization.

Table 1 summarizes some of the findings from systematic investigations in the literature that used *S. pasteurii* for soil stabilization applications by injection. A table of the literature that used other application methods to introduce *S. pasteurii* for soil stabilization is presented in the Supplementary Materials, Table S1.

Although the injection method has proven to be quite effective in small specimens at the laboratory scale, significant problems have been noted at larger scales. Whiffin et al. [10] investigated the impact of vertical injection of *S. pasteurii* along a 5-meter-long sand column. Their findings revealed that while calcium carbonate precipitation occurred throughout the entire treatment length, the concentration profile exhibited non-uniformity. In particular, higher quantities of calcium carbonate were observed at the injection points, with a subsequent decline in concentration along the length of the column. The impact of injection direction was assessed by Paassen et al. [76] in a large-scale experiment involving the horizontal injection of *S. pasteurii* in a soil bed with a volume of 100 m³. The study revealed a considerable variation in the peak strength of the unconfined compressive strength values, again confirming non-uniformity along the path of injection within the treated sand volume. In general, as the distance from the injection points increases, there is a decrease in the calcium carbonate content, which leads to an increase in porosity and a subsequent decline in the mechanical properties. This limitation of MICP application on a large scale arises

from the occurrence of system clogging near the injection sites. This phenomenon is caused by the rapid precipitation of calcium carbonate by the bio-cementing agent, which leads to the closure of pores and hinders the migration of the bio-cementing agent throughout the entire treatment area, resulting in non-homogeneity. Hence, it is anticipated that the utilization of bio-cementing agents with low urease activity or the regulation of bio-cementation kinetics to achieve a slower rate would result in a reduced level of non-homogeneity in treatment, as it would facilitate the effective distribution of the bio-cementing agent along the entire length of the treatment. This disadvantage can be mitigated by employing a two-phase vertical injection procedure (alternating the injection of bacterial suspension and cementation or nutritional solution) and fine-tuning the injection parameters, such as decreasing the injection rate of bacterial suspension and increasing the flow rate of cementation solution [10,77].

Among the several studies that evaluated the impact of particle size on the effectiveness of bio-cementation treatment aided by *S. pasteurii*, three stand out due to their systematic approaches [46,49,117]. Lin et al. [117] studied the effect of grain size on MICP-treated Ottawa sand by *S. pasteurii*, considering fine grains ($D_{10\%} = 0.26$ mm and $D_{50\%} = 0.33$ mm) and coarse grains ($D_{10\%} = 0.58$ mm and $D_{50\%} = 0.71$ mm). The findings of this investigation revealed that the coarse-grain soil had a lower CaCO_3 content in comparison to the fine-grain soil. However, it was observed that the coarse-grain soil demonstrated greater increases in S-wave velocity, peak shear strength, and cohesiveness when compared to the fine-grain soil. Moreover, the results obtained from triaxial testing indicated that the peak deviator stress of the coarse-grained soil containing 1.6% CaCO_3 and the fine-grained soil containing 1% CaCO_3 exhibited an average increase of 93% and 171%, respectively, as compared to their respective untreated specimens.

Hoang et al. [46], on the other hand, investigated this effect on EICP-treated silica (SiO_2)/quartz sand, considering fine grains ($D_{10\%} = 0.26$ mm and $D_{50\%} = 0.36$ mm) and coarse grains ($D_{10\%} = 0.61$ mm and $D_{50\%} = 0.72$ mm). The outcomes of this study highlighted that, similar to MICP-treated sand, the mechanical properties of EICP-treated sand, such as Young's modulus and UCS test, are higher in coarse-grained sand than in fine-grained sand.

As in Table 1, the trend of permeability reduction influenced by grain size indicated that permeability reduction in fine-grained bio-cemented sands steadily declined with the increase in CaCO_3 content, while for the same increase in CaCO_3 , the trend of reduction in permeability declination was comparatively less pronounced in coarse-grained sand [46]. This phenomenon is often attributed to the smaller distances between particles, known as nucleation sites, in fine-grained sand. The precipitation of calcium carbonate fills the small pores in fine grain rapidly, leading to the obstruction of pathways through which bacteria can move to continue the cementation in other pores. In coarse-grained sand, the interparticle spacing is greater. Through the precipitation of CaCO_3 , the contact points between the particles are initially joined, followed by the deposition of additional layers of calcium carbonate on top. This process facilitates the movement of bacteria or enzymes through the pores, enabling them to reach more profound or distant locations.

Mahawish et al. [49] studied the effect of fine (0.075–9.5 mm) and coarse (2.36–16 mm) grain percentages in mixed Pakenham Blue Metal columns on the mechanical properties of treated sand. Five columns with different percentages of fine/coarse grains (A = 0/100, B = 25/75, C = 50/50, D = 75/25, E = 100/0%) were subjected to MICP treatment by *S. pasteurii*. The average calcium carbonate precipitation for columns A and E was determined to be about 7% and 6.5%, respectively, and about 6% for other mixed columns (B, C, and D). However, Column B exhibited the most uniform distribution of CaCO_3 along its length. The unconfined compressive strength test results for all three mixed columns (B, C, and D) were determined to be approximately 0.6 MPa, while for Column A, despite having the highest CaCO_3 content, this value was lowered by half to 0.3 MPa, and for Column E, it was dropped to nearly 0.5 MPa. These findings underscore a significant aspect regarding the impact of grain size. While a higher level of porosity (100% coarse grain in Column A) does facilitate the migration of the bio-cementing agent throughout the treated column and

may result in the highest CaCO₃ content, it does not necessarily yield the best mechanical properties. This is due to the fact that the bacterial solution tends to migrate to the bottom of the column under the influence of gravity, resulting in non-homogeneity throughout the column. Consequently, the top of the column exhibits the lowest CaCO₃ content, while the bottom exhibits the highest, contributing to the overall inferior mechanical properties of the column due to non-homogeneity.

As previously stated, soil stabilization is more advanced than other application fields of bio-cementation when it comes to scaling up the process for field application. Few studies have been conducted on the scaling up of bio-cementation to field scale [1,118–121]; therefore, it is important to highlight the most significant findings from these investigations. Omoregie et al. [1] presented an economic strategy to scale up the production and cultivation of *S. pasteurii* under nonsterile condition using a custom-built stainless-steel stirred tank reactor with a capacity of 3 m³. The scalability of the bacterial cells was investigated by increasing the volume of the seed cultures from 214 L to 2400 L and monitoring their growth for a duration of 90 h. The results of the in situ soil bio-cementation experiment demonstrated that bacterial cells cultivated using this method retained their ability to induce CaCO₃ precipitation even after being immobilized within sand specimens. The presence of CaCO₃ crystal formation within the treated sand particles was confirmed through the analysis of CaCO₃ content and microstructural data, which confirmed that the method presented in this study is as successful as the sterile condition.

Table 1. Selective literature employing *Sporosarcina pasteurii* for soil stabilization treatment via injection method.

		Characterization				Treat. Time (Days)	Comments and Results	Ref.
SEM	EDS	XRD	CaCO ₃ Content	Mechanical Test (MPa)	Permeability Reduction			
X			60 kg/m ³	UCS = 3.52		20	<ul style="list-style-type: none"> CaCO₃ content, and ultrasonic wave velocity showed a linear relation 	[4]
			60–105 kg/m ³	0.2–0.57	slight reduction	5	<ul style="list-style-type: none"> Mechanical: single stage confined drained triaxial test Decrease in porosity ranged from 10 to 2% along the distance from injection point 	[10]
X	X		2.5–16%	UCS = 0.2–2.3		16	<ul style="list-style-type: none"> MICP coarse sand (Young's modulus = 20–250 MPa) 	[46]
			1.5–8%	UCS = 0.45–1.5	X		<ul style="list-style-type: none"> EICP coarse sand (Young's modulus = 75–125 MPa) 	
			2–6%	UCS = 0.2–0.9			<ul style="list-style-type: none"> EICP fine sand (Young's modulus = 25–75 MPa) 	
X	X		1.6–2.5%	X		1	<ul style="list-style-type: none"> Fine sand 	[117]
			0.9–1.1%	X			<ul style="list-style-type: none"> Coarse sand 	
X		X		UCS = 0.2–2.5	5–35%	1	<ul style="list-style-type: none"> Treatment with soluble calcium is more efficient than calcium chloride 	[94]
X			5.3%	CPT = 32.1		14	<ul style="list-style-type: none"> MICP results in fewer but larger calcite crystals Shear wave velocities increased by 600% CPT increased by 500% 	[67]
			X	X		16	<ul style="list-style-type: none"> Horizontal injection 	[76]
X			11%	UCS = 1.3	Increased by 800%	14	<ul style="list-style-type: none"> Bio-cemented clean sand 	[8]
			11%	UCS = 0.3			<ul style="list-style-type: none"> Bio-cemented oil-contaminated sand Portland cement-treated oil-contaminated sand 	

Table 1. Cont.

SEM	EDS	XRD	Characterization			Treat. Time (Days)	Comments and Results	Ref.
			CaCO ₃ Content	Mechanical Test (MPa)	Permeability Reduction			
X						13	<ul style="list-style-type: none"> Various bacteria and several strains of <i>S. pasteurii</i> Production rate of <i>S. pasteurii</i> is 2 orders of magnitude higher than the others 	[122]
X			0.9%	X			<ul style="list-style-type: none"> The liquefaction resistance and pre-triggering behaviors of loose sands improved significantly Shear strengths and stiffness increased 	[123]
				CPT = 3.6–5.1		15	<ul style="list-style-type: none"> Shear wave velocities = 961 and 967 m/s CPT improved by 419–543% 	[124]
			3.6%	Shear strength increased up to 72%			<ul style="list-style-type: none"> Increase in properties by number of treatments Shear strength and slope of failure line in triaxial consolidated drained test improved 	[125]
X	X	X		UCS = 1.47			<ul style="list-style-type: none"> The presented method reduces ammonia discharge by more than 8 times. 	[32]
X			X	UCS = 2.5			<ul style="list-style-type: none"> 8 treatments with monitoring effect of each treatment on CaCO₃ content and mechanical properties. 	[126]
X	X		13%	UCS = 0.9–2		8	<ul style="list-style-type: none"> 16 treatments by solutions contacting different percentages of bacteria 	[53]
X		X	0–4%	UCS = 0.16		10	<ul style="list-style-type: none"> With modest CaCO₃ precipitation (0–4%) in intergranular sand voids, the UCS increased by more than 100 kPa. 	[51]
X	X		7.5%	Shear strength = 0.15	98%	30	<ul style="list-style-type: none"> 15 cycle of treatment (1 per 48 h) The peak strengths increase by 266% 	[68]
X	X	X	2.5–7.3%	Needle = 1.67–5.3			<ul style="list-style-type: none"> Various strains of <i>S. pasteurii</i> 25–44% reduction in riverbank erosion 	[5]
X		X	15.2%	UCS = 1.74			<ul style="list-style-type: none"> Injection with fixation 	[3]
			7.6%	Needle = 0.5			<ul style="list-style-type: none"> Injection without fixation 	
X	X	X		UCS = 0.3	X	1	<ul style="list-style-type: none"> Using seawater supplemented with urea concentrated cementation solution increased the mechanical properties 	[6]

X: Experiment has been performed, UCS: unconfined compressive strength test, CPT: Cone penetration test.

4.2. Building Construction

Bio-cementation has been utilized in the field of building construction, primarily for producing new building materials rather than repairing damaged structures. The most common application method used in building construction is to employ bio-cementing solution as an admixture component of concrete. Table 2 presents an overview of key findings derived from systematic investigations documented in the literature, which have employed *S. pasteurii* to develop self-healing concrete through mixing techniques. As can be observed, there is a notable disparity in the quantity of relevant publications utilizing *S. pasteurii* in comparison to soil stabilization (Table 1 and Table S1). Moreover, it is worth mentioning that in the context of building construction (Table 2), the primary emphasis is placed on the mechanical and permeability characterization of the treated material rather than the assessment of calcium carbonate content, compared to soil stabilization (Table 1).

The experimental approach commonly used in the majority of the literature involves the following steps:

1. Introducing bacteria as an admixture component in concrete.

2. Curing the cast concrete for a period of 28 days.
3. Inducing cracks in the concrete by applying either uniaxial or flexural force.
4. Treating the damaged concrete with a bacterial nutrient media or treatment solution.
5. Monitoring the healing progress of the crack over time.
6. Conducting mechanical and permeability tests to characterize the healed concrete.

S. pasteurii has shown remarkable effectiveness in repairing concrete cracks. A study by Sohail et al. [127] utilizing this bacterium for the purpose of repairing cracks in concrete has demonstrated that not only does this bacterium have the capability to effectively fill cracks up to a width of 4 mm, but it can also successfully adhere two separate pieces of a concrete specimen that have become detached during a flexural test.

Various nutritional media, such as lactose mother liquor [128,129], sodium carbonate [129], calcium nitrate–urea, and calcium chloride–urea [129,130], have been employed in the cultivation of *S. pasteurii* within concrete mixtures. However, due to the variability in environmental conditions, concrete components, and treatment methods, it is unfortunate that a definitive conclusion cannot be reached on this matter. Kim et al. [131] investigated the influence of concrete type on the process of *S. pasteurii* bio-cementation by measuring the relative mass gained as a result of treatment on normal and lightweight concrete, concluding that the relative weight gains observed in both normal and lightweight concrete were comparable. This suggests that the precipitation of calcium carbonate by *S. pasteurii* is not dependent upon the specific type of concrete used, as long as the environmental conditions necessary for bacterial growth (such as concrete pH and temperature) remain consistent.

Table 2. Selective literature employing *S. pasteurii* for new construction material using mixing method.

Characterization					Treat. Time (Days)	Comments and Results	Ref.
SEM	EDS	XRD	Mechanical Test (MPa)	Permeability Reduction			
X		X	X	X	28	<ul style="list-style-type: none"> Bacterial nutrient: calcium nitrate–urea and calcium chloride–urea 	[130]
X		X			91	<ul style="list-style-type: none"> The aggregates were immersed in bio-cementing solution before mixing Visual analysis of induced crack healing 	[132]
X	X	X	CS = 32 CS = 36 CS = 31	7% 8% 6%	91	<ul style="list-style-type: none"> Bacterial concentration 10³ cells/mL Bacterial concentration 10⁵ cells/mL Bacterial concentration 10⁷ cells/mL 	[133,134]
X	X	X	0.2–0.3		28	<ul style="list-style-type: none"> Higher optical density resulted in higher compressive strength (increased 33%) and lower water absorption 	[136]
X	X		CS = 29–42		28	<ul style="list-style-type: none"> Compressive strength (CS) increased by 79.2% Flexural strength increased by 50% 	[137]
X	X		28% increase 41% increase	10.6% 17.9%	120	<ul style="list-style-type: none"> Bacteria proportion: 0.25% of cement weight Bacteria proportion: 0.5% of cement weight 	[138]
			X		28	<ul style="list-style-type: none"> Compressive strength increased by 45% Flexural strength increased by 18% 	[135]
X	X	X	CS = 46		28	<ul style="list-style-type: none"> Cracks up to a width of 4 mm were healed Two separate pieces of a concrete adhered again 	[127]
			CS = 29.2		28	<ul style="list-style-type: none"> Used rubber particles to immobilize the bacteria 	[139]

X: Experiment has been performed, CS: compressive strength.

Although the straightforward method of adding bacterial and nutrient solutions into concrete mixtures is commonly employed, researchers have explored alternative approaches to introduce bacteria into concrete mixtures. Chen et al. [132] employed

lightweight aggregates as carriers for *S. pasteurii* in concrete. In this study, the aggregates underwent several initial immersions in nutrient media (specifically, calcium lactate/yeast extract), followed by multiple drying cycles. Subsequently, the aggregates were immersed in a bacterial solution. After the aggregates were completely dried, they were combined with other components to produce concrete. The comparison of the healing process of induced cracks was conducted on three groups: the controlled group (A) without treated aggregate, the air-cured treated group (B) with treated aggregate but no additional supplement, and the immersion-cured treated group (C) with treated aggregate and immersion in a urea/calcium carbonate solution. The results showed that the precipitation of CaCO_3 by *S. pasteurii* can be reactivated in the presence of appropriate nutrition; in group C, the cracks were completely cured after 91 days, while in group B, only partial curing was observed, and no curing was observed in group A.

When using bio-cementing agents as a mixture component in concrete, it is important to keep in mind that an excessive bacterial concentration in the concrete solution may not always lead to the best permeability or mechanical properties. For instance, an investigation on the effect of *S. pasteurii* concentration (10^3 , 10^5 , and 10^7 cells/mL) in a concrete mixture on the mechanical properties of the concrete demonstrated that the compressive strength of concrete with 10^5 cells/mL bacterial concentration was 11% higher than that of 10^3 cells/mL and 13.5% higher than that of 10^7 cells/mL [133,134]. The observed decrease in compressive strength of concrete at higher cell concentrations of bacteria (10^7 cells/mL) can be attributed to the disruption of matrix integrity caused by excessive bacterial activity.

The study conducted by Zaerkabeh et al. [135] is among the limited number of research efforts that have examined the impact of crack size on the effectiveness of self-healing concrete utilizing *S. pasteurii*. Cracks with widths of 0.5 and 1 mm and depths of 5, 10, and 15 mm were investigated in this study. The study's findings indicate that the flexural strength and energy absorption capability of the specimens dropped as the depth and width of the cracks increased. Notably, the specimen with a crack depth and width of 0.5 exhibited the highest healing efficiency after 7 and 28 days. While the authors acknowledge that impeding the flow of nutrients and oxygen by clogging the pores could potentially lead to a drop in the healing rate among samples tested after 28 days, it is important to note that this study did not take into account the volume of the crack, which could possibly contribute to the observed reduction in healing in cracks with larger dimensions. It is comprehensible that in a suitable environment, the quantity of bio-cementation, like any other chemical reaction, is a function of time. Since the rate of the chemical reaction involving the precipitation of CaCO_3 by *S. pasteurii* for all the studied specimens is constant, the quantity of precipitated crystals will increase over time. Hence, in the case of larger cracks, a more extended period of monitoring the healing process may have yielded more precise conclusions.

5. Conclusions

5.1. Concluding Remarks

The goal of this review is to provide a concise summary of key elements pertinent to the current literature on using *Sporosarcina pasteurii* as a sustainable construction material. The main novelties of this review are that it offers a general overview of the factors to be considered when using bio-cementation (especially for *S. pasteurii*), provides a thorough summary of comparative studies on the subject that presented outstanding results, and sheds light on areas that have been overlooked in the literature and have the potential for future investigation. After a thorough examination and analysis of the most recent prominent works of literature, the concluding remarks of the review article may be summarized as follows:

- The application of bio-cementation has demonstrated notable efficiency in different engineering sectors, exhibiting enhancements in mechanical properties and a reduction in the permeability of construction materials at a laboratory scale. Moreover, the reduction in embodied energy (43–95%), carbon footprint (18–49.6%), and lifecycle cost-effectiveness render bio-cementation a significant step toward sustainable construction.

- Bio-cementation, whether produced by microbial or enzymatic agents (MICP or EICP), is a complex phenomenon whose kinetics can be influenced by various parameters related to the characteristics of bio-cementing agents (rate of urease activity and bio-cementing agent condition), environmental conditions (temperature, pH, and availability of nutrition and nucleation sites), and even the method that has been used to employ bio-cementation. The increase in urease activity rate of the bio-cementing agent, which is influenced by factors, such as the type of bio-cementing agent, nutrition media, pH, and temperature, leads to an increase in the number of precipitated crystals and a simultaneous decrease in the crystals' dimensions. The influence of pH and temperature on bio-cementation depends upon the specific bio-cementing agent employed, as each bacteria possesses its own distinct ideal pH and temperature conditions for enzymatic activity.
- Among the different methods for introducing the bio-cementation agent into the system, the injection method is the primary technique used in soil stabilization. Despite the presence of several drawbacks and obstacles, which appear to be resolvable through the fine-tuning of the injection process, this method appears to be better suited for large-scale applications in this sector. Building construction, on the other hand, mainly uses bacteria or enzyme solutions and nutrition media as admixture components of construction materials.
- *Sporosarcina pasteurii* is widely recognized as the predominant microorganism employed for bio-cementation due to its superior performance in terms of both quantity and quality of the precipitated CaCO_3 , as well as the enhanced permeability and mechanical properties of the treated material. *S. pasteurii* has a biological mechanism that enables it to remain in a dormant state for an extended duration until it is exposed to a favorable environment where it can resume its activity of precipitating calcium carbonate. Moreover, it exhibits the ability to endure severe alkaline environments with a pH level as high as 13.6, as well as temperatures of 55 °C for a duration of 4 h, while persisting in the process of precipitation, although at a reduced rate.
- The results of the comparison between samples treated with microbially induced calcium carbonate precipitation (MICP) and samples treated with enzymatically induced calcium carbonate precipitation (EICP) by *S. pasteurii* indicate that the EICP treatment yields superior mechanical and permeability properties. However, the MICP process is favored by a larger number of investigations due to its cost-effectiveness. One of the reasons for this outcome is attributed to the disparity in size between the bacterium (on the order of micrometers) and the enzyme (on the order of nanometers), which facilitates the migration of enzyme within nucleation sites, leading to improved homogeneity and enhanced favorable properties.
- The granularity of the treated particles is a crucial factor that greatly influences the homogeneity of bio-cementation and the treatment outcomes. An excessive amount of either fine or coarse grains will lead to an uneven treatment outcome, resulting in poor mechanical and impermeability properties. The homogeneity and outcome of bio-cementation are enhanced when a wide range and variation of particle sizes are used.

5.2. Future Research Directions

Upon thorough examination of the research studies included in this review article, as well as those initially selected but ultimately excluded from the bibliometric analysis, it becomes evident that further exploration is required to enhance our comprehension of various elements regarding exploiting bio-cementation. Despite the extensive study of *S. pasteurii* as the predominant bacterium used for bio-cementation, a comprehensive understanding of the process outcomes based on all the influencing parameters for optimization remains elusive due to the multitude of influencing parameters. This is due to the fact that the existing literature primarily focuses on the examination of one or two parameters at a time, while several other parameters vary between different studies. The knowledge gaps and

the direction of future investigations for a better exploitation of bio-cementation can be outlined as follows:

- While there is a large body of scientific literature dedicated to using bio-cementation for new construction materials, exploiting this phenomenon for remediation of existing damaged construction (existing concrete structures) to extend their service lives and avert demolition, thereby decreasing a portion of the construction waste, has not progressed as much. For example, there is a scarcity of research that has explored the potential application of bio-cementation for repairing cracks in concrete structures that do not already contain a bio-cementing agent as an admixture.
- The current literature has approached employing bio-cementation from a construction standpoint, while the complexity of the bio-cementation phenomenon emphasizes the necessity of a multidisciplinary approach to exploiting bio-cementation at the intersection of biology, material engineering, and building construction, which is essential for addressing numerous unresolved inquiries pertaining to the exploitation of bio-cementation. Which bacterial strains or enzymatic agents demonstrate compatibility and suitability for applications in soil stabilization or building construction? Does the soil or construction material offer an ideal environment for the proper activity of the bio-cementing agent? Does achieving the optimal condition for the bio-cementing agent necessarily result in the attainment of optimal material qualities that are suited for the purposes of soil stabilization and construction applications?
- A key step in the process of scaling up bio-cementation is conducting a comprehensive and systematic analysis of the biological composition of bacterial urease activity and the impact of various environmental factors on the bacterial CaCO_3 precipitation quantity and quality to gain a better understanding of the influential factor for one specific bacterium. Being able to describe the kinetics of CaCO_3 precipitation as a function of pH, temperature, and nutrition media provides a more comprehensive understanding of potential results and constraints. For example, conducting a comprehensive investigation on the impact of temperature on the activity of urease in *S. pasteurii*, spanning from $-5\text{ }^\circ\text{C}$ to $55\text{ }^\circ\text{C}$ at $5\text{ }^\circ\text{C}$ intervals, can provide valuable insights into the consequences of freezing, the kinetics of precipitation in relation to temperature, and the temperature thresholds for bacterial viability and urease enzyme denaturation. In this context, using a multidisciplinary strategy in addressing this subject offers a broader and more extensive opportunity for experimental characterization. For instance, optical density (OD), which is a widely utilized method in microbiology for assessing cell viability in the context of microbiologically induced calcium carbonate precipitation (MICP), can offer valuable insights into the performance of bacteria and the kinetics of CaCO_3 precipitation as a function of temperature, pH, and nutrition media, paving the way for significant input information for future numerical models.
- Another underdeveloped aspect in the literature pertains to the numerical simulation models of bio-cementation. The significant constraints on studies are primarily attributed to the complex nature of the process, which occurs across different time and dimensional scales and entails interconnected biological, chemical, hydraulic, and mechanical phenomena. The extremely limited research on this subject concentrates mostly on greater scales beginning with the pore in the treated material, without taking into account interrelated parameters [15,140]. Figure 5 presents a schematic illustration of the proposed flowchart by the author for the interrelated elements in bio-cementation that need to be taken into account as inputs for numerical modeling of this phenomenon, including pertinent disciplines and dimensional scales. The dimensional scale ranges from the nano- or microscale, depending on the use of EICP (nanometers) or MICP (micrometers), to the metric scale of treated construction material or soil. The multidisciplinary nature of bio-cementation involves the incorporation of diverse interrelated parameters from several disciplines at different stages as inputs. First, it is fundamental to establish the rate of urease activity from a biological perspective in relation to environmental conditions, including the initial pH, temperature, and

nutrition media, to predict the rate and amount of CaCO_3 precipitation. As stated in Section 2, the bio-cementation process produces ammonium as a byproduct, leading to a pH increase of 1 to 2 units (Figure 3). This pH alteration subsequently influences the rate of urease activity. Hence, the starting pH and pH variation as a function of time with calcium carbonate precipitation must be addressed when determining the urease activity rate. On the other hand, as CaCO_3 precipitates, the availability of nutrition media for bio-cementing agents reduces with time, affecting urease activity once more. In parallel, parameters regarding treated material, such as application method and grain properties, that are specific for each application field, must be considered. For instance, in the case of soil stabilization by injection, the injection flow velocity and granularity of the treated soil are critical for fluid dynamic studies of injected solution propagation and bio-cementing agent migration. After taking into account all of these distinct characteristics from various disciplines on different dimensional scales, an accurate prediction of the bio-cementation process will be achievable.

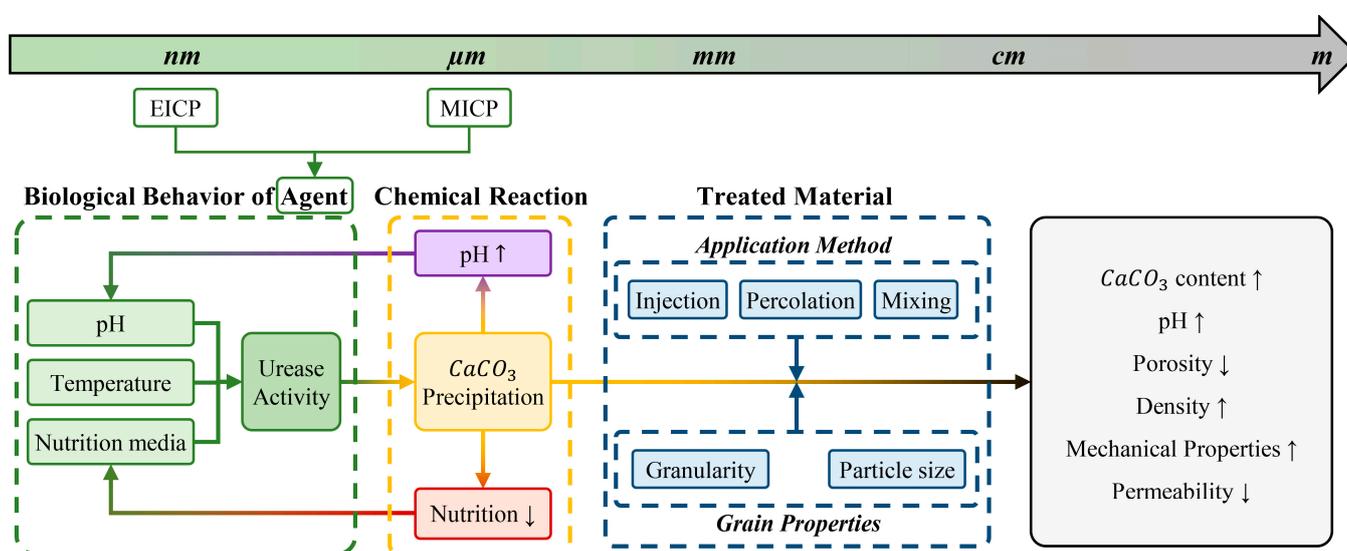


Figure 5. Schematic representation of interconnected parameters in bio-cementation for numerical modeling of this phenomenon at different dimensional scales.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/su151813869/s1>, Table S1. Selective examples of studies employing *S. pasteurii* for soil stabilization via surface percolation and mixing techniques.

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