

Review

State-of-the-Art Review of the Applicability and Challenges of Microbial-Induced Calcite Precipitation (MICP) and Enzyme-Induced Calcite Precipitation (EICP) Techniques for Geotechnical and Geoenvironmental Applications

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Citation: Almajed, A.; Lateef, M.A.; Moghal, A.A.B.; Lemboye, K. State-of-the-Art Review of the Applicability and Challenges of Microbial-Induced Calcite Precipitation (MICP) and Enzyme-Induced Calcite Precipitation (EICP) Techniques for Geotechnical and Geoenvironmental Applications. *Crystals* **2021**, *11*, 370. <https://doi.org/10.3390/cryst11040370>

Academic Editor: Cesare Signorini

Received: 16 February 2021

Accepted: 30 March 2021

Published: 1 April 2021

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Abstract: The development of alternatives to soil stabilization through mechanical and chemical stabilization has paved the way for the development of biostabilization methods. Since its development, researchers have used different bacteria species for soil treatment. Soil treatment through bioremediation techniques has been used to understand its effect on strength parameters and contaminant remediation. Using a living organism for binding the soil grains to make the soil mass dense and durable is the basic idea of soil biotreatment. Bacteria and enzymes are commonly utilized in biostabilization, which is a common method to encourage ureolysis, leading to calcite precipitation in the soil mass. Microbial-induced calcite precipitation (MICP) and enzyme-induced calcite precipitation (EICP) techniques are emerging trends in soil stabilization. Unlike conventional methods, these techniques are environmentally friendly and sustainable. This review determines the challenges, applicability, advantages, and disadvantages of MICP and EICP in soil treatment and their role in the improvement of the geotechnical and geoenvironmental properties of soil. It further elaborates on their probable mechanism in improving the soil properties in the natural and lab environments. Moreover, it looks into the effectiveness of biostabilization as a remediation of soil contamination. This review intends to present a hands-on adoptable treatment method for in situ implementation depending on specific site conditions.

Keywords: enzyme-induced calcite precipitation; microbial-induced calcite precipitation; geotechnical engineering; geoenvironmental engineering

1. Introduction

Improving soil properties has become inevitable when finding available places with soils of considerable strength is difficult. Instead of finding areas with soil of good geotechnical properties, soil improvement using soil stabilization techniques in the desired location seems preferable. Soil stabilization focuses on the improvement of the soil's bearing capacity and the reduction of settlement and deformation [1–4]. Ground improvement is most effectively addressed through soil stabilization. Researchers have tested various techniques of stabilization, and some have been vastly implemented in the field, specifically in the past four decades. Land with good soil performance is becoming scarce due to population growth. Therefore, using techniques to improve the performance of existing soil has become necessary. Among the soil stabilization methods, mechanical and chemical stabilization are widely acclaimed. Mechanical stabilization involves the process of densifying the soil mass by expelling air voids with nominal variation in water content for better

performance, whereas chemical stabilization involves amending the soil with additives to achieve the desired density, reduce permeability, or improve soil strength [5].

Chemical stabilization utilizes cementitious materials like lime, asphalt, and chemicals such as silicates and polymers, and Portland cement. These affect the chemical form of the soil matrix, improving its geotechnical behavior [6]. Chemical stabilization has attracted greater attention due to its effectiveness in soil improvement using traditional binders with a calcium base, like lime, fly ash, and cement, or novel stabilizers, like acids, salts, lignosulfonates, enzymes, petroleum emulsions, resins, and polymers [7]. In this method, the additives must be mechanically mixed with the soil in its natural state. With the influence of a specific chemical stabilizer on the site, the additive mixed must be properly distributed in the soil mass to ensure its effectiveness [8].

Stabilization techniques also include physical methods wherein soil is reinforced to achieve more strength and reduced settlements using reinforcing bars, strips, grids, fibers, and sheets [9]. Due to the rapid population growth, the scarcity of land for construction has increased, leading to construction activities on problematic soils [10]. Using stabilization techniques for problematic soils ensures the safety of structures built on them by improving soil performance against loading. Therefore, soil stabilization serves vital purposes in civil engineering. Apart from increasing the soils' strength, reducing their permeability, improving their bearing capacity, and filling voids, contaminant remediation has also been used to reduce the hazardous effects of pollutants (heavy metals) present in the soils due to anthropogenic activities. Heavy metal contamination in soils is a threat because heavy metals intrude in the food chain and cause hazardous effects [11]. Many techniques are being developed to reduce or recover heavy metals from polluted sites. Physical and chemical methods are proven to be effective in removing a wide spectrum of pollutants. However, the process consumes a lot of energy and may require extra effort to reach the desired level of heavy metal removal [12]. The use of soil stabilization techniques has been proven to be useful in both geotechnical and geoenvironmental applications.

Biologically mediated soil modification is also an emerging trend in soil stabilization. Mitchell and Santamarina [13] explored the possibility of using the biological components of rocks and soils to trigger interests in biological applications in geotechnical engineering. Dejong et al. [14] considered their work as the first of its kind. They also quoted the National Research Council of USA [15] regarding the biogeotechnical field being an important research area in the 21st century. Ants and termites amend soils, making tunnels water-resistant; this shows that biogeotechnical processes happen naturally [16]. Nature has always been an inspiration to humans for exploring possibilities of reaping benefits by replicating natural phenomena. One similar attempt is made in soil stabilization by domesticating microbes to improve soil performance. Mineralization through microbes such as bacteria, fungus, and algae is observed in nature [17], and the mineralization process by using bacteria has various applications in engineering [18]. Bacterial intrusion called microbial-induced calcite precipitation (MICP) in soil treatment improves soils' geotechnical properties through the precipitation of calcium carbonate (CaCO_3), binding soil grains together [19]. Soil stabilization through microbes, which precipitate CaCO_3 , is applied to different soil types like liquefiable soils [20], sand [21–23], sandy soil [24], and tropical residual soils [10]; it is used for the remediation of porous media [25] and the restoration of calcareous stone materials [26]. MICP is even used to seal rock fractures [27], treat wastewater [28], and reduce beach sand erosion [29].

With the understanding of calcite precipitation in the soil through the bacterial method, a similar method of precipitation without bacterial intrusion in the soil was attempted by directly using the urease enzyme, which precipitates calcite. This method of soil stabilization through the precipitation of CaCO_3 with the use of enzymes instead of microorganisms is called enzyme-induced calcite precipitation (EICP) [30–33]. This method also has a wide range of engineering applications for soil treatment such as stabilizing slopes, avoiding erosion due to wind and water, reducing the scouring of soil, checking the seepage beneath levees, improving the bearing capacity of soil, tunnelling, and controlling seismic

settlement [34] and dust [35]. Further biostabilization of soils has also found its way in the remediation of contaminants. This review considers the available research studies conducted on the EICP and MICP techniques and discusses their applicability and challenges in geotechnical and geoenvironmental applications.

2. Overview of the Microbial-Induced Calcite Precipitation (MICP) and Enzyme-Induced Calcite Precipitation (EICP) Methods

In MICP, precipitates of calcium carbonate are produced by a combination of dissolved calcium ions and urea produced by the urease bacteria after hydrolysis [36]. Due to the complexity of the cultivation of urease-producing microorganisms and the uncontrollability of enzymatic activities, urease activity is incited directly with enzymes, specifically with urease [37–39]. The enzyme-mediated precipitation of calcite is achieved without any bacterial activity. The EICP method is used to improvise the geotechnical properties of soils by using an aqueous chemical solution that precipitates calcite within soil voids. The precipitates help in roughening and binding soil grains and even in pore filling, thereby improving the strength and stiffness of the soils. The EICP method is also distinguished from MICP by its use of free urease instead of bacteria. Enzymes can be derived from microbes, fungi, and agricultural sources [40,41].

Hydrolysis of Urea

The process before the precipitation of CaCO_3 in soil voids in biotreatment starts from urea hydrolysis initiated by the urease enzyme. During urea hydrolysis, the decomposition of urea leads to the formation of carbon dioxide and ammonia. The water in the system helps ammonia to dissolve and form hydroxide and ammonia ions. These ions create an environment that allows an increase in the solution's pH. Simultaneously, carbon dioxide dissolves in water and develops ions of bicarbonates and hydrogen due to the increased pH of the environment; carbonates are formed due to the reaction between bicarbonate and hydroxide ions, forming carbonate ions and calcium carbonate in the presence of calcium ions; the calcium carbonate formed is precipitated because of its low dissolution rate in water [42]. Urea hydrolysis can be imitated either through the urease produced by bacteria or directly by using the free urease enzyme. Therefore, the reactions that take place in the soil is common in both MICP and EICP, but these two methods differ in terms of the source that initiates the hydrolysis [43]. Equations (1) and (2) show the chemical reactions that represent urea hydrolysis, leading to the precipitation of CaCO_3 [44].

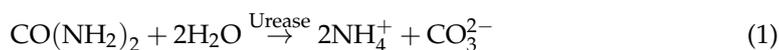


Figure 1 presents the EICP and MICP mechanisms. The precipitation of CaCO_3 by microbes was tested with and without urea by Golovkina et al. [45], who inferred that two metabolic routes—autotrophic and heterotrophic—were responsible for the precipitation of CaCO_3 in the soil. Precipitation with urea was initiated with the usual urea hydrolysis, and the urea-free medium also successfully precipitated calcite at low pH values with different bacteria strains.

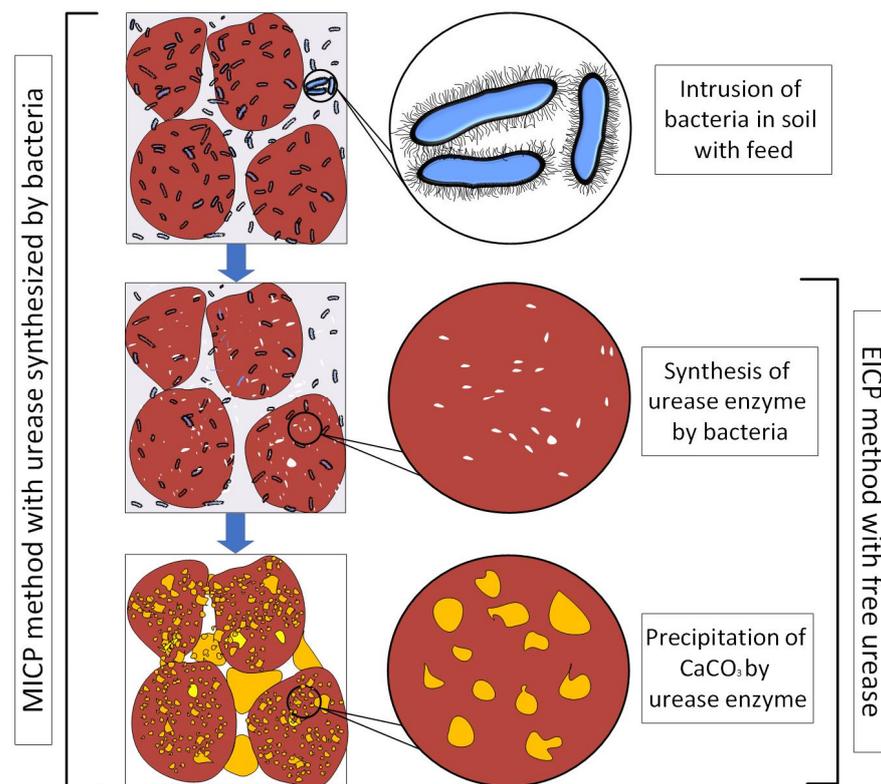


Figure 1. Mechanisms of microbial-induced calcite precipitation (MICP) and enzyme-induced calcite precipitation (EICP) in the CaCO₃ precipitation.

3. Bacterial Precipitation of CaCO₃ in Soils

Bacteria can adapt to varying environmental conditions due to their physiology and genetics, which is because they have existed in nature since three and a half billion years ago [46]. Some important features of microorganisms are that they bear cells with a simple structure without an enclosed nucleus. With multiple chromosomes and unique chemical compositions, microbes are characterized and classified by their cell wall, nutrients, RNA, DNA, and type of biochemical changes [47,48]. Bacteria are the most widely found microbes in soils. A bacterial cell has a diameter in the range of 0.5–3.0 μm , with an elongated, spiral, or spherical shape [49]. The bacterial activity in producing calcite for soil treatment involves various bacteria. Burbank et al. [21] studied CaCO₃ precipitation through biological mediation using indigenous bacteria and found them effective in increasing the liquefaction resistance of sands. They concluded that using indigenous bacteria is advisable to make soil bioremediation more economically feasible. Lee et al. [50] studied the improvement of soil properties using organic materials and found a 1.5–2.5 times increase in soil strength when compared with samples without an organic stabilizer. The stabilizer used was an organic acid material named Con- α , which was developed by Osaki Corp. in Japan. It allows microbe proliferation with aging. The importance of using this organic material is to ensure safety for the environment. pH tests confirmed that the organic acid was eco-friendly. The tests were conducted by preparing samples mixed with 3% and 6% of the organic biostabilizer by weight of the soil, which were tested for different ages. The authors concluded that the pores in the soil were filled with matter produced by the microbes, improving the soil's strength. Although MICP has potential for soil improvement, upscaling this method, optimization and training/educating the technicians on its effective applications are identified as challenges in its implementation [51]. To grade the worth of MICP, the rate of CaCO₃ precipitation is said to be 60 kg/m³ of soil [52]. MICP is carried out by using bacteria such as *Sporosarcina pasteurii* (*S. pasteurii*)/*Bacillus pasteurii* [21–25,50,52–57], *Idiomarina insulisl-sae* [24], *Pseudomonas putida* [54], *Bacillus cereus* [58], *Bacillus sphaericus* [59], and indigenous

bacteria [60]. MICP is even used for the improvement of the performance of construction materials [54], sediment stabilization [61], and reduction of coastal erosion [62].

Soon et al. [10] used MICP for the improvement of the engineering properties of soils. The species of bacteria used was *Bacillus megaterium*, combined with other cementing reagents. They found that the CaCO_3 precipitates were effective in soil stabilization, capable of improving shear strength, and even useful in reducing the hydraulic conductivity of soil and sand. Proto et al. [63] studied the reduction of the permeability of saturated sand through the formation of biofilms on the surface of the sand grains, biofilms being the accumulation of cells and extracellular polymeric substances in an organic process. The process of bioaugmentation was incited by strains of non-native bacteria injected in the sand, and bacteria already present in the sand contributed to the precipitation. The use of biofilms resulted in a considerable decrease in the permeability of the soil. Bioaugmentation is a commonly used method for removing contaminants from soil mass; this process is initiated by allochthonic or autochthonic microbes against nondegradable organic matter from soil [64]. The transformation of harmful compounds into different forms by using bacteria should be possible, showing bioaugmentation [65]. The use of additives, along with microbes, has also been tested for the improvement of soil performance. Zhao et al. [66] used fiber felt scrap (activated carbon) with the MICP technique to treat sand. They observed that unconfined compressive strength (UCS) and tensile strength improved, showing the possibility of using a discarded scrap waste material along with MICP treatment to improve soil strength.

4. Enzyme Usage and Sources for Soil Treatment

The most widely studied enzyme source for soil treatment is the jack bean plant, technically termed *Canavalia ensiformis*. This plant is a draught-resistant species classified in the Fabaceae family [34]. Larsen et al. [67] reported that calcite precipitation increases ten times with the use of the jack bean meal instead of pure urease. The urease enzyme was first crystallized in 1926 by James B. Sumner [68–70]. Oliveira et al. [71] conducted a study on the effect of soil type on the precipitation of calcium carbonate by EICP treatment in the soil by jack bean urease for its improvement. They found that the precipitation of CaCO_3 increased the strength of the soil by 40–106%, but the test proved ineffective for organic soils. Renjith et al. [5] used a commercially available enzyme-based additive named “Eko soil” for the construction of unpaved roads in Australia. They found that the treatment methods could be used for cost-effective and sustainable unpaved roads. They also surveyed other enzymes that were commercially available or manufactured from fermented matter, which is converted into a chemical, liquid, or organic form. Javadi et al. [72] used urease enzyme extracted from watermelon seeds. They found that the theoretical maximum precipitation of calcite was around 64%, which was considered promising for soil treatment using urease extracted from watermelon seeds. It is also important to note that using enzymes for soil stabilization is expensive; the cost of enzymes is equal to 90% of the total cost of materials used [73,74].

Use of Additives in the Biotreatment

The use of additives, along with the urease enzyme, in the biotreatment has also been tested to improve the precipitation process for better soil performance. Almajed et al. [75] used non-fat milk powder as an additive to improve the urease activity and obtained surprising results wherein the amount of CaCO_3 precipitated and the UCS values were better than those for soils treated with enzyme solutions without non-fat milk powder. It was noted that the amount of calcite precipitated is not regarded as an indicative factor of increase in strength; rather, the precipitation pattern governs the improvement in the geotechnical properties of soils; that is, even low carbonate precipitation with a suitable pattern may lead to higher strength compared to high carbonate precipitation. The enzyme treatment is performed with an aqueous solution consisting of urea, calcium chloride, and urease enzyme in deionized water for mixing/injecting in the soil for calcite

precipitation [76,77]. Hamdan and Kavazanjian [78] also used non-fat milk powder as a stabilizer, along with urease, in the enzyme solution to test its effectiveness in fugitive dust control. They observed that the treatment with the enzyme resulted in resistance to wind erosion. Putra et al. [79] used magnesium chloride as a substitute in the enzyme treatment to precipitate CaCO_3 . They found that the ratio of precipitation was 90% of the theoretical maximum, which was obtained with the addition of a small quantity of magnesium chloride. The use of magnesium resulted in a lower precipitation rate, resulting in the higher injectivity of the enzyme solution. It changed the shape of CaCO_3 precipitates, simultaneously precipitating aragonite along with calcite. The use of additives to improve the efficacy of the enzyme treatment on soils has become an important part of research. Yuan et al. [39] used soybean urease for silt improvement in flooded areas and with urease. Additional materials, like glutinous powder of rice, brown sugar, and skim milk in powdered form were used to reinforce the urease activity. Hommel et al. [80] developed a numerical model for the EICP method to simulate the outcomes for different dosages of the enzyme solutions in any experimental setup. They developed a model that could give qualitative outcomes for the experimental setup modeled in the program. Therefore, EICP and MICP can be tested for their proposed outcomes using a numerical model before conducting the experiments physically.

5. Geotechnical Applications of the Biocementation Technique

The use of biostabilization methods wherein CaCO_3 precipitation helps in improving soils has attracted the interest of geotechnical engineers substantially [81–83]. The biotreatment of soils further needs suitable environmental conditions to achieve the desired outcomes through the precipitation of CaCO_3 [54,84]. However, the use of an enzyme-based stabilization method depends on factors like type of soil, method of construction, curing, and temperature, which may result in poor outcomes unless a suitable adjustment is not made to control the hindrances as per the type of enzymes [5].

5.1. Biotreatment Techniques

Mujah et al. [44] reported that MICP is effectuated by the injection, surface percolation, and premixing methods. In the injection method, the treatment solution is injected in the soil. In the premixing method, soils are mixed with the bacterial solution before dumping the soil in its place to serve the intended purpose. In the surface percolation method, the cementation solution is made to be absorbed in the soil from the surface. Wiffin et al. [81] tested the biotreatment through the injection method on a 5 m long column of sandy soil. They observed that the precipitation of CaCO_3 was not even along the length of the column. Sotoudehfar et al. [85] studied the factors influencing MICP applied through the injection method. They used a specially designed pump for injecting the cementation fluid into the soil. They found positive outcomes with their injection method of implementing MICP on soils. The injection method of the biotreatment was carried out by two phase injection procedures wherein initial bacterial strains were injected; later, bacterial feed was injected. Stocks-Fischer et al. [25] reported that injecting bacteria and reagents together may result in clogging at the injection site, especially when the flow rates of the fluids are low in the soil. The injection technique may be suitable when the treatment fluid is of low viscosity. In contrast, injection or biogrouting may need substantial pumping energy to achieve the desired soil strength [86]. The inoculation of bacterial strains in soils is also practiced for contaminant removal from soil mass [65].

Almajed et al. [40] studied the EICP treatment through percolation and premixing methods applied on Ottawa 20–30 sand. They interpreted their findings by comparing both methods of treatment and found that premixing was not effective in maintaining the intactness of the sand specimen, whereas the percolation method portrayed better results. An intact specimen, which could be easily tested for its strength and percolation method, also provides good interparticle bonding. Neupane et al. [87] used percolation for EICP on sand. They observed an almost uniform distribution of calcite precipitates in sand

at 5 °C, whereas precipitation was reduced to 5% at a temperature of 23.5 °C. It can be understood that implementation techniques also play a vital role in the biotreatment of soils, and specific methods of implementation can be devised depending on the soil type and the environmental conditions.

5.2. Effect of Biotreatment on the Unconfined Compressive Strength (UCS) Test

The biotreatment of soils is well understood for its degree of effectiveness by UCS values [36,88–90]. Ali et al. [91] stated that the UCS test is most trusted to ascertain the effectiveness of soil stabilization methods. Sharma and Ramkrishnan [92] studied soils (fine grained) treated with MICP and observed the improvement in the UCS value of soil. They also inferred that the particle packing plays an important role in improving soil strength and even leads to improved bearing capacity, reduced settlement and permeability, and diminished shrink-swell characteristics of soils. The development of pore pressure can also be stopped with soil treatment. Strength enhancement in soils treated with biocementation methods is mainly achieved because of the adhesion of soil grains due to calcite precipitates in soil voids. Figure 2 shows a comparison of UCS values obtained for the soils before and after the biotreatment.

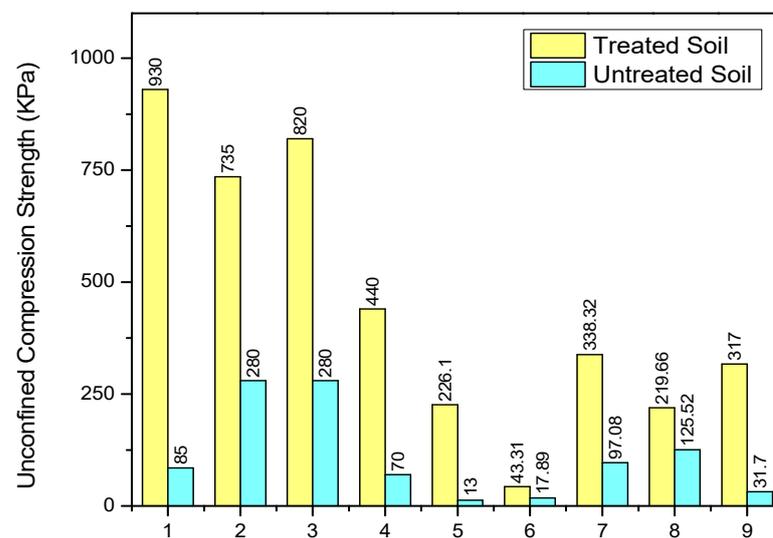


Figure 2. Comparison of unconfined compressive strength (UCS) results for soil samples before and after biotreatment. 1—Sotoudehfar et al. [85], 2—Wani and Mir [93], 3—Wani and Mir [93], 4—Moghal et al. [94], 5—Moghal et al. [94], 6—Xiao et al. [95], 7—Sharma and R [92], 8—Sharma and R [92], 9—Park et al. [96].

Table 1 shows the UCS test results obtained by researchers for different soils treated with MICP/EICP techniques. Yasuhara et al. [31] used free enzyme (urease) supplied by Kishida Chemical to treat sand through CaCO_3 precipitation. They found that the experiments showed the effectiveness of the enzyme treatment on UCS samples and permeability. Strength gain in the soils depends on the amount of calcite precipitated. Notably, for the substantial improvement in the stiffness and strength of soils treated with a biostabilization technique, a minimum of 4% of calcite precipitation per mass of treated soils is required [96].

Table 1. Unconfined Compressive Strength (USC) results obtained by different researchers after the biotreatment of soils.

SI No	Bacteria Type	Type of Soil	Maximum UCS Value Obtained after Treatment (kPa)	UCS Value for Untreated Soil (kPa)	Reference
1	<i>Sporosarcina pasteurii</i>	Poorly graded sand	930	85	[85]
2	Jack Bean Urease	Ottawa 20–30 sand	88.8	-	[40]
3	<i>Bacillus subtilis</i> <i>Bacillus pasteurii</i>	Dredged soils	735 820	280	[93]
4	Pararhodobacter sp.	Fine grained sand Coarse sand Mixed sand	1330 2870 2800	-	[97]
5	Jack Bean Urease	Silica sand	1745	-	[75]
6	Jack Bean Urease	Red soil Black soil	440 226.1	70 13	[94]
8	<i>Sporosarcina pasteurii</i>	Fine to medium-grain sand	12,400	-	[98]
9	Urease Enzyme	Silica sand	380	-	[99]
10	Urease Enzyme	Sand	1600	-	[100]
11	Urease Enzyme	Silica sand	600	-	[79]
12	Urease enzyme	F-60 silica sand Ottawa 20–30 sand	529 391	-	[38]
13	<i>Sporosarcina pasteurii</i>	Soft Clay	43.31	17.89	[95]
14	<i>Sporosarcina pasteurii</i>	Sand (commercially available)	14,000	-	[101]
15	<i>Sporosarcina pasteurii</i>	Fine grained soil (CL) Fine grained soil (CH)	338.32 219.66	97.08 125.52	[92]
16	Jack Bean Urease	Poorly graded silica sand	555	-	[102]
17	In-situ soil bacteria	Poorly-graded sands	5300	-	[103]
18	Urease enzyme from watermelon seeds	Mikawa sand	3000	-	[104]
19	Jack-bean extract	Nakdong River sand	317	31.7	[96]
20	Jack Bean urease	Ottawa sand	1700	-	[105]
21	Jack Bean urease	Ottawa 20–30	1600	-	[106]
22	Terrazyme	Clay with low plasticity	1073	-	[107]

5.3. Reduction of Hydraulic Conductivity by Biotreatment

The use of biotreatment methods for permeability reduction in soils has led to effective results [10,63,90,108–112]. One of the reasons that leads to the reduction in soil permeability is the cementation of soil grains with precipitates of CaCO_3 , leading to the blockage of connected pores in the soil mass. Although permeability reduction due to biotreatment depends on the size of soil grains, finer soils have miniscule flow paths due to the proper packing of soil grains; even the size of precipitates plays an important role in reducing permeability. Sometimes, suspended precipitates also get accommodated in the voids of the soil mass, thereby contributing further in the reduction of flow paths [108,113]. Cuthbert et al. stated that permeability reduction due to the precipitation of CaCO_3 depends on the quantity of precipitates; that is, with more precipitates, permeability reduction will be higher [114]. Ferris et al. [115] studied the use of bacterially precipitated calcite as a plugging material in porous media and proved a permeability reduction in the sand tested. They concluded that a 40% increase in bacteria paved the way for a 70% reduction in sand permeability, which can be attributed to greater precipitates from more bacteria, leading to

a higher percentage of CaCO_3 precipitation in the soil mass. Reduced permeability after the soil treatment by calcite precipitates is also due to the reduction of the pore throat at the points of contact between the soil grains where precipitation occurs [82]. Gui et al. [116] used the MICP method in porous media for bioclogging. They found that permeability reduction was greater than 72%. The main reason they identified for the reduction of permeability was the formation of biofilms on the sand grain surfaces. Figure 3 shows the flow paths in a soil mass and the blockage of the flow paths by biofilms on soil grains, plugging, and sealing of pore throats with calcite after the biotreatment.

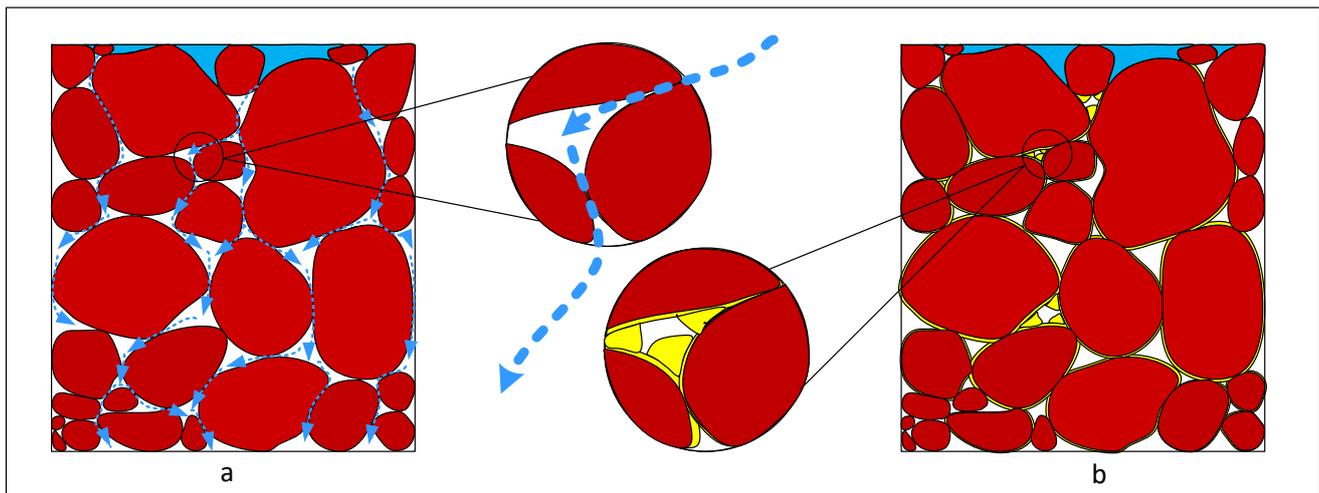


Figure 3. Schematic representation of soil mass: (a) Flow paths allowing water to percolate through soil mass. (b) Representation of the blockage of pore throats by calcite precipitation and the formation of calcite biofilms on soil grains, leading to narrowing/blockage of flow paths and reducing permeability after biotreatment.

Nemati et al. [109] compared the microbial and enzymatic processes applied for permeability reduction. They found that bacterial precipitation may include a degradable biomass developed as a plugging agent in soil pores, which may dissolve or decompose with time or after exposure to moisture, whereas enzymatically precipitated calcite proves to be a durable plugging agent that contributes to permeability reduction and is, hence, more convenient for geotechnical applications. Rittmann [117] suggested that reduction in permeability is possible because of the formation of biofilms, which reduces pore sizes after coating, clogs flow paths on the units of porous medium, and increases the friction factor of the porous medium after clogging. Chittoori et al. tested the effect of porosity, consolidation, and the unit weight of expansive clays. Their study was important in the wake of soil treatment with microbes because pore size and size of pore throat play important roles in microbial treatment [118]. Figure 4 shows the maximum reduction of permeability in percentage achieved by different researchers in their studies after the biomineralization of soils. It can be inferred from Figure 4 that permeability reduction is achieved through biocementation, which is also promising in applications like lining the base of water bodies and seepage control in water retaining structures.

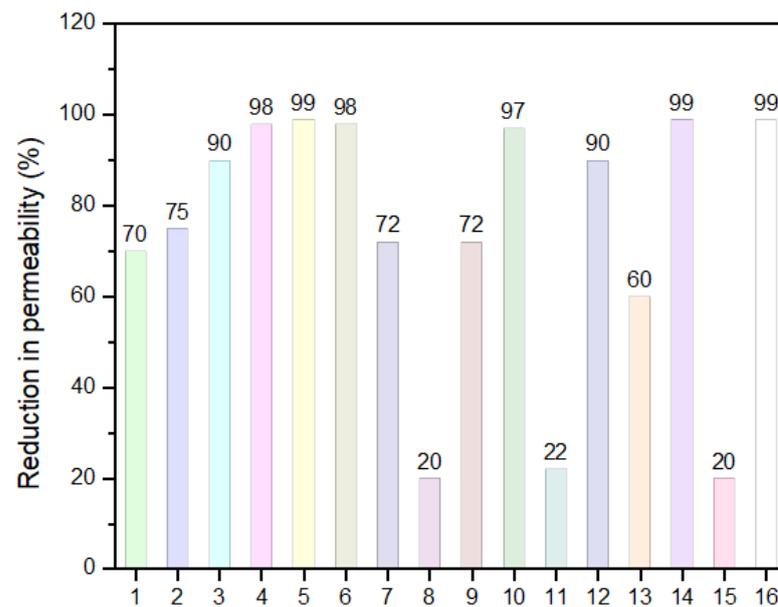


Figure 4. Maximum permeability reductions in percentage achieved by different researchers in their studies after soil biomineralization. 1—Yasuhara et al. [100], 2—Whiffin et al. [81], 3—Soon et al. [119], 4—Nemati and Voordouw [32], 5—Moghal et al. [120], 6—Handley-Sidhu et al. [110], 7—Zamani and Montoya [108], 8—Ferris et al. [115], 9—Gui et al. [116], 10—Proto et al. [63], 11—Ragusa et al. [121], 12—Cunningham et al. [122], 13—Van Paassen [123], 14—Ivanov et al. [124], 15—Al Qabany and Soga [125], 16—Ivanov and Chu [126].

5.4. Liquefaction Control by Biotreatment

Earthquakes and explosions make soils vulnerable and liquefy them, causing serious damage to the structures. Poorly graded and saturated sands are potential targets to liquefaction [127]. Major threats encountered due to the liquefaction of soils are landslides, damaged underground sewage lines and tunnels, and quicksand effects, although liquefaction helps in preventing seismic waves from reaching the Earth's surface since it produces a damping effect to the waves [128]. Liquefaction control is achieved by various techniques. Densification or compaction of existing soil is also among the methods adopted, but this method poses a threat to adjacent structures [129]. Biocementation has been proven as an effective method in controlling liquefaction in soils since calcite precipitation reduces permeability in soil [115]. Burbunk et al. [20,21] quoted that MICP improved resistance to liquefaction. Water in the voids of soils develops pressure transmitted through flow paths and tends to detach the soil grains apart or facilitate in the possible space in the vicinity. This pore water pressure contributes to the factors leading to the liquefaction of soils. Soil grains, when gelled together after biocementation, are less prone to liquefaction because of the disruption of flow paths and, later, due to permeability reduction and reduced pore water pressure [130]. Zamani and Montoya [131] tested the use of MICP on the permeability and shear of sand with silt and found a reduction in permeability depending on the amount of fines in the sample. Figure 5 shows the development of pore pressure in soil voids due to pore water and the development of resistance to the pore pressure through precipitates of calcite.

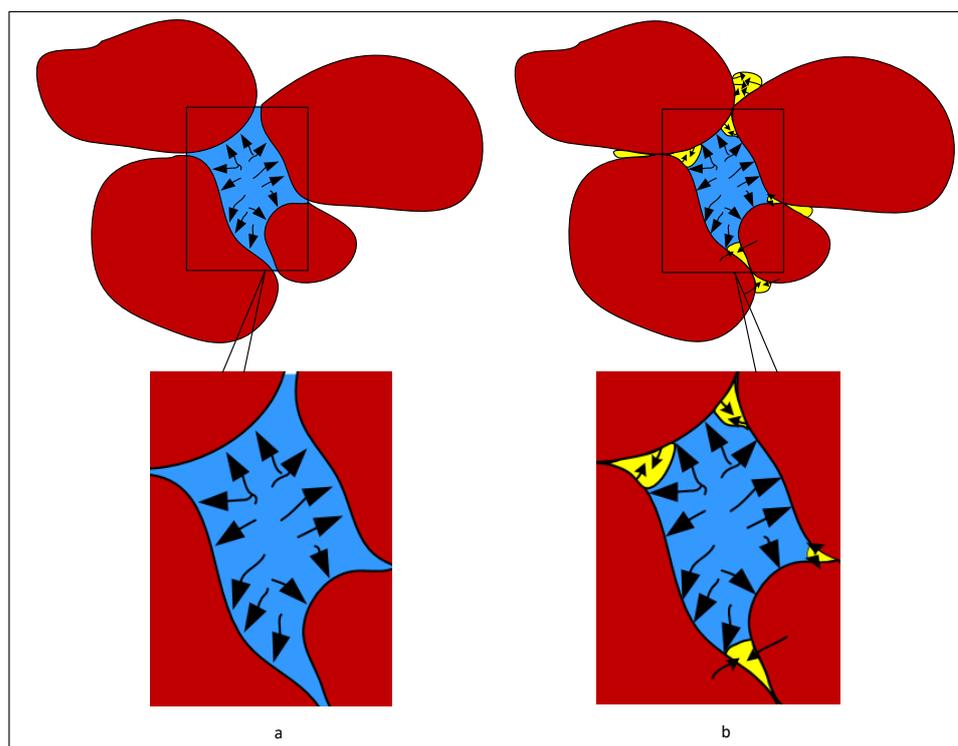


Figure 5. Representation of pore pressure in soil void: (a) Pore pressure developed by pore water pushing soil grains away from each other, leading to the loosening of the soil mass. (b) Cementation of soil grains by bioprecipitated calcite offering resistance to pore pressure.

6. Biotreatment of Soils for Geoenvironmental Applications

Bacterially precipitated CaCO_3 has also been used for capturing heavy metal contaminants to reduce their hazardous effects by converting the heavy metal traces into carbonates [12,132–135]. Bioremediation through urease in soil is effective for contaminant remediation [136]. Although natural calcite is used as an adsorbent for removing ions of heavy metal from contaminated water [137,138], the use of calcium for remediating heavy metal contaminants in soil is also practiced by researchers [139–141]. Natural calcite used as an adsorbent is very rare, and even the quality of naturally available calcite is not feasible for adsorption. Therefore, calcite precipitated by microbes was tested for the purpose of adsorption [142]. Kulczycki et al. [143] used bacterial ferrihydrite for the sorption of cadmium and lead and found that the precipitates of ferrihydrite were effective in providing sites for the heavy metal ions to sorb. Pan et al. [144] studied the microbial strategy for lead remediation. They inferred from their study that use of microbes was cost-efficient and environmentally-friendly as a lead remediation method. Velmurugan et al. [145] studied the kinetics of lead absorption by *Penicillium* sp. MRF-1 in a contaminated mining site in South Korea. Their study covered the use of this metal-resistant fungus strain for the remediation of $\text{Pb}(\text{II})$ within the dimensions of time of exposure, pH, and temperature. They concluded that *Penicillium* sp. MRF-1 was an inexpensive and conveniently cultivable fungus for the removal of Pb from contaminated solutions. Moghal et al. [94,120] used the enzyme treatment for adsorption and desorption studies for cadmium, nickel, and lead contaminants and found that the urease enzyme was effective in precipitating the carbonates of cadmium, nickel, and lead. They also obtained encouraging results in the level of desorption of heavy metals even after washing the contaminated soils with harsh extractants like ethylene diamine tetra-acetic acid (EDTA) and citric acid. The sorption studies, on the other hand, depicted better results in providing the sites on the soil grains to sorb. Sorption and desorption studies were conducted for individuals and for cocktail solutions of contaminants. These studies obtained appreciable results, encouraging the application of these techniques in situ. Nathan et al. [146] used the EICP method for heavy

metal remediation in paper pulp deinking. They found that the enzymatic bioremediation is effective in reducing the hazardous effects of heavy metals. It also established that the urease enzyme is a nickel-based enzyme [147] and suggested that calcite precipitates by urease enzyme are active in showing affinity to the nickel contaminant, encapsulating them in the precipitates, and converting them into nickel carbonates [94].

Lauchnor et al. [148] studied the co-precipitation of strontium (Sr) in the porous media along with calcite precipitated by the MICP technique. They inferred that Sr precipitation was effective, thereby indicating the effective implementation of this method on site for the remediation of Sr. Mitchell and Ferris [149] tested the use of calcite precipitated with the MICP method to coprecipitate Sr in contaminated water and found that calcite precipitated by the MICP technique was exceptionally effective in the remediation of groundwater. Sr was also remediated by the formation of SrCO₃ in microenvironments of soil mass, leading to the reduced effect of radio nucleoids [150]. Wang et al. [151] studied the effect CaCO₃ on immobilizing heavy metals and observed that CaCO₃ was successful in serving the purpose. Precipitates of calcite in the soil mass contributing to heavy metal immobilization can also be attributed to the number of heavy metal ions in the soil mass. If there are fewer heavy metal ions, then the sites for the ions to settle down will be sufficient, leading to better results in terms of the immobilization of heavy metals. Varenya et al. [152] studied lead retention using the MICP method and found that the precipitates of CaCO₃ could be effective in the remediation of lead. They concluded that the MICP method has the potential to be applied in arid areas where phytoremediation cannot be used to remove heavy metals. The MICP method can be effective in reducing the hazardous effects caused by heavy metals like arsenic, cadmium, chromium, copper, and lead [153]. Therefore, the effectiveness of the biomineralization method can be well understood. The bioremediation of contaminants is a promising technique to reduce adverse effects caused by heavy metals. Table 2 shows a brief list of heavy metals remediated by the biostabilization method.

Table 2. List of contaminants remediated by the biostabilization method.

Applied On	Bacteria Used	Reference
Toxic metals	Sporosarcina luteola	[154]
Lead-contaminated mine wastes	Pararhodobacter sp.	[97]
Lead	Bacillus (pumilus and cereus)	[155]
Lead (II)	Rhodococcus opacus	[156]
Zn(II), Ni(II) and Cr(VI)	Trichoderma viride	[157]
Cobalt and copper	Lyngbya putealis	[158]
AA 6061 nuclear alloy	Bacillus cereus RE 10	[159]
Au(III)	Bacillus subtilis	[160]
Lead	Pseudomonas aeruginosa	[161]
Cd, Ni and Pb	Urease Enzyme	[94,120]
Cr ⁶⁺	Rhodococcus erythropolis	[162]
Cu and Pb	Comamonas testosterone, Enterobacter ludwigii and Zoogloea ramigerais	[163]
Copper	Stenotrophomonas maltophilia	[164]
Nickel	Lysinibacillus sp.	[165]
Pb and Cu	Bacillus thioeparans	[166]
Cr(VI)	Bacillus cereus	[167]
Cr(VI)	Cellulosimicrobium funkei	[168]
Cd(II)	Bacillus cereus RC-1	[169]
Polycyclic aromatic hydrocarbons (PAHs)	Pseudomonas plecoglossicida J26	[170]
Cadmium	Exiguobacterium undae	[171]

Effect of Biotreatment on Mine Tailings/Dust Control

The various applications of the biotreatment of soils include the control of permeability, improvement of the bearing capacity, strength and stiffness development, and the control of dust due to erosion [172]. The air quality in the majority of cities worldwide is becoming a grave concern due to the increase in population and urbanization [173,174]. In this scenario, measures to curb the deterioration of air quality are developed to safeguard the environment. Dust contributes to the deterioration of air quality. According to Watson et al. [175], the major sources of air pollution in the major cities of the U.S. are vehicular emissions and dust from roads. With dust's severe impact on the air quality, methods to reduce dust emission are employed. These methods include using dust suppressants, spraying water, and providing wind shield walls against dust emission [176]. Chang et al. [177] reported that water spraying to control dust serves the purpose for a maximum duration of 4 h. Using water for dust suppression impacts water reserves, since water offers a temporary remedy and needs repeated application. Additionally, chemically activated suppressants for dust control are highly corrosive and hamper the environment. Sustainable and eco-friendly dust control techniques are on high demand [176]. Dust control using biotreatment can be achieved in the same way that sand solidification in the desert can be carried out; potential dust sources with dust particles can be dealt with through biocementation [178].

Sun et al. [176] conducted a study on dust near a quarry site in China by developing a simulation of rainfall erosion and by conducting field tests. Their test methods involved ascertaining surface strength, which is most vulnerable to wind erosion. Surface hardening was obtained by spraying biotreatment solutions on the surface; after spraying a thin and hard calcite on the surface, a crust of soil was formed. They confirmed that the cementation of dust particles using CaCO_3 precipitates through the enzyme treatment reduced dust pollution and that implementing EICP for dust control could be efficient during sandstorm and rainfall. Meyer et al. [56] used *Sporosarcina pasteurii* to treat two soil types to control air pollution due to dust. The treated soils were made to pass through a wind tunnel, and the amount of reduction in soil mass after exposure in the wind tunnel was observed to express the amount of wind erosion. The results obtained from this work showed that microbially precipitated calcite was very much effective in controlling soil erosion, as proven by wind tunnel experiments. Naeimi and Chu [179] compared the effectiveness of dust control through the biotreatment method and conventional techniques. *Sporosarcina pasteurii* was used in their study to treat sand against dust emission. The comparison was done on the same sand treated with calcium lignosulfonate, water, and calcium chloride. Their results showed that the biotreatment of soil exposed to wind in the wind tunnel improved erosion resistance, and only 1.5% mass loss was observed. Other treatment methods showed greater loss in mass after wind tunnel testing; hence, the use of biomediated soils was the best treatment method used in their study. Table 3 provides different bio-stabilizers adopted for dust control in different non-plastic materials.

Table 3. Bio-stabilizers adopted for dust control in different non-plastic materials.

Specimen Tested	Bio-Stabilizer	Reference
Coal dust	MICP (Urease microbes)	[180]
Coal dust	MICP (<i>Staphylococcus succinus</i>)	[181]
Desert soil	MICP (Indigenous bacteria)	[182]
Sand	MICP (<i>Sporosarcina pasteurii</i>)	[179]
Sand (well-graded)	MICP (<i>Sporosarcina pasteurii</i>)	[56]
F60 silica sand	EICP (Urease enzyme)	[183]
Ottawa F-60 fine grained uniform silica sand Well graded silty fine sand Mine tailings	EICP (Urease enzyme)	[78]

The erosion of deposits of mine tailings caused by wind erosion is one of the most serious environmental concerns [184,185]. Wind carrying mine tailings poses a threat to water bodies nearby and deteriorates air quality, which means serious risks to human and animal health [186]. Controlling dust by suppressants sprayed on the deposits is a common method. Dust suppressants agglomerate the fine particles and check the possibility of dust/finer mine tailings escaping with the wind movement; agglomerated dust particles form dense deposits on the ground, trapping dust sources beneath [187]. Chen et al. [188] conducted a study on the reduction of dust from mine tailings with a biopolymer coating on deposits. They found that the biopolymer coating was effective in mitigating mine tailing dust. Govarthanan et al. [189] used bacteria for the mineralization of lead contaminants found in mine tailing. They found that precipitates of calcium carbonate mineralized by bacteria were effective in lead bioremediation. Bacteria used in their study were effective in changing nitrates of lead to silicon oxides and sulfides of lead, thereby reducing the severity of lead on the atmosphere. Zamani et al. [190] studied the effect of MICP on mine tailing stability and found that microbial precipitates of CaCO_3 were effective in improving the stability of slopes of mine tailing materials. It can be observed from the literature that the remediation of heavy metals and dust control from mine tailings are well addressed by the biocementation process.

7. Limitations of Biocementation Techniques

Miftah et al. [43] reviewed the effectiveness of the MICP and EICP techniques in soil improvement and expressed that these methods could be effective in many geotechnical applications. However, certain concerns limit the effectiveness of these techniques. In the MICP method, concerns such as the type of soil, environmental issues, and the uniform treatment of soil mass are factors that create problems for its application. In the EICP method, the cost of enzymes happens to be too high since 57–98% of the cost of enzyme solutions is incurred on the urease enzyme. The soil type also plays an important role in governing the effectiveness of the biotreatment. The MICP method is restricted to the subsoil, and other regions of the soil may not provide a feasible environment to the bacterial growth. MICP does not show good results when used on very fine soils because comparatively larger sizes of bacteria cannot be accommodated in the pores of fine soils. On the other hand, EICP does not pose any hindrance in its application due to its size. Miftah et al. [43] also discussed the environmental concerns related to the use of MICP. This technique leaves microbes in soils after treatment, which means that it may require the permission of the concerned authorities and regular inspection to ensure that the energy of microbes is not hazardous to the surroundings. Furthermore, the release of ammonia through MICP is dangerous to people and the ecology of the area where it is applied, especially to the air and water. Additionally, the increase in pH may develop potential corrosion, and further contamination of groundwater due to chloride may be possible after the precipitation of CaCO_3 . On the other hand, urease used in the EICP technique may not have a long-term impact on the environment because it becomes degraded after a certain time period. The use of microbes for soil treatment needs a specific environment in the soil mass for their cultivation, and the storage of bacterial strains is an expensive process. With these limitations in the use of alternate means for calcite precipitation, EICP seems to be better than MICP [191,192]. It has also been observed that high concentrations of calcium chloride and urea hinder the bacterial activity, reducing the amount of calcite precipitation. Conversely, using enzymes can very well be possible with high concentrations of calcium chloride and urea, which paves the way for a greater amount of calcite precipitation [109]. Therefore, the EICP technique is preferable over MICP. Yasuhara et al. [100] mentioned that maintaining bacteria for their cultivation requires technical expertise. Controlling bacterial activity also poses a challenge in the MICP method; the EICP technique is free of this constraint.

8. Conclusions

Biostabilization of the soil is an emerging trend with relatively simple onsite applications; the application of the stabilizer of a particular type onsite is carried out by injecting the stabilizer in the work area. Biostabilization of soils through EICP and MICP have the potential to meet the ever-growing demands of setting new infrastructure and remediating contaminants in the soils. Furthermore, the challenge of reducing environmental pollution and developing sustainable techniques can be achieved by the implementation of these techniques. The development of precipitates in the voids of the soil is helpful in reducing hydraulic conductivity. The application of MICP/EICP has stretched to the extent that ponds can be created in regions with soils of high permeability by relying on CaCO_3 precipitates. Salient observations made from the existing literature are summarized below.

- The development of biostabilization methods has been proven to be sustainable, eco-friendly, and effective in soil treatment, leading to the improvement in the geotechnical performance of soils such as reduced permeability, reduced porosity of soil mass, improved bearing capacity, control of soil erosion/dust, mitigated liquefaction of soils, seepage control, stabilized slopes, and contaminant remediation.
- The possibility of the intrusion of bacteria in soils for calcite precipitation is limited due to their sizes. Soil pores with sizes less than 0.5μ cannot accommodate the microbes for the process of calcite precipitation since sizes of microns range from $0.5 \mu\text{m}$ to $3 \mu\text{m}$. Enzyme particulates have sizes of about 12 nm, which can make the precipitation of calcite more convenient, even in finer clays.
- Soil treatment with MICP/EICP may increase chloride and ammonium ion (formed during hydrolysis) concentrations in the groundwater due to the precipitates of CaCO_3 . It even causes an increase in the pH of the surrounding groundwater, triggering corrosion for structures built on them. The applicability of these techniques also pose challenges such as the type of soil to be treated and the associated costs. Further studies on biotreatment can address these issues and aid in developing better application methods of biotreatment.
- Between the two methods, EICP is preferred over MICP as it requires less monitoring. The literature suggests that precipitates developed through MICP are vulnerable to moisture and may dissolve. MICP requires the environment to be maintained for proper bacterial growth and the production of urease enzymes. On the other hand, in EICP, the use of free urease is more promising for the calcite precipitation in the voids of soil grains. It provides a convenient approach to soil treatment because of its ease of application and lower maintenance in comparison to the MICP method.

Author Contributions: Conceptualization, A.A.B.M. and A.A.; Methodology, A.A.; Formal analysis, A.A.B.M.; Investigation, K.L.; Resources, A.A.B.M.; Data curation, K.L.; Writing—original draft preparation, M.A.L.; Writing—review and editing, M.A.L., A.A.B.M. and A.A.; Visualization, A.A.; Supervision, A.A.B.M.; Project administration, A.A.B.M.; Funding acquisition, A.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work is funded by College of Engineering Research Center and Deanship of Scientific Research at King Saud University in Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The content presented here was sourced from existing published literature, hence, this clause is not applicable.

Acknowledgments: The authors acknowledge the College of Engineering Research Center and Deanship of Scientific Research at King Saud University in Riyadh, Saudi Arabia for their financial support for the research work reported in this article.

Conflicts of Interest: The authors declare no conflict of interest.

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