Review

State-of-the-Art Review of Enzyme-Induced Calcite Precipitation (EICP) for Ground Improvement: Applications and Prospects

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Abstract: The global construction industry consumes huge amounts of mined materials that are considered unsustainable for earth resources. In addition, Portland cement which is a key element in concrete and most construction materials is considered one of the main contributors to worldwide CO₂ emissions. On the other hand, natural cemented soil deposits are examples of sustainable structures that have survived decades of severe environmental conditions. Mimicking these natural biological systems provide an alternative to the current practices of construction materials production. Enzyme-induced carbonate precipitation (EICP) is a bio-inspired technique based on the precipitation of calcium carbonate for enhancing the geo-mechanical properties of soils. In this technique, calcium carbonate acts as a cementitious agent that binds the soil particles together at the points of contact, hence, increasing the strength and stiffness of treated soils, while relatively reducing the soil permeability and porosity. The achieved enhancements make EICP useful for applications such as ground improvement, construction materials, and erosion control over traditional binders. This paper presents a state-of-the-art review of EICP for ground improvement including the fundamental basics of EICP treatment. The paper also discusses the chemical and physical factors affecting the performance of EICP such as enzyme source, enzyme activity and solution constituents. Moreover, the paper reviews the different methods and testing techniques used in the application of EICP for soil treatment. Furthermore, the paper compares EICP with other biomineralization techniques in terms of performance and applicability on ground improvement. Finally, the paper discusses the research gaps and existing challenges concerning the commercialization and large-scale implementation of the technology.

Keywords: bio-cementation; soil stabilization; ground improvement; bio-grouting; bio-inspired treatment; urea hydrolysis; calcium carbonate; calcite precipitation

1. Introduction

Recently, more sustainable and environmentally friendly solutions have been demanded to mitigate the adverse effects of pollution on the environment. Current construction and soil improvement practices are major sources of carbon dioxide emissions worldwide due to the extensive use of ordinary Portland cement (OPC). Over the last decade

or so, extensive research has been undertaken to develop eco-friendly binders for soil improvement as alternatives to OPC [1–5]. Among all existing soil binders, bio-cementation via carbonate precipitation has shown great promise as a bio-inspired technique to enhance soil geo-mechanical properties. Bio-cementation is inspired by living organisms that use organic and inorganic compounds to build strong and rigid materials [6]. Several techniques can be used to induce bio-mediated or bio-inspired carbonate precipitation, including urea hydrolysis, microbial denitrification, and sulphate reduction [7,8]. However, the hydrolysis of urea is the most advanced mechanism for inducing carbonate precipitation and the most often discussed in the literature due to its relative simplicity [9–14].

Urea hydrolysis utilizing bacteria (*sporosarcina pasteurii*) as a source of urease enzyme for soil cementation was first discussed by Whiffin [10] and later called “microbial-induced carbonate precipitation (MICP)”. Various studies have examined the potential use of MICP in addressing many challenges in granular soils, such as erosion resistance, slope stability, under-seepage of levees, and bearing capacity of shallow foundations [1,13,15–18]. However, several drawbacks of MICP have limited its field application, such as the need for multiple cycles of treatment to reach sufficient carbonate precipitation and the relatively large size of exogenous bacteria, which makes it limited to the soil in which the pore throats are bigger than bacterial size [3,19,20]. In addition, since bacteria are living organisms, a suitable and sensitive environment is required for bacterial growth and enzyme production including the temperature, PH, and oxygen availability for some bacterial species [21–23].

To overcome the abovementioned problems associated with MICP, free urease enzymes derived from a plant source was first suggested by Nemati and Voordouw [9] and used as a catalyst in hydrolysis. This hydrolysis technique is usually referred to as “enzyme-induced carbonate precipitation (EICP)”. Unlike MICP, the free urease enzyme used in EICP has a size of the order of 12 nm and is soluble in water [19], which contributes to increasing the groutability of the enzyme solution inside the soil pores. Moreover, the use of free enzymes obviates the need to provide nutrients for bacterial activity, thus, facilitating the field applicability and reducing the treatment costs [3,9]. In addition, since no living organisms exist in EICP, it is not affected by the cellular processes or metabolic rates specific to microbial organisms [24].

This paper presents a state-of-the-art review of the use of EICP for improving soil geo-mechanical and physical properties, and the associated potential geotechnical and construction applications. An extensive experimental dataset for various geotechnical properties of EICP treated sand is compiled, including unconfined compressive strength (UCS), shear strength parameters, and hydraulic conductivity. Furthermore, these engineering properties of EICP-treated sands are analysed and correlated to various factors. The analysis of the compiled data from the literature is expected to provide insights into the best practices and eventually help researchers to identify research gaps within the technology. This objective is achieved by underlining the factors affecting the hydrolysis via EICP, and comparison of EICP and MICP. Finally, the advantages, limitations, and future research directions for bio-cementation via EICP are presented with a focus on presenting envisioned applications for the technology.

2. Bio-Mineralization via Enzyme Induced Carbonate Precipitation (EICP)

2.1. Development of EICP Technique

Nemati and Voordouw [9] were the first to propose the use of free urease enzyme derived from a plant source as a catalyst in carbonate biomineralization for geotechnical applications. In their study, the enzymatic reaction to produce carbonate was used to reduce the soil permeability and clogging of soil pores for possible application in oil recovery. The use of enzymatic reaction to improve sand soil mechanical properties was first proposed by Yasuhara et al. [25,26]. In their studies, they have shown substantial improvement in soil unconfined compressive strength (UCS) after treatment of soil with multiple cycles of enzymatic solution. In 2015, Kavazanjian and Hamdan [3] were the first to introduce the term (EICP) for the biomineralization process using plant-driven urease enzyme in

urea hydrolysis. In their study, EICP solution was injected in sand columns to improve soil mechanical properties.

Since its development, EICP has been used in several other applications such as dust suppressants for wind erosion control [27–32], mitigation of liquefaction of sand [33], bio-bricks [34], surface water erosion control [35], and concrete crack healing [36]. In the pie chart shown in Figure 1, the number of research studies conducted on the use of EICP in geotechnical, construction, and building materials are categorized based on the applications. As shown in the figure, most of the studies conducted explored the use of EICP for improving the mechanical properties of sandy soils at the laboratory scale. Few recent studies explored the use of EICP cementing solution in field applications as a grout for ground improvement [37]. Moreover, few recent studies explored the life cycle assessment (LCA) of EICP as a suppressant for wind erosion control [31] and as a grout for ground improvement [37].

Figure 1. Pie chart showing the number of research works utilizing EICP in geotechnical engineering applications in addition to construction and building materials applications.

2.2. EICP Overview

In general, the process of urease-aided carbonate mineralization (regardless of the enzyme source used) is derived by the urease catalysing the hydrolysis of urea. This reaction produces ammonia (NH_3) and carbamate which are unstable and subsequently degrade to produce a second ammonia molecule and carbonic acid (H_2CO_3), as shown in Equation (1) [38].

These products in the presence of water give bicarbonate, ammonium, and hydroxide ions, respectively, as shown in Equations (2) and (3) [39]:

The production of hydroxide ions from reaction (3) results in an increase in pH, which in turn leads to the formation of carbonate ions, as shown in Equation (4):

This process can be summarized in Equation (5), as shown in Figure 2.

Figure 2. Schematic for hydrolysis of urea process using urease enzyme.

The increase in pH in the presence of dissolved Ca^{2+} creates favourable conditions for ions to merge, leading to calcium carbonate precipitation, as summarized by Equations (6) and (7).

The overall process for EICP is summarized in Figure 3; carbonate precipitation takes place by the supply of carbonate ions and the alkalinity resulting from urea hydrolysis. Active calcium carbonate crystals (precipitates) bind soil particles together as they accumulate at the soil contact points, thus, the produced cementation between the soil particles increases. However, carbonate precipitation may occur in locations other than grain contact points producing inactive crystals that do not contribute to soil cementation (Figure 3).

Urease Enzyme Source

As mentioned earlier, the EICP technique uses a free urease enzyme as a catalyst for the hydrolysis process. Urease can be extracted from either bacterial [24] or plant sources [9]; however, the extraction of urease from plant sources is the most common method for EICP applications. Several plant sources used for urease enzyme were found in the literature, including watermelon seeds, soybean, pumpkin seeds, winged bean seeds, and jack bean seeds [40]. The activity of the urease enzyme derived from different plant sources is summarized in Table 1. It should be noted that the Jack bean seeds have been used as a rich source of urease due to their high activity and commercial availability [41]. In 1926, Sumner [42] was the first to isolate the urease enzyme from jack beans (*Canavalia ensiformis*). In his work, he proposed that enzymes could be proteins devoid of organic coenzymes and metal ions. Blakeley [43] showed that urease is an enzyme in the form of a

protein that contains a nickel ion cofactor. Figure 4 demonstrates the octahedral shape of the urease enzyme [43].

Figure 3. Overview of bio-inspired calcite precipitation using EICP.

Table 1. Urease activity based on its plant source [43].

Enzyme Source	Reported Enzyme Activity (mg NH_3 /g/h at 30 °C)
Soya Bean Seeds	360
Watermelon Seeds	355
Pumpkin Seeds	755
Jack Bean Seeds	4871

Figure 4. Micrographs of crystals from jack bean urease [43].

Pure urease can be costly, accounting for 57–98% of the total cost of the EICP cementing solution [44]. In an attempt to reduce the treatment cost, some researchers examined the use of crude urease enzyme extracted in the lab from jack bean [45], watermelon [46,47], soybean [48], and bacterial cells [24,49,50] as cheap alternatives to costly lab-grade urease

enzymes. Urease was extracted and purified from watermelon seeds through blending, filtration, and acetone fractionation. This method was able to produce carbonate precipitation in soil but was not cost-effective for large-scale applications due to the need for high energy and labour for de-husking the watermelon seeds [47]. Few studies have investigated the possibility of using jack bean meal or industrial grade urease for soil improvement [51–53]. Furthermore, Hoang et al. [24,49] employed a unique sonication approach that lyses *Sporosarcina pasteurii* bacteria cells in order to release their intracellular urease enzyme. The rapid growth of bacteria outside the soil can be promising for the commercial production of the enzyme. Khodadadi et al. [45] investigated the use of crude jack bean extracted with low purity in deionized water as the source of the enzyme without the need for extra fractionation steps or chemical extraction solution to get the purified extracts. Javadi et al. [54] investigated the use of lyophilization through freeze and drying the crude enzyme extract to a powder to facilitate storage and transportation of the extracted enzyme. Results have shown that the lyophilized crude extract has maintained its activity for up to a year with minimal loss in activity and longer than commercial urease enzyme.

It should be noted that the effectiveness of the EICP depends highly on the urease source and activity, which widely vary with the environment pH, temperature, and concentration. Several researchers have used a variety of additives to the EICP cementing solution to improve the EICP performance such as biopolymers, hydrogels, and milk [55–57]. Appendix A Table A1 shows a comprehensive summary of the studies found in the literature in which different urease sources were used in the enzymatic bio-mineralization and the corresponding reported activity for each urease source. Appendix A also shows the additive used to stabilize urease and enhance the EICP soil treatment in each study.

3. Factors Affecting Carbonate Crystallization in EICP Treatment

The urea hydrolysis involves the breakdown of urea in water. The result of this reaction forms calcite in the presence of a calcium source. The reaction can be 10^{14} times faster than the chemical (un-catalysed) urea hydrolysis in the presence of a catalyst (urease enzyme) [43]. The improvement in EICP treatment is based on the precipitation of calcium carbonate within the treated soil matrix. It is thus important to shed light on the crystallographic patterns, such as size, shape, and distribution of precipitates, since they play a significant role in defining the mechanical properties of EICP bio-cemented soils [44,58,59]. The main factors that affect crystallization are the temperature, urease activity, pH level of the cementing solution, and concentration of cementation solution. These factors are discussed in some detail below.

3.1. Effect of Urease Enzyme Activity and Concentration

Both the enzyme concentration and urease activity have a significant effect on EICP treatment. The enzyme concentration is routinely represented as grams of enzyme per liter of cementing solution (g/L). The urease activity unit is usually represented as U/g, which means the μmol of urea hydrolysed per minute by 1 g of urease; this property depends mainly on the enzyme source and extraction method [40]. The literature on EICP highlighted the importance of finding the optimum amount of urease concentration to fully consume substrate (urea) in the solution. This optimization is essential to achieve high hydrolysing efficiency and reduce the cost of treatment. Several researchers have conducted the optimization based on increasing the precipitation ratio (PR%), defined as the carbonate precipitated mass over the maximum theoretical carbonate precipitation mass, calculated based on the EICP solution constituents. Several studies on the optimization of enzyme concentration have utilized electrical conductivity (EC) measurements of the EICP cementing solution as an index of the ionic strength of the solution (concentration of the chemical constituents).

As shown in Figure 5, Neupane et al. [60] found that the precipitation ratio, in the case of EICP solution with an equal molar of urea and calcium chloride with a calcium

chloride concentration of 0.5 M, rapidly increased with the increase in urease enzyme concentration until reaching a urease concentration of 2.0 g/L. Increasing the concentration of enzyme more than 2.0 g/L led to a slow and gradual increase in precipitation ratio up to a maximum of 90% at a concentration of 3.0 g/L. Further increase in the enzyme concentration led to a slightly lower precipitation ratio. However, in the same study and for a higher concentration of calcium chloride (1 M) of equimolar EICP solution, the increase in enzyme resulted in a continuous increase in PR%. Moreover, Almajed et al. [61] reported that an increase in the enzyme concentration, up to 6 g/L, led to an increase in the precipitation ratio regardless of the EICP solution. Almajed et al. [61] reported an optimal enzyme concentration of 3 g/L. Finally, Song et al. [2] showed a similar trend of increasing the precipitation rate with the increase of enzyme concentration (see Figure 5).

Figure 5. Change in precipitation ratio vs. enzyme concentration (data collected from several sources).

Carmona et al. [62] claimed that using 8 kU/L of urease concentration, the increase in urea–CaCl₂ concentration beyond 0.5 mol/L lowered the amount of hydrolysed urea, thereby yielded a lower mass of calcium carbonate precipitation. Carmona et al. [62] attributed this to the increase of reagents concentration that may inhibit the catalysation capacity of urease. Moreover, the same amount of urease concentration (8 kU/L) mixed with urea–CaCl₂ concentration of 0.25 mol/L led to less calcite precipitation; thus, an excessive amount of the urease enzyme compared to the concentration of urea–CaCl₂ may suppress its catalysing efficiency according to this study.

Song et al. [2] studied the effect of urease concentration to evaluate the precipitation rate and quantify calcium carbonate formation with respect to time. They showed that the calcium carbonate precipitation rate can be increased by the urease concentration increase. Ahenkorah et al. [63] performed SEM imaging on calcium carbonate tube precipitates. SEM images showed that CaCO₃ precipitates from a solution containing low active urease enzyme (3500 U/g) have a disordered and anhedral calcite crystal, while the high active urease enzyme (40,150 U/g) resulted in euhedral and agglomerated rhombohedral calcite morphology.

Generally speaking, the effect of increasing the enzyme concentration can be illustrated using enzyme kinetics, which was first proposed by Michaelis and Menten [64] to study the factors affecting the speed of the enzyme reaction [65,66]. According to Michaelis and Menten's [64] model, the increase in the enzyme concentration results in a linear increase in the initial reaction velocity (V_0), as shown in Figure 6a. The EICP reaction velocity, after mixing the enzyme with substrate: urea, can be monitored by continuously measuring the concentration of the hydrolysis product (NH_4^+) formation over time [67].

Few studies have used this approach in evaluating the EICP kinetics and mostly relied on PR. Miftah et al. [68] monitored the NH_4^+ concentration over time, as shown in Figure 6b, showing slower rate hydrolysis of urea (production of NH_4^+) with time. The slower rate of hydrolysis with time can be attributed to the consumption of the substrate (urea) with time and therefore it becomes a limiting factor or due to denaturalization of enzyme due to the presence of inhibitors. If three enzyme concentration levels (A, B, and C) are considered, as shown in Figure 6a, an increase in the initial reaction velocity (V_o) will be obtained. This increase in reaction rate due to the increase in enzyme concentration results in a faster rate of hydrolysis and reduction of time needed to reach 100% PR. This may illustrate the different optimum enzyme concentrations reported from different studies, as most of these studies did not consider the reaction kinetics and evaluated the EICP outputs after constant time. For example, Almajed et al. [61] and Neupane et al. [60] evaluated the PR after 72 h assuming the reaction is concluded. However, as illustrated in Figure 6a, this may not be always true and it may need a longer time for the reaction to conclude especially at lower enzyme concentrations. Therefore, it is recommended in future studies to include the enzyme kinetics in the optimization process based on monitoring NH_4^+ production over time, not just carbonate precipitation mass and precipitation ratio.

Figure 6. A typical enzyme-catalysed reaction (a) initial reaction velocity versus enzyme concentration (typical reaction based on Michaelis and Menten model) (b) product formation over time (data adopted from Miftah et al. [68]).

3.2. Chemical Constituents Concentrations

Optimizing EICP chemical constituents is desirable to achieve a cost-effective cementing solution while maintaining a high amount of carbonate precipitation. It is believed that the amount of precipitation and reagents concentration has a significant effect on the hydrolysis process. Various studies have reported different optimum concentrations of chemical constituents for the EICP solution to maximize carbonate precipitation. Nemati & Voordouw [9], and Nemati et al. [69] reported an increase in carbonate precipitation with the increase in urea and calcium chloride concentration up to a certain level. Yasuhara et al. [26] reported that using a higher amount of calcium source led to a higher degree of precipitation formation. However, Neupane et al. [60] reported that using a lower concentration of constituents (equimolar of urea and calcium chloride) can lead to a higher precipitation ratio; they reported that 0.5 mol/L had a precipitation ratio of 80%, which was higher than the PR achieved at 1.0 mol/L. Chandra & Ravi [70] studied the optimum combination of urea and calcium chloride in a bench-level tube test and evaluated the precipitation by gravimetrically measuring the amount of precipitated calcium carbonate. They prepared several equimolar concentrations of urea- CaCl_2 of 0.25, 0.5, 0.75,

1.0, and 1.25 mol/L with 0.2 g/L of urease enzyme. It was concluded that the increase in urea–CaCl₂ concentration may inhibit the activity of urease and reduce the precipitation amount of calcium carbonate, hence, reducing the hydrolysing efficiency. The fact that the increase of urea–CaCl₂ in the concentration may inhibit the activity of the urease enzyme was also reported by Carmona et al. [62]. Almajed et al. [61] correlated the efficiency of the urease enzyme with the solution EC that is controlled by the ratio and concentration of urea–CaCl₂. It was found that for each enzyme concentration, there is a critical EC value representing the maximum concentration of calcium chloride and urea, which results in nearly complete precipitation. Moreover, beyond a certain threshold value for the EC reading, the precipitation ratio dropped drastically. Finally, Ahenkorah et al. [63] found that the rate of change in electric conductivity increases with the increase in urease enzyme concentration regardless of the activity.

The effect of the substrate (urea) concentration in the EICP cementing solution can be illustrated using the enzyme kinetics model proposed by Michaelis and Menten [64]. In this model, the rate of hydrolysis is considered as hyperbolic with the change of the hydrolysis rate (*V*) increases with the substrate concentration [*S*] (urea concentration in this case) until it reaches an asymptote at *V* = *V*_{max}, as shown in Figure 7a. Where *V* is defined as the rate of change in the product concentration [*P*] (NH₄⁺ in this case). Equation (8) defines the rate of hydrolysis based on the Michaelis constants *K*_m, and *V*_{max}.

$$V = \frac{d[P]}{dt} = \frac{V_{\max} \cdot [S]}{K_m + [S]} \quad (8)$$

This model suggests that the increase in substrate (urea) concentration increases the rate of hydrolysis which agrees with the reported behaviour in several studies (e.g., [9,69]). However, the rate of urea hydrolysis (*V*) is also affected by inhibitors that may affect the rate of carbonate precipitation and even denaturalize the urease enzyme. These inhibitors can be categorized as competitive inhibitors where *V*_{max} remains the same and non-competitive inhibitors where *V*_{max} changes depending on the concentration of inhibitor, as shown in Figure 7b [67]. Recently, Ahenkorah et al. [71] introduced a normalized enzyme concentration term [*E*_s] (defined as enzyme concentration multiplied by enzyme activity) to explain the effect of Enzyme and substrate concentration [*S*] on the PR%. PR% was found to exhibit a non-linear correlation with the ratio [*E*_s]/[*S*] with an optimum value of 20 kU/mol beyond which no increase in PR% was reported.

Figure 7. A typical enzyme-catalysed reaction (showing the effect of inhibitors in an enzyme-catalysed reaction) (a) initial reaction velocity versus substrate concentration [*S*] (typical reaction based on Michaelis and Menten model); (b) product formation over time (data for normal enzyme adapted from Miftah et al. [68]).

Several studies have shown that ammonium ions (a by-product from the hydrolysis process) in the EICP solution act as a non-competitive inhibitor [64,72,73], as shown conceptually in Figure 7. This may illustrate the reported reduction in carbonate precipitation beyond a specific threshold of substrate (urea) concentration (e.g., [61]). This may also be illustrated more in Hamdan's [58] study in which a series of experiments were conducted to define the optimum ratio of (urea to CaCl_2) in EICP solution. Hamdan [58] compared the ureolysis efficiency at low (0.1 to 0.6 M) and high (1.0 and 6.0 M) substrate concentrations, and the test was conducted by preparing EICP solutions with varying concentrations and ratios between the reagents. Although a high concentration solution yielded more calcium carbonate than a low concentration, it was found that the lower concentration had a better ureolysis efficiency (higher PR). Figure 8 shows the relationship between the initial ratio of urea to CaCl_2 and the pH of the EICP cementing solution. Hamdan [58] showed that this drop in pH has a role in reducing the hydrolysis rate. High pH leads to an increase in calcium carbonate precipitation that limits the transformation of ammonia into the acidic form (NH_4^+), thereby preventing the reversal of calcium carbonate formation.

Figure 8. Relationship between initial CaCl_2 -urea ratio, and pH for high and low substrate concentration [58].

The above studies may suggest that an increase in substrate (urea) concentration will increase the hydrolysis reaction rate to a specific threshold value for the substrate concentration (not clearly defined in the literature). After reaching this threshold, a reduction in hydrolysis rate due to the increase in non-competitive inhibitor concentration (NH_4^+) was reported. However, further studies are still required to thoroughly define this threshold based on the substrate (urea concentration), urea: CaCl_2 , and urease enzyme activity and source.

3.3. Temperature

Few studies have investigated the effect of temperature on the behaviour of EICP treated soils. Nemati & Voordouw [9] reported that an increase from 20 to 50 °C enhanced the production rate of calcium carbonate from 0.038 to 0.34 g/L/h. In another study by Zhao et al. [74], a 26% decrease in permeability was measured for EICP-treated soil at 30 °C compared to that at 22 °C. Arab et al. [53] have studied the effect of temperature during the curing of EICP treated bio-specimens. They reported that specimens cured at 10 °C exhibited the lowest UCS results with an average UCS of 669 kPa while specimens cured at 25 °C and 40 °C had a UCS of 1411 and 1537 kPa, respectively. They also attributed this reduction in compressive strength at lower temperatures to the reduction in urease activity which has resulted in lower carbonate precipitation.

3.4. pH Level

The entire process of calcium carbonate precipitation during hydrolysis is highly dependent on the overall pH level of the whole environment. A sufficiently large increase in the environment pH (alkalinity) is necessary to shift the carbonate equilibrium from CO_2 to HCO_3^- to CO_3^{2-} and then precipitate CaCO_3 in the presence of Ca^{2+} . This change in the pH level occurs due to the formation of OH^- , which is a by-product of ammonium generation (NH_4^+) that raises the pH and provides favourable conditions for calcium carbonate precipitation [1,75]. Jacob [76] explained the relationship between carbonate precipitation and pH in water, showing that generating more carbonate CO_3^{2-} usually occurs at a high value of pH. Furthermore, maintaining a high pH value (>9.0) helps in increasing calcium carbonate saturation and drives the precipitation reaction towards completion. Speciation of ammonia-ammonium shifts toward ammonium, which increases the pH and decreases the possibility of reversing the reaction, since calcium carbonate dissolves in an acidic environment at a pH lower than 7.0 [75]. Moreover, the activity of the urease is also dependent on the system pH. The optimum pH that maintains the urease activity is found to be 6.9 [77].

4. Soil Cementation via EICP

The observation of sand-forming sandstones through the precipitation of calcium carbonate paved the way for exploring new methodologies under the biogeotechnology branch such as EICP. Several recent studies have demonstrated the transformation of loose sand into bio-cemented sand (see Figure 9). In this section, several studies that showed the successful application of EICP as a cementing binder are presented.

Figure 9. Comparison of sand before (right) and after (left) bio-cementation via EICP.

4.1. Application Methods

To achieve the full potential of EICP-based soil improvement, carbonate cementation must be concentrated at the contact points of soil particles. The improper distribution of carbonate precipitation may lead to failure in soil cementation. Several methods have been proposed in the literature to introduce EICP cementing solution into the soil matrix, including: (1) injection method; (2) mix-and-compact; (3) surface percolation; and (4) spraying. The soil grain and pore size have a significant effect on selecting the proper application method of EICP cementing solution and each method has also been used for specific applications.

4.1.1. Injection Method

Hamdan [58] used the injection method to treat Ottawa (20–30) silica sand columns using the EICP cementing solution. The soil was injected dry and saturated, and the UCS test results of treated soil were found to be in the range of 35 to 125 kPa. Kavazanjian et al. [78] used EICP cementing solution as a grout for soil nails. The injected cementing solution consisted of 0.87 M urea, 0.5 M $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.85 g/L low-grade urease enzyme, and 4.0 g/L stabilizer (non-fat milk). The EICP solution was injected into perforated PVC tubes

with a diameter of 9.5 mm and a length of 330 mm using a 60 mL syringe. The tubes were buried in a wooden box full of F-60 silica sand. After 5 days of curing, the stability of the treated soil mass was examined using weights applied to the top of the box after disassembling one of the wooden box faces. The stabilized soil mass in the box remained stable without any sign of cracking or instability. However, uneven carbonate distribution along the soil column was reported. Neupane et al. [60] also utilized injection and reported an increase in precipitation near the injection point, which led to further decrease in porosity and effectiveness of precipitation in latter injections, thereby requiring higher pressure in the injection tube to maintain the rate of flow downstream of the treated soil column. Martin et al. [37,79] utilized a tube-à-manchette (TAM) permeation grouting technique to inject EICP cementing solution to create 0.3 m diameter $\times$ 0.75 m long columns of treated sands. A target value of 500 kPa established from laboratory testing was achieved in the field after four cycles of treatment.

One of the issues in the bio-cementation solution for both (MICP and EICP) used for grouting is the low viscosity of the solution since the cementing solution is water-based [80]. This reduces the efficiency of injection as the solution drain off rapidly. Therefore, several researchers proposed using biopolymer as additives to improve groutability of the cementing solution and uniformity of carbonate precipitation. The treatment with biopolymers alone was proposed for the compaction grouting or deep cement mixing (DCM) method due to its high viscosity [80]. Consequently, biopolymers were shown to improve the EICP cementing solution viscosity without affecting the hydrolysis process [81]. Examples of these biopolymers are sodium alginate [82], and xanthan gum [44,81,83]. In addition, the pre-injection of sodium bentonite evidenced a uniform improvement for columnar and mass EICP stabilization [58].

4.1.2. Mix and Compact

In this method, the EICP cementing solution is mechanically mixed all together with the soil to be treated and then compacted to the desired compaction level. This method is suitable for several field applications such as pavement construction and surficial soil improvement. Yasuhara et al. [26] pre-mixed the urease powder with Toyoura sand to achieve a homogenous mix before injecting the soil with the reagents of EICP. This application method achieved UCS of up to 1.6 MPa after several injections. Hamdan [58] used the mix-and-compact approach to treat sand specimens and reported a compressive strength from 392–529 kPa compared to 35–125 kPa in the case of injection. In both Almajed [44] and Almajed et al. [61], a similar approach to Hamdan [58] was followed to treat Ottawa (20-30) silica sand. Almajed [44] showed that the EICP treated soil strength via mix and compact is highly dependent on the soil relative density and soil particle size.

4.1.3. Surface Percolation

Surface percolation is simply applied by pouring the EICP cementing solution on the top surface of the sample. Hamdan [58] used the surface percolation method to treat sand columns of medium-coarse Ottawa (20-30) silica sand and medium grain F-60 silica sand. The UCS test results of the treated soils were in the range of 38–210 kPa with a carbonate content of 1.6–2.0%. Similar to the injection method, multiple applications were required to increase the carbonate precipitation and increase the compressive strength of the treated soil. Almajed et al. [61] reported that the surface percolation method resulted in UCS of 100 kPa at 1.2% CaCO_3 and one treatment cycle, whereas four treatment cycles led to 465 kPa at 4.6% CaCO_3 . Based on the analysis of SEM images of the treated soils, Almajed et al. [61] showed accumulation of precipitates between the soil particles along the flow path of the cementing solution during percolation, which may suggest that the surface percolation method facilitated nucleation and, consequently, the growth of calcite crystals between soil particles, hence providing better binding than the mix-and-compact method (Figure 10). However, the mix-and-compact method showed a more uniform distribution of carbonate precipitation compared to the surface percolation technique (Figure 10).

Figure 10. Comparison of calcium carbonate precipitation distribution based on application method: (a) mix and compact (carbonate content: 1.8%), (b) percolation (carbonate content: 1.79%); and (c) injection (carbonate content: 1.8%) [44].

4.1.4. Spraying

EICP spraying is usually used when the cementing solution is intended to be kept at the surface of the treated soil where only strengthening of the surface is required. Consequently, this application method is suitable for water erosion resistance and fugitive dust control. Knorr [29] applied a spraying method for dust control using two sprayers for the urea–CaCl₂ and enzyme solutions to avoid any losses in the precipitation since the precipitation process starts within minutes after mixing. Knorr [29] sprayed 200 mL of EICP cementing solution on 23 cm pans full of soil and observed that the soil surface could withstand up to 24.4 m/s of wind speed without any detachment in a wind tunnel test. Hamdan and Kavazanjian [27] used a hand-held plastic bottle sprayer to apply EICP cementing solution on the soil surface. They separated the solutions of urea–CaCl₂ and enzyme and compared them with a pre-mixed EICP solution. Contrary to Knorr [29], the premixed EICP solution showed better erosion resistance than the separated solution. The pre-mixed EICP solution showed no sign of soil detachment at a wind speed of 25 m/s. Almajed et al. [32] sprayed both EICP cementing solution and sodium alginate biopolymer on several types of soils for wind erosion resistance, and substantial improvement was achieved compared to soils sprayed with EICP cementing solution alone. Miao et al. [84] demonstrated how EICP can withstand several cycles of wetting and drying after EICP spray application while still having nearly the same wind erosion resistance ability. It was shown also that increased multiple passes of spray from 2 to 6 can have a respectable reduction in weight loss after wind tunnel exposure. Wu et al. [30] successfully utilized urease extracted from soybean to spray EICP cementing solution on coal dust from mining activities to suppress fugitive dust.

5. Geotechnical Properties of EICP Treated Soils

The purpose of any ground improvement technique is to improve certain geotechnical characteristics of soils (e.g., increase soil shear strength, reduce settlement, reduce soil permeability, slope stability, increase soil bearing capacity, and improve soil resistance to liquefaction) for construction or infrastructure developments. Therefore, successful ground improvement aims to alter the geotechnical property of soil in a controlled manner to achieve the desired response. In this section, the effect of EICP on several geotechnical properties of bio-treated soils is discussed.

5.1. Hydraulic Conductivity

The hydraulic conductivity of treated soils is an important parameter in evaluating the success of soil improvement techniques. The application of EICP treatment binds the soil particles and thus relatively decreases the hydraulic conductivity of treated soils. However, the extent of the reduction can be controlled based on the cementing solution

used and the number of treatment cycles. Soil hydraulic conductivity is related to the soil packing density and the associated porosity since the soil mass behaviour depends on the macroscale particle-particle interactions [85].

The pore occupation in EICP treatment is a result of CaCO_3 crystals that fill the space between soil particles, which causes a change in the soil pore volume and hence reduces soil hydraulic conductivity. Nemati and Voordouw [9] investigated the use of EICP to modify the soil porosity for oil recovery applications. They showed that the permeability of treated soil depends on the formation type of the carbonate and carbonate content, which depends on the concentration of enzyme and reactants concentration (urea and calcium chloride). Following the injection of the treatment mixture, the soil permeability decreased about 92% compared to untreated samples. A second injection of the treatment solution was applied and further decreased soil permeability by 73%, resulting in a total reduction in soil permeability of 98%. The study concluded that EICP treatment is very efficient in reducing the permeability of treated soils, especially for the sweeping of water in oil recovery applications.

Yasuhara et al. [26] found a linear relationship (on a semi-log scale) between the hydraulic conductivity and carbonate content. Neupane et al. [60] adopted a multi-physics reaction simulator to study the change in soil porosity over time during the calcite precipitation reaction; their simulation was capable of predicting the reduction in soil porosity and change in hydraulic conductivity.

Finally, Hoang et al. [24] compared the bio-cementation treatment of sandy soil permeability using both MICP and EICP for 8, 12, and 16 cycles of treatment. They found that increasing the treatment cycles increases the accumulation of carbonate crystals, which further clog the soil pores and decrease the soil hydraulic conductivity. The hydraulic conductivity of the specimens treated using the EICP cementing solution at 16 cycles showed a maximum reduction of 90% compared with the original untreated soil, while a 99% reduction in the hydraulic conductivity was attained in the case of MICP-treated soils at the same number of cycles.

5.2. Treated Soil Strength

Several testing protocols have been used in the literature to evaluate the enhancement in EICP-treated soil strength depending on the envisioned application. The shear and compressive strength of EICP treated soils are discussed in this section.

5.2.1. Unconfined Compressive Strength (UCS)

UCS is one of the most common tests used to evaluate bio-cemented sand in general soils due to the simplicity and efficiency of this test [5,10,24,26,58,60,61,86–90]. Several factors can affect the UCS magnitude, including cementing solution, soil type, and the number of treatment cycles. Several researchers have found a strong correlation between the carbonate content and UCS of EICP-treated soils [44,53,58,61,91]. Therefore, in order to improve treated soil compressive strength, several researchers have used multiple cycles of treatment with EICP solution to increase the amount of carbonate precipitation and hence increase soil compressive strength. Figure 11 shows data compiled from the literature for UCS against carbonate content. As shown in the figure the UCS shows a general increasing trend with carbonate content. As shown in the figure, the reported UCS values for EICP-treated soils found in the literature range between 17 to 6500 kPa for carbonate content in the range from 0.5% to 17%. This scatter in the data is attributed to the different conditions used in each study to produce the EICP treated soils, including the number of treatment cycles, EICP chemical constituent concentrations, soil type, soil density, and use of additives that enhance EICP cementing solution performance.

Figure 12 shows the effect of the number of EICP treatment cycles on the UCS of EICP treated soil. To achieve UCS of 6500 kPa 29 cycles of EICP treatment was needed. The data points from Ahenkorah et al. [92] showed a different trend with improved gain in treated soil strength with the number of EICP cycles. This reported improvement may be

attributed to the use of non-fat powder milk as an additive to the EICP cementing solution in their study.

Figure 11. Effect of carbonate content on the UCS of EICP treated soils data adopted from several studies [3,24,26,45,49,53,56,61,68,79,81,83,87,92–99].

Figure 12. Effect of the number of EICP treatment cycles on UCS of EICP treated soils data adopted from several studies [24,26,49,61,69,79,93,94].

Figure 13a shows the UCS of EICP treated soils using only one cycle of EICP cementing solution against carbonate content. In this case, the range of UCS is 17 kPa to 2900 kPa with a carbonate content in the range from 0.5% to 8.9%. The carbonate content is expected to be low in the case of one cycle of treatment and therefore the UCS is much lower compared to the case of multi-cycles of treatment. The scatter in the data collected in the case of one-cycle is attributed to the fact that researchers have used different techniques to enhance the EICP cementing solution to reach enough UCS in one cycle. Each improvement technique may have a different trend in terms of carbonate content against UCS. Figure 13b,c show the data for carbonate content against UCS for treated soil with modified and non-modified EICP cementing solution, respectively. The data in Figure 13b shows a good correlation between the UCS and compressive strength. Almajed et al. [56] study results show that a substantial increase in the UCS results at a much lower carbonate content of less than 1% CaCO_3 was observed. They attributed this improvement to the casein proteins non-fat milk that bind the calcium ions in the EICP solution and then work as nucleation sites for the carbonate precipitation. Nevertheless, the results from the Almajed et al. [56] study have shown much higher compressive strength at such lower carbonate content compared to other studies that have also used non-fat milk powder in the EICP cementing solution. In Arab et al. [53] study, non-fat milk powder was used with relatively high molarity of urea (3 M) in the EICP cementing solution with urea to calcium chloride ratio of (1:0.67). As shown in Figure 13b the use of high molarity substrate has resulted in relatively higher carbonate precipitation and compressive strength. Figure 13c shows the results of the carbonate content versus UCS for EICP basic solution without any additives (i.e., basic EICP constituents of urea, calcium chloride, and urease were used). As shown in the figure the general increase in carbonate content has resulted in higher UCS. Lee and Kim [95] used high urea and calcium concentration to induce higher carbonate precipitation. While Zhao et al. [87] utilized batch reactors to induce carbonate precipitation in one cycle.

(a)

Figure 13. Cont.

Figure 13. Effect of carbonate content on the UCS of EICP treated soils using one cycle of EICP cementing solution (a) all data for one cycle treatment; (b) modified EICP cementing solution, and (c) non-modified EICP cementing solution. Data adopted from several studies [3,51,53,56,61,69,79,81,83,87,95–97].

Finally, other researchers have explored the use of different additives to enhance the compressive strength of EICP-treated soil. Almajed [44] reported a 600% increase in the UCS values obtained by adding 1.1% (*w/w*) of xanthan gum biopolymer to the soil. This significant increase in treated soil strength was attributed to the presence of the xanthan gum, which increased the bonding between soil particles. However, it should be noted that the treated sample in this study was left to dry for one month due to high water retention of the xanthan gum.

Arab et al. [34] showed up to a 400% increase in soil compressive strength using sodium alginate biopolymer along with EICP. The use of fibres along with EICP to enhance EICP treated soil UCS was investigated [100]. The authors have utilized sisal fibre with EICP to treat Ottawa (20-30) silica sand, and the results on the UCS showed an optimal fibre ratio and fibre size of 0.3% (w/w) and 10 mm, respectively. The maximum strength of EICP-treated sand with the optimal amount of fibres was four times greater than the samples treated with EICP alone without fibres.

5.2.2. Triaxial Shear Test

The triaxial shear test is a reliable method to determine the shear strength parameters for soils in general. Hamdan [58] tested both Ottawa (20-30) sand and Ottawa (F-60) silica sand treated with EICP using a triaxial test under drained conditions. Multiple cycles of EICP soil treatment were used with a one-week interval between the cycles. The triaxial testing program was conducted at a confining pressure of 60 kPa. The p-q plot failure envelopes for Ottawa (20-30) treated silica sand showed a significant increase in drained soil strength up to 100 kPa at 2% calcite precipitation, with a relative density of 60%. On the other hand, the p-q plot failure envelope of Ottawa (F-60) treated silica sand showed an increase in treated soil drained shear strength of up to 125 kPa at 1.6% of calcite precipitation with 35% relative density. Almajed [44] conducted an undrained triaxial test on EICP-treated Ottawa (20-30) sand samples at three confining pressures: 50 kPa, 100 kPa, and 150 kPa. When tested at 50 kPa, the test yielded a tensile failure pattern, whereas, for 100 kPa and 150 kPa, a shear failure was observed. In this testing program, the effective friction angle (ϕ') and effective stress cohesion (c') were calculated as 34.6° and 332.9 kPa, respectively for EICP treated soil compared to $\phi' = 29^\circ$ and $c' = 0$ for untreated soil as summarized in Table 2. Song et al. [101] reported an increase in treated soil cohesion combined with a reduction in treated soil friction angle as a result of the increase in the induced carbonate content. The maximum shear stiffness achieved for EICP treated soil at 150 kPa confining pressure was 1012 kPa compared to 790 kPa for the untreated soil at the same confining pressure.

Gao et al. [48] conducted triaxial consolidated undrained (CU) tests on both EICP-treated and untreated silty sand soil treated at different levels using three levels of confining pressure (100 kPa, 150 kPa, and 200 kPa). The results from his testing program are summarized in (p-q) space in Figure 14. As shown in Figure 14a, as the carbonate precipitation increases both initial stiffnesses at low strain and (q), which treated specimens can endure before yielding, significantly increase. Figure 14b shows the triaxial test results in a (p-q) space with the failure envelope slope k_f for untreated soil and k_f^* for EICP treated soil at the peak q_p . As shown in the figure, when the effective stress path first reaches failure envelope (k_f^*), breakage of calcite cementation at particle-particle contacts initiates. As the carbonate bonds continue to break the shear strength of the treated soil starts to degrade until it completely fails. While untreated soil exhibited monotonic failure with positive pore pressure buildup due to undrained conditions. The EICP treated soils have exhibited positive pore water pressure development as well however as the cementation pressure increases specimens start to develop dilative response with reduction in pore water pressure development. As bonds continue to break the shear resistance continues to decrease until the soil has completely failed, and the benefit of cementation is lost.

During shearing, the EICP-treated specimen reached a peak strength that degrades with the increase in the axial strain to a residual (q) compared to the untreated specimen that generates no initial peak strength and the strength gradually increases to failure as the stress path reaches the failure envelope k_f (Figure 14a). It is important to note here that the failure envelope (k_f) is shifted due to carbonate precipitation to k_f^* having almost the same angle (α) of 21° with an increase of the intercept (a) to 38.43 kPa.

Figure 14. Experimental results for the CU triaxial response of untreated and EICP-treated specimens (data adopted from Gao et al. [48]). (a) q versus axial strain, (b) triaxial test results in a (p - q) space.

Table 2. Values for the shear strength parameters based on triaxial testing.

Study	Carbonate Content Range	Confining Pressure Range	C'	ϕ'
Almajed [44]	0.7–1.2%	50–150 kPa	332.9 kPa	34.6°
Song et al. [101]	1–2.2%	50–150 kPa	176.1 kPa	28.8°
Gao et al. [48]	3.9–7.4%	100–200 kPa	42–40 kPa.	23–19.3°

Simatupang and Okamura [33] conducted a study to investigate the effect of the degree of saturation on the behavior of EICP-treated sand during the precipitation process using a series of undrained cyclic triaxial tests. In their study, the bio-cemented sand specimens have shown significant liquefaction resistance with a reduction in the excess pore water pressure during the cyclic loading. In this study, it was found that the liquefaction resistance of EICP treated soils is highly stress-dependent. This was manifested with a reduction in liquefaction resistance as the confining pressure increased.

5.2.3. Direct Shear Test

Aishwarya & Christy [102] examined the effect of EICP treatment on the shear strength of sandy soils using a direct shear test. They found that EICP soil treatment had doubled the shear strength of untreated soil at a normal stress of 50 kPa. Interestingly, the increase in carbonate precipitation beyond a specific threshold has led to a decrease in shear strength, which was attributed to unstable carbonate within the soil pores that are not contributing to bridging soil particles together.

Moreover, Putra et al. [103] utilized a direct shear test to evaluate soil treated with modified EICP using magnesium sulfate. A cohesion of 53 kPa was achieved at 4.1% precipitation with minimum effect on friction angle. The friction angle reported was about 19° after treatment compared to 20° for untreated sand.

5.2.4. Splitting Tensile Strength (STS) and Flexural Strength

Few studies have studied the tensile and flexural strength of soils treated with EICP solution. Arab et al. [34] studied the flexural strength of EICP treated soil beams using a three-point flexural test. The study was conducted using three EICP cementing solutions with three different concentrations of urea with a constant CaCl_2 to urea ratio. The flexural strength increased with the increase in carbonate content. Ahenkorah et al. [92] studied the STS of EICP treated soils using multiple cycles of equimolar (0.50 M) urea, calcium chloride

(CaCl₂). The results show an improvement in STS as the carbonate content increased (Figure 15).

Figure 15. Splitting tensile strength (STS) versus carbonate content (data adapted from Ahenkorah et al. [92]).

5.2.5. Shear Wave Velocity

Shear wave velocity, V_s , is a soil mechanical property used as an index of both bio-treated soil stiffness and strength [2,11,12,97–105]. It can be measured by non-destructive techniques such as bender element and resonant column, which enable continuous measurement of the development in shear modulus throughout the soil treatment process. Song et al. [2] measured the shear wave velocity and electrical conductivity of EICP-treated sand to assess the development of shear stiffness with time during the curing period. The shear wave velocity was measured using two anchored bender elements for three specimens, each with different urease concentration while maintaining the equimolar concentration of urea and calcium chloride at 1 M. For the three tested specimens, the shear wave velocity tended to increase during the precipitation of the calcium carbonate and started to converge to the maximum value when the precipitation was completed. It was concluded that the increase in urease concentration increases both the shear wave velocity and the rate of shear wave velocity gain (R) (Figure 16). Obeidy et al. [105] used a piezoceramic bender element to measure the shear wave velocity of EICP-treated sand and found that the shear wave velocity has increased from 80 m/s for untreated soil to 120 m/s for treated soil after only one cycle of treatment injection.

Figure 16 shows clearly the effect of enzyme kinetics on the development of soil shear wave velocity in EICP treated soils. As shown earlier in Figure 7, increasing the enzyme concentration increases the rate of hydrolysis and this may explain the increase in shear wave velocity gain rate (R).

Figure 16. Shear wave velocity versus time for different urease enzyme concentrations data from (Song et al. [101]).

6. Main Factors Affect EICP Treated Soil Properties

6.1. Effect of Soil Particle Shape and Particle Size Distribution

The bio-cemented soils and granular soils behaviour, in general, is dependent on the soil relative density [61,106,107], particle shape [108], and particle size distribution [104,105]. This is attributed to the change in the voids volume and the number and the distribution of contact points within the soil matrix. Almajed [44] treated two types of soil (Ottawa sand 20-30 and F 60 sand) using the same recipe of EICP solution. The results showed higher strength and better carbonate distribution for treated Ottawa sand (20-30) (coarse sand) compared to (F-60) sand (finer sand). Rounded particles seem to support the carbonate precipitation to adhere to the contact point and this leads to a slight increase in carbonate content [108–110]. However, the effect of particle shape on the compressive strength of bio-cemented soils is debatable since untreated sand soils with angular particles would have higher shear strength parameters compared to soils with rounded particles.

To show the significance of the soil particle shape, morphology, and chemical composition, Krishnan et al. [111,112] investigated the same silica soil with the same gradation (Ottawa sand 20-30) but from three different sources, and the results showed that the strength varied over a wide range. These results emphasize the importance of the particle shapes, morphology and biochemical properties of the sand on the mechanical performance of EICP treated sands.

6.2. Effect of Soil Type

Most EICP studies have focused on clean sands with no fines. Few studies reported the successful application of EICP in silty and clayey soils with fine content of more than 40% [48,70]. Chandra and Ravi [70] found that EICP was not effective in treating silty soils due to its low pH value (measured to be 5.0). Low pH creates an acidic environment that negatively affects the hydrolysis of urea and precipitates less calcite. In another study by Oliveira et al. [113], silty soil showed a significant improvement in the EICP precipitation ratio; however, the silty soil in this study had a pH value of 7.75. Oliveira et al. [113] further found that the initial pH of treated soil is indicative of the treatment efficiency; besides the treatment was reported to be ineffective for soils with low pH values such as organic clays. Calcium carbonate precipitation was found to decrease the stiffness and strength of organic soil by almost 50%. Oliveira et al. [113] hypothesized that organic matter formed a

coating on the soil particles, which hindered bonding between calcium carbonate crystals and soil particles.

Zango et al. [114] investigated the strength of EICP treated residual clay soil typically used as clay liner using several EICP cementation solutions at different moulding water contents. The UCS of EICP treated clay was reported to increase with increment in the molarity of urea-CaCl₂ solution. The highest UCS reported was 643.5 kPa using 1.00 M cementation solution prepared dry of optimum moisture content.

6.3. Nucleation Sites for Carbonate Precipitation

Many factors are involved in the amount and distribution of calcium carbonate minerals in the soil pores resulting from urea hydrolysis, such as the application method, enzyme activity, soil degree of compaction, and soil type. Bio-crystallization of a carbonate crystal during the hydrolysis process is a two-step process, where the nucleation of a crystal is followed by crystal growth derived by the difference in chemical potentials of the liquid and the solid crystal [115]. In the MICP treatment, in addition to the bacteria metabolic reactions that catalyse the hydrolysis of urea, it is believed that bacteria catalyse the nucleation of calcium carbonate by reducing the required activation energy barrier [116]. Microbial cell surfaces can bind Ca²⁺ ions due to their net negative charge [117] and this helps in forming effective bridges between the soil contact points provided by the microorganisms. However, EICP lacks nucleation sites, which allows the solute molecules to diffuse across their surface lattice instead of moving randomly around. The absence of these nucleation sites may adversely affect the carbonate precipitation distribution in the soil pores and, as a result, make the carbonate precipitation occur randomly within the soil matrix. Zehner et al. [115] showed that sand grains act as nucleation sites while observing calcite nucleation and growth in real-time via confocal laser scanning microscopy.

Almajed et al. [61] proposed using calcite seeds to improve the nucleation sites, allowing the precipitate molecules to diffuse across their surfaces. Almajed et al. [61] further examined samples treated with calcite seeds through SEM and showed that seeded solution had densely aggregated rhombohedral calcite crystals compared to the disordered morphology of the precipitates from the unseeded solution. Moreover, Zehner et al. [115] showed improvement in crystal nucleation and growth in the presence of calcite seeds.

Several researchers have proposed using non-fat powder milk as an enzyme stabilizer [28,29,56,105]. Almajed et al. [56] hypothesized that the non-fat powder milk may also catalyse the nucleation of calcite crystals that helps in improving carbonate precipitation in EICP treatment. As shown earlier in Figure 13b, several researchers reported that adding non-fat powder milk to the EICP solution significantly increased the compressive strength of EICP-treated soil at a much lower carbonate content. They attributed this increase partially to the nucleation points formed by the non-fat milk protein. Khodadadi et al. [45] studied the effect of specific urease activity (U) defined as urease activity by the total amount of protein (U/mg of protein). The specific activity can be considered as an index of the urease purity (the higher the number the higher the urease enzyme purity). Interestingly, they found that the UCS of bio-cemented sands using crude extraction with low purity was higher than those treated with high purity commercial enzymes (i.e., high specific activity) at the same conditions as shown in Figure 17. These results may suggest that organic impurities (such as proteins) in the bio-cementation solution may enhance the effectiveness of EICP by introducing nucleation sites for the carbonate crystals and reducing the energy barrier necessary for nucleation [115].

Figure 17. The effect of urease enzyme-specific activity [U/mg protein) on EICP treated sand UCS (data adopted from Khodadadi et al. [45]).

6.4. Carbonate Crystals Morphology

The microstructure of EICP-treated soil can be assessed visually using SEM images by observing morphology, shape, and the location of the carbonate precipitation within the soil matrix. This enables the possible correlation between the carbonate crystals morphology and the performance of EICP-treated soil. As mentioned previously, some non-active calcium carbonate particles just precipitate within the soil pores and do not contribute effectively to the treated soil strength. The carbonate crystals that enhance the strength of soil dramatically are those that form effective bridges between the soil particles. Song et al. [2] conducted a study to examine the effect of precipitation microstructure on the mechanical properties of treated soil and the morphology of calcium carbonate structure. They concluded that not only the amount of carbonate but also the location of the carbonate has a significant effect on treated soil mechanical behaviour (Figure 18). Almajed et al. [61] studied the morphology of carbonate precipitation for soils that contain calcite using SEM images. They concluded that small size rhombohedral calcite crystals are more likely in EICP treated soils that already have calcite. Moreover, they concluded that disturbance in calcite crystal formation is expected in the presence of inhibiting matter such as magnesium ions and organic compounds. Martin et al. [118] evaluated the effect of the carbonate crystals size and shape on the mechanical performance of EICP treated soils. They concluded that soils that attained higher UCS had shown calcite crystals that are agglomerated at the soil inter-particle contact locations. This improvement in crystals morphology was attributed to the use of not-fat milk in the EICP cementing solution. Zehner et al. [115] used microscope sample cell to study precipitation processes in EICP in real time in the presence of calcite seeds and sand grains. The precipitated crystals was further characterized utilizing Raman microspectroscopy and SEM. In their study dissolved chalk solution (DCS) was used as a source of calcium, it was observed that mainly calcite crystals were formed. Moreover, it was found that multiple cycles increased the contact area between aggregates and sand grains promoting effective bridges between the sand grains

Figure 18. SEM images for two urease concentrations: (a) 0.5 g/L; and (b) 0.1 g/L [2].

7. Comparison between EICP and MICP

EICP and MICP are two faces of the same coin since they both result in the precipitation of calcium carbonate via urea hydrolysis. On the contrary, these methods are different when it comes to the crystallization of CaCO_3 and the mechanism of precipitation. In MICP, bacteria such as *Bacillus pasteurii* or *Sporosarcina pasteurii* are used as a source of urease compared to urease enzyme derived from a plant source in EICP or bacterial source in bacterial enzyme-induced carbonate precipitation (BEICP). Comparing MICP and EICP at the microscale can be a complicated process; the behaviour of free enzyme and living bacteria can vary depending on the soil type, cementing solution concentration, and enzyme source. However, a comparison in terms of performance and efficiency obtained from each method is achievable through routine shear and compressive strength testing.

Nafisi et al. [119] compared the macroscale and microscale behaviour of MICP- and EICP-treated soils through conducting triaxial testing, shear wave velocity, and SEM. They observed that EICP-treated soil needs less calcium carbonate precipitation to achieve the same shear wave velocity obtained from MICP-treated soil. Moreover, Nafisi et al. [119] compared the shear responses under drained conditions for EICP- and MICP-treated specimens at the same level of shear wave velocity. It was found that MICP specimens result in higher shear strength and larger dilative strain; however, MICP needed a higher number of injections to achieve the same shear wave velocity obtained from EICP samples. In order to induce enough carbonate precipitation in both bio-cementation techniques, several researchers have used cycles of treatment. In MICP, several strategies have been proposed to achieve higher carbonate precipitation [4,14,83,120]. These strategies include several cycles of two-phase injection of the microbial culture, followed by injection of the cementing solution containing the urea, calcium chloride, and nutrition needed for the microbial activity. However, few researchers have proposed techniques to induce carbonate after only one cycle of treatment. Cui et al. [121,122] proposed the use of (one-phase-low-pH method) in which EICP solution consisting of urea and calcium chloride with urease solution of pH = 6.5, is injected into the soil for one phase treatment of sand. Arab et al. [53] and Lee and Kim [95] proposed the use of high concentration EICP solution (up to 3 M urea concentration) to induce enough carbonate precipitation after one cycle of treatment.

Zhao et al. [87] utilized a batch reactor to induce carbonate precipitation in one cycle using both MICP and EICP. They found that using microbially produced urease is superior to using free urease enzymes when it comes to the enhancement of soil mechanical properties. It was found that under the same enzyme activity for both methods, MICP-treated soil had much better performance than EICP-treated soil; MICP-treated samples had an unconfined compressive strength of 1.76–2.04 MPa, while EICP samples had only 0.33–0.43 MPa.

To understand the effect of carbonate content on the UCS for both techniques (i.e., MICP and EICP), the results of carbonate content against UCS results were compiled from several studies in the literature and summarized in Figure 19. Figure 19a shows a comparison of EICP treated soils versus MICP treated soils after only one cycle of treat-

ment. It can be concluded that higher UCS results were achieved in EICP-treated soil at lower carbonate precipitation compared to MICP-treated soil.

(a)

(b)

Figure 19. Comparison of UCS for both MICP and EICP treated specimens versus carbonate content (a) one treatment cycle; (b) multiple treatment cycles (results collected from different studies, MICP-diamond and EICP-circle) data adopted from several studies [5,15,24,49,61,69,79,92–94,98,123].

Figure 19b shows the comparison for the case of multiple treatment cycles. It can be seen that, in general, higher carbonate content was achievable in the case of MICP, which resulted in higher UCS results for MICP-treated soils. To achieve such higher carbonate contents, a higher number of injections are required in the case of MICP-treated soil. The above comparison between EICP and MICP demonstrated that EICP is advantageous to MICP in terms of efficiency in the case of one cycle of treatment and higher compressive strength is achievable at lower carbonate content. However, higher carbonate content was achieved using multiple treatment cycles with higher UCS as well for the case of MICP. Similar conclusions were reached by Ahenkorah et al. [92] by comparing EICP to MICP treatment at the same conditions. In their study, a higher increase in UCS and carbonate content was achieved for soils treated with MICP compared to EICP after the same number of treatment cycles. However, higher uniformity of carbonate precipitation over the length of the treated soil columns was achieved in the case of EICP treated specimens with higher splitting tensile strength compared to MICP treated specimens at the same carbonate content.

8. Envisioned Applications of Soil Bio-Cementation via EICP

Even though the treatment of EICP is not yet commercialized and is still under development, many envisioned applications can be suitable for this promising technique in the future, serving as a sustainable solution for several engineering applications. This includes fugitive dust mitigation [27–32], surface water erosion control [29], creation of subsurface barrier excavation stabilization, soil nailing [78], liquefaction mitigation [33,46], and foundation support, among others [44,58]. Besides, EICP has been recently investigated to heal cracks in cement mortars [36] and combined with cement to improve cement-treated soil behavior [111]. Moreover, EICP can mitigate earthquake-induced soil liquefaction, improve soil slope stability, and immobilization of divalent cation contaminants [108]. More recently, in a trial to scale-up the EICP for field applications, Martin et al. [37,79] utilized a tube-a-manchette injection system to inject EICP cementing solution in the field. This demonstrates the ability of the EICP to be used for soil improvement in the field using conventional installation methods. Finally, Nething et al. [124] successfully implemented biomineralization in 3D printing a rigid and stable bio-cemented sand structure using urease active calcium carbonate powder.

8.1. Advantages of EICP for Soil Improvement

- EICP can promote sustainability and provide a product that has a less harmful impact on the environment than other conventional methods. When compared to Ordinary Portland Cement (OPC), carbonate cementation can replace OPC for a variety of ground improvement applications while reducing carbon dioxide emissions. EICP is likely a less energy-extensive solution than OPC.
- Urease used in EICP is a powder-like material that is soluble in water with a size of around 12 nm per subunit, which facilitates penetration into fine-grained soils [19].
- Using a free urease enzyme to hydrolyse urea eliminates some of the complications and restrictions related to the microbial technique. Some of these restrictions include microbial cell transport in soil, oxygen availability for deep soil treatment (for bio-stimulation), the need to provide nutrients for bacterial activity, and interaction with other microorganisms. These restrictions can be easily avoided when employing a free urease enzyme for the process of hydrolysis [19,122].
- Another potential benefit of EICP is that it can be transported to the site as a dry powder without any strict measures regarding temperature or handling. However, in the case of MICP, If the bacteria are grown off-site, they would have to be transported as a liquid or slurry (probably refrigerated), which would increase the cost. Even if ureolytic bacteria are cultivated on-site, custom-built tools and reactors have to be utilized [125].

- EICP is a flexible treatment that can be adjusted for many engineering applications, as mentioned earlier. A slight change in the mixture and concentration of the solution can give a different response in terms of shear strength, permeability, and shear wave velocity [121].

8.2. Challenges of EICP for Soil Bio-Cementation

Although EICP is a promising solution for many engineering applications, the treatment method is not simple, and several issues need to be addressed by the research community to further enhance the EICP field application and maximize its benefits, including:

- Environmental concerns regarding the contamination of groundwater caused by the production of ammonium chloride (NH_4Cl) as a by-product of the EICP process. Ammonium is potentially harmful and has a strong odour and can endanger the water supplies [29,126]. Furthermore, the choice to flush the ammonium from the soil is likely to be uneconomical [19,31]. To solve this problem, it was suggested to treat the cementation solution from ammonia-rich effluent before discharge and then back-feed the ammonia as a fertilizer to the surrounding plants [17]. Zehner et al. [115] successfully utilized crushed industrial quality limestone as a source of calcium instead of CaCl_2 . This process eliminated the production of chloride salts that may pollute water and lead to steel corrosion. However, this issue is still under investigation and considered one of the major challenges that face the wide applications of these techniques in the field.
- The distribution and homogeneity of the EICP treatment should be addressed as well as the soil compatibility and particle size. Since the EICP solution is water-based with low viscosity, it is hard to control the treated soil mass. Attempts to increase the viscosity of the treatment solution using biopolymers or copolymers could be the solution to immobilize the treatment solution and enhance its distribution along the soil matrix [81]. It is also crucial to control the rate of precipitation to improve the spatial distribution of carbonate within the soil pores [122].
- Scale-up bio-cementation for field soil grouting applications is still under investigation. Few studies have investigated the use of industrial-grade economical chemical constituents for the use in the hydrolysis process [125,127]. Recently, Khodadadi et al. [45] have shown that crude extracted urease enzyme from jack beans through a simplified process that involves few steps that can be conducted in situ. The process was used for mid-scale EICP injection in the field in demonstration of the possibility of scaling the process up [115]. However, more economical strategies need to be developed to scale up the production of EICP for grouting in field applications.
- Replacing bacteria with free enzymes in EICP leads to the loss of nucleation sites and potentially fastens the decay of urease activity as the enzyme may break down easier when not protected by the bacterial cell wall. However, a recent study by Almajed et al. [56] reported that adding non-fat dry milk powder provides nucleation sites for the calcium carbonate and enhances the stability of the urease, which works as an alternative to the bacterial cell wall. It is believed the protein in the milk can bind the calcium ions in the EICP solution, resulting in aggregate calcite or precipitate that acts as a nucleation site for carbonate precipitation [128]. However, the effect of the protein in the EICP cementing solution needs further investigation.
- Long term durability of EICP treated soils needs further investigation. For example, Song et al. [129] studied the effect of loading cycles on the shear wave velocity of EICP treated soils. They concluded that the stiffness of EICP-treated soils degrades when stress increases beyond debonding stresses, and this stress threshold depends mainly on the void ratio. Additionally, the post erosion behaviour of EICP treated soil in light of available literature of the behaviour of untreated gap-graded sandy soil [130].

8.3. Future Research

EICP falls in the category of bio-geotechnical engineering, which is an emerging and promising multidisciplinary field that involves earth science and microbiology ecology, structure, geotechnical, and chemical engineering. Although EICP has proven itself as a reliable method for soil treatment, it is still in its infancy and needs to be addressed in terms of maximizing the efficiency of treatment and reaching an optimum implementation that can lead to its commercial use.

Several studies have investigated the behaviour of soil treated with EICP, both at the microscale and macroscale. However, further research in terms of upscaling and implementing field-scale tests will help to identify the optimal application method and the effect of complex environmental conditions. Another aspect of field application of this technique is the understanding of the durability of EICP-treated soils when exposed to environmental conditions, which in turn will assist in understanding the durability of the treatment process and how tolerable it is to freeze–thaw, wet-dry, temperature, and humidity variations. It is critical to understand the urease production, handling, storage, and overall efficiency for in situ implementation and optimizing the performance for the required application in terms of optimum concentration of the solution and chemical environment affecting the overall process.

Enzyme activity is a major player in the whole process. It is important to advance characterizing the behaviour of the urease catalyst for more improved ways to quantify urease activity based on the enzyme protein mass, depletion rate, sorption properties, and solubility of the free urease enzymes. Moreover, the saturation level of soil may have a significant level on the precipitation distribution, which reflects the strength of EICP treated soils.

The groutability of EICP is a major concern, since the EICP cementing solution is water-based. Hydrogels, biopolymers, and copolymers have been investigated as additives to enhance the retention of the EICP cementing solution in the soil matrix, the calcite distribution, and the minimization of soil segregation and cementation medium. These aspects certainly need more work to clarify the use and benefits of EICP.

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Appendix A

Table A1. Summary of Previous Research Papers on EICP.

Application	Reference	EICP Cementing Solution				Soil Type	A Measure of Soil Improvement and General Remarks
		Urea	CaCl ₂	Jack Bean Urease	Additives		
Improve Soil Compressive Strength	Yasuhara et al. [26]	0.5 and 1.0 mol/L	0.5 and 1.0 mol/L	0.5 and 1.0 g/300 g of sand (activity not reported)	-	Toyoura sand	UCS in the range 400 kPa to 1.6 MPa
	Park et al. [96]	5/50 to 25/50 g/mL	10/50 g/mL Calcium Chloride, Calcium Hydroxide, or Calcium nitrate.	0 to 25 mL Jack Bean extract	-	Local sand from Nakdong River	Achieved UCS ranging from 30 to 317 kPa
	Neupane et al. [60]	1 mol/L	1 mol/L	15 g/L At 2970 units/g activity	-	Homogenous silica sand specimens	UCS of around 380 kPa
	Kavazanjian and Hamdan [3]	1.38 M (mix and compact) 1.36 (injection)	1.58 M (mix and compact) 0.765 (injection)	0.40 g/L at 200 U/g activity (mix and compact) 0.44 g/L at 26,100 U/g activity (injection)	non-fat dry milk at 4.0 g/L	Ottawa 20/30 silica sand	UCS of 529 kPa at 0.8% axial strain and 425 kPa at 1.8% for mix and compact treatment
	Putra et al. [98]	0.5 g/L	0.5 g/L	1 g/L	-	Keisa sand	Achieved UCS of 60 kPa at 3% carbonate precipitation
	Carmona et al. [62]	0.25 to 1.25 M	0.25 to 1.25 M	0.12 g/L (4 kU/L)	-	Poorly graded sandy soil (ASTM D2487)	UCS of 140 kPa at 0.25 M solution
	Zhao et al. [74]	1.5 M	1 M	0.450 mg/mL at activity of 15,000–10,250,000 units/g	1 mL/mL of solution of polyacrylate gel	Ottawa F-60	Hydrogel enhanced EICP was able to withstand up to 4.8×10^3 265 kPa
	Putra et al. [97]	0.5 mol/L	0.25 to 0.5 mol/L Calcium Chloride or 0 to 0.25 mol/L Magnesium Chloride	1 g/L at Activity of 2950 U/g	-	Dry silica sand	Achieved 0.6 MPa at 8.3% of carbonate precipitation
	Putra et al. [99]	0.5 and 1 mol/L	Mixtures of Calcium Chloride, Magnesium Chloride and Magnesium Sulfate at 0.04 to 1 mol/L	1 or 2 g/L	-	Dry silica sand	Maximum UCS of 555 kPa was achieved at 10% carbonate precipitation
	Oliveira et al. [113]	0.25 to 0.5 M	0.25 to 0.5 M	4 kU/L to 8 kU/L of activity 34,310 U/g	-	Sandy, silty, and organic soils	Strength gain: sandy soil (25 kPa), silty soil (250 kPa), organic soil (50 kPa)

Table A1. Cont.

Application	Reference	EICP Cementing Solution				Soil Type	A Measure of Soil Improvement and General Remarks
		Urea	CaCl ₂	Jack Bean Urease	Additives		
	Almajed [44]	0.875, 1.75 and 2.65 M	0.5, 1 and 1.5 M	0.85 g/L at activity of $\approx$ 1500–2500 U/g	1.1% xanthan gum (<i>w/w</i> %) of sand and 4.0 g/L of dry nonfat milk	Ottawa 20/30 silica sand	The addition of xanthan gum resulted in UCS of 1461 kPa when mixed with a powder with the soil
	Almajed [61]	1 M	0.67 M	3 g/L at activity of 3500 U/g	-	Ottawa 20/30 sand	UCS of 1268 kPa after 4 cycles of treatment
	Chandra & Ravi [70]	0.5 mol/L	0.5 mol/L	8 kU/L at activity of 40,150 U/g	-	silty sand (SM), clayey sand (SC), and silt (ML)	SM & ML achieved UCS of around (200 kPa), SC achieved UCS of (400 kPa)
	Beser [94]	20 g/L	49 g/L	5 g/L (activity not reported)	-	2095 granular silica sand	EICP achieved 66% and 77% UCS of the 28-day well cement and Type I cement mortars
	Almajed et al. [100]	0.5 M	0.875 M	0.85 g/L at Activity of 3500 U/g	-	Ottawa 20/30 sand	UCS in the range of 25 kPa to 296 kPa when sisal fiber was mixed with soil
	Pasillas et al. [81]	1 M	0.67 M	3 g/L (activity not reported)	Glycerol 50% (<i>v/v</i>) xanthan gum 0.25% (<i>w/v</i>)	Ottawa 20/30 and F-85 sands	The addition of xanthan gum reduces UCS by 25%. While glycerol does not produce an intact sample
	Beser [94]	20 g/L	49 g/L	5 g/L (activity not reported)	0.2% FORTA super-sweep fine fiber or 3 g/L nutrient broth	2095 Granusil silica sand	addition of nutrient broth to the EICP samples increased UCS from 2 to 6 MPa while the addition of fiber does not have a significant effect
	Hoang et al. [24]	0.3 M	0.3 M	Bacterial enzyme specified by activity only (4–5 mM urea/min)	-	Ottawa 20/30 silica sand	Achieved an unconfined compressive strength of 1691 kPa using bacterially derived urease for EICP
	Pandey [83]	60 g/L	111 g/L	5 g/L at activity of 678.70 μ M urea/min	7.5% (<i>w/w</i>) PVA	Ottawa graded sand Additionally, Recycled glass particles	EICP for 100% Ottawa graded sand results in 192 psi. Adding 20% of recycled glass reduces UCS by 70%. Adding PVA reduce UCS to 151 psi

Table A1. Cont.

Application	Reference	EICP Cementing Solution				Soil Type	A Measure of Soil Improvement and General Remarks
		Urea	CaCl ₂	Jack Bean Urease	Additives		
	Almajed et al. [56]	1.00 to 0.37 M	0.67 to 0.25 M	0.85 g/L at activity of 3500 U/g	4 g/L of powdered milk	Ottawa 20/30 sand	Adding powdered milk to the solution increases the compressive strength from 0.12 MPa to up to 1.82 MPa
	Almajed [131]	1 M	0.67 M	3 g/L at activity of 3500 U/g	4 g/L of milk and 300, 500 or 700 of Biochar mixed with sand	Local Crushed clean silica sand	Adding biochar to EICP require 4 cycles to achieve UCS of obtained of EICP alone
	Krishnan et al. [112]	1 M	0.67 M	3 g/L urease at activity of 4200 U/g	4 g/L nonfat milk powder	Ottawa 20/30	Achieved UCS of up to 1.6 MPa at carbonate precipitation of 1.3%
	Rohy et al. [91]	1, 2 and 3 M	0.67, 1.34 and 2 M	3, 9 and 6 g/L of active Jack bean meal at activity of 1500 U/g	4, 8 and 12 g/L of nonfat dry milk	ASTM C778 graded silica sand	3 M solution treatment at a curing period of 14 days resulted in UCS of up to 3 MPa
	Refaei et al. [82]	1, 2 and 3 M	0.67, 1.5, and 2 M	3, 6, and 9 g/L at activity of 1500 U/g	4, 8 and 12 g/L of non-fat dry milk and sodium alginate at 0.25, 0.5 and 1% (<i>w/w</i>) of soil	ASTM C778 graded silica sand	2 M solution at 6 g/L urease and sodium alginate of 1% resulted in a maximum UCS of 1800 kPa
	Hoang et al. [49]	0.15 mol/L and 0.3 M	0.15 mol/L and 0.3 M	The bacterial enzyme at 5 mM (urea/min)	-	coarse- and fine-grained sand	BEICP-treated sands ranged from 400 to 1500 kPa
	Almajed et al. [110]	0.5, 1 and 2 M	0.335, 0.5 and 1.34 M	1.5, 3 and 6 g/L bean meal urease enzyme 1500 U/g	4 g/L nonfat dry milk	Ottawa 20/30, filtration, Alrasheed, and Al-Nafud Sand	EICP treatment for the sand after one cycle resulted in higher compressive strength than the sands stabilized using 10% OPC
	Cui et al. [121]	1.0 M;	1.0 M;	40 U/mL (Extracted from Bacteria)	-	Ottawa 20/30 sand	EICP resulted in 3 MPa compared to 1 MPa at the same carbonate content of 9%
	Martin et al. [37]	1.5 M	1 M	9,900 U/L of Urease	6 g/L nonfat milk powder	local quarry sand	Achieved an average compressive strength of 154 kPa
	Lee & Kim [95]	0.75, 1.5 and 3 M	0.75, 1.5 and 3 M	70 & 140 g/L (Soybean Solution) at Activity of 0.4705 Ammonium mg/L/min	-	Local Sand from Hantan River—South Korea	Achieved UCS up to 860 kPa
	Ahenkorah et al. [92]	0.5 M	0.5 M	0.25 g/L at activity of 40,150 U/g	4 g/L nonfat dry milk	Adelaide Industrial (AI) sand	Achieved UCS of up to 4.23 MPa

Table A1. Cont.

Application	Reference	EICP Cementing Solution				Soil Type	A Measure of Soil Improvement and General Remarks
		Urea	CaCl ₂	Jack Bean Urease	Additives		
	Yuan et al. [132]	4.2 M	2.8 M	100 g/L of Urease	Skim Milk powder or Rice Powder or Brown sugar (2 to 32 g/L)	Local soil from the Yellow River flood area in Kaifeng	Achieved average UCS of 1489.3 kPa for modified EICP with up increase of up to 32.62% over nonmodified.
	Martin et al. [118]	1 M	0.67 M	12.6 kU/L	4 g/L nonfat milk powder	Ottawa 20/30, F85, Glass Beads and Local washed Quarry	Reached UCS of 1600 kPa at 1.2 Carbonate Content for Ottawa 20/30
	Miftah et al. [68]	1 M	0.67 M	1.25 to 15 mL/L Crude Urease Extract at Activity of 465 U/mL	4 g/L nonfat milk powder Additionally, 0%, 5% and 10% of Seawater	Local beach sand from Famagusta Bay—Cyprus	Achieved UCS of 0.255 MPa for control samples and 0.224 MPa for samples treated with 10% Seawater
	Pratama et al. [133]	0.5 mol/L	0.5 mol/L	20 g/L at Activity of 1668.7 U	-	Keisha sand	Achieved UCS of 600 kPa after 3 cycles
Shear Wave Velocity	Song et al. [2]	1 M	1 M	0.1, 0.5 and 1 g/L at activity of 40,318 U/g	-	uniformly graded natural sand,	0.8 ms to 1.5 ms reduction in electric conductivity and 800 m/s of shear wave velocity for 1 g/L of urease
	Obeidy et al. [105]	NA	0.1 and 0.2 M	NA	NA of dry non-fat milk	Ottawa 20/30 sands	Achieved 600 m/s of shear wave velocity for sand columns
	Song et al. [101]	1 to 1.5 M	0.5 and 1 M	0.5 to 0.9 g/L of Urease	-	Ottawa 50/70 or Jumunjin	EICP treatment increase the sand's shear stiffness of up to 30 times after 6 h of treatment.
	Nafisi et al. [104]	333 mM	100 mM	0.55 g at activity of 200 unit/g	0.3 g of nonfat powder milk	Ottawa 20/30 or Ottawa 50/70	Achieved shear wave velocity of up to 700 m/s
Permeability Reduction	Nemati & Voordouw [9]	12 g/L	30 g/L	0.01, 0.03 and 0.1 g/L at 26,100 U/g	4 g/L	Sand 70% & glass 30% (w/w%)	98% reduction in permeability after 2 injections
	Nemati et al. [69]	36 g/L	90 g/L	0.3 g/L (activity not reported)	-	Sand 70% & glass 30% (w/w%)	62% decrease in the permeability when using EICP
	Larsen et al. [51]	88, 110 and 132 mol/L	200, 250 and 300 mol/L	Urease active bean meal of 25 g/L at activity of 150 kU/g	25 g/L bentonite or use of large-grained bean meal	-	The addition of bentonite highly increase the plugging efficiency from 1 to 2.9 bar
	Yasuhara et al. [26]	0.5 and 1.0 mol/L	0.5 and 1.0 mol/L	1 and 2 g/300 g of sand (activity not reported)	-	Toyoura Sand	Permeability of treated sand is reduced from 10 ⁻¹ to 10 ⁻³ cm/s
	Neupane et al. [60]	0.5 mol/L	0.5 mol/L	2 g/L at activity of 2950 U/g	-	Sandy soil	Reduction of porosity from 0.41 to 0.382

Table A1. Cont.

Application	Reference	EICP Cementing Solution				Soil Type	A Measure of Soil Improvement and General Remarks
		Urea	CaCl ₂	Jack Bean Urease	Additives		
Water and Vapor Retention to Enhance Groutability	Handley-Sidhu et al. [52]	400 and 200 mM	400 mM and 200 mM	0.25 & 0.5 g/L (activity not reported)	-	borosilicate beads	Permeability was reduced from 9.99 to 0.175 Darcy
	Putra et al. [98]	0.5 g/L	0.5 g/L	1 g/L	-	Keisa sand	Hydraulic Conductivity reduced to less than 0.02 cm/s
	Hoang et al. [24]	0.3 M	0.3 M	Bacterial enzyme specified by activity only (4–5 mM urea/min)	-	Ottawa 20/30 silica sand	one-log decrease in permeability after sixteenth cycle treatment
	Hoang et al. [49]	0.15 mol/L and 0.3 M	0.15 mol/L and 0.3 M	Bacterial enzyme at 5 mM (urea/min)	-	coarse- and fine-grained sand	Permeability reduction from 10 ⁻¹ to 10 ⁻² was achieved after BEICP treatment
	Hamdan et al. [125]	0.6 and 3.0 M	0.4 and 2.0 M	0.44 g/L for high activity (26,100 units/g) and 0.85 g/L for low activity enzyme (200 g/U)	4.0 g/L of unspecified stabilizer (0.1 to 5%) of guar gum and xanthan gum) and 5% to 30% of polyol-cellulose hydrogel	F-60 fine-grained silica sand	17% reduction in vapor pressure at 2% guar gum and 47% reduction while using guar gum.
	Pasillas et al. [37]	1 M	0.67 M	3 g/L (activity not reported)	Glycerol 50% (v/v) xanthan gum 0.25% (w/v)	Ottawa 20/30 and F-85 sands	The addition of xanthan gum enhanced water retention the most at retaining 74% of the original solution
Direct Shear Test	Aishwarya & Christy [102]	1 and 2 M	0.5, 1, and 2 M	1 mg of this urease active the meal can hydrolyze 3 mg of urea in 30 min at 37°C	-	Gujarat and Yamuna sand	Shear strength increase in the range of 40–50%
	Putra et al. [103]	1 mol/L	0.9 to 1 mol/L Calcium Chloride and 0 to 0.1 Magnesium Chloride	2 g/L	-	Silica sand	Achieved a cohesion of 53 kPa at 4.1% carbonate content
Fugitive Dust Control	Bang et al. [28]	0.333 mol/L	0.1 mol/L	0.5 units/mL	0% to 1% 1 L solution.	Poorly graded sand	EICP resulted in 0.01% weight loss at 9 m/s wind speed
	Knorr [29]	1 and 2 mol/L	0.375 to 2 mol/L	0.5 g/L (activity not reported)	4 g/L	Arizona silty-sand, medium-grained silica sand, and mine tailings	Achieved average detachment velocity of >25 m/s

Table A1. Cont.

Application	Reference	EICP Cementing Solution				Soil Type	A Measure of Soil Improvement and General Remarks
		Urea	CaCl ₂	Jack Bean Urease	Additives		
Cyclic Triaxial Test	Hamadan & Kavazanjian [27]	0.075 to 3 M	0.05 to 2 M	0 g/L and 0.45 g/L at activity of 26,100 U/g	4 g/L of powdered nonfat milk	uniform, fine-grained, clean Ottawa F-60 silica sand, well-graded native Arizona silty fine sand; and mine tailings obtained from a site in southern Arizona	Achieved a detachment velocity of >25 m/s for the 3 treated soil types
	Almajed et al. [32]	1 mol/L	0.67 and 1 mol/L	3g/L at activity of 1500 U/g	4 g/L of powdered milk and 0.5%, 1% or 2% of sodium alginate (<i>w/w%</i>) of solution	Nafud desert sand	Combinations of EICP and Sodium alginate had a 0% erosion rate compared to other combinations
	Miao et al. [84]	0.75 mol/L	0.75 mol/L calcium acetate	Urease from soybeans at 4000 U/L	-	Tengger desert sand	EICP Treated 29.1 m/s. Higher spray passes lead to more wind erosion resistance
	Martin et al. [37]	1.5 M	1 M	9900 U/L of Urease	6 g/L nonfat milk powder	local quarry sand	Needle penetrating test resulted in 348 N surface strength of treated sample compared to 35 N for the control sample
	Hamdan [58]	400 mM and 1M	300 mM and 1 M	2.0 g/L	-	Ottawa 20/30 sand and Ottawa F-60 silica sand	20–30 silica sand resulted in a strength increase of 100 kPa at 2% carbonate content whereas F-60-treated silica sand increased up to 125 kPa at 1.6%
	Simatupang and Okamura [33]	0 to 0.3 mol/L	0 to 0.3 mol/L	0 to 35 g/L (activity not reported)	-	Tohoku Keisha No. 4	Only 1% of carbonate content is required to double the liquefaction resistance for up to 0.4
	Almajed [44]	1 M	0.67 M	3 g/L at activity of 3500 U/g	4 g/L non-fat dry milk	Ottawa 20/30 sand	Internal friction angle and effective stress cohesion were calculated as 34.58° and 332.96 kPa

Table A1. Cont.

Application	Reference	EICP Cementing Solution				Soil Type	A Measure of Soil Improvement and General Remarks
		Urea	CaCl ₂	Jack Bean Urease	Additives		
	He et al. [50]	0.5 mol/L	0.5 mol/L	Bacterial enzyme at 0.33 mL/mL (<i>w/w</i>) of solution (activity not reported)	-	Ottawa sand (ASTM graded) with quartz powder	10 passes of treatment solution resulted in the highest deviator stress results of 1750 kPa
	Gao et al. [48]	0.25 and 0.5 mol/L	0.25 and 0.5 mol/L	40 and 130 g/L Soybean concentration at an activity of 6.5 and 13.2 mM/min	-	Quartz sand and Ottawa sand	Achieved maximum axial stress of 250 kPa after 15 treatments.
Crack Healing in Concrete	Dakhane et al. [36]	0.6 and 1.2 M	0.5 and 1 M	1 g/L at activity of 200 U/g	2 g/L of non-fat dry milk	-	33% enhancement of flexural strength for mortars treated with EICP
Tensile Strength	Ahenkorah et al. [92]	0.5 M	0.5 M	0.25 g/L at activity of 40,150 U/g	4 g/L nonfat dry milk	Adelaide Industrial (AI) sand	Achieved a splitting tensile strength of 1 MPa at 11.6% carbonate content.
Water Erosion	Cuccurullo et al. [128]	2 mol/L	2 mol/L	Crude Soybean Extracts	-	Silty Clay from (Bouisset brickwork factory – France)	Treated soil reported significantly lower weight loss due to water erosion and did not experience any cracks or swelling.
	Ossai et al. [35]	1 and 2 M	0.67 and 1.34 M	3 g/L	4 g/L nonfat dry milk	Two natural quartz sand and Ottawa 20/30	The average percent loss of fines due to pre-rinsing was 1.6% for natural sands and almost 0% for Ottawa 20/30
Biobricks	Arab et al. [34]	1, 2, and 3 M	0.67, 1.34, and 2 M	3 g/L	4 g/L nonfat dry milk and 0. To 1.5% of soil dry mass Sodium Alginate	Graded silica sand ASTM C778	Achieved UCS of 1800 kPa, and flexural strength of 2200 kPa and thermal conductivity of 0.25 W/mK, and initial rate of absorption of 16.29 g/min/50 cm ²

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