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Biostabilization techniques and applications in Civil Engineering: State-of-the-Art

Mahi Patil [a], Parishi H. Dalal [a], Sowmya Shreedhar [b], Trudeep N. Dave [a,*], Kannan K.R. Iyer [a,*]

[a] Department of Civil Engineering, Institute of Infrastructure, Technology, Research and Management, Ahmedabad 380026, India
[b] Delft University of Technology, Delft, the Netherlands

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ABSTRACT

Biostabilization is an emerging environmental friendly stabilization method for improving the properties/performance of civil engineering materials/structures. The present work focuses on various biostabilization methods, the processes, pathways involved, and their applications in civil engineering, which have been explored through laboratory and field-scale studies. Different microorganisms, enzymes, and nutrient dosages used, and the effect of treatment on improvement in compressive strength, reduction in permeability, and other properties have also been discussed. A critical assessment of earlier studies has shown that the increase in compressive strength varies exponentially with calcium carbonate precipitation. It has also been observed that improvement in compressive strength in different studies varies significantly, and efforts have been made to understand the reasons for this variation. The work also discusses the factors controlling upscaling of the biostabilization process and its prospective applications in various infrastructure projects. The detailed assessment presented in this work may help engineers and researchers to understand the challenges associated with biostabilization methods, thereby, leading to their successful implementation in future applications.

1. Introduction

The performance of civil engineering structures such as buildings, industrial structures, towers, foundations, slopes, embankments, and landfills; during their lifetime, are significantly affected by different environmental and anthropogenic factors/effects such as temperature and humidity variation, wind forces, tidal effects, chemical interaction in the marine environment [1,2], microbial activity within soil, discharge of pollutants from industries, acid rain, sulphate attack [3–8]; and natural calamities such as earthquakes, tsunamis, cyclones and freezing/thawing effects in cold regions [2,9]. The effects on the civil engineering infrastructure include the development of cracks, expansion, deterioration of concrete [7], reduction in strength [10–13], corrosion of steel [14], pollution of the rivers, seas, and other water bodies due to industrial effluent discharge [15,16], pollution of soil and groundwater due to percolation of leachate from landfills and use of chemical fertilizers [17], erosion of soil [18], seepage through structures and soil [19], as well as total and differential settlement of infrastructure facilities. The impacts of these effects on various infrastructure facilities include an increase in their maintenance and life-cycle cost, and functional redundancy of these facilities earlier than expected [20–22].

In order to improve the performance of various civil infrastructure facilities, efforts have been made in earlier studies to reduce the permeability and water seepage using different materials, to control the crack formation and its coalescence in concrete and other materials, to improve the resistance of construction materials against salts, chloride, sulphate, and other environmental agents; to stabilize soil for improving its bearing capacity and reduction in settlement characteristics for embankment and other soil structures, and to control contamination of the surrounding environment by immobilization of contaminants. Permeability (viz., rate of flow of fluid through materials) can be reduced through grouting methods such as water-saturated cement grout [23], swelling clay grout [24], colloidal silica grout [25], bentonite grout [26], ultrafine Portland cement grout [27], cement-fly ash grout with fibers and superplasticizer [28], and acrylamide grout [29]. Grouting has also been widely used for soil stabilization [30,31]. Recently, Grouted Vertical Barrier (*GVB*) has been employed for swiftly reducing the seepage characteristics (viz., seepage rate, exit hydraulic gradient, and uplift force) of small hydraulic structures [32] and earthen dams [33]. However, the adverse effects of conventional grouting

* Corresponding authors.
E-mail addresses: mahi.patil.19pc@iitram.ac.in (M. Patil), parishi.dalal.20pc@iitram.ac.in (P.H. Dalal), s.shreedhar@tudelft.nl (S. Shreedhar), trudeepdave@iitram.ac.in (T.N. Dave), kannaniyer@iitram.ac.in (K.K.R. Iyer).

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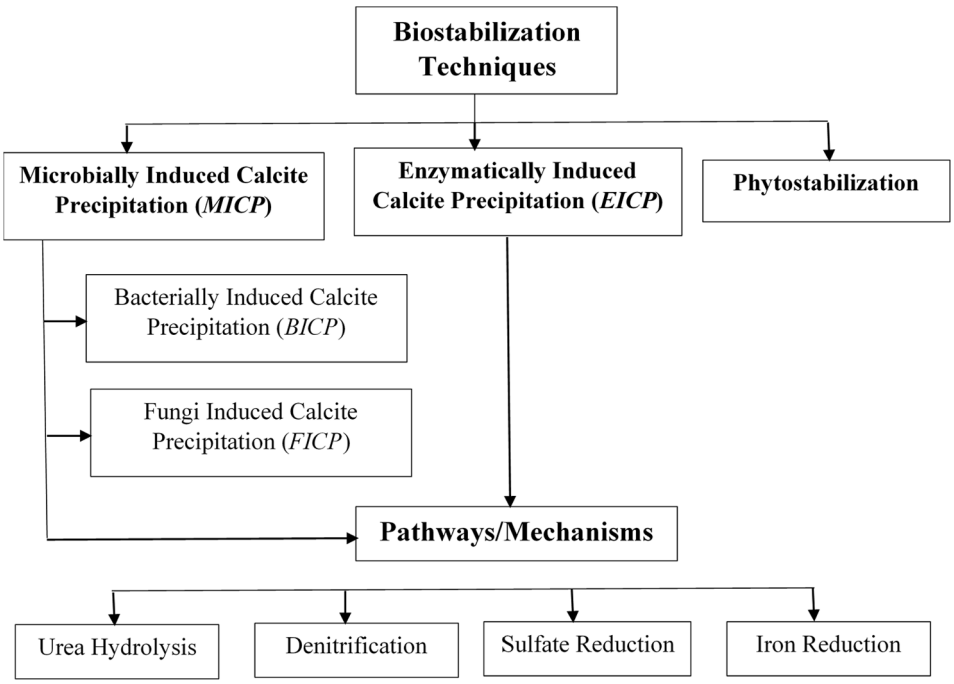

Fig. 1. Biostabilization techniques and their mechanisms.

methods on the environment due to harmful materials have been a concern [23]. Moreover, most grouting methods require high pressure and energy, which renders these methods costly [24,29]. Researchers have also reported other methods, such as the utilization of polystyrene or kaolinite particles, which have resulted in some reduction in permeability [34].

Improvement in strength and controlling the cracks in concrete can be achieved by altering the composition of concrete with a higher amount of binder material [35] such as Portland cement. Efforts for treatment of cracks (viz., healing of cracks) and improvement in strength and durability of construction materials have been made by other methods such as grouting [25,26,29], the addition of steel fibers [36], and carbon nanofibers [37]. Kavazanjian and Hamdan stated that Portland cement is also widely utilized for ground improvement applications, especially for cohesionless soils [38]. For cohesive soils, lime has traditionally been utilized for improving the properties of soils [39,40]. However, it has been noted that 7% of global CO_2 is generated from the production of cement itself [41]. Also, the utilization of lime results in the depletion of natural limestone. Research has also been attempted to stabilize the soil using materials such as rice husk ash, fly ash, sludge from rubber factories, and/or sugar manufacturing plant, and slag from steel industries, through solidification of the soil [42–45]. However, except fly ash, other materials have been seldom used in practice due to environmental concerns and pre-treatment requirements to remove the undesirable components from these materials [30,39,45].

In a nutshell, the utilization of various conventional methods mentioned above for stabilization and improving the performance of civil infrastructure facilities possess various limitations such as being less effective, energy-intensive, time-consuming, or costly. Moreover, some of these methods cause the depletion of natural resources and/or environmental pollution [23,24,29,34,41]. Due to these limitations of conventional methods, biostabilization has gained attention as an environmentally sustainable method for stabilizing materials. Biostabilization is a biochemical technique to produce calcium carbonate, which has binding characteristics. It can be used for improving various properties of civil engineering materials and structures such as strength [46–48], permeability [46,49,50], repair of fractures in the rock and concrete [51,52], and crack healing [53,54]. Biostabilization is an eco-friendly and cost-effective technique [55] and is relatively less time-consuming for application [56]. Although extensive laboratory studies have been conducted to evaluate the efficacy of biostabilization for different civil engineering materials, their field implementation has been assessed by limited studies [57–59]. The present work discusses in detail the process of biostabilization, its methods, pathways, and the applications of biostabilization in civil engineering. The effects of biostabilization on improving various properties of civil engineering materials have also been evaluated in the study. The paper is further divided into the following sections: biostabilization techniques, application of biostabilization for improving different properties of civil engineering materials, field/large scale applications of biostabilization, and focus areas for future research on biostabilization.

2. Biostabilization techniques

An overview of different biostabilization techniques is presented in Fig. 1, which includes Microbially Induced Calcite Precipitation (*MICP*), Enzymatically Induced Calcite Precipitation (*EICP*), and Phytostabilization. *MICP* further can be classified into Bacterially Induced Calcite Precipitation (*BICP*) and Fungi Induced Calcite Precipitation (*FICP*). Both *MICP* and *EICP* techniques of biostabilization can be achieved by one of the four pathways/mechanisms: urea hydrolysis, denitrification, sulphate reduction, and iron reduction.

2.1. Microbially induced calcite precipitation (MICP)

MICP refers to the generation and precipitation of calcium carbonate by microbial cells and biochemical activities [60]. *MICP* has obtained considerable attention in recent years due to the involvement of natural processes in the mechanism and its various potential applications [61]. A study by Boquet et al. [62] noted that bacteria present in soil could promote calcium carbonate deposition. These microbes consume organic matter in the soil to evolve carbon dioxide (CO_2), which subsequently dissolves in water to produce bicarbonate and carbonate under favourable environmental conditions. *MICP* can occur in four major ways: urea hydrolysis, denitrification, sulphate reduction, and ferric iron reduction [63–65]. The cost of cultivating denitrifying

bacteria is comparatively high due to its slow production rate and longer growth period. The major disadvantage of the denitrification pathway is its lower precipitation rate of $CaCO_3$ [66,67]. However, unlike ureolytic bacteria, denitrifying bacteria can grow and function in situ under oxygen-deprived conditions, thus enabling long-term on-site remediation [66,68]. In the sulphate reduction process, toxic gas (H_2S) is produced, which adversely affects the environment and human beings. The iron and sulphate reduction seem to be undesirable pathways due to the low solubility of the essential oxidizing substrates [67,68]. In comparison to these pathways, urea hydrolysis is simple and easy to control, and precipitates higher calcium carbonate in a short period [65]. Moreover, urea hydrolysis is the most economical pathway as it is a rapid process with no additional requirement of nutrients for the long-term maintenance of bacterial activities [47,48,69–71]. Hence, this mechanism has been widely adopted for *MICP* and its related applications [47,72–74].

2.1.1. Urea hydrolysis in MICP

During urea hydrolysis, urease catalyzes the hydrolyzed urea into ammonium and carbonate [47,60,63,72–79]. One mole of urea gets hydrolyzed to form one mole each of ammonia and carbamic acid, which then is spontaneously hydrolyzed to form one mole of ammonia and carbonic acid. The equilibration of NH_3 and H_2CO_3 (formed during the above reactions) in water forms bicarbonate, two moles of ammonium, and two moles of hydroxide (OH^-) ions. The OH^- ions tend to increase the pH, which helps to shift the bicarbonate equilibrium and the formation of carbonate ions is achieved as depicted in Eq. (1) [60,80].

$$HCO_3^- + H^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2H_2O \quad (1)$$

The release of NH_4^+ increases the surrounding pH, and the reaction spontaneously produces calcium carbonate [60,72,81]. If a sufficient concentration of CO_3^{2-} and Ca^{2+} are present in the solution, then precipitation of $CaCO_3$ will occur at the cell surface of microbes according to Eq. (2) [60,65]. Various calcium sources that can be used in the mechanism are calcium chloride, calcium nitrate, and calcium acetate; however, the influence of calcium sources is limited to the structure of crystals [78,79].

$$\text{Cell-}Ca^{2+}CO_3^{2-} \rightarrow \text{Cell-}CaCO_3 \quad (2)$$

The chemical reactions associated with the precipitation of minerals are influenced by urease through the various parameters such as pH, concentration of calcium, and dissolved inorganic carbon [60,63,77–79], which are responsible for the concentration of carbonate ions (CO_3^{2-}). Another important parameter is the availability of the nucleation sites, which provides a platform for continuous and stable calcium carbonate formation [78,82]. The microorganisms in these biochemical reactions play two major roles: (a) providing urease for the process of urea hydrolysis, and (b) provision of nucleation sites for the precipitation of minerals, viz., calcium carbonate [83].

2.1.2. Types of MICP

Bacterially induced calcite precipitation (*BICP*) and Fungi induced calcite precipitation (*FICP*) are two types of *MICP*. Bacterially induced calcite precipitation (*BICP*) refers to precipitation resulting from the reaction taking place between the microbes (bacteria) and the environmental conditions to which they are subjected. *BICP* is a type of *MICP* that is usually performed under an open environment, without any need for specialized cell structures. The bacterial cell body acts as a nucleation site in the process [84,85]. Perito and Mastromei explained certain metabolic pathways for *BICP*, which include both the autotrophic and heterotrophic pathways [61]. Important groups of bacteria that are involved in the autotrophic pathway [86] are methanogenic archaebacteria, sulphurous (purple and green bacteria) or non-sulphurous bacteria, and cyanobacteria [63,86]. These bacteria use carbon as the energy source and produce organic matter, wherein carbon is mostly obtained from dissolved or gaseous carbon dioxide. Therefore, these pathways enhance the local carbon dioxide depletion, either of the medium or the environment, in the bacteria's close vicinity. When Ca^{2+} ions are present in alkaline or neutral media, such CO_2 depletion promotes calcium carbonate precipitation. For aqueous environments like freshwater or marine ones, photosynthesis-induced calcification using cyanobacteria is one of the common pathways for *BICP* [63,87,88]. In photosynthesis, cyanobacteria produce carbonate while consuming bicarbonate, thereby making the surrounding environment alkaline, and leading to precipitation of carbonate by calcium ions in water. The heterotrophic pathway includes two simultaneous processes for carbonate precipitation by bacteria [86]. The first process is the active precipitation by ion exchange through the cell membrane; wherein, calcification produces protons as a source of nutrients and for the bicarbonate uptake [86,87]. This type of precipitation can be linked to the transport of ions through cellular membranes, and generates minerals and protons as a product of the reaction. The various advantages of using *BICP* include: bacteria are usually harmless, their collection and isolation are not very complex, and they are easy to culture and manage in laboratory conditions [89].

Amongst the microbes, other than bacteria, fungi are also reported to induce calcite precipitation, and this process is called fungi-induced calcite precipitation, *FICP* [89,90]. Fungi species are saprophytic, and they produce a large amount of organic acid, enhancing the dissolution of rocks and the neo-formation of oxalate and carbonate crystals [91]. It has been reported that the precipitation of calcite crystals occurs on fungal hyphae, viz., slender filaments formed during the growth of fungi, which act as a nucleation site [89]. Further, the hyphae grow into mycelia branches, which further form a mat on the mineral substrate [91]. These mycelial structures and biomass, act as bio-based fibres for calcite precipitation or bio-cementation [90,91]. In *FICP*, urea is hydrolyzed by the urease enzyme produced by fungi species, which results in the interaction of carbonate and calcium ions. These calcium ions surrounding the fibrous mycelia bind with carbonate ions and accumulate at the nucleation sites, ultimately resulting in calcite precipitation, as the pH of the medium increases due to the dissolution of $Ca(OH)_2$ [89,90].

Recent studies have reported that engineered growth of the vegetative part of a fungus (mycelium - a mass of hyphae: thin, tubular, branching, thread-like networks in soil) can modify shear behaviour [92], and improve the unconfined compressive strength of sands [93]. An explorative experimental work reported in recent studies [92] has demonstrated that the mycelia network of *Pleurotus ostreatus* (fungus) can induce soil water repellence, reduce infiltration, lower field-saturated hydraulic conductivity, alter water retention curve, and significantly improve the erosion resistance of the sand. So far, studies have been conducted using a limited number of fungal species (*Pleurotus ostreatus* and *Rhizopus oligosporus*), grown in the sand at a bench scale and under controlled conditions. Although at preliminary stages of development, the reported outcomes seem promising. Further investigations are required to elucidate the mechanisms of mycelium-mediated ground improvement and its associated limitations and methodological optimisation towards field-scale applications for suitable geotechnical problems. Another recent study has stated the advantages of using fungi over bacteria [89], which includes the ability of fungi to sustain a deleterious environment better than bacteria. Further, bacteria in some cases may require an aseptic environment and expensive growth (nutrition) media to produce microbial spores as compared to fungi [65,94]. Overall, limited studies have explored the fungi species as compared to bacteria and bio-based enzymes for calcite precipitation.

2.2. Enzyme Induced Calcite Precipitation (EICP)

In Enzyme-induced calcite precipitation (*EICP*), urease enzyme in the presence of calcium and urea catalyzes the urea hydrolysis process to precipitate calcium carbonate ($CaCO_3$) in a water-based solution. Urease enzyme can be extracted from various microorganisms (bacteria, fungi,

Table 1
Sources of Urease Enzyme for EICP Technique.

Origin/Source of enzyme	Enzyme Activity*	References
Type III: From Jack Beans (*C. ensiformis*)	26,100 U/g	[97]
Jack bean meal	2,950 U/g	[94,98]
Jack bean meal	2,970 U/g	[99]
Jack Bean and *B. pasteurii*	60 U/mg and 194 U/mg	[100]
Sword Beans	–	[95]
Jack bean	34,310 U/g	[47]
Type III Jack Bean and Low-activity Jack Bean	26,100 U/g and 200 U/g	[101]
Sporosarcina pasteurii ATCC-11859	25.4 mM/min	[102]
Low grade Jack Bean	≈200 U/g	[38]
Calzyme Labs and Bean Meal	149 U/mg and 1.7 U/mg	[103]

*U/g- Units per gram, U/mg- Units per milligram, mM/min- milli-Mole per minute.

and algae), plant, and animal species. Jack Bean has been reported to be the commonly used plant species as an enzyme source [95,96]. Enzyme/Urease activity can be understood as the amount of urease consumed to hydrolyze urea and thus decides the efficiency of the process. The activity of an enzyme in the soil is a function of numerous activities associated with different biotic and abiotic components [95,96]. The different sources of urease enzyme commonly used for *EICP* treatment by earlier researchers are presented in Table 1 [38,47,94,95,97–103].

Enzyme-induced carbonate precipitation is similar to that of microbially induced precipitation. *EICP* does not include any living organisms, but it imitates *MICP,* and the enzyme is usually extracted from microorganisms or plant species. Hence, it is also considered a bioinspired process. *EICP* as compared to *MICP*, has a rapid rate of carbonate precipitation, high carbon utilization efficiency from the substrate for the formation of $CaCO_3$; and as it does not involve living organisms, the need of nurturing urease-producing microbes is eliminated [38,101]. Unlike *MICP*, the *EICP* does not involve complex or sensitive processes of harvesting bacteria and storing them. It is carried out by urease enzyme production and extraction; and mixing of urea, calcium source and urease enzyme with the materials to be improved [47]. These advantages make *EICP* appropriate for various surface treatments and other applications, employing carbonate precipitation that has a specific short time, within which they need to sustain themselves effectively [101]. Sometimes, the yield of carbonate precipitation is reduced, possibly due to the tendency of pure urease to co-precipitate with carbonate minerals [103]. The typical mechanism of *MICP/EICP* via urea hydrolysis is illustrated below in Fig. 2.

2.3. Factors affecting calcium carbonate precipitation during MICP/EICP

Urease activity and the amount of calcium carbonate precipitation in all the above-mentioned mechanisms are dependent on different environmental factors [60,77,104,105]. The various factors affecting calcium carbonate precipitation are discussed below:

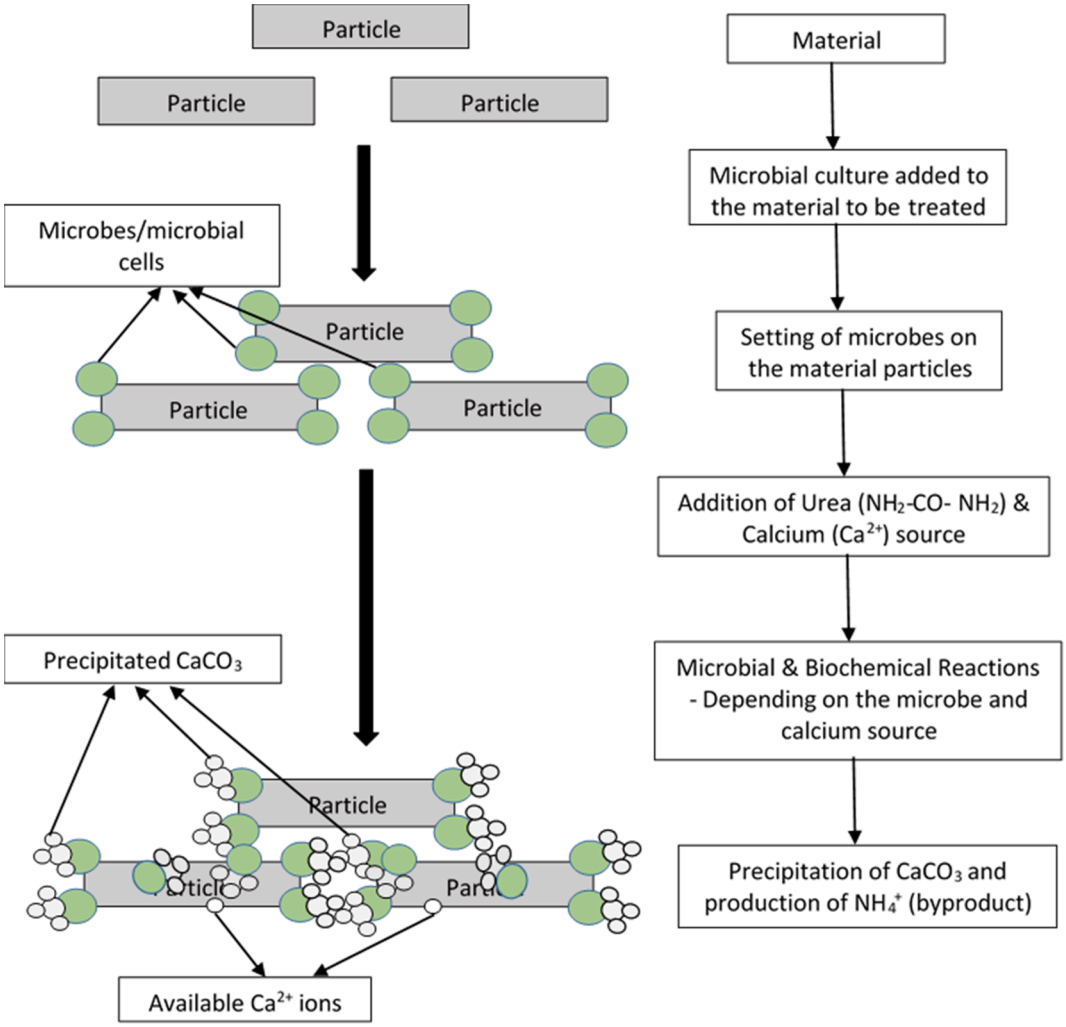

Fig. 2. Typical mechanism of MICP/EICP via urea hydrolysis.

Table 2
Phytostabilization for slope stabilization.

Major Properties/ Process	Plant Species	Soil stabilized	References
Root Tensile Strength	Shrubs- *Artiplex lentiformis, Lycium andersonii, Larrea tridentate, Allenrolfea occidentalis, Rosa canina, Cotoneaster dammeri, Juniperus horizontalis, Rhodomyrtus tomentosa, Melastoma sanguineum, Inula viscosa, Spartium junceum, Genista cinerea, Thymus serpyllum* Herbs- *Achnatherum calamagrostis, Aphyllantes monspeliensis* Trees- *Schefflera heptaphylla, Reevesia thyrsoidea, Pinus nigra, Quercus pubescens* Grass- *Vetiveria zizanioides*	Fine Sand (Stream-bank), Highly Saturated Soil (Slopes), Highly Consolidated Clays (Creek Catchment Area), Jurassic Black Marls, Landfill Slope, Clayey Soil, Other Soil	[117–123]
Root Cohesion	Shrubs- *Artiplex lentiformis, Lycium andersonii, Larrea tridentate, Allenrolfea occidentalis, Rhodomyrtus tomentosa, Melastoma sanguineum, Spartium junceum* Trees- *Schefflera heptaphylla, Reevesia thyrsoidea*	Fine Sand (Stream-bank), Highly Saturated Soil (Slopes), Landfill Slopes	[119,122,123]
Erosion Control /Mitigation	Shrubs- *Genista cinerea, Thymus serpyllum* Herbs- *Achnatherum calamagrostis, Achnatherum calamagrostis* Trees- *Pinus nigra, Quercus pubescens,*	Soil (Jurassic Black Marls)	[121]
Soil Shear Strength	Shrubs- *Retama sphaerocarpa, Anthyllis cytisoides* Rush- *Juncus acutus* Grasses- *Brachypodium retusum, Piptatherum miliaceum, Vetiveria zizanioides*	Jurassic Limestone, Dolomite Mountains with Calcareous Piedmonts, Cretaceous Deposits, Miocene Marls, Keuper Gypsum Deposits, Clayey Soil, silty clayey sand (Riverbank)	[118,124,125]

• For calcite precipitation, high pH (8.7–9.5) is mandatory, as lower pH will tend to dissolve carbonate rather than precipitating it [75,85,106]. Further, the urease activity is faster at pH value ~ 9 [96,107].

• The optimum temperature required for the ureolysis process ranges from 20 to 37 °C [81,108,109].

• Calcite precipitation is directly proportional to the bacterial cell concentration, as cells provide the site for nucleation, and concentration up to 10^6-10^8 cells/ml is desired [75,108].

• Type of microorganism (viz., bacteria or fungi) plays an important role in urease production [107], and relatively constant rate and better urease activity by fungi species have been reported as compared to bacterial species [90].

2.4. Phytostabilization

Several plant varieties have been explored in stabilizing slopes, improving slope durability, preventing and controlling soil erosion, reducing surface run-off, and minimizing slope failures by planting the desired plant/grass species on the surface/slope. This biotechnical/bio-engineering strategy of soil stabilization (phytostabilization) has gained attention as an eco-friendly, sustainable, and low-cost technique [110–113]. The roots of the plant species grow stronger and penetrate deeper with time, thereby binding strongly with the desired material (viz., soil, waste dumps), and enhancing the strength and stability of the soil and waste dump slopes [114]. This approach has recently gained global recognition, and some studies have been conducted in this direction.

Studies have reported the application of various plant species such as *Cynodon Dactylon, Zoysia Matrella*, *Leucaena leucocephala, Acacia mangium, Melastoma malabathricum, Sisam tree roots, Pinus radiate, Kunzea ericoides, Shorea robusta* (popularly known as Sal)*, Karanj*, *and Azadirachta* (popularly known as Neem) for phytostabilization of soil and mine waste dumps [110,112–116]. Phytostabilization demands low/no maintenance cost and hence proves to be an economically viable solution for slope stabilization. A summary of plant species used, soils stabilized, and improvement in properties reported from phytostabilization studies is presented in Table 2 [117–125].

In addition to phytostabilization, plant species like *Agrostis capillariscapillaries*, *Thlaspi caerulescens*, *Ni-Alyssum montanum*, and grass have been utilized for phytoremediation to remove/reduce contaminants, heavy metals, organic and inorganic matter, and certain undesired compounds from the contaminated soils, wastewater, and sludge; while also helping in preventing the erosion and leaching of pollutants [126,127]. Additionally, plant growth-promoting microorganisms (*PGPMs*) have been utilized to improve soil fertility, nutrient quality, plant and crop yield. Further, *PGPMs* may also enhance phytostabilization by decontaminating the polluted soil, thus speeding up the process of plant growth [128,129].

2.5. Biostabilization in civil engineering

Several applications of biostabilization techniques in civil engineering have been suggested in recent years [48,56,60,69,74,78], such as reduction of building settlement by stabilization of soil to enhance the stability of underground constructions or tunnels, retaining walls, dams, and embankments; scour/erosion prevention to increase resistance to erosive forces of water flow and/or heavy wind, increase in the resistance to erosion of the soils present beneath pipelines, gravity foundations, offshore structures, and coastal areas and rivers; slope and erosion protection for dam and levee (embankment) safety, as an impermeable barrier to divert or to stop the subsurface contaminant transportation and protect groundwater, and/or reactive permeable barrier in mining operations and other environmental applications. Other applications explored the improved resistance to liquefaction by cementation of subsurface, enhancing self-healing properties of soils and other building materials, the formation of grout curtains to reduce the migration of heavy metals and organic pollutants for preventing piping action (cavity formation) in earth dams and dikes, reduction of infiltration from the ponds and leakage in construction sites or landfill.

Researchers have employed different *MICP/EICP* treatment methodologies depending on the materials to be biostabilized and the type of microbes used. The optimization of any particular treatment methodology is based on the number of trial studies. The treatment used by researchers for stabilizing concrete, cement-mortar, and ash follows two methodologies, (a) Uniform mixing of dry mix (sand & cement) with a grown microbial culture for a defined water-to-cement ratio and casting of specimens of desired shape and size, followed by demolding and curing of specimen in reagent medium/solution for a desired period or until the day of testing [78,79,130–141]. (b) Surface Treatment: Mixing of dry mix and casting of specimens of desired shape and size is achieved, and bacterial culture media and reagent media of desired concentrations are then prepared. Both the media/solutions are then sprayed on the prepared specimen for desired days/no. of treatment cycles. Alternatively, the specimen are first immersed in the microbial culture for up to 1 day for inoculation, then removed, wiped clean, and

immersed in reagent solution of desired concentration for desired days/ no. of treatment cycles. At the end, after treatment/curing demolding of specimens for the respective testing [50,93,142–144].

The treatment for stabilizing different soils also follows two methodologies. In the first method, the microbial culture is thoroughly mixed with soil, which is packed into molds/plastic columns (bottom blocked with Whatman filter paper) in layers along with compaction to prepare a soil specimen of desired shape and size. The columns/molds are then fed with reagent media (sprayed/ sprinkled/ injected) of desired concentration for desired days/no. of cycles. At the end of curing period, the biostabilized specimens are demolded and subjected to respective tests [38,47,71,90,94,109,145–147]. In the second method, the required soil specimen is prepared and placed in the treatment chamber as required, microbial culture of desired concentration is then injected into the prepared specimen at some flowrate, and microbes are allowed to set into the sample for 6–12 h. Later, reagent solution of desired concentration is injected into the specimen with same flowrate for desired days/no. of cycles. At the end of curing period, the biostabilized specimens are demolded and subjected to respective tests [46,59,64,74,95,102,148–154]. For the treatment of the bricks (prepared from ash and other materials), the bricks are first oven dried at 50° C and cooled at room temperature, and then immersed in microbial culture media of desired concentration for 4 days. The bricks are then removed and cured by sprinkling reagent media for desired days/no. of cycles, and the biostabilized bricks are then subjected to respective tests [155–159].

The above-mentioned methodologies have been followed on the specimens of different shapes and sizes cast using customised and/or standard molds, viz. cube (70.6 mm and 150 mm), mortar prisms (40 mm × 40 mm × 160 mm), cylindrical specimens (38 mm × 76 mm, 15 mm × 50 mm), petri dish samples (90 mm), PVC molds (50 mm × 150 mm), consolidation test ring molds (75 mm × 20 mm), direct shear test box molds (60 mm × 60 mm × 20 mm) and brick molds (228 mm × 107 mm × 169 mm). Further, different methods such as X-Ray Diffraction (*XRD*), Energy Dispersion Spectroscopy (*EDS*), Energy Dispersive X-Ray (*EDX*), Scanning Electron Microscopy (*SEM*), Environmental Scanning Electron Microscopy (*E-SEM*), Fourier-transform Infrared Spectroscopy (*FTIR*), Thermogravimetric Analysis (*TGA*), CO_2 Volume Evaluation (*CVE*) Method, HCl Titration Method, Calcimeter, Acid-rinsed Method, Inductively Coupled Plasma Optical Emission Spectrometry (*ICP-OES*) and U-tube Manometer Method, have been used to determine the amount of calcite precipitation in the biostabilized samples of different materials [38,46,50,59,64,74,78,79,93,130–144,155–159]. Earlier studies have also explored the effect of biostabilization on different materials properties for civil engineering applications and the details are discussed in the following section.

3. Biostabilization for improving different properties of materials

Several researchers have made efforts towards the application of biostabilization (predominantly *MICP* and *EICP*) in civil engineering, as summarized in Tables 3 and 4 [38,41,46,50,53,54,57,59,64,65,68,71,74,78,79,90,94,95,97–99,102–104,109,130–134,147–150,159,160–169]. It can be observed from Tables 3 and 4 that researchers have studied various properties/parameters such as compressive strength, compressibility characteristics, hydraulic conductivity, shear strength parameters, swelling characteristics, tensile strength, stiffness, crack healing, density and void ratio, electrical conductivity, erosion characteristics, freeze and thaw effects, leachability, setting time and water absorption for cementitious materials. Studies on slope stability and stabilization of materials have also been reported. Researchers have also reported a method known as Calcite In-situ Precipitation System (CIPS), developed by CSIRO, Division of Exploration and Mining, Australia; wherein two water-based solutions are mixed to produce calcite or calcium carbonate crystals [161].

The efficiency of biostabilization techniques can be attributed to the

Table 3
Summary of application of biostabilization for improving engineering properties of materials.

Properties modified	Techniques used	Microorganisms used	Application material	References
Compressive Strength	*MICP, FICP*, Calcite In-situ Precipitation System (*CIPS*), *EICP*	Bacterium- *Sporosarcina pasteurii, Bacillus* sp. *CT-5, Bacillus megaterium*, Other Bacillus sp., *CIPS* Solution, *Bacillus pseudofirmus, B. cohnii*alkaliphilic, *Bacillus subtilis, Acinetobacter* sp. *SC4*, Bacteria Spores Powder, Fungi- *Penicillium chrysogenum CS1*, Enzyme-Urease, Substrates- Calcium Lactate, Calcium Formate, Calcium Nitrate	Cementitious Materials, Fly Ash-amended Mortar and Concrete, Sandy Organic Silt, Sand, Expansive Soil, Fly Ash and Rice Husk Bricks, Ottawa 20/30 Silica Sand, Sand + Silt Soil, Calcareous Sand, Silica Sand, F-60 Silica Sand, Ottawa 50–70 Sand, Fine Grained Soil, Itterbeck Sand, MSW Incineration Fly Ash, Loose Sand	[38,46,53,57,59,64,71,74,78,79,90,95,98,99,102–104,130–134,149–150,161,167]
Consolidation Parameters	*MICP*, Calcite In-situ Precipitation System (*CIPS*)	Bacterium- *Sporosarcina pasteurii*, CIPS Solution	Sandy Organic Silt, Calcareous Sand, Silica Sand	[149,162]
Hydraulic Conductivity	*FICP, MICP, EICP*	Bacterium- *Bacillus megaterium, Sporosarcina pasteurii, Bacillus sphaericus* Fungi- *Aspergillus niger*, Enzyme- Urease	Cementitious Materials, Fly Ash-amended Mortar and Concrete, Ottawa 20/30 Silica Sand, Sand + Silt Soil, Sand, Glass Beads, Lateritic Soil, Itterbeck Sand, Dry Ottawa Sand, Sand (With Radioactive Effluent), Gravel–Sand Mixtures, Ottawa 50–70 sand, Loose Sand	[46,50,78,79,95,97,102,109,147–148,160,163,168]
Shear Strength Parameters	*MICP*, Calcite In-situ Precipitation System (*CIPS*), *FICP*	Bacterium- *Sporosarcina pasteurii*, *CIPS* Solution, Fungi- *Penicillium ostreatus*, Substrate-Calcium Acetate and Calcium Nitrate	Sandy Organic Silt, Calcareous Sand, Silica Sand, Ottawa 50–70 sand, Quartz Sand, Sand, Mixture of Silica Sand and Lignocel, Fine Grained Soil	[59,64,68,92,104,149,150,162,166,168,169]
Stiffness	*MICP, EICP*	Bacterium- *Sporosarcina pasteurii*, Enzyme- Urease	Cement Mortar, Ottawa 50–70 sand, Itterbeck Sand, Loose Sand	[46,53,95,104,166]
Swelling Characteristics	*MICP*	Bacterium- *Sporosarcina pasteurii*	Expansive Soil	[167]
Tensile Strength Parameters	*MICP, EICP*	Bacterium- *Sporosarcina pasteurii*, Polyurethane (*PU*), Enzyme- Urease	Concrete, Loose Sand	[57,95]

Table 4
Summary of application of biostabilization for improving various parameters/material properties.

Parameter/ Property modified	Techniques used	microorganisms used	Application material	References
Crack Healing (Self-healing)	*MICP, EICP*	Bacterium- *Sporosarcina pasteurii, Bacillus pseudofirmus, B. cohniialkaliphilic, Bacillus subtilis, Acinetobacter* sp. *SC4*, Bacterial Spores,Polyurethane (*PU*), Enzyme- Urease, Substrates- Calcium Lactate, Urease, Calcium Formate, Calcium Nitrate	Cementitious Materials (Concrete, Cement, Cement mortar), Concrete(With Aggregate Material), Chalk Reservoir, Dry Ottawa Sand	[41,53,54,57,103,109,131,132,169]
Density and Void Ratio	*MICP*	Bacterium- *Sporosarcina pasteurii*	Cement	[133]
Porosity	*MICP, EICP*	Bacterium- *Sporosarcina pasteurii*, Enzyme- Urease	Fly Ash Concrete, Gravel–Sand Mixtures, Siliceous Sand, Itterbeck Sand, Loose Sand	[46,94,95,147,162]
Electrical Conductivity	*MICP*	Bacterium- *Sporosarcina pasteurii*	Sandy soil	[164]
Resistance to Erosion	*MICP*	Bacterium- Bacillus sp., *Sporosarcina pasteurii*	Sand, Bar Sand, Gravel–Sand Mixtures, Sandy Soil, Ottawa 50–70 Sand	[71,74,147,148,163]
Freeze-thaw Effect	*MICP*	Bacterium- *Sporosarcina pasteurii, Bacillus megaterium*	Cement Mortar, Fly Ash and Rice Husk Bricks	[53,159]
Leachability	*MICP*	Bacterium- *Sporosarcina pasteurii*	MSW Incineration Fly Ash	[134]
Setting time	*MICP*	Bacterium- Bacteria Spores Powder, Substrates- Calcium Formate, Calcium Nitrate, Calcium Lactate	Cementitious Materials	[132]
Slope Stability	*MICP*	Bacterium- *Sporosarcina pasteurii*	Sandy Soil, Siliceous Sand	[74,150]
Solidification/ stabilization	*MICP, FICP*	Bacterium-*Sporosarcina pasteurii*, Fungi- *Penicillium chrysogenum CS1*	MSW Incineration Fly Ash, Natural Soil and Aqueous Solution (with lead & Chromate)	[65,134]
Water Absorption	*MICP*	Bacterium- *Sporosarcina pasteurii, Bacillus* sp. *CT-5, Bacillus megaterium, Acinetobacter* sp. *SC4, Bacillus sphaericus*	Cementitious Materials, Fly Ash-amended Mortar and Concrete, Fly Ash and Rice Husk Bricks	[50,78,79,103,130,133,159,162]

concentration of calcium carbonate precipitated, which primarily depends on the microorganisms utilized, and other factors such as material to be treated, nutrient concentration, and the treatment duration. It has been inferred, that the bacterium *Sporosarcina pasteurii* has been popularly used (52%) for biostabilization compared to other microbes, possibly due to ease of its availability and high rate of calcite precipitation. Fig. 3 presents the distribution of different microorganisms and other bio-based enzymes/substrates used for biostabilization, as observed by the authors based on the detailed literature review carried out in this work.

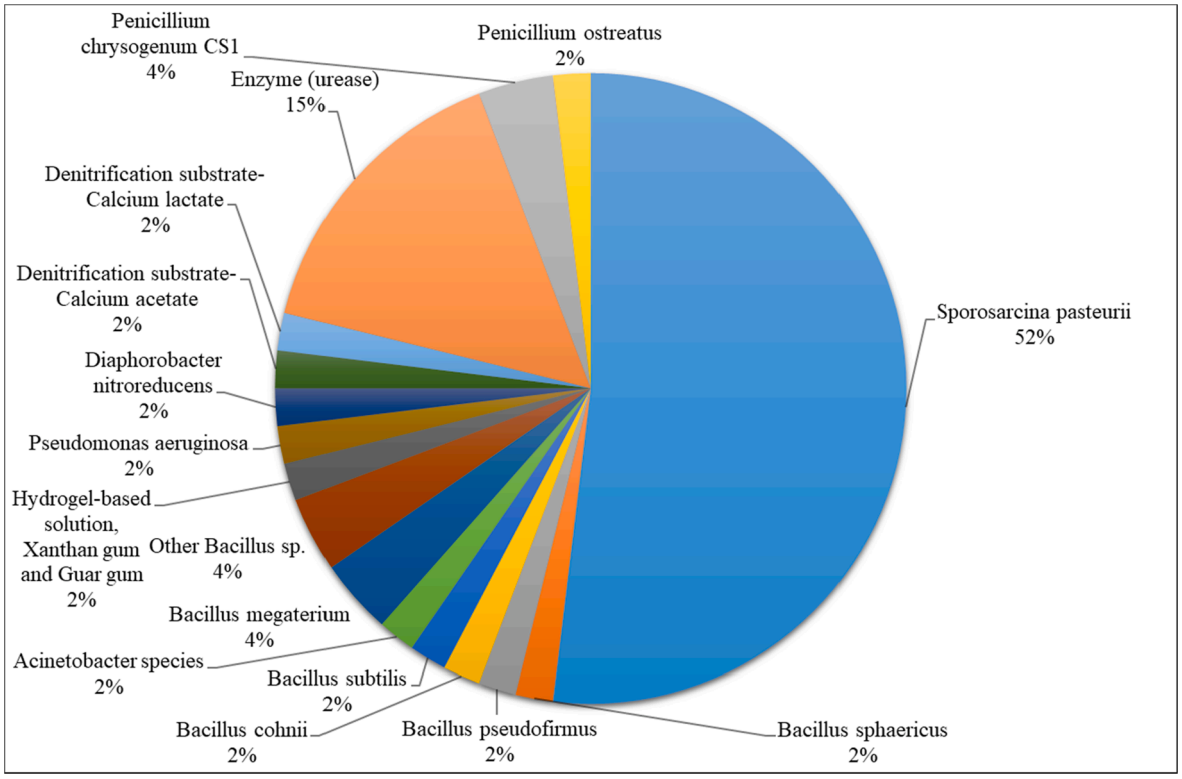

Fig. 3. Percentage distribution of microorganisms and other bio-based enzymes/substrates utilized for biostabilization.

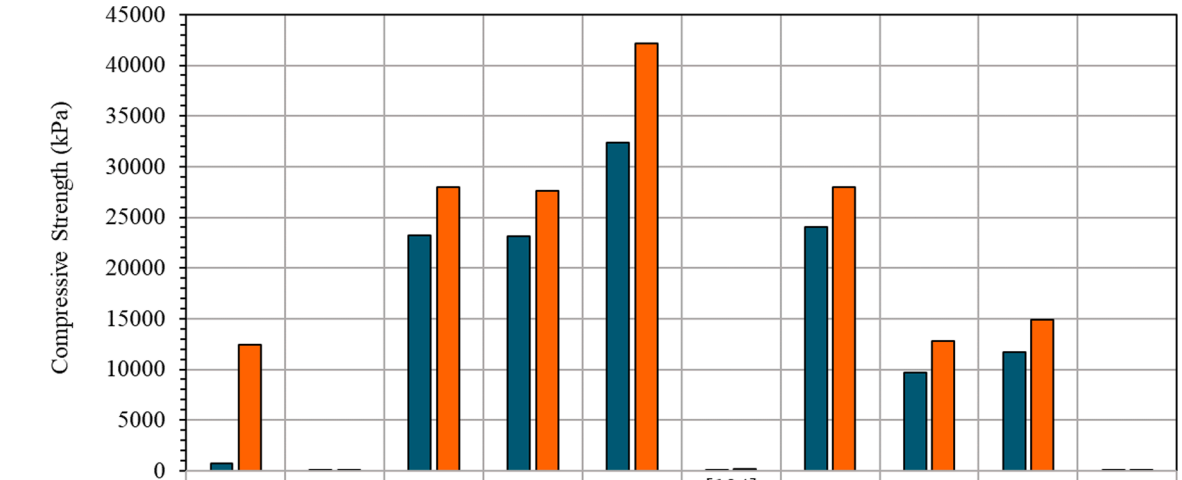

Fig. 4. Improvement in compressive strength (CS) after MICP/EICP treatment Where, S- Sand, C- Concrete, CM- Cement Mortar, FAMC- Fly Ash- amended Mortar & Concrete, FAC- Fly Ash Concrete, RHAB- Rice Husk Ash Bricks, FAB- Fly Ash Bricks, ES- Expansive Soil, MSWFA- Municipal Solid Waste Fly Ash.

3.1. Improvement in compressive strength and permeability properties using MICP/EICP

Many calcite precipitation studies focussed on the determination of compressive strength (*CS*) of materials, while fewer studies focussed on permeability; whereas, limited studies are available on other parameters/properties. Some researchers have reported the improvement in compressive strength before and after treatment, as presented in Fig. 4 [59,71,79,133,134,159,166,170]. The materials such as sand (other than cementitious materials) achieved lower compressive strength; however, the percentage improvement in compressive strength was higher for sand. Van Paassen et al. [59] reported a maximum improvement of 1671.4% for fine to medium sand, using *Sporosarcina pasteurii*, with a treatment period of 16 days. Chen et al. [71] treated sand using *Bacillus* species for 6 days and achieved around 205% improvement in compressive strength. Sandy soil being coarser, exhibited a maximum scope for improvement in compressive strength. It may be noted in this context that bacteria and fungi have a typical size ranging from 0.5 to 5 μm and 2–10 μm, respectively, making their transportation difficult in silty or clayey soils (fine-grained soils). Hence, *MICP* is deemed ineffective in these soils [68,171]. For cementitious materials (cement, cement mortar, and concrete), around 15–30% of

Table 5
Summary of factors responsible for calcite precipitation.

Materials treated	Microorganism/enzyme Used	Concentration of Injecting Substances	No. of Cycles /Injections /Treatment Period	% CaCO3 Precipitated	Reference
Porous Media and Sand	Enzyme-Urease (Jack Beans)	Urease- 0.01 to 0.1 g/l Urea & $CaCl_2$- 36 / 90 g/l	No. of injections- 3, Treatment duration- 72 h	7%	[97]
Itterbeck Sand	Bacterium-*Sporosarcina pasteurii*	Bacterial injection- OD600- 1.583, Activity- 0.23 mS/minUrea & $CaCl_2$- 1.1 M	Treatment duration- 124 h	10.50%	[46]
Sand	Bacterium-*Sporosarcina pasteurii*	Act- 1.1 mol-urea/L/hUrea & $CaCl_2$- 1 mol/L	Treatment duration- 16 d	27.30%	[59]
Rice Husk Ash Bricks	Bacterium-*Bacillus megaterium*	Bacterial culture- OD600- 1.0, 10^6 cells/ mlActivity- 692.5 U/ml	Treatment duration- 3 weeks	31%	[159]
Fly Ash Bricks	Bacterium-*Bacillus megaterium*	Bacterial culture- OD600- 1.0, 10^6 cells/ mlActivity- 692.5 U/ml	Treatment duration- 3 weeks	31%	[159]
Loose Sand	Enzyme-Urease (Sword Beans)	Urease- 1 gUrea & $CaCl_2$- 1 mol/L	No. of injections- 4, Treatment duration/curing time- 24 h	10%	[95]
Sandy Soil	Bacterium- *Sporosarcina pasteurii*	Urease- 42.6 mMUrea & $CaCl_2$- 1 M	Treatment duration- 24 h	2.80%	[164]
Ottawa 20–30 Silica Sand	Enzyme-Urease (Jack Beans)	Urease enzyme- 0.44 g/L, Activity- 26,100 U/ gUrea & $CaCl_2$- 1.36 M & 0.765 M	Treatment duration- 25–30 d	4.30%	[38]
Silica Sand	Enzyme-Urease (Jack Beans)	Urease- 15 g/L, Act- 2970 U/gUrea & $CaCl_2$- 1 mol/L	Treatment duration- 24 h	12%	[99]
Sandy Soil	Bacterium-*Sporosarcina pasteurii*	Bacterial Culture- O.D600-1.0Urea & $CaCl_2$- 0.7 M	No. of cycles- 18 (3/d), Treatment duration- 6 d	12%	[74]
Silica Sand	Enzyme-Urease (Jack Beans)	Urease enzyme- 1 g/L, Act- 2950 U/gUrea & $CaCl_2$- 0.5 mol/L & 0.25–0.5 mol/L	Treatment duration /Curing period- 5 d	8%	[98]
Sand with Radioactive Effluent	Fungi-*Aspergillus niger*	Bacterial conc.- 500 mL, O.D600- 1.0,	Treatment duration- 68 d	1.03%	[148]
Siliceous Sand	Bacterium-*Sporosarcina pasteurii*	O.D600- 2.3Urea & $CaCl_2$- 1 mol	–	15.17%	[150]

*U/g- Units per gram, U/ml- Units per millilitre, mM- milli-Mole, mS/min- millisiemens/minute.

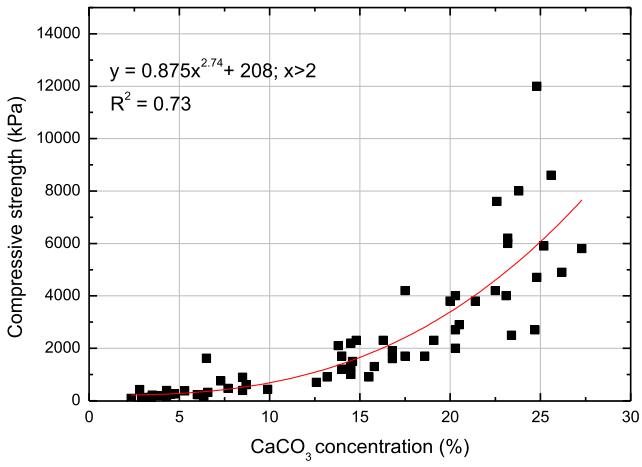

Fig. 5. Compressive strength versus $CaCO_3$ concentration.

improvement was noted, and for fly ash, about 25–30% improvement was observed.

The compressive strength and percentage improvement achieved with respect to untreated material can be attributed to the calcite concentration, nature of the material, pore space distribution, and the bonding of precipitated calcite with the particles of material treated. $CaCO_3$ forms part of relatively weaker bonds in cementitious materials compared to stronger bonds such as C-S-H (calcium-silicate-hydrate) and C-A-H (Calcium-aluminate-hydrate). Hence, calcite precipitation is not able to enhance strength significantly. Based on the above-discussed studies, it can be inferred that *MICP/EICP* treatment is more effective for stabilizing sand and similar coarser fraction of non-cementitious materials.

Table 5 summarises the $CaCO_3$ concentration achieved by researchers in previous studies, along with the factors affecting/responsible for precipitate concentration. A maximum $CaCO_3$ concentration of 31% was noted by Dhami et al. [159] followed by Van Paassen et al. [59], who achieved $CaCO_3$ precipitation of 27.30%. Dhami et al. [159] used *Bacillus megaterium* to treat rice husk and fly ash bricks, with a treatment duration of 3 weeks; whereas, Van Paassen et al. [59] treated sand using *Sporosarcina pasteurii* for 16 days. Higher $CaCO_3$ concentration in the former case can be attributed to the material treated (viz., ash), which possesses a higher surface area due to its fineness for better calcite precipitation, and the treatment duration was almost a week higher than the latter. On the other hand, Gui et al. [148], in their study, obtained about 1.03% $CaCO_3$ concentration after the treatment of sand with radioactive effluent using fungi sp. *Aspergillus niger*. It was observed that studies that used bacterium species with comparatively higher treatment duration exhibited better calcite precipitation.

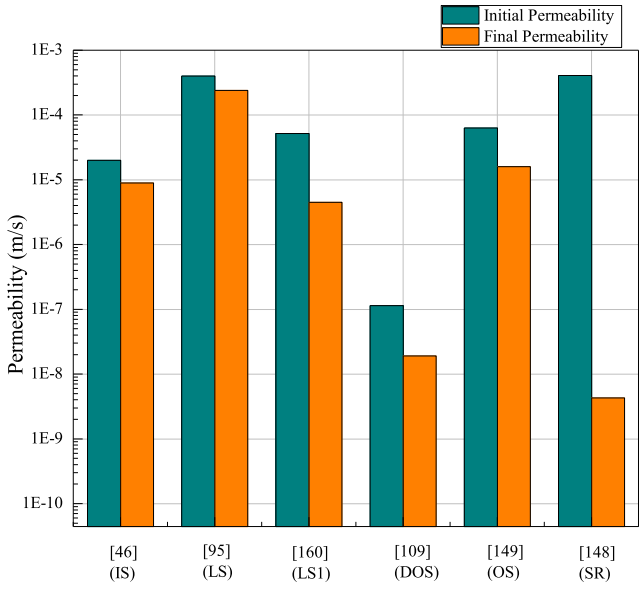

Fig. 6. Reduction in permeability before and after the treatment IS - Itterbeck sand, LS - Loose sand, LS1 - Lateritic soil, DOS - Dry Ottawa sand, OS - Organic soil, SR - Sand with radioactive effluent.

In order to correlate achieved compressive strength (kPa) with $CaCO_3$ concentration (%), based on the studies reported by earlier researchers for soils [38,59,95,98,99,172], Fig. 5 has been plotted to depict this relationship. The figure exhibits a mild increase in compressive strength up to almost 10% of $CaCO_3$ concentration, and then a relatively exponential increase in compressive strength is noted with a further increase in $CaCO_3$ concentration. The figure also depicts the predictive equation correlating the compressive strength with % $CaCO_3$ concentration. Some scatter in compressive strength is observed for $CaCO_3$ concentration higher than 20%, nevertheless, the corelation between compressive strength and $CaCO_3$ concentration is evident. Similar to compressive strength, an improvement in permeability (reduction) has also been studied by some researchers. The permeability before and after treatment, based on earlier studies, has been plotted in Fig. 6. A maximum reduction in permeability of almost 100% was achieved in dry Ottawa sand, treated using *Sporosarcina pasteurii* for a treatment duration of 24 h, with three treatment cycles and 1.6 M of cementation solution [109]. Other researchers [148,149,160] achieved a reduction in permeability in the range of 75–91%. Yasuhara et al. [95] treated loose sand; however, they achieved a lower reduction (40%) in permeability, possibly due to employing the *EICP* technique (Urease enzyme) and comparatively less treatment duration (24 h), which resulted in reduced efficiency.

3.2. Improvement in other properties using MICP/EICP

Limited studies have been carried out for improving other properties of civil engineering materials (properties other than strength and permeability) by employing *MICP/EICP*. The details of these studies have been summarized below:

3.2.1. Water absorption

Dhami et al. [159] utilized *MICP* (*Bacillus megaterium*) for reducing the water absorption of fly ash (*FA*) and rice husk ash (*RHA*) bricks. The water absorption (%) of an untreated brick specimen of rice husk ash and fly ash were 15% and 13.5%, respectively. For bacterially treated *RHA* and *FA* specimen, water absorption was noted as 8% and 7.5%, respectively (viz., 46% and 44% reduction in water absorption was achieved, respectively). The water absorption of the treated specimens was less than the conventional red brick, which exhibits a water absorption of about 12–12.5%. Cement mortar cubes, when treated with *Bacillus species CT-5* absorbed nearly six times less water after 168 h, as compared to the untreated cubes [78]. Another study noted that when mortar cubes are treated using *Sporosarcina pasteurii* [130], a significant reduction in water absorption for mortar specimen cured for 28 days has been observed. Further, a 42% reduction in water absorption was observed on treating limestone/limestone-based mortar with *Acinetobacter species SC4* [95].

3.2.2. Crack healing (Self-healing)

Algaifi et al. [173] studied the efficacy of *MICP* (*Lysinibacillus sphaericus*) for self-healing of cracks in cement paste. The model study predicted the self-healing of cracks to be completed in 60 days. However, Scanning Electron Microscopy (*SEM*) analysis indicated that the complete healing of cracks was achieved in 70 days. The difference in prediction and actual healing of cracks has been attributed to the reduction in porosity, which would reduce the available oxygen,

calcium, nutrient, and nitrogen for the *MICP* process. Jonkers et al. [54] utilized *Bacillus strain B2-E2-1* to heal cracks, and it has been concluded that 100% crack healing is achieved by *MICP* treatment within 2 months, while the self-healing reduces to 33% in control environment conditions without bacterial treatment. Fig. 7 shows typical scanning electron microscopy (*SEM*) images to depict the cementation due to *MICP* induced $CaCO_3$ precipitation, which helps in bridging cracks.

3.2.3. Setting time

Luo and Qian [132] studied the influence of various bacteria-based self-healing agents (*RB*-calcium lactate bacteria spore powder, *JB*-calcium formate bacteria spores powder, *NB*-calcium nitrate bacteria spores powder), on the hydration kinetics of the cementitious material. It has been observed that with *RB* content up to 3%, initial setting time (time at which cementitious materials begins to harden) reduced from 136 minutes to 68 minutes, and final setting time (time at which a 5 mm^2 needle no longer penetrates the surface) increased from 216 minutes to 338 minutes. The addition of *JB* and *NB* accelerated the initial setting time and reduced the final setting time. Hence, it has been concluded that self-healing agents *JB* and *NB*, accelerated the hydration while *RB* delayed the hydration. This can be attributed to the higher hydration rate of calcium formate and calcium nitrate in *JB* and *NB* and the lower hydration rate of calcium lactate in *RB*.

3.2.4. Bio-clogging for reducing hydraulic conductivity and Porosity:

Gui et al. [148] have adopted *MICP* (*Aspergillus niger*) to resist the radioactive percolation and studied the effect of bio-clogging by microbes against radioactive percolation. Results exhibited a 74% reduction in the hydraulic conductivity and an adsorption rate of about 90% at the end of 68 days of experiments. This indicated that the utilization of *Aspergillus niger* resulted in an effective covering of the sand surface and blocked the seepage path due to bio clogging by $CaCO_3$ precipitation. Whiffin et al. [46] treated sand using *MICP* (*S.Pasteurii*) and found that the reduction in porosity is proportional to the amount of $CaCO_3$ precipitated, and the maximum reduction in porosity of 10% was reported for the corresponding highest $CaCO_3$ precipitation.

3.3. Benefits of biostabilization using MICP/EICP

Biostabilization techniques such as *MICP/EICP* are eco-friendly and sustainable techniques for treating/stabilizing different properties of soils and other civil engineering materials and/or structures. If the favourable conditions prevail, *MICP/EICP* application may take a shorter time, and trials prior to actual application would help optimize the effectiveness and duration of treatment [174]. Recent studies have indicated that *MICP/EICP* can be successfully integrated with different geosynthetics/geo-materials, viz., geo-tubes, bio-based granular geo-material [175,176], and can also be employed using conventional methods, e.g. conventional oil-field method [174,177]. *MICP/EICP* treatments can also be applied in inclement as well as sunny weather [178,179] and the biochemical reactions involved in the process can also occur in the presence of hydrocarbons [177,180,181]. Studies have also indicated that the bacterial strains used in biostabilization can extract carbon dioxide from the air for $CaCO_3$ precipitation [182], and along with soil stabilization, it also promotes vegetation/plant growth [175,179,183]. *MICP/EICP* can also utilize non-ureolytic bacteria for material stabilization that consume other organic compounds such as lactate instead of urea to form carbonate ions [182]. *MICP/EICP* process can be further enhanced by using genetically modified bacteria as it will increase bacteria survivability and enzyme activity [182], which suggests a high potential of improvement in the efficacy of *MICP/EICP* treatment in future. The economic feasibility of the process can be improved by using waste from plants and animals as nutrients, which helps to reduce cost and make the process more sustainable [176,182,183]. Studies have also suggested utilization of industrial-grade chemicals, viz., urea & calcium chloride, for improving the cost-effectiveness of *MICP/EICP* treatment methods [178,183].

4. Field/large scale application of biostabilization

Improvement in soil properties has been observed based on *MICP* (predominantly) and *EICP* treatment in many laboratory studies. Upscaling of these processes from the laboratory to field scale requires an understanding of geotechnical, hydrological, chemical, and biological conditions at the site. Field applications (upscaling) of bio-mediated treatment processes for soil stabilization pose various challenges that need to be addressed before implementation, viz., understanding soil and pore fluid interactions, controlled distribution of mediated calcite precipitation, and permanence of the cementation [184]. Though many laboratory studies have advanced the understanding of *MICP*, its viability as an engineering solution remains to be established, due to limited field studies.

In the last decade, some researchers have attempted field-scale studies [51,59,185–189] for biostabilization (*MICP* and *EICP*) to treat sandy soil, to seal leakages in levees (earthern dams), to improve the erosion resistance of loose deposits of sand, to provide surface stabilization for dust control and future re-vegetation, to reduce fractured rock permeability in the subsurface, to improve the stability of bore-hole installed in gravel for laying gas pipeline, and sand stabilization using surface percolation. A recent study, based on field-scale demonstration, reported that *MICP* is quite effective for reducing permeability and sealing of fractures in sandstone [174]. The *MICP* treatment was carried out using *S. pasteurii*, with injecting solution consisting of urea and calcium for sealing the fracture in sandstone at 340.8 m below the ground surface. The *MICP* treatment was performed through a 24.4 cm diameter well, and the microbes and injecting solution were delivered using conventional oil-field technologies, to seal the fractures successfully. Calcite precipitation was noted even about 1.8 m above the location of the fracture. The study also suggested the suitability of *MICP* treatment for other applications such as well-bore cement sealing and unconventional oil and gas-related applications.

Another study explored the surface percolation method for treating a 2.3 m high coastal sandy slope (1 V: 3.3H) covering an area of 1 m × 8 m [178]. *S. pasteurii* bacteria, cultured aerobically in ammonium-yeast media, was utilized and cementation solution was prepared with a 3:1 ratio of urea and calcium chloride. The soil was inoculated with microbes and retained for 3 h, followed by spraying of cementation solution at pressure varying from 69 to 138 kPa and discharge rate of 32.55 L/hour. The treatment was given for 10 days at a rate of two cycles per day. Dynamic Cone Penetration (DCP) and calcite precipitation measurement were used as an indication of soil stabilization/improvement. The study concluded that maximum calcite precipitation was observed in the top 5 cm depth (5–5.5%) and reduced with depth (1% at 18–20 cm depth). DCP test results indicated that the penetration index (P_R) prior to the treatment ranged from 6 to 13 cm/blow in the top 10 cm of the soil layer, while it varied from 4 to 8 cm/blow at higher depths. The post-treatment values of P_R ranged from 1.25 to 6 cm/blow in the upper 10 cm and 3 to 6 cm/blow for higher depths, with lower P_R values indicating soil improvement. The study concluded that higher initial soil hydraulic conductivity would aid better calcite precipitation and distribution in *MICP* treated soil. It has also been noted that for slope stabilization, steeper slopes would help the treatment solution to penetrate to higher depths easily and would yield a relatively well distributed depthwise calcite precipitation pattern in soil.

Hodges and Lingwall [183] presented a case study on the field-scale application of surface *MICP* treatment through bio-augmentation in rural South Dakota. Treatment was carried out at three site locations with an aim of providing short-term surface erosion protection. *MICP* treatment solution (with vegetation seeds) was applied with different concentrations using the surface spraying method. The treatment was applied for three weeks, and the soil strength was evaluated at frequent intervals, while the vegetation was monitored for three months after the

treatment. It was observed that *MICP* was effective for soil strengthening at all the three sites. The study concluded that the test sites have good erosion resistance, however, vegetation growth was not very effective.

Kirkland et al. [177] demonstrated field application of *MICP* in Indiana, USA, at a water injection well used for secondary oil recovery, with an aim to mitigate poor waterflood efficiency (viz., minimizing leakage of residual hydrocarbons from the hydrocarbon reservoir to the surrounding areas). *S. pasteurii* microbial culture and urea calcium media were injected into the well to promote *MICP* through a 0.2 m open hole drilled up to 774.4 m below ground surface, and a 7.3 cm tubing was used to accommodate a 14.2 L dump bailer used to deliver the *MICP* promoting fluids to the desired zone. After 25 inoculum injections (approx. 360 L) and 49 calcium media injections (700 L), the injectivity of the system had decreased from 5.7 L/min/MPa to 1.6 L/min/MPa (70% reduction). Pumping tests indicated that the treatment was stable even two weeks after the completion of the treatment, and it was concluded that *MICP* treatment partially sealed the leakage/undesirable pathways and calcium content determined was around 40%. The study concluded that *MICP* can be applied using conventional techniques to reduce permeability, mitigate/seal leakage paths and to improve waterflood efficiency in oil/gas wells. The study also confirmed that the *MICP* treatment is effective even in soil/rock strata with hydrocarbons.

Another field study examined the potential of *MICP* for reducing wind erosion of desert soil by conduction of *MICP* treatment on bare sandy land located in Ulan Buh Desert, China [179]. *S. pasteurii* (ATCC 11859) was used to induce calcite precipitation, while urea (agriculture grade) and calcium chloride (industrial grade) were used to prepare the cementation solutions of different concentrations. Fifty-four test plots, each measuring 2 m × 2 m were treated, bacterial suspensions and cementation solutions (1:1) were mixed and sprayed on to the surface with a total volume of 1, 2, 3, and 4 L/m^2 (for 0.1 M, 0.2 M, 0.4 M, and 1 M concentrations, respectively) for 30 days. At the end of the treatment, *MICP* stabilization was evaluated by assessing the wind erosion and penetration resistance, and the residual bearing capacity was evaluated as 326.8 kPa for *MICP* treated soil and 22.6 kPa for untreated soil. The exposure of treated soil to natural/local weather conditions indicated a negligible depth of wind erosion after 30 days. The erosion depth of the *MICP* treated sandy land relative to that of the untreated land was 4.5% (after 90 days) and 5.1% (after 180 days). The research work concluded that *MICP* can be used to produce a lightly-cemented crustal layer over a loose and cohesionless desert soil to mitigate wind erosion in arid and semi-arid areas.

Earlier studies have also reported a comparison of lab-scale studies and field application [174–176,178,183,190]. Laboratory studies being done at small scale, the necessary parameters can be fairly controlled in laboratory scale studies, while the parameters may be different in field conditions, e.g. injection pressure and boundary conditions in field would differ from laboratory conditions [174,183,190]. In case of lab

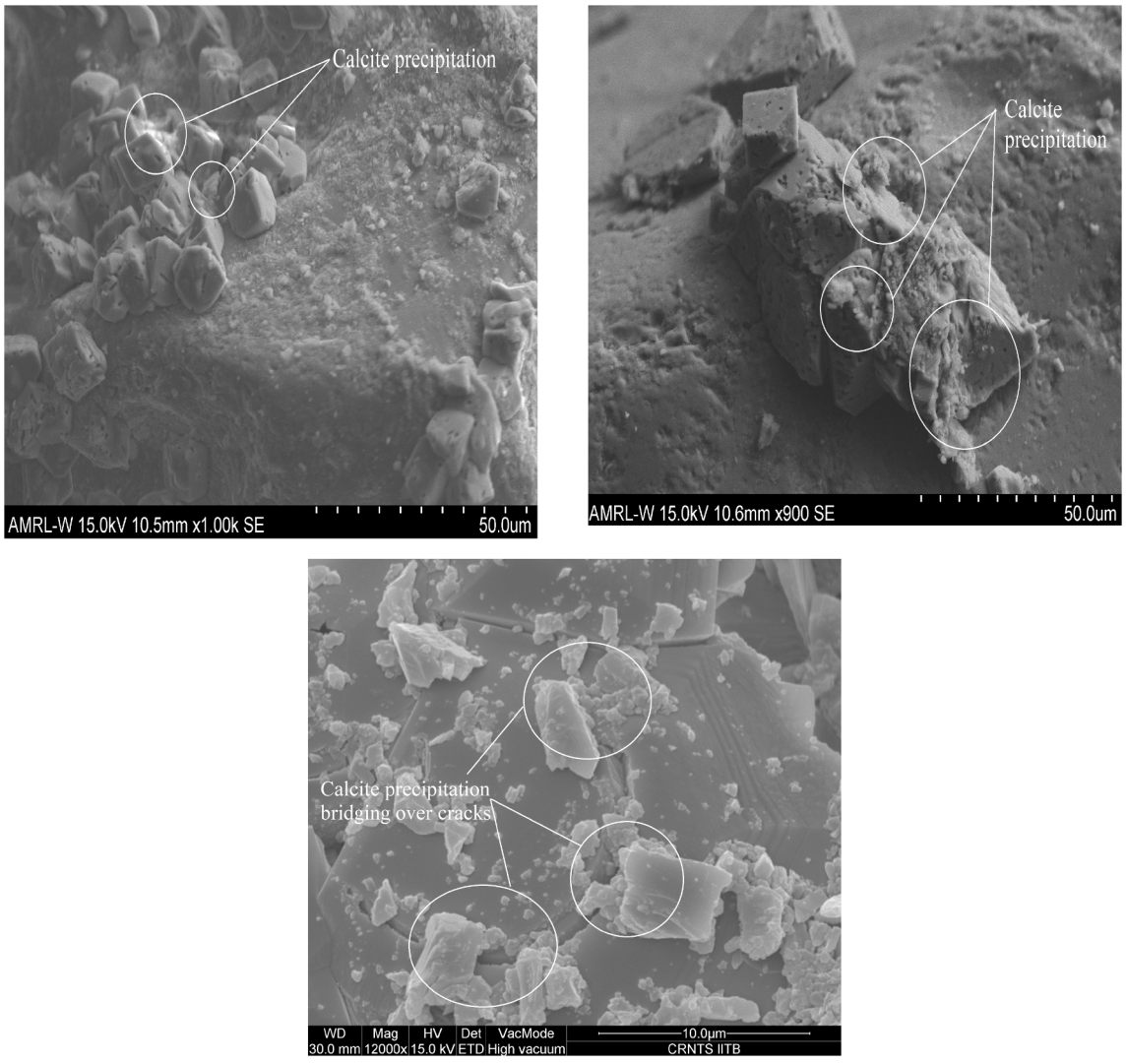

Fig. 7. Typical *SEM* images depicting microbially induced calcite precipitation (Images Courtesy: AMRL, University of Strathclyde and CRNTS, IIT Bombay).

Table 6
Upscaling Criteria for Biostabilization Techniques.

Upscaling criteria	Parameters considered	Effects	Recommendations by researchers	References
Soil Compatibility	Natural variability and heterogeneity of soil/material (particle size, mineralogy), Hydraulic boundary conditions, Pore space geometry	The distribution of bio-chemical amendments and the reactions they stimulate, The transport of microbes and nutrients, Calcium carbonate precipitation	Site specific investigations to verify if MICP is a viable option prior to field implementation	[38,59,68,94,147,167]
Pore Fluid Composition	Chemical composition of the pore fluid	Can hinder the bio-treatment reaction network	Understanding the aqueous chemistry of the groundwater and injection solutions prior to the field implementation	[38,53,148,150]
Uniformity	Creating uniform cementation, Degree of cementation, Uniform cementation gradient/ stiffness gradient, Spatial distribution of the treatment	Results in non-uniform stiffness of the material, Affects the efficiency/Success of the treatment	Larger concentrations of microbes to induce greater rate of precipitation, Reversal of injection direction	[47,74,102,131,148,167]
Permanence	Stability of the treatment, Compatibility of the precipitated calcite with the long-term environment, Favorable environments	Stability/Solubility of precipitated calcium carbonate, Affects the efficiency/Success of the treatment	Provision of favorable conditions/environment, Continuous monitoring during the service life of the treated material/ treatment process	[47,63,65,66,71,90,149,159,165]

scale studies, the optimum concentration/quantity of microbes and injecting solution can be decided by conducting a number of trials under different conditions, while during field application, the precise parameters related to microbes and injecting solution can be finalized only after the successful multiscale applications and predictions through numerical/computational models [174–176,178,190]. Small/laboratory scale application of *MICP/EICP* results in relatively lower heterogeneity in the treatment and clogging of pores (calcite precipitation) as compared to field application, wherein, practical difficulties such as bio-clogging and heterogeneity of calcite precipitation have been encountered. Studies have also reported that major calcite precipitation takes place around the injection points [191,192]. Researchers have noted that during field application, the cost of nutrients could affect the overall cost of *MICP* treatment significantly, and efforts are necessary at laboratory scale to explore more cost-effective nutrients for *MICP* treatment [179,192]. Further, upscaling of *MICP* treatment in the field would result in enhanced release of by-products such as ammonia, and its reduction/capturing requires the attention of researchers, while this problem has not received much attention during laboratory-scale studies [192,193]. Hence, field-scale application face challenges during

Table 7
Challenges and Limitations Related to Pilot and/or Field Scale Application of Bio-stabilization Techniques.

Challenges	Causes	Effects/Limitations	Mitigation options identified by researchers	References
By-products of the process (Ammonium, nitrate, hydrogen sulfide)	Substantial volumes of chemical reagents and microbial solutions when used for field applications	Induces toxic effects on human health, vegetation and atmosphere	Flushing treatment- to mitigate/get rid of the by-products	[41,56,59,68,160,167]
Cost of the treatment	The processes are material consuming, Implementation of the process requires preliminary investigations at small and pilot scale before upgrading, Injection and extraction wells could represent a non-negligible part of the final cost	At large scale the technology may be expensive, The cost of the treatment may also increase depending on the material	Cost analysis prior to field application	[56,59,68,83,94,133,194–196]
Feasibility	Parameters such as injection flow rate, number of treatments, volumes, concentrations are key factors that controls the process, These parameters must be priory analyzed in laboratory, which can be time and cost consuming	Clients are easily prone to use conventional soil improvement techniques as all parameters are controlled and have shown their efficiency over years	Statistical studies must be conducted including rigorous assurance/quality control process, Monitoring operations during treatment and maintenance norms should be considered for re-treatment/healing processes	[38,47,56,94,150,197]
Performance	Heterogeneity of the treatment along the soil matrix	Non-uniform improvement of the material, Reduced efficiency of the process	Uniform treatment could be achieved when controlling variables (number of injections, method of injection, concentrations of reactants and flowrate of injection) will be fully understood	[53,63,74,94,148,159]
Lifetime/service period	Unfavorable conditions (temperature, pH, weather)	Degradation of the precipitated calcium carbonate, Need for the repetitive treatment- adds to cost	MICP is expected to be stable for more than 50 years if alkaline conditions are provided, The calcite must be assessed to evaluate its long-term degradation, Regions with unfavorable conditions must be focused	[41,99,150,165]

upscaling from laboratory studies [51].

Keeping in view the challenges for field-scale application, DeJong et al. [184] reported some upscaling principles, for successful implementation of the treatment at field scale, as summarised in Table 6. It has been inferred that the important upscaling criteria which need special attention include soil compatibility, pore fluid composition, ensuring uniformity of the cementation process, and stability of the treatment (permanence). From the field-scale applications attempted in earlier studies, major challenges/limitations that need to be addressed before upscaling have been discussed in Table 7. The causes, effects, and approaches for mitigation of various challenges such as removal of undesired by-products, ensuring feasibility and performance of the treatment at site, and long-term reliability of *MICP* treatment needs to be addressed.

5. Future research on biostabilization

Biostabilization techniques have brought a new revolution in geotechnical engineering and other civil engineering applications. However, further research is required to make these processes environmentally safe, cost-effective, and address the challenges related to field application [63,67]. The major challenges that require the attention of researchers are stated below:

- Urea hydrolysis is the most widely used process for calcite precipitation, and it yields ammonium and chlorine (from $CaCl_2$) as a byproduct. Ammonium, when oxidized, creates an acidic environment that promotes the dissolution of precipitated $CaCO_3$. Hence, further research is required to reduce unwanted byproducts [56,59,67,83].
- Although bio-clogging improves soil properties, the bio-film formed in soil pores affects the mass transfer rate, the concentration of nutrients and microbes between the biofilm, and flow through the soil pores [69,184]. An in-depth understanding of this process is required for better field implementation of the biostabilization (viz., *MICP/EICP*) techniques.
- The depthwise penetration of microbes and essential nutrient media in soil is limited by the minimum soil pore size ranging from 0.5 to 2 μm, which makes the process limited to relatively coarser soil types (silty sand, sand, etc.) with suitable hydraulic conductivity [69].
- Microbial processes are generally slower and are usually more complex due to the dependence of microbial activities on environmental parameters such as pH, temperature, type of soil, the concentration of microbes, nutrient media, and the amount and rate of calcite precipitation. Hence, effective control of these parameters is mandatory for achieving higher efficiency [69,107].
- Durability and homogeneity of microbially treated soil are the two major concerns during large-scale field applications using biostabilization techniques. Further, there is a need to explore alternative inexpensive nutrient sources, as compared to those used in the laboratory environment, for field application of biostabilization techniques [83,107].
- There is a need for the development of a monitoring methodology, which would help to monitor the entire process during the treatment, and throughout the service life of a treated soil zone [184].

6. Concluding remarks

It is a well-acknowledged fact that the development, improvement, and management of biostabilization processes needs an interdisciplinary approach. A large number of studies have reported improvement in compressive strength; some studies have reported the reduction in permeability of the soil, while other properties have also been studied by limited researchers. These studies indicate the potential of *MICP/EICP* treatment for improving the properties of civil engineering materials. The factors affecting the compressive strength and its improvement have been attributed to the calcite concentration, nature of the material, pore space distribution, and the bonding of precipitated calcite with the particles of material treated. Further, exponential corelation between the compressive strength of material and the amount of calcium carbonate precipitation has been noted. The maximum percentage improvement in compressive strength up to 1671.4% has been reported for sandy soil, while the improvement for cementitious materials has been reported in the range of 15–30%. Further reduction in permeability varying from 40% to up to almost 100% has been reported in earlier studies.

The limited studies carried out for upscaling the biostabilization process to field-scale revealed that ensuring the viability of solution considering field soil compatibility, control on pore fluid composition, ensuring uniformity of cementation, and permanence of the solution are important aspects to be considered. Further, there is a need to carry out research to reduce the cost of treatment and production of undesired by-products. Nevertheless, despite these challenges, biostabilization using *MICP/EICP* can be considered a potential alternative to the conventional stabilization techniques in civil engineering, as it is environmentally friendly, sustainable, and economical, with good potential for a wide range of applications.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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