REVIEW

A review of the use of bio-based substances in soil stabilization

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Abstract

Conventional techniques of soil stabilization involve using additives such as lime and cement. However, these methods take up a great deal of energy and cause considerable environmental pollution. Recently, bio-additives have been taken into account as sustainable development, cost-effective, and environmentally acceptable alternatives to chemical stabilizers in geo-environmental applications. In these techniques, bio-chemical activities, including bio-cementing, bio-clogging, bio-coating, and bio-encapsulation, are employed to stabilize soil particles. The present study aims to examine the impact of bio-stabilizers type and quantity on the geotechnical characteristics of soil for soil stabilization. For this purpose, the biochemical performance of various biological methods of soil stabilization (e.g., bio-microorganisms; bioenzymes; and biopolymers) is first presented. Then, the behaviors of bio-substances in all types of soils are investigated through a comprehensive review of previous research. Afterward, the biochemical behavior of bio-additives and their properties, mechanism, application, and interaction with soil particles are investigated on a microscopic and macroscopic scale. Next, the most effective factors in bio-stabilization are determined and evaluated. Finally, the essential recommendations for choosing the kinds and amount of bio-additives for soil stabilization are offered based on the soil type. The findings of this study indicate that the performance of bio-stabilizers is based on the percentage and type of bio-additives, soil type, and the quantity of electrostatic forces generated during cementation and hydrogel production. In addition, among various bio-additives, S. pasteurii and Bacillus sphaericus, TerraZyme, Xanthan gum, and Guar gum showed the best performance by increasing mechanical/shear strength by up to 300% and decreasing permeability, compressibility, and/or shrinkage properties. Furthermore, temperature, curing time, and soil pH were determined as crucial factors in establishing interlocking forces between soil particles and choosing the appropriate biomass.

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1 Introduction

Some soils may exhibit inadequate compressive, shear, and tensile resistance to the imposing stresses. Therefore, it is necessary to enhance the strength of such soils beneath building sites and sub-layers of transportation roads (Vincevica-Gaile et al., 2021). In addition, when buildings are constructed on problematic soils, the soil must possess adequate strength against loads and stresses. However, this soil strength might not exist on the construction site. Thus, it is essential to enhance the bearing capacity and mechanical properties of the soil (e.g., cohesion and friction). In this regard, soil stabilization is the most frequent method to improve the mechanical properties of soil (Huang et al., 2021).

Since ancient times, several methods have been used to stabilize soils for structural construction. The Romans and Chinese used various techniques for soil stabilization in their buildings and roadways, which date back more than 5,000 years (Aamir et al., 2019; Shalchian & Arabani, 2022). These improvement methods include i) incorporation of chemical additives such as lime, bitumen, fly ash, cement, and synthetic polymer that can alter the chemical properties of soils and ii) mechanical techniques such as compaction, ground anchorage, and nailing, blasting, vacuum consolidation, reinforcement that can modify the mechanical characteristics of soils (Jha & Sivapullaiah, 2020; Lakhanpal & Chopra, 2018). In addition to the mentioned procedures, a combination of chemical and mechanical methods (cement, polymers with preloading) has occasionally been used to enhance the soil's qualities (Onyejekwe & Ghataora, 2015). However, employing conventional methods for soil modification may involve significant drawbacks due to their adverse environmental impacts. Cement, for instance, which is among the most well-known stabilizers, is one of the key climate challenges since both the production of cement and the use of cement contribute to the acceleration of global warming by emitting a significant amount of carbon dioxide (CO_2) into the atmosphere. In addition, using conventional stabilizers leads to the exhaustion of natural resources (Firoozi et al., 2017; Shalchian & Arabani, 2022). Therefore, problems concerning sustainable development and environmental concerns can be addressed by implementing soil stabilizing techniques using materials that are both affordable and environmentally compatible. In the past few years, biological soil stabilization approaches have emerged as a viable option among several strategies for soil improvement. In geo-environmental science, biological methods have been introduced as environmentally friendly techniques, and studies have shown promising results in this field (Chang et al., 2020; Choi et al., 2020; Rajoria & Kaur, 2014). These biological compounds have been introduced as an alternative to conventional soil treatment methods (Ramdas et al., 2021; Sharaky et al., 2018).

Biomaterials, such as microbial precipitation, bio-enzymes, and biopolymers, are eco-friendly materials following sustainable development requirements (Jang, 2020). Numerous bio-substances and bio-compounds in nature can stabilize and bind the soil particles together through biochemical processes according to the previous attempt of review articles (Baziar et al., 2021; DeJong et al. 2014b; Ghosh et al., 2021). However, one of the challenges in selecting the required biological substances is finding low-cost appropriate biological agents that cover a large area to stabilize existing soils. Microorganisms, bio-enzymes, and biopolymers are the most suitable bio-additives for stabilizing and improving soil properties such as compressive strength, stiffness, erosion resistance, shear behavior, and hydraulic conductivity (Mallikarjun et al., 2022; Soldo et al., 2020; Umar et al., 2016). However, reviewing the literature shows that various effects, biochemical interactions, mechanisms, and limitations of these bio-based substances on the various type of

soils (granular and cohesive) and their impacts on the mechanical properties of stabilized soil have not been completely comprehended (Jang, 2020; Ramdas et al., 2020). In addition, no thorough research has compared the behavior and effects of various bio-stabilizers on cohesive and granular soils. This shortcoming may stem from the fact that soil (either stabilized or unstabilized) exhibits a complex mechano-dynamic behavior influenced by numerous factors, including the soil's physical and chemical properties, bio-content, the amount of confining pressure, and environmental factors. Bio-stabilizers and their applications in soil stabilization are a multifaceted, interdisciplinary green technology that encourages interdisciplinary research and collaboration among environmental and geotechnical engineers, road and pavement engineers, green chemists, biotechnologists, and geo-micro-biologists (Mekonnen et al., 2020; Rajoria & Kaur, 2014). Therefore, geo-environmental engineers are urged to investigate the mechanical characteristics and behavior of stabilized soil with bio-substances (Ramdas et al., 2021, 2022).

The present study aims to investigate the mechanism, interaction, and performance of soil stabilization in combination with biological materials to determine the type and percentage of compounds required to achieve optimal performance for the stabilization of different soils following sustainable and environmentally friendly geotechnics. To this end, the biochemical features and characteristics of bio-enzymes, bio-microbes, and biopolymers were analyzed, followed by examining the mechanisms, biochemical interactions, and impacts of these biological materials as soil stabilizers. Next, the influence of biological materials in soil stabilization was reviewed on a large/small scale based on biological soil stabilization, and the research gaps in their mechanisms were discussed. In addition, the benefits, limits, and potential future uses of each biocomposite for soil stabilization are discussed. Finally, the type and dosage of biological stabilizers recommended for various soils are outlined.

2 Material and method

2.1 Article selection criteria

The selection criteria for the papers to be reviewed are provided in Table 1. Methodological filters and considerations were given to English articles that primarily addressed the use of bio-materials for soil stabilization. Papers published in languages other than English were not included. This systematic review examined studies published between 1995 and 2023. The included publications were those that investigated the enhancement of soils' geotechnical qualities by incorporating the most abundant microorganisms, bio-enzymes, and biopolymers and were published in peer-reviewed journals. This paper presents information

Table 1 The criteria for article selection

Criterion	Inclusion	Exclusion
Language	English	Non-english
Type of literature	Research, technical, discussion and review articles	Book, book chapter
Countries and region	All over the word	None
Timeline	From 1995 to 2023	Before 1995

obtained from previously published papers. The primary objective was to enlighten the readership about recent developments in geotechnical engineering about soil stabilization using more environmentally friendly and sustainable materials.

2.2 The results of research

The following databases, which included 320 documents, were searched: Google Scholar (175), Springer (95), and ScienceDirect (50), providing the most results for the studied topic. The papers underwent scientific peer assessment to ensure the reliability of the collected data. According to their data and/or references, 71 of the acquired articles were omitted because they did not meet the inclusion criteria, and 44 were excluded because they were duplicates. The ultimate revision yielded 141 eligible articles on the implementation of the most sustainable bio-based materials to improve soils' engineering characteristics, 9 of which were case studies and the remaining were experimental investigations. In addition, 66 essential publications were included to critically examine the global status of research on the usage of bio-compounds in geoengineering. At the beginning of the study, first-level keywords such as *soil stabilization*, *bio-compounds in soil*, and *soil treatment* were used; at the next level, more precise terms such as *soil stabilization with a bio-microbe, soil stabilization with a bio-polymer,* and *soil stabilization with a bio-enzyme* were applied. Sections 3 and 4 discuss the bio-based substances and their biochemical reactions. Sections 5, 6, and 7 provide a detailed review of research on soil stabilization utilizing microorganisms, bio-enzymes, and bio-polymers based on their type, number of applications, usage, and historical trend in the soil stabilization process. Then, the application of bio-materials at macro- and micro-scales and their cost are assessed.

3 Bio-based substances

Several substances derived from bio-sources (e.g., microorganisms, bio-enzymes, and biopolymers) have been examined as potential replacements for conventional chemical stabilizers in developing green and road geotechnical infrastructures. These substances have gained more attention regarding their high rate of bio-chemical activity, rapid growth, cheap curing, and non-toxicity in the soil matrix. Each biomaterial possesses unique chemical properties and induces distinct biochemical reactions in the soil matrix. Therefore, deeper comprehension of these bio-based materials and technologies is necessary for their increased use in soil stabilization (Choi et al., 2020; Ikeagwuani & Nwonu, 2019). As shown in Fig. 1, soil stabilization using biomaterials can be accomplished using microorganisms, bio-enzymes, and biopolymers**.** The use of microorganisms to achieve soil stabilization through bio-cementation through microbial precipitation (MICP), also known as "bio-grout", is among the most prevalent methods. This method uses bacteria's metabolic activities to produce calcite precipitation ($CaCO_3$). Accordingly, this biochemical interaction between microorganisms in the soil matrix causes soil particles to bind, resulting in higher soil compressive and shear strength (Cheng & Shahin, 2019). In other words, by adding microorganisms to the soil and their biochemical interactions with soil grains, the bio-cemented process binds the soil grains and strengthens the interlocking forces between soil particles (Kalkan, 2020; Robert & Chenu, 2021). In biological engineering, the so-called biocatalyst bio-enzymes can control how fast chemical reactions happen and how much energy is needed to switch from one product to another (Mekonnen et al., 2020).
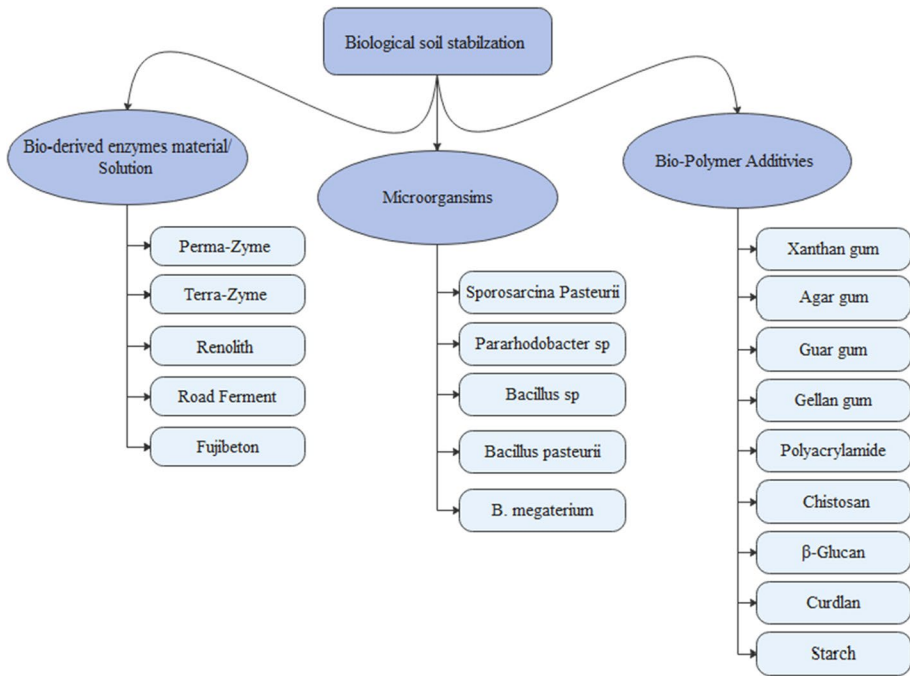
Fig. 1 Soil stabilization with biomaterials

Bio-enzymes are protein molecules that catalyze chemical reactions in the soil to form cement bonds that stabilize soil structure and reduce water absorption tendency (Ganapathy et al., 2017). Biopolymer stabilization modifies the soil's properties by producing a "hydrogel" hydration. After dehydration, water molecules tend to escape from polymer chains, forming complexes of polymer chain attachments. In addition, during the drying process, hydrogels transform from "rubber gel" to "glass" (Muguda et al., 2017). Bio-enzymes are biomass materials derived from natural enzymes that work by interacting with clay particles to form a rock-like surface. Grains stick together and form a dense soil matrix (Joshi & Solanki, 2019; Kushwaha et al., 2018).

Bio-enzymes are proteins made up of a chain of amino acids that combine small parts to make a molecule and can improve the function of a molecule that already exists (Mekonnen et al., 2020; Rafique et al., 2016). Bio-enzymatic solutions increase water's hydration process by reducing water's surface tension. As a result, the thickness of absorbed water around the soil particles reduces, leading to the breakage of water molecules around the particles. Bio-enzymes use their chemical properties to absorb water and fill cavities, allowing clay grains to stick together and form a dense soil matrix (Joshi & Solanki, 2019; Kushwaha et al., 2018). On the other hand, based on the type of monomers making the polymers, biopolymers for soil stabilization can be categorized into polysaccharides, proteins, and nucleic acids. Based on their response to heat, biopolymers can also be classified as elastomers, thermoplastics, and thermosets. Generally, some biopolymers are water-sensitive, and wetter soils show poorer biopolymer-soil adhesion. As a result, when gel-type biopolymers are hydrated, they form hydrogels with significantly less tensile strength than compressive strength. Protein-based biopolymers are less prevalent than polysaccharides due to their higher cost and require

greater water (Ibrahim et al., 2019; Kakkalameli et al., 2022; Krebsz et al., 2017). The engineering sciences have used at least eight types of biomaterial processes and mechanisms, each defined according to its operating mechanism. Figure 2 illustrates the procedure and effectiveness of biomaterial-based soil stabilization. In recent years, four prominent applications have been considered in geotechnical engineering: bio-clogging, bio-cementation, bio-coating, and bio-encapsulation (Lee et al., 2019). Bio-clogging or biological clogging in soil or porous matrix is a process to fill in the pores and voids in the soil/matrix to reduce the hydraulic conductivity of the soil or porous matrix significantly. Also, bio-cementation is the process of producing particle-binding compounds on-site through microbial activities to increase soil shear strength. The bio-encapsulation and bio-coating techniques involve enclosing and shielding soil microorganisms and particles to preserve their biochemical activities and decrease the water absorption tendency (Chu et al., 2014; Ivanov & Chu, 2008; Ivanov et al., 2019; Li et al., 2016).

Currently, liquid-type non-conventional stabilizers have surpassed conventional stabilizers used to improve pavement and soil beneath shallow foundations. These non-conventional additives were introduced as attractive and competitive materials since they can be incorporated in-situ into the soil matrix and rapidly sprayed or pressure-injected into the soil pores in the case of deep mixing. Although conventional stabilizers such as cement and lime are effective for enhancing poor soils with enough clay content, the enhancement rate in compressive and shear strength with the addition of cement is higher than that of lime after 1 and 2 weeks of curing, respectively. Cement requires a high quantity of additives (3–7%) to achieve better strength. In this respect, the optimum percentage found from previous research ranges from 3 to 8%. Therefore, excessive dosage of lime may result in acidic soil, which is undesirable. Combining appropriate bio-based stabilizers is an efficient method for decreasing the additive dosage of conventional stabilizers (Ramdas et al., 2021). In addition, compared to other conventional and other bio-stabilizers and bio-microorganisms, bio-enzymes, and bio-polymer,
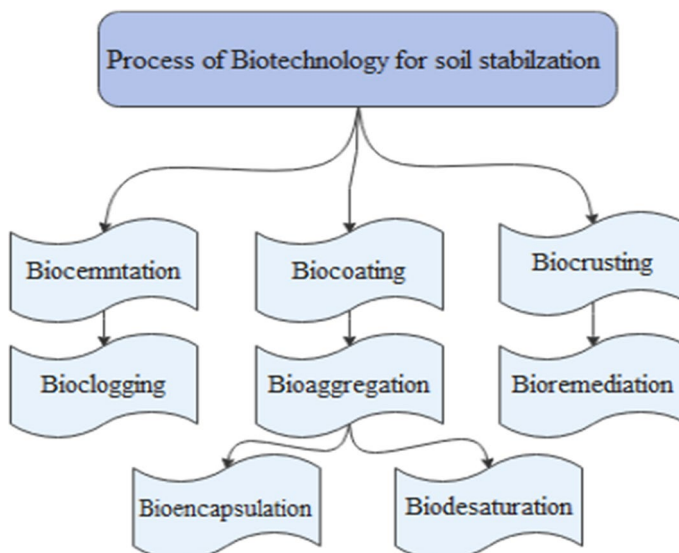

Fig. 2 Biotechnology process in engineering

it requires less dosing, does not need specialist workers, boosts photosynthesis in plants, and creates only a small amount of disturbance (Ramdas et al., 2020).

The bio-chemical activities of each biomaterial for soil stabilization and improvement vary due to different biochemical compounds. In the following sections, the behavior of each bio-based substance is discussed separately according to its functions, availability, and historical applications.

4 Biochemical reaction of bio-based substances

4.1 Microorganisms

Precipitation of calcite uniformly within soils through biological activity is among the widely used methods to raise the pH that causes supersaturated environments defined as microbial-induced calcium carbonate precipitation (MICP). In recent years, MICP has been presented as a new branch of geotechnical engineering to improve soil mechanical and dynamic properties due to nontoxicity and low cost (Mujah et al., 2017). Conventional grouting methods for soil stabilization employ particulate (cement/lime), which can be costly and environmentally unfriendly (Qu et al., 2022). As an alternative to conventional soil improvement approaches, the MICP method should be considered for addressing geotechnical problems, including liquefaction and erosion, and boosting mechanical qualities like adhesion and friction (Kalkan, 2020). In this method, microorganisms are ubiquitous in the top and bottom soil layers and have been active for millions of years. There are often more than 10^9 cells per gram in the surface layers of soil, although the population density of microorganisms declines with depth (Schulz-Bohm et al., 2018). The number of microorganisms that can be exploited as biological agents is high, but their presence in the project site's soil may be negligible. In the MICP method, the microbial population is often boosted by injecting nutrients (bio-stimulation) or augmented by injecting additional microorganisms (bio-augmentation) (Cheng & Shahin, 2019; Taghavi, 2021). In either case, the objective is to increase the activity level and/or concentration of the microbial population to a level sufficient to initiate and sustain a bio-chemical reaction. The key contributions made toward the development of MICP approaches are outlined in the following section based on their historical order. Gollapudi et al. (1995) may have been the first to use MICP for geotechnical applications. They attempted to prevent the leaching of groundwater contaminants in extremely permeable channels by mixing a mixture of bacteria (Bacillus pasteurii) with sand (Gollapudi et al., 1995). The second attempt using bio-microorganisms to improve soil compressive and shear strength was made by (Rahman et al., 2020). To date, various microbes have been applied in soil matrix stabilization (Mohapatra & Pradhan, 2021). The simplest and most common process for generating microbial carbonate precipitation is using a urolytic bacteria with urease's enzymatic activity as a catalyst for biochemical reactions. In this process, the bacterium is cultivated in vitro and then injected into the soil (Mujah et al., 2017; Yi et al., 2021). This biological method employs urolytic bacteria abundant in soil and the natural environment. This method can produce controllable cementation at a much faster rate (less than 24 h) than chemical cementation in soil environments (Tang et al., 2020). The conceptual model of this process is illustrated in Fig. 3. Compared to other stabilizers, the MICP mechanism binds neighboring soil particles, thereby adhering and cementing them together in a chain of biochemical reactions (Chittoori et al., 2021). The series of general biochemical reactions entails the hydrolysis of urea by bacteria, the production of
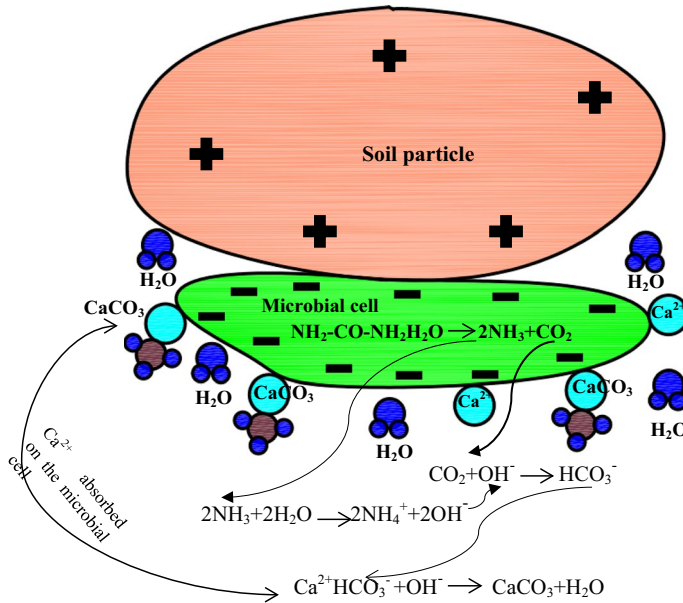
Fig. 3 Schematic view of the biological precipitation of calcite inside the soil matrix
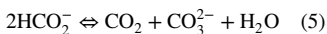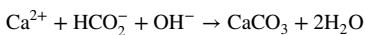
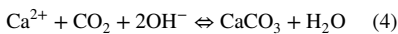ammonia and carbon dioxide, and ultimately the biological deposition of calcium carbonate ($CaCO_3$) that occur within the soil mass.

Due to the presence of hydroxide ions on their cell walls, bacterial cells have a negative charge and adhere to the surface of soil particles containing a relatively high nutrient concentration. However, calcium ion with calcium salt is absorbed by the bacterial cell wall's negative charge. Bacteria emit mineral carbonate and ammonium ions in reaction to adding urea to the environment (Jiang et al. 2022a). This response can lead to local super saturation in the presence of calcium ions, resulting in the formation of non-uniform carbonate deposition on the cell wall, which improves the soil's mechanical properties. In the presence of substrate and adequate microbial activity, cementation covers all cell surfaces. There are other microbial mechanisms for cementing soil masses that are presented in Table 2, including 1) Amino Acid Amino Acidification, 2) Denitrification, 3) Dissimilatory Sulfate Reduction, 4) Photosynthesis, 5) and Methane Oxidation. During amino acid metabolism, CO_2 and ammonia are produced through microbial activity (Mujah et al., 2017).

These microorganisms are, therefore, frequently urease-positive bacteria. Aerobic bacteria are favored because their cellular respiration produces carbon dioxide, which increases when pH rises owing to ammonium formation (Naveed et al., 2020). According to the literature,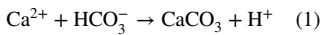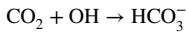 most urease-positive bacteria used in geotechnical studies belong to the species shown in Fig. 1 (DeJong et al., 2010; Smit et al., 2022; Yu, 2021).

4.2 Bio-enzymes

Bio-enzymes are fermented enzymatic components derived from plant extracts. These enzymes are organic matter obtained as a concentrated liquid comprised of protein molecules (Mekonnen et al., 2020). Using bio-enzyme agents as soil stabilizing additives

Table 2 Chemical reactions of various mechanisms for the MICP process

Amino acids $+ O_2 \rightarrow NH_3 + CO_2 + H_2O$
$NH_3 + H_2O \rightarrow NH_4 + OH^-$
$CO_2 + OH \rightarrow HCO_3^-$
$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+$ (1)

$(CH_3COOH)_2Ca + NO_3^- \rightarrow CaCO_3 + \frac{4}{5}N_2 + 2CO_2 + 3H_2O + OHk$ (2)
$6CaSO_4 + 4H_2 + 6CO_2 \rightarrow CaCO_3 + 4H_2S + 2S + 11O_2$ (3)
$CH_4 + O_2 \Leftrightarrow CH_3OH + H_2O$
$CH_3OH \rightarrow CHOH$
$CHOH + H_2O \rightarrow HCOO^- + H^+$
$HCOO^- + H_2O \Leftrightarrow HCOOH + OH^-$
$HCOOH \rightarrow CO_2$
$Ca^{2+} + CO_2 + 2OH^- \Leftrightarrow CaCO_3 + H_2O$ (4)
$Ca^{2+} + 2HCO_3^- \rightarrow CaCO_3 + CO_2 + H_2O$
$Ca^{2+} + HCO_2^- + OH^- \rightarrow CaCO_3 + 2H_2O$
$2HCO_2^- \Leftrightarrow CO_2 + CO_3^{2-} + H_2O$ (5)
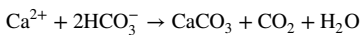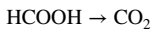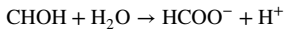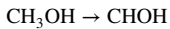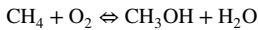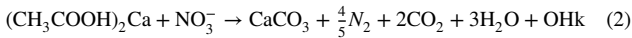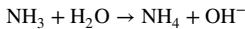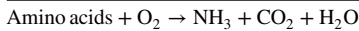
has been the subject of intense research regarding their high manufacturing capability, low cost, environmental safety (non-toxicity), and relatively wide applicability compared to conventional chemical stabilizers (e.g., Portland cement, hydrated lime, and bitumen), which are required in large amounts to stabilize soils. Research has demonstrated that biological enzymes and their derivatives are resistant to precipitation and can be used in any climate (Thomas & Rangaswamy, 2020). Because of the large specific surface area and cation exchange capacity that influence the soil matrix's expansion and shrinkage, mechanical stabilization methods cannot entirely remove the water layer that covers the particles (Kushwaha et al., 2022; Mekonnen et al., 2020). This propensity of soil masses to take in water is a consequence of hydrogen bonding (oxygen or hydroxyl molecules absorbing hydrogen from water), van der Waals gravity, and the bipolar gravity of the charged surface (Taha et al., 2013). Although these enzyme-based soil stabilizers offer numerous advantages over conventional chemical stabilizers, it is unclear how and under what circumstances these substances operate. Stabilizing methods with these substances suffer from restricted use, and their particular chemical makeup makes their long-term effectiveness difficult to predict. Bio-enzymes are biocatalysts that accelerate unaltered biochemical reactions. Consequently, it is essential to conduct a study to provide objective scientific evidence for applying novel enzymes as a product in soil stabilization. Applying enzyme stabilization for roads originates from using enzymes to substances used to treat the soil to improve agricultural applications in 1967. A procedure modification resulted in producing a material that is acceptable for stabilizing weak roadbeds. Enzymes, when added to soil, enhance the particles' wetting and adhesion capabilities. Bio-enzymes react with soil molecules to generate a cementing link that stabilizes the soil structure. At a larger application scale, the treated soil can form a dense, strong, hard, and water-repellent bond layer and structure that can be used as a soil stabilizer (Pooni et al., 2019). Bio-enzymes stimulate biochemical reactions in the soil to form a cementitious bond that stabilizes soil structure and decreases the soil's natural tendency to absorb water. Moreover, they increase

soil density, thereby lowering water retention and erosion. Bio-enzymes can also be used in a wide range of soils regarding their better interaction of enzymatic components with ionic soils, as long as a small amount of clay particles are present in the soil mass (Khan & Taha, 2015). Bio-enzymes are commonly sold as a concentrated liquid that must be mixed with aqueous solutions on the construction site before being spread on the soil or injected into deeper soil layers to stabilize them (Mekonnen et al., 2020). Bio-enzymes are sensitive to temperature such that they operate optimally at low temperatures (35 °C) and degrade at higher temperatures. The basic mechanism of the bio-enzyme activity in clay structure considerably lowers the adsorbed water layer and the double water layer. According to the researchers, bio-enzyme soil stabilization can be performed in two distinct methods (Muguda & Nagaraj, 2019). The first stabilization approach employs cations as an ion exchange agent, a process similar to cation exchange that reduces the thickness of the double water layer in stabilized soil (Velasquez et al., 2006). In the second stabilization method, components of the enzyme material in the soil matrix are combined to initiate a series of reactions between the enzymes and large organic molecules present in the soil masses, leading to the formation of a reactive mediator. This process also involves the clay mineral bio-encapsulation. These organic molecules have large flat surfaces resembling clay particles and can coat the clay minerals to neutralize the negative charge and reduce the clay's water-absorption capacity (Scholen, 1995). Bio-enzymes can be absorbed into the pores between the layers of organic molecules, resulting in a decreased sensitivity to moisture and a reduction in soil particles swelling. As represented in Figs. 4 and 5, clay particles contain high concentrations of cations to compensate for the negative surface loads caused by a bond fracture and isothermal replacement. The hydroxyl (-) and hydrogen (+) ions are separated when a bioenzyme reduces the dipole moment of a water molecule. The hydroxyl ion is subsequently broken down into oxygen and hydrogen, and the hydrogen hydroxyl atom is converted into hydronium ions. Generally, it can absorb or repel positive or negative charges depending on the conditions. A sufficient amount of positively charged metal ions (e.g., sodium, potassium, aluminum, and magnesium) are present in the absorbed water layer to ensure uniformity of charge with respect to negative soil electrical ions.

Regarding the effect of bioenzyme components on the reduction of the electric charge of the water molecule, there is a sufficient negative charge to exert enough pressure on positively charged metal ions in the absorbed film or layer of water. Consequently, this process
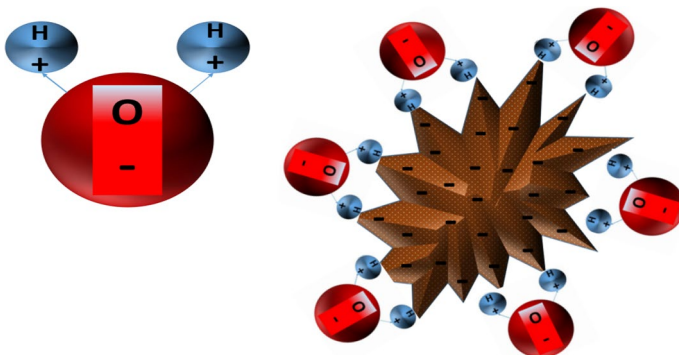

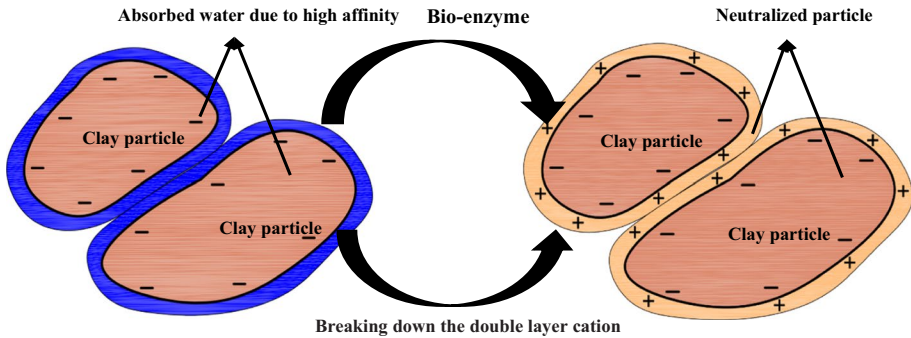**Fig. 4** Surface charge of clay particle

Fig. 5 Mechanism of soil stabilization with bio-enzyme

reduces the electrostatic potential of soil particles (Saini & Vaishnava, 2015; Shankar et al., 2012). When this reaction occurs, the metal ions are converted to free water, which is washed away or evaporated. Consequently, the thickness of the water layer surrounding the particles is diminished. In other words, the particles lose their ability to expand, and the soil's mass solidifies, resulting in soil stabilization and preparation for building and road construction. Adsorbed water surrounding soil particles has been identified as a key issue when discussing soft soils. Bio-enzyme can diminish or even eliminate the soil's adsorbed water layer and enhance the soil's cohesiveness and compaction characteristics. Bioenzyme soil stabilization creates a high-density, hard, water-resistant layer that provides an ideal soil surface for paving roads and controlling dust or erosion compared to other stabilizers (Mekonnen et al., 2020; Renjith et al., 2020).

4.3 Biopolymer

Since more than half a century ago, the polymer industry has been supported by fossil fuels. Nevertheless, the future shortage of petroleum resources (Sorrell et al., 2012) is an undeniable reality. Due to the enormous depletion of fossil fuel resources, sustainable development is now of paramount importance. Therefore, prominent scientific and political leaders have urged the fundamental change to "bio-based material". The history of admixtures began in the 1920s with the introduction of the lignosulfonate biopolymer for ordinary Portland cement concrete plasticization. This biopolymer was the first functional polymer employed on a large scale in the building industry (Plank, 2004). Despite their many potential advantages over traditional chemical stabilizers, how and under what prerequisites these substances operate is still unknown. The applicability of these substances' stabilizing processes is limited, and their specific chemical composition makes it challenging to anticipate their long-term efficacy. In recent years, research on using bio-based polymers has increased dramatically, although these materials still represent a small portion of the polymer industry (Cywar et al., 2022). Biopolymers are a class of polymeric materials that are made by living organisms. Recently, these materials have gained attention in research and engineering fields because of their abundance, biocompatibility, and distinctive features.

Biopolymers are chain-like molecules made up of repetitive chemical blocks that can be dissolved in the environment and are produced from renewable sources (Christian, 2020). These materials are substances derived from natural sources like polysaccharides

(e.g., cellulose), proteins like gelatin, casein, silk, and marine prokaryotes. Biopolymers can also be produced by synthesizing bio-derived monomers (e.g., polylactic acid) or microbial activity (Chang et al., 2020). Biopolymers of microbial origin that function as water-insoluble gels (Fig. 6) can be used to improve soil properties, enclose bioremediation areas, and reduce soil liquefaction (Latifi et al., 2017). Biopolymers' non-toxicity, relative affordability, and biocompatibility have increased their application in engineering, medical devices, energy, and food packaging (Edebali, 2021; Mohiuddin et al., 2017). Engineers have recently considered biopolymers in civil engineering, particularly geotechnical engineering, because of their biocompatibility and environmentally friendly behavior. Owing to different biochemical processes, the mechanism and process of clay soil stabilization with sandy soils differ when using biopolymers. When a biopolymer solution is introduced to sandy soils, the biopolymer solution's suspended particles enter the pores between the sand particles and coat their surfaces (Chang et al., 2020). The presence of silica and silicate, which increase the hydrophilicity of sand grains, accelerates the process of biopolymer interaction with sand particles. When the surface of the sand particle comes into contact with biopolymers, the biopolymers cover the soil particles and form a strong shell and bridge, thereby initiating the stabilization process (Fatehi et al., 2021; Jang, 2020). In contrast, with time and water evaporation, biopolymers increase soil bond strength and bring soil particles closer together, decreasing pore spaces. Therefore, the fewer voids between soil particles, the greater the interaction of the biopolymers with each other and the soil particles (shorter bond chains), and the greater the strength of the soil masses to applied stresses. The sand particles form stronger bonds with increasing the amount or
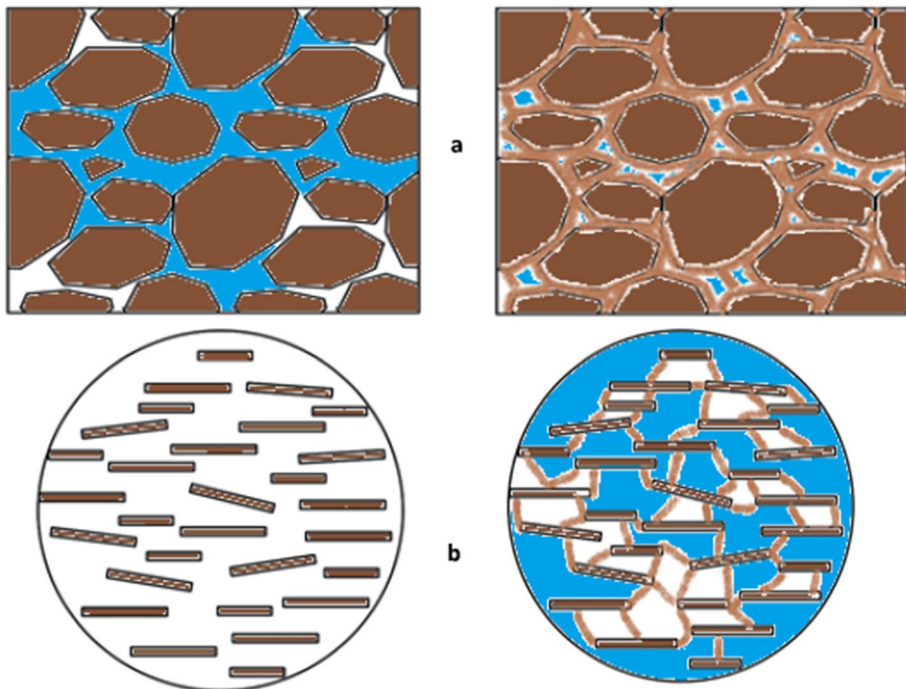

Fig. 6 Bio-capsulation and bio coating of soil aggregates with biopolymer in **a** clay and **b** sandy soil

concentration of biopolymers (up to their optimal content) (Chen et al., 2019; Fatehi et al., 2021; Refaei et al., 2020). On the other hand, clayey soils show a different mechanism by adding biopolymers. Here, chemical bonds are formed by different electrostatic forces, hydrogen bonds, ionic bonds, or van der Waals bonds, which can change over time due to their ionic structures. It is noteworthy that kaolinite clay soils have negative and positive ions on their surfaces and montmorillonite soils typically have negative ions. As a result, they also interact with biopolymers due to the electrical charges of biopolymers, natural cations within the clay, and the amount of charge on soil particles. In general, the biopolymer covers the soil particles in clay soils (bio-capsulation and bio-coating) due to the bond between the layer that absorbs water and ions.

5 Effect of microorganisms on soil stabilization

5.1 Sporosarcina pasteurii (S. pasteurii) microorganism

Examining the impact of adding 1 mol of S. pasteurii microorganisms on the permeability and shear strength of a 5-m sand column in the Netherlands revealed that the samples' strength did not improve significantly at low concentrations of calcium carbonate (under). However, higher amounts of calcium carbonate resulted in a significant increase in strength compared to virgin sand, which had a strength of 570 kPa due to the injection mode of calcium carbonate with the maximum amount and at a distance of approximately 1 m. In this study, the minimum amount of calcium carbonate required to initiate microbial activity was determined to be 60 M. Besides, the permeability study revealed that the maximum amount of calcium carbonate 105 M reduces permeability by 90% (Whiffin et al., 2007). Applying S. pasteurii for soil bio-grouting in a large-scale study (100 m^3) revealed that the values of soil stiffness increase with increasing compressive strength and shear wave. In this study, the estimated calcium carbonate values ranged from 12.6 to 27.3% of soil weight, with a peak compressive strength of 12.4 MPa. Additionally, the maximum modulus of Young of 8.5 GPa was observed (Van Paassen et al., 2010). The results of a study for stabilizing and reducing the potential erosion of sandy soil with S. pasteurii and considering three parameters, namely concentration, heat and humidity, and sampling preparation (wash or unwashed), demonstrated that a concentration of 1×10^7 cells/mL in 3 mol has the best performance for reducing soil erosion potential up to 70%. It was also proved that at humidity levels under 20%, soil erosion potential decreases as temperature rises. In addition, it was observed that when water and microorganisms are added to unwashed soil, crust-like layers cover the soil particles, which has a significant impact on the interaction between most particles and reduces erosion (Meyer et al., 2011). It was also found that by injecting S. Pasteurii into a sand column with a 61 cm height and a 2.54 cm diameter, the hydraulic conductivity decreased by approximately 60% in 36 days (Cunningham et al., 2011). The effects of wave, erosion and compressive strength were considered on fine-grained coastal sandy soils of North Carolina in America using a laboratory model and S. Pasteurii microorganisms. It was estimated that the amount of erosion due to the wave entering the stabilized soil is significantly reduced due to the cementation of materials and increased soil cohesion (Shanahan & Montoya, 2014). According to Zhao et al. (2014), the compressive strength due to the addition of S. Pasteurii is highly dependent on parameters such as urea/bacterial concentration, curing time, and sand type. This study determined that the compressive strength of stabilized and unstabilized soils is in the range of

1.76–2.04 MPa and 0.33–0.43 MPa, respectively, indicating a 5 times increase in soil compressive strength. Furthermore, in terms of improving soil engineering properties, it was noticed that the MICP process catalyzed by bacteria is much more effective than the urease-catalyzed process (Zhao et al., 2014). The impact of Pasteurii bacteria on the consolidation and shear strength of organic soil was assessed in a comprehensive study. Soil stabilization resulted in pH changes in the range of (8.3–9.3), which was a key parameter in $CaCO_3$ precipitation. By conducting three stages of consolidation experiments, it was determined that the changes in porosity ratios for stabilized soils were reduced. It was also revealed that coefficient of volume compressibility (M_v) and coefficient of compressibility (C_C) are greater for the virgin samples than those for the stabilized samples. This result suggest that the compressibility of the stabilized samples is reduced by filling the pores of organic soil with solid particles of calcite, which causes cementation between organic soil particles. Moreover, bio-filling between soil particles decreased permeability by 8% and increased cohesion strength and friction angle to 2.4 kPa and 8°, respectively (Canakci et al., 2015). Shear strength and stiffness of stabilized sand of Ottawa province in Canada by MICP were evaluated using triaxial tests under undrained and drained shear. The stress–strain behavior revealed that with increasing activity of Pasteurii, the strain hardening behavior changes to the softening behavior, and the corresponding transition from global to local failure occurs in the soil mass. Besides, this process increased the dilation of loose sand. Furthermore, it was found that the shear strength of sandy soil, the rate of stiffness reduction due to cement degradation before failure, and the softening behavior of samples after failure depended on the effective stress path and drainage conditions. The peak stress ratio increased from 1.3 for unstabilized sand to 1.9 for cemented sand (with a shear wave velocity of 1400 m/s). Moreover, the peak shear strength (q_u) increased with increasing the cementation level (Montoya & DeJong, 2015). A laboratory model was used to simulate tidal cycles, erosion, and failure mechanisms of stabilized sandy coastal slopes with MICP. Tidal wave cycles were simulated with a gentle flow and applied to sandy soil mass to evaluate shoreline stability, resulting in significant separation of soil particle volume from the slope surface by tidal waves. However, stabilization of sandy soil with S. Pasteurii showed that using 0.7 M of $CaCl_2$ and urea solution could dramatically enhance slope stability up to a steep slope of 53° and a slope angle prone to erosion up to 35° and can minimize slope erosion (Salifu et al., 2016). For low and high concentrations (0.1 to 1 mol) of S. Pasteurii mixture, wind tunnel tests at a speed of 45 km/h were performed to evaluate the erosion and weight loss of stabilized soil samples, and the results were compared with the weight loss of control samples. The findings revealed that the weight loss of stabilized samples relative to the weight loss of the virgin samples was 1.29% for low bacterial mix concentrations and 0.16% for high concentrations, indicating a significant improvement in erosion control in stabilized specimens. In addition, the penetration resistance of stabilized samples with high concentrations of the bacterial solution reached 56 kPa. SEM and x-ray analysis of the morphology of sedimentary $CaCO_3$ crystals revealed that $CaCO_3$ is mostly precipitated as Vaterite crystals that form point-to-point contacts between sand particles (Maleki et al., 2016). Studying the dynamic response and hysteresis loops using triaxial shear tests on stabilized soil with Pasteurii revealed that sand stabilization with this microorganism can improve soil engineering parameters such as liquefaction and seismicity resistance. In this study, the use of bacterial solution and nutrient salt proved to be effective for reducing the curing and precipitation time to 1–2 days (Han et al., 2016). Response and monotonic mechanical behavior of Ottawa sand in Canada stabilized by MICP method were investigated using four stabilization levels (unstabilized, slightly stabilized, medium stabilized, and highly stabilized) and three levels of effective

confining pressure (100, 200, and 400 kPa). The results showed that stiffness, peak shear strength, and dilation increase with increasing calcite content at the effective confining pressure. In addition, the results demonstrated that the improvement of peak and residual friction angles and the initial elastic modulus before rupture (E_i) depend on the amount of cementation and the effective confining pressure (Feng & Montoya, 2016). In an experimental study, a combination of undrained compaction tests, calcite mass measurements, and SEM tests demonstrated that the strength of bio-cement sand strongly depends on the cement surface (or calcite content). Also, it was found that the effective friction angle and effective cohesion of bio-cement sand change linearly and exponentially, respectively, with increasing calcite content. Furthermore, the obtained results proved that S. Pasteurii has a significant effect on the stress path and failure behavior of stabilized sand (Cui et al., 2017). Using cyclic triaxial and shear wave tests with the addition of S. Pasteurii to sandy soil indicated that the mechanical behavior of sandy soil samples with a higher shear wave velocity is more resistant to liquefaction. In other words, stabilized specimens with greater shear wave velocities before inducing significant strains and increased pore pressures indicate an increase in failure cycles. Moreover, it was demonstrated that the behavior of stabilized sands under identical cyclic stress ratios differs. This result suggests that the behavior of stabilized soils is influenced not only by precipitation mass but also by the pattern of calcite sediment distribution within the mass (Feng & Montoya, 2017). A case study was conducted to evaluate the influence of soil stabilization with S. Pasteurii and its ability to reduce the sensitivity of lateral soils on a Brazilian road to soil saturation due to rainfall. The average vertical permeability coefficient decreased from 1.15×10^{-7} m/s for unstabilized to 1.92×10^{-8} m/s for stabilized samples, indicating an 83% reduction (Smith et al., 2017). The use of microbial carbonate precipitation to control surface and subsurface erosion in the laboratory via mixtures of sand and kaolin was assessed through a series of internal erosion tests. To this end, a rigid-walled column erosion-testing device was used that allowed for independent control of MICP stabilization. The results showed that soil stabilization with MICP improves erosion and volumetric shrinkage of sand and clay mixtures because of carbonate precipitation by direct adsorption /coating of fine particles and cohesion on the contact surface of coarse particles. It was also stated that the performance of MICP is more effective at preventing internal erosion in a mixture of sand and clay with a higher pore ratio due to the increased porosity and, consequently, the increased precipitation of calcium carbonate. Another issue that was proven in this study was that the difficulty in moving bacteria and injecting chemicals into the sand and clay mixtures washes away the calcium carbonate produced, which reduces the overall carbonate content and stabilization efficiency (Jiang et al., 2017). In another attempt, the results of the erosion control experiments demonstrated that stabilization with MICP could reduce cumulative erosion weight, erosion rate, and axial strain compared to virgin soil (Jiang & Soga, 2017). As an alternative to $CaCl_2$ in the MICP process, researchers sought to develop a method for producing soluble calcium ions from two waste sources. For instance, the proposed limestone powder was obtained from aggregate mines, and acetic acid was obtained from rapid pyrolysis of lignocellulosic biomass. Based on the obtained results, the engineering properties of bio-cemented sand with mentioned compounds (e.g., permeability, compressive and tensile strength) depend on the calcium carbonate content. In this study, bio-clogging permeability decreased from 1×10^4 to $8.17–1.52 \times 10^6$ m/s (Choi et al., 2017). The results of soil column tests on 14 different soil types showed that a wide range of soil engineering properties is improved by MICP stabilization. After stabilization, the soil column samples had compressive strength in the range of 0.22–5.34 MPa, shear wave velocities from 151 to 1469 m /s, and a 3.5 times reduction in hydraulic conductivity. In addition, the

relationships between soil improvement after stabilization, soil properties and calcite content showed that D_{10} particle size and fine particles might significantly affect the compressive strength and hydraulic conductivity of the soil, while the shear wave velocity was less sensitive to these cases (Gomez & DeJong, 2017). Another research investigated the effects of the oxygenation process and oxygen on the stabilization of MICP catalyzed by S. Pasteurii under three aeration conditions: aeration box conditions, limited air conditions, and open-air conditions. The results showed that dissolved oxygen in the limited air reactor decreased over time and was depleted in about 7 days. In addition, dissolved oxygen in the open air decreased by 50% after 7 days of stabilization due to the presence of surface air. The results also showed that the unconfined compressive strength (UCS) values of sand from Xiamen, China, and $CaCO_3$ could be 100 times different, depending on oxygen supply conditions (Li et al., 2018b). The strength parameters of lateritic soil belonging to Anambra State (Nigeria) stabilized with Pasteurii were evaluated at various concentrations of 1.5×10^8, 6×10^8, 1.2×10^9, 1.8×10^9, and 2.4×10^9 ml, with a curing time of 12 h. Based on the obtained results, a suspension concentration of 1.2×10^9 ml is optimal for lateritic soil, as it can increase compressive strength up to 3 times (Osinubi et al., 2018). In a laboratory study, Sporosarcina ureae, an aureolytic microorganism, was compared with other aureolytic and non-aureolytic organisms of the genera Bacillus and Sporosarcina in terms of their ability to produce carbonate by urolytic MICP to strengthen and stabilize the soil. The results showed that the optimal pH for the growth of S. ureae is 9. Also, according to Fig. 7, the majority of microbial activity takes place under normal water conditions, which can increase soil shear strength from 15.77 to 135.80 kPa (Whitaker et al., 2018).

An experimental study was conducted to investigate the stabilization of loose sandy soils with S. Pasteurii. The results showed that solutions should be added gradually to the soil matrix to prevent the accumulation and flocculation of calcite precipitation on the surface of the sand (Sharaky et al., 2018)**.** Characterizing plasticity and shrinkage of lateritic soil with the addition of S. Pasteurii revealed a general decrease in the values of Atterberg limits with increasing concentrations of S. Pasteurii. In this research, 75% of S. Pasteurii and 25% of cementitious reagent in suspension density of 2.40×10^9 with maximum calcite content of 6% presented the greatest increase in soil plasticity index (Osinubi et al., 2019). The possibility of culturing urolytic bacteria with low-cost reagents and increasing soil stabilization was assessed. To this end, standard laboratory media were replaced with
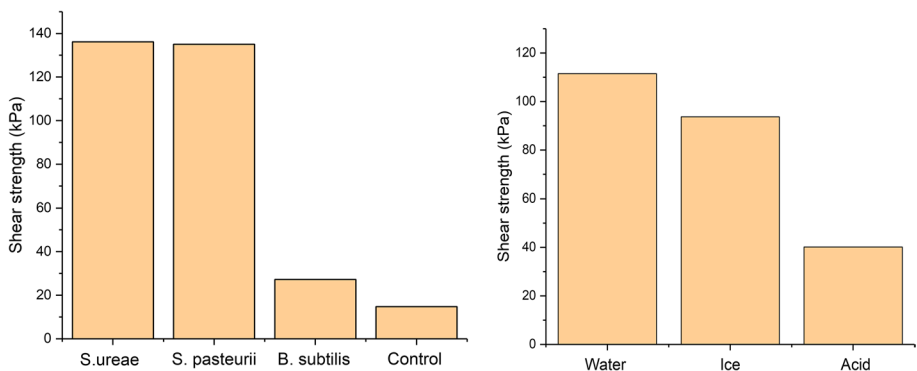

Fig. 7 Direct shear strengths of treated sands with Sporosarcina ureae in flood (water), freeze–thaw (ice) and acid rain (acid) simulations

growth media made from deionized water and tap water for the culture of S. Pasteurii. Surface penetration was applied to perform a bio-cementation mechanism on sand columns using different concentrations (0.25–1 mol). The soil was exposed to room temperature (26 ± 2 °C) for 3 weeks after 92 h of stabilization. Eventually, the results showed that the surface strength of the combined samples was in the range of 4,826–11,448 kPa (Omoregie et al., 2019). The effect of bacterial culture medium and the effect of time (curing) on wind erosion control in sandy soil were investigated in laboratory work. Sugar cane molasses (10%), Tryptic Soy Broth (as a bacterial culture medium of S. Pasteurii), urea solution (2%), and calcium chloride were used as experimental samples. Evaluation results revealed that the erodibility of the stabilized samples in the wind tunnel below the average speed of 16 m/s decreased from 52 to 0% (Nikseresht et al., 2019). In a laboratory study, the UCS and hydraulic permeability of soil from India were considered to examine the formation of mineral sediments between soil particles caused by the metabolic process of S. Pasteurii. The results revealed that calcite minerals can reduce the volume and diameter of pores between soil particles and create a dense microstructure in the soil mass by reducing the distance between soil particles. Moreover, it was observed that uniformity in microbial injection decreases hydraulic conductivity and increases UCS and sample stiffness (Vempada et al., 2020). Furthermore, it was found that S. Pasteurii can significantly improve stabilized sand's penetration and abrasion resistance by considering the effects of cement solution concentration and the number of stabilization cycles. It is noteworthy that this bacterium is an aureolytic bacterium for microbial solution preparation. According to this study, the formation of microorganism surface crusts on sandy soil walls becomes more apparent with increasing the number of treatments and dosage (Fig. 8) (Katebi et al., 2021). In another study, the stabilization of siliceous, silty, and calcareous sandy soils by bio-cementation with S. Pasteurii bacterial solutions was investigated at various levels of addition. The results demonstrated that the curing time of the solution has a significant effect on the physical and mechanical properties of sandy soils and the formation of calcite precipitation by bacterial activity, which leads to the cohesion and bond of soil particles. Moreover, it was proved that a high content of precipitated calcium carbonate increases shear and compressive strength by 4 to 7 times while reducing soil permeability (Mohamed 2021). The impact of urease activity and different bacterial concentration densities on cementation uniformity in coarse sands was studied. The result showed that the larger the
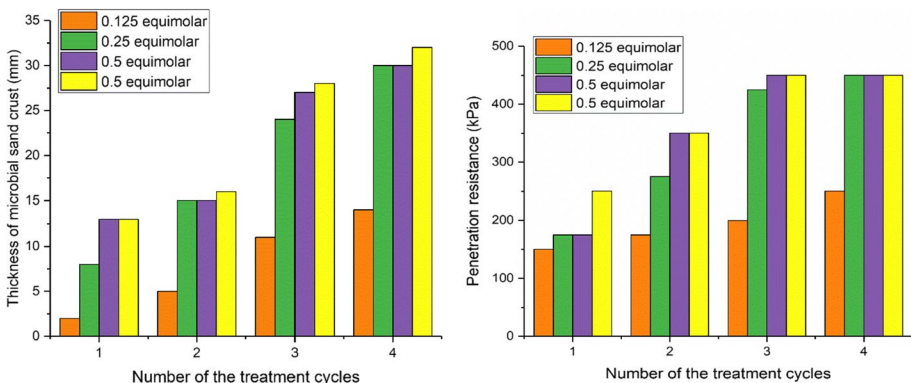

Fig. 8 Thickness and Penetration resistance of the produced sand samples

space between soil particles, the longer it takes bacterial activity to improve mechanical parameters. Moreover, SEM analysis showed that slower rates of MICP reactions result in more efficient and uniform cementation (Konstantinou et al., 2021). A novel and simple method was presented based on the flocculation of S. Pasteurii bacterium to maximize bacterial retention in porous silica sand. The experiments were conducted in two columns of 300 mm and 1000 mm sand that were uniformly distributed. In this research, 10 mM Ca^{2+} and 20 mM urea were determined to be the optimal conditions for maximizing the highest urease retention rate of up to 85%. These conditions lead to more than 80% of the total urea conversion rate and 8–9% more calcium carbonate production. In addition, the range of soil strength in all stabilized columns is observed to be 40–50×10^3 kN/m^2 (Yang et al., 2022).

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5.2 Bacillus sphaericus microorganisms

Cheng et al. (2013) conducted a series of laboratory tests, including permeability, compressive strength, triaxial unsaturated consolidation, and durability, to determine the influence of Bacillus sphaericus microorganisms on sandy soil. The results showed that shear strength (cohesion and friction angle), stiffness, and shear modulus increase up to 150% with increasing calcium carbonate content in different degrees of saturation (20–100%). Moreover, the failure mechanism showed that in stabilized soil, tensile cracks appear first at the specimen's top and bottom, and then failure occurs. In Fig. 9, SEM analysis indicates that in a state of complete saturation, rhombic calcium carbonate crystals are formed in this process. The accumulated crystals are not only in the pores between the particles but also on the surface of the particles or suspended in empty spaces, resulting in inadequate cohesion between the soil particles. However, due to the homogeneous adsorbed solution on the surface of the sand particles caused by the surface tensile force, the effect of bio-coating on soil particles is readily apparent for stabilized sands at 20% saturation (Cheng et al., 2013). Applying a compressive strength test, the results of a laboratory investigation determined that the UCS of sand stabilized with Bacillus sphaericus increases; however, the extent of this improvement depended on the concentrations of calcium chloride and urease (0.1,
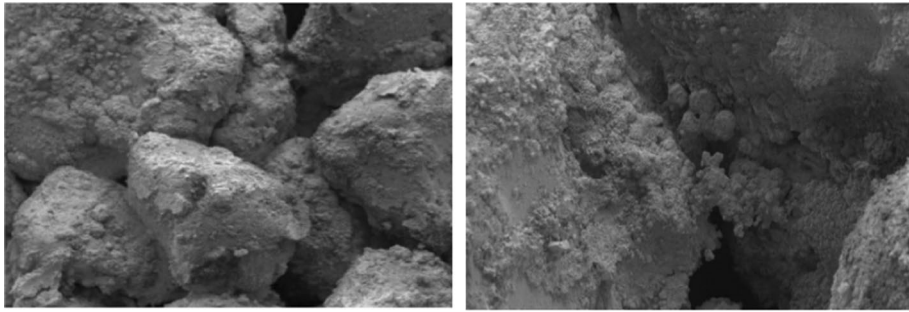
Fig. 9 Formation of CaCO$_3$ crystals for samples treated

0.25, and 0.5 mol). The permeability test results also revealed that using a solution with a high concentration of calcium urea chloride leads to a rapid decrease in permeability during the early stages of precipitation formation, whereas using a solution with a low concentration leads to a gradual and more uniform decrease in permeability (Qabany & Soga, 2014).

Adding Bacillus sp to sandy soils reduced soil permeability from 10^4 m/s to 10^{-7} m/s when adding calcium carbonate (average 2.1 kg/m^2). Furthermore, bending and compressive strength tests revealed that the shear strength, cohesion, and tensile strength have an increasing trend in flexural strength values ranging from 90 to 256 kPa, which are approximately 90 to 250 times higher than virgin soil. Compressive strength also increased up to 932 kPa (Chu et al., 2014). A new method for cementing sandy soils in marine environments on the Australian coast was developed by modifying the calcium carbonate formation technique. The proposed method involves washing urease-activated bacteria with high salinity tolerance. Next, a mixture of urea and seawater through porous sandy soil is used to release bacterial carbonate from the urease reaction and precipitate insoluble and semi-soluble carbonate calcium salts. According to the compressive strength results in this study, this parameter increased to 300 kPa, which is nearly twice as much as the MICP stabilization in which calcium and urea are highly concentrated. It was also found that the permeability of all stabilized samples was approximately 30% lower than that of control samples, indicating a reduced drainage ability (Cheng et al., 2014). The combined effect of fibers in the proportions of 0.4 and 0.8% and microorganisms of Bacillus sphaericus showed that using fibers results in more interlocking forces in the process of calcium carbonate precipitation in the soil matrix (Fig. 10). Fibers can also bridge between sand particles, pores, and the cementation of calcium carbonate. According to the findings, the unconfined compressive and tensile strengths of fiber-reinforced sand increased by 138 and 186%, respectively. Moreover, compared to virgin sand, the permeability decreased by 126%, and the brittleness by approximately 50% (Choi et al., 2016).

The effect of fundamental parameters (i.e., urease concentration, temperature, rainwater leaching, oil pollution, and ice-freeze cycle) was investigated on the activity of Bacillus sphaericus in sandy soil by conducting a UCS test. The results indicated that the formation of crystalline masses, which fill the spaces between the sand particles, plays a substantial role in enhancing compressive strength. In this research, the optimal stabilization of bio-sediment was achieved at 25 °C. It was also found that rainwater was detrimental to the biosynthesis process and that bacterial cohesion could be reduced using the two-phase injection method by rinsing with low ionic strength water (i.e., tap water, rainwater). The results also demonstrated that the conventional stabilization of MICP by the two-phase

Fig. 10 SEM photos of generation calcium carbonate on soil and fiber
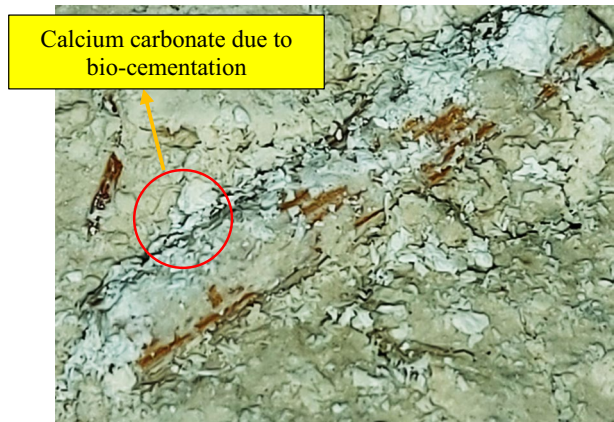
injection method is ineffective for stabilizing oil-contaminated soils. However, the method of pre-mixing biological microbes with soil can significantly increase the UCS values and stiffness of oil-contaminated soils. Moreover, the durability test of MICP-stabilized samples proved that this method has high durability against freezing and thawing. This property was attributed to the points of contact between the particles and the connection to each other due to the formation of sedimentary crystals (Cheng et al., 2017). Bacillus sphaericus was used to improve the strength and erosion resistance of low-plasticity clay (CL) soil in Fars Province (Iran) based on the controlling variables (i.e., curing time, bacterial cell density, precipitation agent concentration, and temperature). In this investigation, pinhole experiments were considered to examine the level of dispersion and erosion of soil samples. Here, the decrease in pH during microbial activity and the resulting reduction in the thickness of the double water layer, and the stabilization of exchangeable sodium ions, were considered the primary mechanisms for reducing the erosion potential of soil samples (Moravej et al., 2018). Direct shear creep experiments were carried out to examine the mechanical creep properties of siliceous sand of China and calcareous sand of China in which microbial solution was injected three, six, or nine times to form the MICP process. Compared to virgin sand, it was observed that the creep deformation of silica sand with MICP has a decreasing trend. In these experiments, the creep deformation of stabilized sand reduced by up to 88% by 9 times injection. SEM also revealed that calcium carbonate produced by microorganisms in soil pores and greater physical contact between sand particles are significant factors influencing the creep behavior of stabilized silica sand (Yuan et al., 2022). According to the results of an experimental study, the optimum cementation solution (0.5 M) can increase soil stability and penetration rate and reduce erosion (Namdar-Khojasteh et al., 2022).

5.3 Pararhodobacter sp microorganism

In a laboratory study, Pararhodobacter sp bacteria were examined, and the impacts of different parameters such as heat, curing, solution injection distance, Ca^{2+} concentration, sodium concentration in solution, and test period were investigated on UCS. After 28 days of curing, the compressive strength of samples was found to increase up to 10 MPa. The test conditions were a curing temperature of 30°C, distance injection of 1 day, and Ca^{2+}

concentrations in a cementation solution of 0.3 mol (Danjo & Kawasaki, 2016). In addition, multiple regression analysis conducted for the expressed variables led to the following experimental formula:

$$q_{\text{ud}} = 48.3C_{\text{ea}} + 0.456D - 19.51 \tag{1}$$

The impact of bioremediation in contaminated coarse and fine-grained sand was evaluated using Pararhodobacter sp. The obtained results proved that bio-microorganism could improve the compressive strength of soil from 1.38 to 2.88 Mpa. It was also observed that the optimum bacterial concentration (10^9 cfu/mL) could play a significant role in reducing the contamination rate due to the absorption and treatment properties (Mwandira et al., 2017). In a laboratory study, the effect of the addition of Pararhodobacter sp and various factors were investigated on the engineering properties of stabilized soil. These properties included temperature and curing temperature, solution injection distance, Ca^{2+} concentration, bacterial population, re-injection of bacteria, and sand particle size. The obtained results revealed that after 14 days with a curing temperature of 30 °C, an injection interval of 1 day, and Ca^{2+} concentrations in the cement solution of 0.5 M, the compressive strength of the samples reached 10 MPa (Ggnn & Kawasaki, 2017). Multiple regression analysis also revealed that the conditions for estimating the compressive strength formula as a test period, D (days), and concentration of Ca^{2+} (Eq. 2):

$$q_{\text{ud}} = 13.99C_{\text{ca}} + 0.37D - 0.09 \tag{2}$$

The results of an experimental study proved that the incorporation of Pararhodobacter sp. in contaminated kiln slag (KS) can reduce permeability and boost compressive strength due to the bio-cementation process (Mwandira et al., 2019). In a large-scale study in Japan, the rate of bio-cementation and soil erosion of slope were investigated. The results indicated that due to the bio-cementation, the hydraulic conductivity of the slope decreased from 1.9×10^{-2} cm/s to 1.85×10^{-3} cm/s (by 90%). It was also found that Pararhodobacter sp could inhibit the excess infiltration of rainwater and improve surface runoff (Gowthaman et al., 2019). Conducting compressive tests on clay sand gathered from an engineering project in Hong'an City, China, proved that the addition of Pararhodobacter sp can increase UCS up to 198%. In addition, SEM evaluation showed that crystallization and mineralization of MICP can form a 3D network of structure and bridge the gaps (Hu et al., 2022).

5.4 Bacillus megaterium microorganism

In an experimental study, the effects and changes in the properties and behavior of silty soil (ML) were investigated by adding B. megaterium at a concentration of $10^8 \times 38$ cfu/ml. The results demonstrated that the shear strength and hydraulic conductivity of soil increased by 60% and decreased by 90%, respectively. In addition, the minimum amount of calcium required for modification and improvement of mechanical and physical soil parameters was 1 or 15 kg/m^3 (Soon et al., 2014). The efficacy of MICP in enhancing shear strength and decreasing soil hydraulic conductivity was also examined. The variables tested in this study included soil types (tropical residual soils and sand) and soil density (85%, 90%, and 95% of the corresponding maximum density). This study demonstrated that microorganisms could effectively increase shear strength and decrease hydraulic conductivity for both soil types. However, soil mechanical parameters improved differently depending on soil density, soil type, and stabilization conditions, and shear strength improvement ratios for residual soil samples were significantly higher than for sand samples. However, due to

particle–particle contact within the pore spaces of the soil mass, sand samples exhibited a greater reduction in hydraulic conductivity than residual soil samples (Soon et al., 2014). Another study investigated the combined effect of bio-cementation (BC) as an additive with fly ash (FA) to improve the geotechnical properties of expansive soils obtained from a slope along the Wuhan-Xian expressway in Hubei Province (China). To this end, a fixed 5% amount of Bacillus megaterium was blended with four concentrations of fly ash, 10%, 25%, and 50%, respectively. The results of compressive strength tests conducted on soil samples containing 25% FABC revealed that the compressive strength of expansive soil increases to approximately 50%. In addition, the analysis of SEM and XRD microstructures of expansive soil indicated that MICP is effective at enhancing soil strength parameters due to the decrease in liquid limit (LL) and plastic limit (PL) (Li et al., 2018a). The soil permeability of landfill located in Anambra state in Nigeria stabilized with B. megaterium indicated that the cementation and bio-filling of pores can significantly reduce the hydraulic conductivity and leachate up to 70% (Etim et al., 2022).

5.5 Bacillus pasteurii microorganism

Bacillus pasteurii microorganisms with initial values of $10^6 \times 2$ cells/mL were used to stabilize sandy soil. DeJong (2006) et al. determined that adding these microorganisms can increase soil's monotonic undrained bearing capacity and shear stiffness. For this purpose, they performed a series of laboratory tests, including Bender elements and Isotropically consolidated undrained triaxial compression. In addition, the response of values revealed that by adding microorganisms and due to the cementation, the soil's behavior could transform into a non-collapsible condition. Moreover, variations in the shear wave velocity demonstrated that this parameter initially decreases and then gradually reaches its maximum value. Also, as bio-microorganisms' activity reduces, this parameter tends to approach zero (DeJong et al., 2006). The dispersion potential of clay obtained from a mine in the eastern north of Iran was investigated by adding sodium hex metaphosphate and conducting shear strength, dual hydrometer, pinhole, and chemical tests. Also, biological stabilization methods with Bacillus sphaericus and Bacillus pasteurii were investigated to determine the influence of bacterial activities on soil improvement parameters. According to the obtained results and chemical properties, the role of carbonate produced in enhancing mechanical properties and erosion, soil chemical (Na ion exchange), and mechanical (cohesion and friction angle) properties was greater in B. pasteurii than in B. sphaericus (Abbaslou et al., 2020).

Microorganisms, particularly Sporosarcina pasteurii and Bacillus sphaericus, by producing bio-cementation using microbial-induced calcite precipitation, can significantly improve the mechanical properties of soils, including compressive strength, bearing capacity, and soil shear strength, up to 250%. In arid environments, bio-cementation can reduce soil hydraulic conductivity, settlement, shrinkage, seepage, and rainfall penetration. Bio-clogging and bio-cementation are two of the most important applications of bio-microorganisms. Through the biological activity, the bio-clogging process in coarse grain soil leads to the clogging of voids between soil spaces, which influences the pore spaces and permeability of the soil. The bio-cementation process results in the particle connecting, bonding, and interlocking of soil particles, thereby enhancing the soil's resistance. Micro-aerophilic bacteria that can thrive in the absence of oxygen are the most suitable for the bio-stabilization of soil. However, the effectiveness of bio-cementation and microorganisms in improving soil strength greatly relies on physical and environmental factors. In

addition, research has shown that bio-cement may be treated in a wide range of environmental conditions, including severe pH, high temperatures, and void size. According to the findings, the optimal concertation of microbes depends on soil texture and physical and chemical parameters, and the optimal pH ranges 7–9.5. In addition, temperature plays a key influence in numerous bacterial activities, with the highest levels of activity occurring between 30 and 60°C. Furthermore, the size of bacteria is an important component with a significant effect on soil stabilization. The optimal size ranges between 0.5 m and 3 m and corresponds to reduced movement of microorganisms in the soil matrix.

6 Effect of bio-enzyme on soil stabilization

6.1 TerraZyme bio-enzyme

A comprehensive study was carried out on soil stabilization with TerraZyme and its effectiveness on base and subsoil soils. In this study, only short-term changes in soil properties were evaluated. The results showed no significant change in soil properties in the early days in cohesive soils, although the soil performance improved gradually (Lacuoture & Gonzalez, 1995). TerraZyme was used in a case study to improve a road in Malaysia that had severe problems during the monsoon season or after heavy rains. After two monsoon seasons of monitoring various parts of the road, it was noticed that the road was in excellent condition with no surface damage or erosion, despite being subjected to heavy rainfall. Hence, the road section did not need to be repaired, and it was suggested that this bio-stabilizer could improve road resistance parameters in various climates while causing minimal damage during hot and humid seasons (Zamri et al., 1999). Field studies and experiments in Brazil using TerraZyme as a biological enzyme stabilizer for road stabilization using Dynamic Cone Penetration test revealed that adding biological enzyme increased the strength of sandy soils, silty clay, sand silt, plastic and non-plastic clay, sandy loam, and clay mixed with clay of Brazilian roads (Brazetti & Murphy, 2000). A thorough investigation was conducted on TerraZyme's efficacy on lateritic and clay soils in the Kerala region (India). In this research, eight weeks were spent observing, recording, and comparing the reaction of enzyme-stabilized and control soil samples. The obtained results showed that the addition of TerraZyme increased the California Bearing Ratio (CBR) by 136 to 180 times compared to the initial value in all soil types studied (Isaac et al., 2003). In addition, stabilizing two types of high plasticity clay (CH) and CL soils with varying concentrations of TerraZyme (0.01–0.1%) demonstrated that using bio-enzymes can increase soil compressive strength due to bio-encapsulation of clay minerals. This improvement is attributed to the cation exchange of monovalent cations for covalent cations, the chemical decomposition of clay mineral structure, and reduction of double water thickness. Furthermore, the addition of bio-enzymes proved to decrease the interlayer adsorption of organic molecules, resulting in a decreased sensitivity to moisture (Tingle & Santoni, 2003). Shankar et al. (2012) investigated the effect of different doses of TerraZyme on the soil of Dacshina laterate (a region in India) with a liquid limit and plasticity index of 25 and 6%, respectively. Experiments on lateritic soils with varying amounts of sand revealed a modest improvement in the physical properties of lateritic soils. However, by increasing the bio-enzyme dose (200 ml/2m^3 of soil), a 300% increase in CBR, 450% increase in compressive strength, and a 42% reduction in permeability were achieved after four weeks of curing. Additionally, it was noticed that bio-enzyme did not enhance soil cohesion (Ravi Shankar

et al. 2009). The efficacy of bioenzyme in a case study of India's National Highway was investigated by conducting UCS and CBR experiments on three soils (sand, clay, and silt) at different enzyme doses, with liquid limit and soil plasticity index of 28 and 30, 46 and 6, and 5 and 6%, respectively. The results showed that after 4 weeks of curing, CBR increases by 157–673%, while UCS increases by 152–200% (Venkatasubramanian & Dhinakaran, 2011). According to a case study, using the TerraZyme bioenzyme as a stabilizer lowers the cost of road construction by 18 to 26% (Rajoria & Kaur, 2014). Changes in the compressive strength of expansive soils caused by the addition of Terrazyme (0.25 to 4 mol) were investigated. The results showed that the higher the amount of bioenzyme, the greater the increase in compressive strength, which increases to about 200% (Agarwal & Kaur, 2014). According to a laboratory study, the optimal dose of TerraZyme (2.5m³/200 ml) can increase soil-bearing capacity and decrease soil plasticity. According to this study, the bio-enzyme reacts with the adsorbed water layer of clay particles and reduces the water thickness of the double layer surrounding the soil particles. As a result, it lowers the voids between soil particles, thereby reducing soil permeability and soil swelling capacity (Saini & Vaishnava, 2015). Compaction parameters, Atterberg limits (liquid limit and plasticity limit), and soil strength of CL soil were investigated using 3 bio-enzymes, including DZ-1X, EarthZyme, and TerraZyme, in a laboratory comparison. Overall, it was found that the parameters did not change significantly due to a lack of changes, interaction, and chemical activity of bio-enzymes with soil (Khan & Taha, 2015). The addition of different doses of TerraZyme in combination with 500, 700, 900, and 1000 ml/m³ clay proved that increasing the bio-enzyme dose significantly increased the CBR value of soil samples. It was also mentioned that the curing time significantly impacts the bioenzymes' performance and efficiency (Panchal et al., 2017). Examining silty soil and silty sand with different bio-enzymes (e.g., TerraZyme, Road Ferment, and urease) at concentrations of 0.2–3 g/L and curing times of 7 and 21 days proved that all three bio-enzymes increased the erosion resistance of SC-SM soil up to 1,380%, whereas ML erosion resistance improved marginally (Shafii et al., 2019). The functional mechanism of TerraZyme in different doses on kaolinite soil was determined by measuring soil plasticity, enzyme-stabilized compressive strength, and curing age. The results revealed that by prolonging the curing time, the soil plasticity index decreases while the plastic and shrinkage limits increase. As a result, the plasticity and shrinkage indexes decrease, which means less propagation of cracks. In addition, evaluating the compressive strength properties revealed that the addition of bio-enzymes could increase soil strength by up to 30 times (Muguda & Nagaraj, 2019). Laboratory research revealed that the Terrazyme with the combination of 1% cement could have a significant effect on the strength parameters and soil plasticity characteristics. Table 3 reveals that the liquid limit of untreated soil is 91%, and it decreases to around 50.2 when 1% cement is added to clay soil. When a tiny percentage of 0.04 ml/kg of terrazyme was added to the soil–cement mixture, the liquid limit increased to 53%. Because of the hydrophilic properties of bioenzyme at low levels, there is a modest rise. After this phase, in the presence of 1% cement and 0.06 to 0.11 ml/kg of terrazyme, the liquid limit of the soil decreased to 48.7% compared to the liquid limit of the untreated soil. The same trend is observed for Plastic limit in 7 and 28 days. The plasticity index was 58% for clay soil, but after treatment, it decreased to 16.4%. The curing period has no noticeable impact on the consistency limits of soil–cement-terrazyme combinations. The decrease in plasticity index results in lower moisture absorption.

According to Table 4, increasing the TerraZyme dosage from 0.04 to 0.06 ml per kilogram (ml/kg) on the 1st day of curing improved the compressive strength of clay in the presence of 1% cement up to 233.9%. Then, by increasing the dosage of TerraZyme up

Table 3 Effect of Terrazyme on consistency limits of soil–cement-terrazyme mixtures (Thomas & Rangaswamy, 2021)

Combinations	Dosage of Terrazyme	Liquid limit %		Plastic limit %		Plasticity index %	
	Milliliter per kilogram (ml/kg)	7 days	28 days	7 days	28 days	7 days	28 days
Soil	0	91.0	91.0	33.0	33.0	58.0	58.0
Soil + 1% cement	0	50.2	49.5	30.5	30.1	19.7	19.4
Soil + 1% cement + terrazyme	0.04	53.0	51.7	33.1	32.3	20.0	19.5
Soil + 1% cement + terrazyme	0.06	49.2	48.5	33.0	32.0	17.2	16.7
Soil + 1% cement + terrazyme	0.08	49.0	48.5	32.0	31.8	17.0	16.6
Soil + 1% cement + terrazyme	0.11	48.7	48.1	31.9	31.7	16.8	16.4

to 0.11 ml/kg, the improvement percentage dropped to 192.15% on the 1st day. This is because the interaction between soil and bio-enzyme decreased, while the bio-bio-contact increased. The same trend was observed on different days of curing, from 7 to 28 days. Compressive strength improved after 28 days of curing for all dosages. An optimal dose of 0.06 ml/kg of TerraZyme reached 354.07% after 28 days of curing, which is approximately 354% greater than the strength of the untreated soil. Evidently, the highest level of biochemical reactions was attained with time (Thomas & Rangaswamy, 2021).

In another attempt, stabilizing residual soil of the north part of Sri Lanka with Terrazyme in various doses (0.5, 1, 2, and 3%) and compressive strength tests demonstrated that adding bioenzyme greatly improves the engineering properties of residual soil and adding 2% bioenzyme, compressive strength increased to 250% (Fazal et al., 2021). In a case study, various dosages of TerraZyme bio-enzyme (0.05 to 0.15 ml/kg) were employed to stabilize highway soil in Srinagar, India. After seven days of testing and soil sample collection, it was determined that adding 0.1 ml/kg to 1 m^3 of soil increased its compressive strength and the California bearing ratio by 40% (Zargar et al., 2022). In another study, the integration of TerraZyme in clayey soil as a stabilizer showed that 0.08 ml/kg of the bio-enzyme could increase soil density and reduce settlement due to the bio-filling effect between soil particles (Kumar et al., 2022). The results of an experimental study demonstrated that the bio-enzyme-treated soil is stiffer than the untreated soil. Furthermore, 0.98 ml/kg of TerraZyme was the optimum treatment dosage, improved compressive strength up to 122.89%, and reduced the settlement of the stabilized soil (Kolhe & Dhatrak, 2022).

Table 4 Effect of Terrazyme on the strength of soil–cement-terrazyme mixtures (Thomas & Rangaswamy, 2021)

Combinations	Dosage of Terrazyme	Increase in compressive strength at different curing days (%)				
	Milliliter per kilogram (ml/kg)	1 day	7 day	14 day	21 day	28 day
Soil + 1% cement + terrazyme	0.04	191.54	207.53	222.78	237.18	303.62
Soil + 1% cement + terrazyme	0.06	233.89	250.61	265.10	326.90	354.07
Soil + 1% cement + terrazyme	0.08	204.40	223.72	241.18	255.27	324.94
Soil + 1% cement + terrazyme	0.11	192.15	216.66	223.72	236.35	309.93

6.2 Renolith bio-enzyme

The results of a laboratory study showed that the addition of Renolith does not significantly increase soil compressive strength by up to 10% but does increase soil compressive strength by up to 20% in soils containing 15% Renolith bioenzyme. This difference was attributed to the increased interaction of enzymes with soil particles (Suriyacht and Pratomvong 2003). Evaluating the effect of 1–5% Renolith on GW soil showed that the CBR of the soil increased from 2.54 to 100% (Jovanovski et al.). The addition of cement (1–10%) and Renolith in various concentrations (1–5%) to sandy and silty soils demonstrated that bioenzyme increases soil density and CBR values. Therefore, it was concluded that adding Renolith to the soil enables high CBR values to be achieved with a lower cement percentage. Moreover, it was determined that the addition of bio-enzyme at optimal concentrations of up to 3% could reduce soil permeability to desirable levels (Singh & Garg, 2015). In the same year, a laboratory study on the addition of cement and Renolith bio-enzyme in proportions of 2–4%, 10, and 2.5% showed that the bioenzyme, due to its chemical activity, could enhance the shear strength of Black cotton soil by up to 44% (Amarnth 2017). Adding Renolith to cement demonstrated that when the amount of cement remains constant, increasing the amount of bioenzyme causes the compressive strength to initially increase and then decrease. In this study, the highest rate of strength increase was 0.22 MPa. As shown in Fig. 11, the optimal values for cement and bioenzyme are 3 and 0.2%, respectively. Besides, studying the failure behavior showed that due to bio-enzyme polymerization, the hydrophobic mechanism prevents the development of cracks and causes a self-healing process at the soil surface, which results in the best performance of 0.3% (Que et al., 2017).

Therefore, it is suggested that 3% Renolith (by weight of lime) and 4% lime (by weight of soil) are the most effective contents for improving soil (Muwumuza & Kasekende, 2021). Kushwah et al. (2022), evaluating the compressive strength and erosion parameters, reported the suitability of Renolith biochemical activities for soil and subgrade stabilization. The bio-stabilized surface permeability test confirmed that the bio-enzyme also

Fig. 11 Effect of bio-enzyme on compressive strength
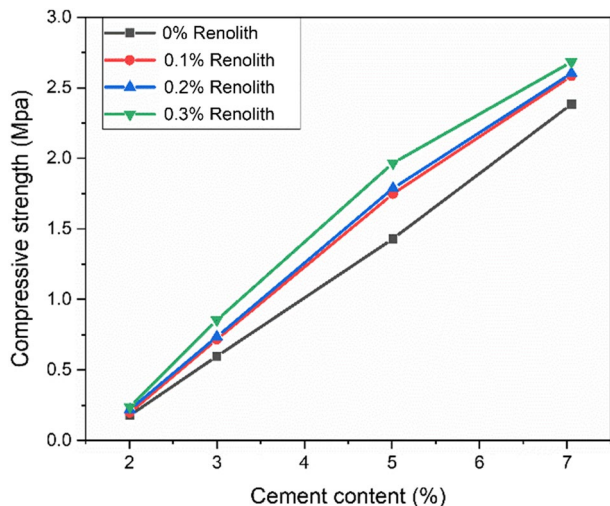
functions as a waterproofing agent and can be used to control dust on low-traffic uncoated surfaces in the local road in south India (Kushwaha et al., 2022).

6.3 Perma-zyme bio-enzyme

Adding and mixing 1:33,000 (v:v) Perma-Zym bioenzyme and 3:7 (v:v) lime to fine-grained soil, coarse soil, and loam soil and performing compressive strength tests showed that Perma-Zyme could significantly improve the strength of fine and coarse soils in 7–60 days. Also, this bio-enzyme showed no effect on silt soil strength. The 28-day compressive strength and permeability of stabilized specimens were examined to see if Perma-Zyme could be used to protect ancient earthen ruins. The compressive strength of the Perma-Zyme-stabilized samples was higher than the mother samples. Moreover, the permeability coefficient of the stabilized soil was lower than the permeability coefficient of the mother soil, indicating improved impermeability (Peng et al., 2011). Two silty soils (ML and SM) were stabilized by the manufacturer-recommended dose of Permazyme 11-X bioenzyme. According to the compaction test results, only a 4 and 1% increase in dry density were observed for ML and SM soils, respectively. After 28 days of treatment, samples of both soils were tested for compressive strength, and no improvement was observed. Similarly, testing for freezing–thawing, wet-drying, and leaching yielded negligible and no improvement, respectively (Milburn & Parsons, 2004). In a laboratory study, the effectiveness of the two bio-enzymes Terrazyme and Permazyme at varying concentrations (1–10 g/L) on strength parameters (CBR and UCS) and specific density of sand, clay, and silty soils was examined. This study revealed a slight increase in the maximum dry density of coarse-grained soil and a slight decrease in the maximum dry density of fine-grained soil. Due to cation exchange, the effect of bioenzyme on the enhancement of fine-grained soil was found to be significantly greater than that of sandy soil. The results also showed that for fine-grained soil, the compressive strength parameters are between 2.75 and 4.5 times compared to virgin soil for an optimal concentration of 0.25% (AbouKhadra et al., 2018). The characteristics of compressive strength, stress–strain, and soil deformation were determined by performing triaxial CU and compaction tests on SP-SM and SM soils in combination with specific doses of Permazyme. The compaction test results indicated that the maximum dry density (MDD) of enzyme-stabilized SP-SM did not improve. However, a moisture content of up to 33% was necessary to achieve MDD. Enzyme stabilization, on the other hand, led to slightly increased soil compaction (SM), which was inconsistent with previous findings. It was also found that the enzyme for soil SP-SM reduced the MDD by 5–15% and for soil SM by 3% (Ionescu & Petrolito, 2019). Compressive strength tests on clay and silt revealed that the biological enzyme not only strengthens the soil when added alone but also improves the strength of the cement-soil mixture. In addition, XRD experiments showed that no new compound is formed in the mass during the solidification process when only biological enzymes are added to the soil, suggesting that this is a physical effect. SEM proved that the bio-enzyme could also improve pore arrangement, pore morphology, and soil pore size. It also reduced the directional fractal dimension, probable entropy, and fractal dimension of pore shape, resulting in better pore placement. In addition, the pore size becomes more uniform (i.e., a higher sorting), and particle density rises (Li et al., 2020).

6.4 Ferment, fujibeton, and other bioenzymes

Bergmann (2000), in a study on biological enzymes, showed that the bioenzyme requires clay to strengthen the soil. It was claimed that at least 2% clay is required for successful soil stabilization and that 10–15% clay yields excellent results. In this study, CBR increased by 37, 62, 66, and 100% after one, two, three, and four weeks, respectively, compared to the percentage of virgin soil (Bergmann, 2000). The effectiveness of soil stabilization with bio-enzyme was evaluated using CBR laboratory tests. Based on the obtained results, the CBR test is a relatively weak indicator for evaluating direct soil strength for laboratory conditions and evaluating soil strength parameters (Tolleson et al., 2003). Another study was conducted to assess the suitability of bio-enzymes as soil stabilizers on five types of soils ranging in clay content from low to high. The results showed that biological enzyme stabilization compared to clay content results in a slight to moderate improvement in soil physical properties. This slight improvement could be due to the soil's chemical composition, which has low bioenzyme reactivity. In contrast, the improvement in CBR and compressive strength of silty to sandy soils increased by 65–252% after curing for four weeks. Overall, this study suggested that the thickness of the pavement design was reduced by 25–40% (Shukla et al., 2003). In an experimental study on two clays stabilized with two different bio-enzymes, the triaxial shear strength test revealed that elastic modulus in Soil I with 96% fine-grained and a plasticity index of 52% did not increase significantly. Nevertheless, it was more effective for soil II, with 60% fine-grained and a plasticity index of 9.4%. It was also found that the soil stiffness of soils I and II increased by approximately 69 and 77%, respectively, and soil shear strength increased from 9 to 39%. Also, it was demonstrated that soil type, its biochemical properties, and the percentage of fine-grained particles have a significant impact on bio-enzyme activity (Marasteanu et al., 2005). Laboratory research was conducted on using bio-enzyme stabilization in three types of clay: high plasticity, low plasticity, and low plasticity silt (ML). Four weeks of stabilization resulted in an enhancement in CBR and a decrease in saturated moisture from 40 to 21% in CH soil. Besides, it was observed that the compressive strength increases by 100% (Kaur & Sharma, 2006). According to a case study and the stabilization of subsoil (SM-SC) with Eco-Soil enzyme in an Australian project, the bio-encapsulation mechanism when mixing soil with bioenzyme can increase soil compressive strength by 101% (Renjith et al., 2017). Studying the effects of three types of bio-enzymes (namely DZ, EAR, and TER), on density and CBR parameters showed that these enzymes provide only a minor improvement for the soil (Penambahbaikan et al., 2017). The findings revealed that by adding Ferment and Fujibeton bio-enzymes to aggregates from local roads in India, the compressive strength value was increased from 170 to 435 and 553 kPa for 28 days of curing. Also, CBR results were increased from 1.2 to 2.4 and 5.4% for soaked CBR and from 4.5 to 8.5% for unsoaked CBR. Moreover, the free swell index reduced from 130 to 65% (Chitragar et al., 2021).

Bio-enzymes are naturally non-toxic, organic, and biodegradable, according to the research undertaken and conclusions of the present analysis. These substances are harmless to humans, animals, fish, and plants as they are made of biodegradable organic materials. The results indicate that bio-enzymes catalyze biochemical processes on the surface of soil particles. Therefore, they increase soil density and reduce water retention between and around soil particles and grains. This study demonstrates that bio-enzymes, particularly TerraZyme, can significantly improve the mechanical properties of soils by increasing compressive strength, bearing capacity, soil shear strength, and CBR value by as much as 270% due to an increase in cohesion, bonding, interlocking, and friction forces. In addition,

bio-enzymes could reduce the soil's hydraulic conductivity, swelling, settlement, shrinkage, and erosion by up to 50%. Bioenzyme decreases the surface tension of water, thereby reducing the complete and quick absorption and dispersion of moisture. As a result, it enables hydrated clay particles to be compressed into and fill the soil's spaces and form a compact stiffer layer. Particularly, TerraZyme reacts with the large biological substances in the soil to generate a reactive intermediate, which interacts with the clay lattice. Therefore, it breaks down the clay matrix and generates the bio-coating and bio-encapsulation effect, which limits further water absorption and the resulting loss of density. This outcome is accomplished by altering the substrate molecules of clay (polarity of electro-negativity and ion exchange of the atoms). By releasing pore water from clay particles, the soil's mechanical and index characteristics are altered, resulting in a matrix that is more dense, cohesive, and stable. Increased soil density lowers water conductivity and restricts water passage through the soil. The reaction regenerates the enzyme, allowing it to participate in the treatment of soil. The destructive action of organic ions on the clay lattice often facilitates this process. However, the effectiveness of bio-enzyme in boosting soil strength relies highly on physical and environmental parameters. In this respect, research has shown the potential of bio-enzymes to use in a wide range of environmental conditions, including extreme pH, high temperatures, and void size. The optimal dosage of bio-enzyme depends on soil texture and soil physical and chemical parameters and varies between (0.1–1 ml/per 5 kg of soil). Meanwhile, the optimal clay content is between 12 and 30%. In addition, temperature significantly affects various bio-enzyme activities, with the highest activity levels occurring between 20 and 40 °C.

7 Effect of biopolymer on soil stabilization

7.1 Xanthan gum and guar gum biopolymer

In another study, the effect of various biopolymers (i.e., Xanthan, Polyhydroxybutyrate (PHB), Guar gum, Polyglutamic Acid (PGA), and Chitosan) was investigated on the permeability and clogging of sandy soils in Ottawa (Canada). The results revealed that PHB had the best bio-clogging effect in reducing permeability. In addition, it was proposed that these biopolymers could be used alone or in combination to stabilize groundwater pollution, prevent the release of subsurface pollutants, and enhance oil recovery in the ground (Khachatoorian et al., 2003). Another study evaluated the interaction of Guar gum and Xanthan gum biopolymers in various percentages (0.5–2%) on sandy soil and kaolinite's hydraulic conductivity parameter. The results showed that biopolymers reduce the initial hydraulic conductivity of silty sand from 1×10^{-6} to 1×10^{-10} cm^2/s due to clogged pores caused by biopolymers (Bouazza et al., 2009). The Atterberg limits of kaolinite soils were studied by adding Xanthan gum and guar gum biopolymers at concentrations ranging from 0.25 to 2.5%. According to the results, the soil liquid limit (LL) generally increases with increasing concentration of porous fluid biopolymer due to increased viscosity and existing cation exchange capacity. Five mechanisms between clay particles, cations, and biopolymers were also considered to interpret the results of this study. i) The biopolymer-induced accumulation of clay particles decreases the liquid limit. ii) Polymer crosslinking induced by divalent cations substantially increases the viscosity of the biopolymer solution and, consequently, its liquid limit. iii) The formation of an interconnected clay-polymer network via cation bridges and hydrogen bonds increases the liquid limit. iv) Reducing

the thickness of the electrical double layer on the surface of the clay decreases the liquid limit. v) Reducing the adsorption of more monovalent cations than biopolymer molecules also decreases the liquid limit (Nugent et al., 2009). Al Kavazanjiian et al. (2009), through a series of laboratory tests, demonstrated that biopolymer mixtures and additives (1–3%) for dense soils could significantly improve the resistance of sandy and silty soils to wind-induced separation. Conducted tests demonstrated that applying bio-polymer emulsion forms a crust layer around soil particles, making them more resistant to surface water erosion (Kavazanjian et al., 2009). Cones fall and undrained shear strength tests of mine sandy soil with the addition of 0.5%-2% Xanthan gum (1–3%) guar gum separately revealed that increasing the amount of biopolymer increases the soil liquid limit and shear strength. This improvement in soil strength parameters was attributed to the high viscosity of the polymer fluid and the biopolymer's bond with soil particles. Guar gum was also found to be more effective than Xanthan gum in increasing undrained shear strength due to its higher viscosity and stronger bonding influence. Moreover, Eqs. (3) and (4) were proposed for predicting the undrained shear strength (s_u) of sandy soils by comparing undrained shear strength data (Chen et al., 2013).

$$s_u = 1.227 \exp\left(-6.08\frac{w}{w_L}\right) \tag{3}$$

$$s_u = 64.5 \exp(-3.21 LI) \tag{4}$$

Adding two biopolymers of Xanthan gum and Guar gum to mine soil and examining the penetration and erosion test (Fig. 12) revealed that the higher the concentration of biopolymers, the greater the resistance to erosion and penetration (Chen et al., 2015). The addition of 1% Xanthan gum to sandy and clay soils in a laboratory study demonstrated that the direct interaction between the biopolymer and the clay produces a relatively hard gel solution that acts as a cementitious adhesive between the sand particles. Also, biopolymer interactions (i.e., hydrogen or electrostatic bonding) with the charged surfaces of clay soils were confirmed to increase soil strength with increasing amounts of fine-grained soil. In

Fig. 12 Average maximum penetration force for soil with the addition of biopolymer
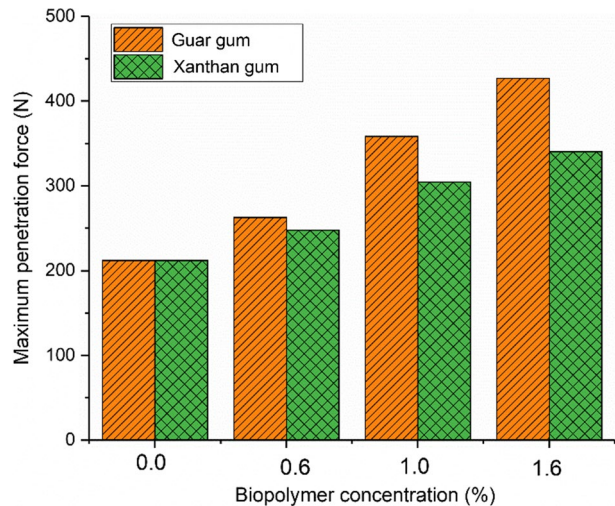
this study, the tests conducted on stabilized soils revealed that the compressive strength and elastic modulus did not diminish over time (Chang et al., 2015a).

The behavior of two biopolymers (namely, Xanthan gum and Guar gum) with different concentrations in the range of 0.25–2% were evaluated on a mixture of two types of cohesive soil (silt) and non-cohesive soil (sand) during curing periods (1–10 weeks). A substantial increase in shear strength (greater than 5 times) and a permeability reduction of up to 64% for both soil types were observed. However, this improvement was more readily apparent in this biopolymer due to Guar gum's greater viscosity. Also, by decreasing the amount of water (hydration) in the soil during the treatment period, the structure of biopolymers changed from a gel-like to a glassy and crystalline state. Consequently, the biopolymer accumulated within the soil pores, and the soil became more resistant due to the biopolymer concentration and the pore space (Ayledeen 2016). Another study was conducted to determine the mechanical parameters of collapsible soil. To this end, a series of laboratory tests, including compaction, collapsibility potential, and shear parameters, were carried out, considering different concentrations of biopolymers (Xanthan gum, Guar gum). In this study, the experiments were performed in two curing periods, after mixing soil with biopolymer and after one week. The analysis of the results demonstrated that both biopolymers could be used as soil stabilizers to improve the collapse parameter. Overall, collapsibility potential was significantly reduced from 9 to 1% when the soil was mixed with 2% biopolymer. In addition, after one week of curing, as the concentration of Xanthan gum increased from 0 to 2%, the cohesion increased from 8.5 to 105 kPa, resulting in an overall increase in soil shear strength (Ayledeen 2017). Evaluating the dynamic properties of sandy soil stabilized with Xanthan gum and Gellan gum using 1 and 2% resonance columns revealed that the stabilization of sand with Gellan biopolymer increased the shear modulus. Also, it was found that due to the fibrous structures of the Gellan biopolymer in the sand pores, the energy loss is greatly increased, and the damping ratio exhibited an increasing trend, which is more pronounced for the Xanthan than for the Gellan (Im et al., 2017)**.** Afterward, mechanical tests of compressive strength, direct shear tests, and one-dimensional consolidation tests were performed on Xanthan gum-stabilized montmorillonite and kaolinite soils at various curing times. In the end, the optimal amount of biopolymer was determined to be 1 and 1.5% for montmorillonite and kaolinite, respectively. In 90 days of curing, the maximum reduction values for C_c and C_s parameters were 76 and 73%, respectively. In addition, microstructural analysis experiments conducted for 28 days revealed the formation of new cement components as a result of chemical reactions between biopolymer and soil particles on the surface. These reactions improved soil behavior by connecting soil particles and filling pores (Latifi et al., 2017)**.** Atterberg limits of clay-sand mixtures stabilized with Xanthan gum biopolymer (silt clay, kaolinite, montmorillonite, and sand) were evaluated using three distinct chemical pores (deionized water and 2 mol/L of NaCl and kerosene). It was found that adding 0.1, 0.5, 1.0, and 2.0% of this biopolymer had negative effects on the soil Atterberg limits. In other words, the liquid limit can decrease by the accumulation of particles due to biopolymer or increase by the formation of biopolymer hydrogels, in which the clay type dominates the behavior of Xanthan gum in deionized water (Chang et al., 2019). Comparing the compressive strength of SM soil samples stabilized with ash, cement, and Xanthan gum biopolymer revealed that less strength is obtained in 3 days than with other stabilizers. However, due to the formation of a soil-biopolymer matrix, this biopolymer can increase compressive strength by up to 3 times in 28 days (Lee et al., 2019). A series of laboratory tests, including compaction, consolidation, permeability, and undrained triaxial tests, were performed to determine the impact of Xanthan, Guar gums (0.5, 1, and 2%) on the strength, permeability, and potential for collapse of silty clay. According to

the findings of this study, both types of biopolymers decreased the maximum dry density while increasing the optimum moisture content. Besides, the addition of 2% biopolymer increased the maximum soil strength from 228.7 to 550 kPa because of an increase in Mohr-Columb cohesion stress and a slight decrease in internal friction angle. In addition, it was found that extending the curing period can improve soil cohesion (Dehghan et al., 2019). Using Xanthan gum in various doses (0.5–2.5%) and assessment of compressive strength, consolidation, and shear parameters of expansive soils in a laboratory study demonstrated that the addition of biopolymer decreased maximum dry density and increased optimum water content. Moreover, adding 1% biopolymer to the soil for 28 days enhanced the UCS values by as much as 4 times. Another noteworthy point is that soil compaction is reduced by up to 65% after 28 days of curing. Also, the strength parameters of the stabilized soil were improved by increasing the biopolymer content, which resulted in higher friction angle (5.8°) after 90 days and higher cohesion (up to 496 kPa) (Joga & Varaprasad, 2019). The influence of various Xanthan gum biopolymer dosages on the sand was investigated both experimentally and numerically. The results obtained from the analysis of micro parameters indicated that higher biopolymer content could substantially increase the soil's UCS and tensile strength due to the interaction bond parameter at the microscale. It was also observed that the interior force at the same tensile position increased with increasing biopolymer content (Chen et al., 2020). Overall, it is proved that adding 0.25–5% of gum Xanthan biopolymer to soil can improve soil stiffness (Fig. 13). This improvement can be attributed to the formation of elements and hydrogen bonds in the matrix. In addition, the UCS of soil increased by 131.86% with adding 0.5% biopolymer after 0 days of curing and by 1132.16% after 90 days of curing. This result suggests that curing time is an important factor in further soil stabilization (Sujatha et al., 2021). In a laboratory study, the combination of Xanthan gum biopolymer in sand columns demonstrated that although the addition of biopolymer can lead to the cohesion of sand particles, exposure to moisture significantly affects the sample's strength (Ramachandran et al., 2021).

Chen et al. (2022) conducted a series of compressive strength tests to examine the performance and behavior of the combination of biopolymer and fibers concerning stress–strain properties, strength enhancement, and failure patterns on soft soil. The results

Fig. 13 Stress–strain behavior of Xanthan gum-treated soil immediately after addition of biopolymer
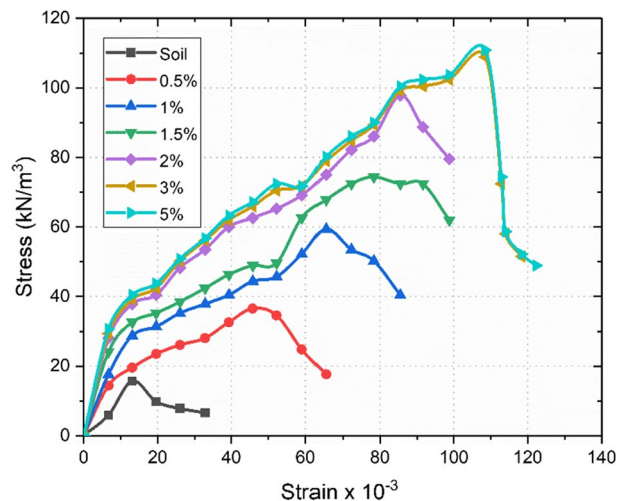
demonstrated that the addition of fibers to biopolymer increases the compressive strength to between 1.5 MPa and 2.5 MPa due to the interaction between these two materials. It was also noticed that the fibers begin to play their role immediately after incorporation into the soil matrix, whereas the biopolymer's effect appears gradually over time. Moreover, biopolymer was found to be responsible for increasing maximum compressive strength and soil brittleness, whereas fibers reduced soil brittleness and enhanced ductility. As displayed in Fig. 14, the biopolymer not only contributes to the bonding strength between particles but also increases the efficiency and contribution of fiber reinforcement interlocking forces in soil (Chen et al., 2022). In the laboratory, soft clay was subjected to a series of tests to determine how applying Guar gum at varying concentrations (0.1, 0.2, and 0.3%) and in combination with lime would affect its properties. The findings indicated that adding 3% lime and 0.3% guar gum increased compressive and CBR values by prolonging the curing time. It was revealed that the cementation between microstructures is the primary factor contributing to the soil's improvement (Onah et al., 2022). Incorporating Xanthan gum into loess soil in Henan Province (China) significantly formed bridge links between soil particles and affected the soil matrix's micropores and mesopores. It was observed that a higher dosage of biopolymer could boost tensile and compressive strength and reduce the ductility of the soil matrix (Jiang et al. 2022b).

7.2 Beta-glucan (β-glucan) biopolymer

Adding β-glucan and carrying out a compressive strength test showed that biopolymer increases soil compressive strength by more than 200% at a concentration of 4.92 g/kg. In addition, it was observed that the highest strength is achieved at a curing temperature of 60 °C (Chang & Cho, 2012). In an experimental study, it was determined that the Atterberg limits, optimum moisture, plasticity index (PI), swelling index (SI), and shear modulus enhance with increasing beta-glucan content in the soil while the compressibility coefficient decreases to 60%. It was also reported that the polymer clogged the pores between soil particles and decreased hydraulic conductivity (Chang & Cho, 2014). In another study conducted in the same year, a series of wind tunnel simulations and laboratory tests proved that using β-glucan and Xanthan gum could increase soil erosion resistance. It was also stated that due to the hydrogel properties, the simultaneous formation of particle adhesion and relatively greater soil porosity in dry conditions increase soil moisture. Therefore, adding this biopolymer can boost vegetation growth in arid regions (Chang et al., 2015c).
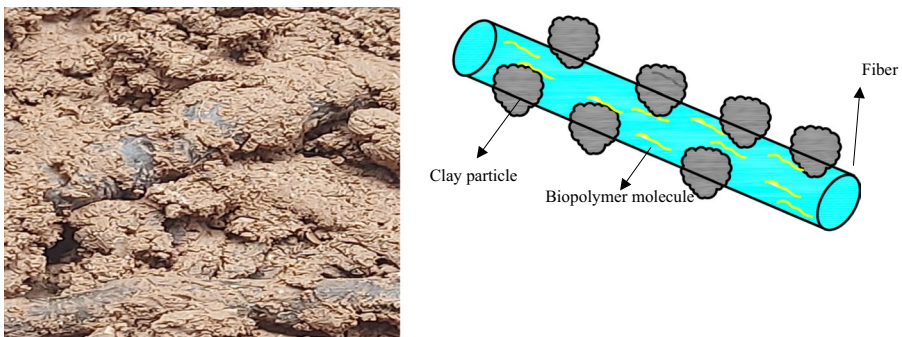

Fig. 14 Single and multiple fibers interaction with biopolymer and clay soil

Stabilization of lean clay with β-glucan biopolymer and the results of triaxial tests demonstrated that by adding 2% biopolymer, the deviatoric stress of stabilized soil is 12 times greater than that of control soil. In studying the stabilization mechanism, SEM revealed the formation of new masses of cement and hydrogels in the soil matrix that tend to bind soil particles together and reduce pore spaces, resulting in increased soil strength (Kumara & Sujatha, 2020). In an experimental study, the effect of β-glucan biopolymer (0.5–3%) on expansive clay was evaluated. In the end, the optimal amount of biopolymer was determined to be 2%. Also, this polymer concentration can increase soil compressive strength by up to 50% due to the biochemical activity of polymers with soil particles and reduce the thickness of the double water layer (Vishweshwaran et al., 2021a). In another attempt the same year, the performance of β-glucan biopolymer (0.5–3%) on clayey sand was evaluated. The results showed that this biopolymer has hydrophilic properties that causes soil compaction and clogs soil pores. Moreover, shear strength tests revealed that adding 1 and 0.5% biopolymer resulted in the highest cohesion value, internal friction angle, and soil shear strength. Furthermore, the durability test revealed that the soil-biopolymer mixture was unaffected even without heat treatment for up to 96 day. Besides, the gel matrix test and hydration test showed that the gel has a constant durability of 150 days and has the ability to reduce cracks on surface in the soil (Vishweshwaran et al., 2021b). In another study, the combined effect of β-glucan biopolymer and kaolinite clay was assessed using Atterberg limits, CBR, compressive strength, and cost estimation. The results demonstrated that, due to the hydrophilic nature of the biopolymer, the liquid and plastic limits tend to increase by adding the biopolymer. In addition, after seven days of curing at the optimal 1% dosage, the compressive strength of the soil increased from 104 to 482 kN/m^2. After 40 days of curing, adding this biopolymer increased soil CBR from 1.39% for virgin soil to 12.16% for stabilized soil (Vishweshwaran & Sujatha, 2021). The application of β-glucan was investigated on the subgrade road in the southern part of India. The obtained result revealed that compressive strength and CBR values could increase up to 45%. Also, the cost savings for a one-kilometer section of pavement were estimated to be 14.3% (Sujatha, 2022). Silty and clean sand was mixed with β-glucan, and the UCS and triaxial strength tests showed that soil could experience higher compressive and failure strain (Soldo et al., 2022).

7.3 Chitosan biopolymer

Several field tests investigating the impact of biopolymers on clay soils in Northern California revealed that adding biopolymers, including Chitosan, Starch Xanthate, and cellulose Xanthate at concentrations up to 120 ppm could reduce soil surface erosion caused by cohesion between soil particles by approximately 78% (Orts et al., 2000). According to another research, adding chitosan to clay soil can increase soil cohesion and compressive strength by up to 3 times and 50% respectively and can slightly improve friction angle (Hataf et al., 2018). The effects of Chitosan biopolymer content, curing time, and conditions on the compressive strength of sandy soils were investigated. The results indicated that adding this biopolymer to sandy soil can potentially increase cohesion between soil particles and can significantly improve the compressive strength of sandy soil by up to 30%. The degradation of biopolymers was also observed to cause a slight decrease in strength over time after increasing the initial soil strength. Moreover, it was stated that better performance is achieved in dry conditions compared to humid and saturated environments. Furthermore, testing the effect of biopolymer on soil pore blockage revealed that

the hydraulic conductivity of sandy soils decreased from 1.6×10^{-6} cm/s to 7.3×10^{-7} cm/s and 5.7×10^{-7} cm/s at dosages of 0.24 and 0.32%, respectively (Shariatmadari et al., 2020). Evaluating clay and sand mechanical parameters by stabilization with cellulose-chitosan biopolymers showed that the compressive strength of soil could be increased by up to 1 MPa (Zinchenko et al., 2022). The combined effect of Chitosan (higher molecular weight and lower degree of distillation, and lower molecular weight and higher degree of distillation) with acidic and alkaline soils confirmed that these biomaterials could have a beneficial effect on the soil compressive strength parameter (Adamczuk & Jozefaciuk, 2022). In an experimental investigation, incorporating chitosan in low plastic silt soil of South India demonstrated about 103% strength improvement after 28 days of curing with no degradation. This study measured parameters including pH, consolidation, compaction, shear strength, and hydraulic conductivity (Kannan & Sujatha, 2023).

7.4 Agar-gum and gellan-gum biopolymer

According to a laboratory study, depending on the biopolymer concentration of the stabilized sandy soil, compressive strength can increase up to 487 kPa using modified Agar (1–4%) and starches (0.5–1%) at different concentrations. As determined by conducting triaxial tests under a wide range of confinement pressures, biopolymers effectively increased stabilized sand's bio-clogging, cohesion, and stiffness parameters. On the other hand, they decreased the friction angle due to the presence of biopolymer gel surrounding the sand particles. The type of failure behavior also demonstrated that adding biopolymer increased the soil brittle failure behavior (Khatami & O'Kelly, 2013). In another attempt, Agar gum and Gellan gum biopolymers (1–3%) were used to evaluate the mechanical parameters of clay (CL) and sandy soil (SP-SM). Also, it was proved that non-thermal and thermal curing could increase the UCS of soil by 2–4 times (Chang et al., 2015b). Evaluating the compressive strength, tensile strength, permeability, and SEM of sandy soil stabilized with Gellan Gum biopolymer at concentrations of 0.5, 1, and 2% revealed that dry soil's friction angle and cohesion could increase to 20° and 103 kPa. In addition, adding this biopolymer proved that the dry compressive strength of soil can be increased by up to 160 times. Furthermore, the pore-filling effects of Gellan hydrogels demonstrated that this biopolymer could reduce the permeability of sands from 1×10^{-4} cm/s to 1×10^{-8} cm/s (Chang et al., 2016a). Evaluating the settlement and shear strength of sandy soils with different overburdens revealed that by adding biopolymer agar (0.5% to 3%) to the soil, shear strength could be increased up to 4 times in 7 days of curing due to an increase in cohesion from 0 to 86 kPa and a 7° increase in friction angle. In this research, the behavior settlement assessment by obtaining vertical displacement during shear deformation revealed a significant reduction in post-stabilization soil settlement. This reduction is attributed to the enhanced interparticles bonding via biopolymer bonding bridges, aggregate formation, particle coverage, and pore filling (Smitha & Sachan, 2016). The use of Gellan gum in clay soil in China increased the undrained shear strength, shear viscosity, and compressive strength (Cheng & Geng, 2021). In addition, after 16 weathering cycles of stabilized clay soil in Korea's glacial tills with 1.5% Gellan gum, it was observed that 82% of the initial shear strength of untreated soil was preserved (Amelian et al., 2022).

7.5 Starch biopolymer

In a laboratory study, heating stabilized sand with starch biopolymer resulted in a gelatinizing process that produced a hard material surrounding the soil particles. This material increased the compressive strength by up to 26 MPa (Kulshreshtha et al., 2017). The combination of sandy soil, fly ash, and starch biopolymer proved to have a significant effect on increasing compressive strength by up to 65% and freezing resistance by up to 22% (Miękoś et al., 2019). It was found that adding starch boosted soil cohesion and friction, decreased hydraulic conductivity, enhanced erosion resistance, and even boosted vegetation growth (Im et al., 2021). Moreover, adding starch to the sand and Korean residual soil revealed that the biopolymer dosage increased the strength and durability against weathering phenomena such as cyclic wetting–drying and freezing–thawing (Lee et al., 2022).

7.6 Polysaccharide biopolymer

Al-Darby (1996) investigated the effect of adding 0.2–0.8% polysaccharide biopolymer on the permeability of sandy soils. This author found that biopolymers can fill the voids between particles and can reduce soil permeability by up to 91% (Al-Darby, 1996). Using Polysaccharide biopolymer enhanced the stability and shear strength of silt loam aggregates by up to 81% and reduced seepage and erosion of soil rates by 21 and 60%, respectively (Lentz, 2015). A series of experimental tests and numerical simulations were carried out to investigate the characteristics of modified sand in Jiangsu Province (China). The results proved that the polysaccharide greatly enhanced the mechanical properties of the natural sand due to the strong inter-particle and bio-cemented network in the soil matrix. Moreover, strong inter-granular bonding connections improved the anti-erodibility of the modified sand and the capacity to resist erosion of the topsoil (Che et al., 2022).

Biopolymers, particularly Xanthan gum and Guar gum, can significantly improve the mechanical properties of soils by increasing inter-particle cohesion, friction, compressive strength, bearing capacity, and soil shear strength by up to four times. This enhancement depends on soil type or soil texture, biopolymer type, biopolymer concentration, and soil moisture content. Because of the hydrogel formation, increased pore-fluid viscosity, and bio-pore blockage, biopolymers can also reduce soil shrinkage, swelling permeability, settlement, shrinkage, and seepage by up to 40%. Biopolymers are useful as a coating agent for coarse-grained soils. This performance can be observed clearly under SEM. In contrast, biopolymers produce agglomerates of tiny particles by electrostatically connecting to larger coarse particles in mixed soils (e.g., sandy and silty mixtures). Studying the interaction between the time of curing, water content, and the strength of biopolymer-stabilized soil revealed that these factors are among the most important determinants of soil strength. As the curing period extends, the gained strength increases and the maximum contact between soil particles and biopolymers occurs between 5 and 10 days. Also, research has shown that the dehydration of hydrogels created in soil matrix by biopolymers can be included in a variety of environmental circumstances. The optimal dosage of biopolymers varies between 0.4 and 2%, depending on soil texture, soil's physical and chemical qualities, the presence of clay or fine particles, and the results obtained. In addition, the temperature has a key effect on the numerous biochemical chain activities of biopolymers, with the highest levels of activity occurring between 25 and 40 °C. Due to the interlocking forces, the presence of clay particles can bridge the gaps and reinforce bio-polymer-soil stability.

8 The micro-scale and macro-scale effects of bio-substances on different soil types

Numerous bio-based chemicals can be included in many types of soils, ranging from expansive clayey soil and cohesive to granular soils (e.g., sand and gravel)**.** When bio-based substances are mixed with soil and interact with its particles in cohesive environments, significant improvements in bearing capacity and compressive strength can be achieved owing to the cementation of soil particles. Applying various bio-based substances, especially in cohesive (e.g., clay, clayey silt, sandy clay, silty clay, and organic clay) and clayey soils (CL, CH, ML, and MH), can significantly lower the level of erosion, settlement, shrinkage, and deformation of the soil matrix. These improvements are attributed to bio-encapsulation, bio-coating, bio-clogging, and bio-cementation phenomena. Changes in soil geotechnical properties may result from physical and chemical-physical interactions between the soil and biomaterials. Moreover, in granular soils, physical contact is prevalent, and the pore size is essential for the movement of bio-stabilizers in the soil method. In the meantime, fine-grained soils experience greater physical and chemical interaction**.** In soil stabilization with microorganisms, the formation of calcium carbonate in the soil's pores increases the density of cohesive, granular, and matrix of soil due to the bio-microorganisms' filling impact. The non-expanding calcite mineral produced by MICP induced a cementation bonding between the soil matrixes. Urea hydrolysis provides the best performance in acquiring $CaCO_3$ bio-chemical activity because of its simple controllability. This reaction can achieve 95% of $CaCO_3$ production in only 10 days. Due to the bio-cementation process, which reduces the soil's affinity and absorbency for water, the liquid limit and plastic limit of stabilized soil can be reduced by as much as 40%. This reduction occurs because bio-cementation decreases the specific surface area of expanding hydrophobic soil minerals (particularly clayey soils), and the quantity of calcite formed on soil particles inhibits soil and water particle reactivity. In terms of mechanical characteristics such as shear, compressive, and tensile strength, microorganisms in both cohesive and granular soil can increase these values by 264, 290, and 350%, respectively. This increase in the mechanical strength of treated soil depends on the micro and macro-interactions cementation between soil particles. It also can cause interlocking, friction, and cohesive forces in the soil matrix (Fig. 15). Also, bio-microorganisms can prevent the formation of cracks in the soil matrix by connecting soil particles and producing a three-dimensional network and reinforcement inside the soil matrix. As a result, a stronger, more homogeneous, and stiffer structure is obtained. The cementation material produced in the soil matrix enhances soil particle adhesion and cohesion, reducing soil consolidation by up to 55%. The formation of calcite in the pores of the soil matrix, which increases the binding and bio-coating of soil particles, can reduce soil swelling and shrinkage by as much as 70%. In addition, bio-clogging of soil particles contributes significantly to lowering soil matrix permeability by up to 75% due to filling inter-particle gaps. Bio-microorganisms contribute significantly to lowering soil erosion by up to 70% in arid and semi-arid deserts and boosting CBR values by up to 300% due to the interlocking, higher interaction between soil particles and binding of particles. In sandy soils, bio-cementation binding soil particles and the reinforcing effect of bio-microorganisms can decrease liquefaction potential by up to 50%.

Bio-enzyme density, surface tension, and viscosity contribute considerably to physical and chemical interactions during the bio-enzyme soil treatment process. In cohesive soils, the thickness of the double water layer is affected by the bio-enzyme solution's dielectric constant and the cation exchange between monovalent and covalent cations. Moreover,
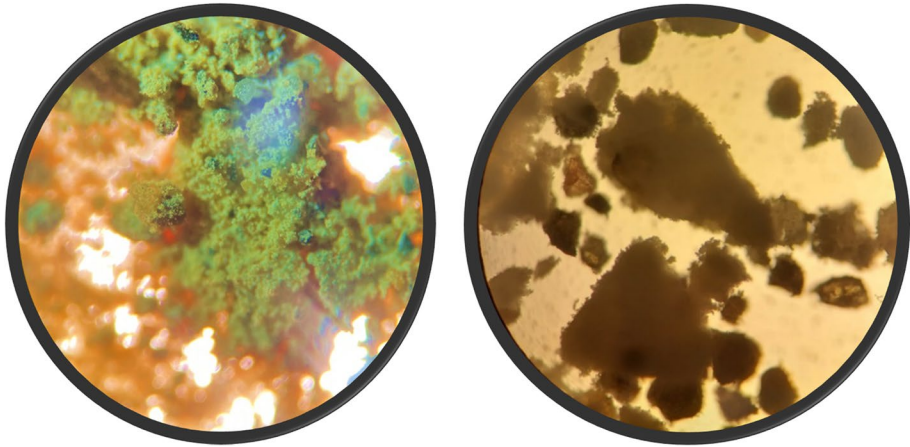
Fig. 15 Production of calcite carbonate on soil particle surfaces

changes in double-layer thickness and the involvement of bio-enzyme solution viscosity in the lubricating of soil particles in coarse-grained soils are essential for soil improvement. When bio-enzymes are injected into the soil, they are absorbed by the clay particles, causing cations to be released, reducing the thickness of the diffuse double layer of clay. Hence, the dry density of soil increases, leading to less water retention or less liquid and plastic limits in a short period of curing time of up to 10 days. Bioenzyme addition to the soil matrix can accelerate the bio reaction between big organic molecules and small clay particles. Large organic molecules have huge flat structures that cover the surface of smaller clay particles, neutralizing their negative charge. As a result, these structures reduce the clay's attraction to water and enhance interparticles cohesion. This bio-coating interaction leads to the binding of soil particles, which can significantly increase shear strength, including cohesion and friction by 281 and 67%, respectively, and compressive and tensile strength by 272 and 60%, respectively, after 28 days of curing for 0.06 ml/kg. As shown in Fig. 16, the failure zone decreases as the percentage of biopolymers rises. Consequently, global failure zones with a high rate of cracks transform into local failure zones with fewer cracks. This improvement in the mechanical characteristics of stabilized soil is attributed
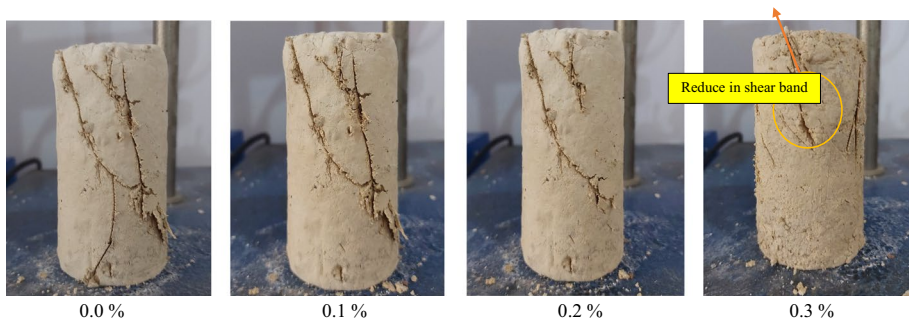

Fig. 16 Changes from overall failure to local failure with fewer cracks in the bioenzyme stabilization

to a cationic exchange between soil particles on a micro and macro scale, which can cause interlocking, friction, and cohesive forces in the soil matrix. Moreover, bio-enzyme can postpone the initiation of cracks and failure in the soil matrix by connecting soil particles and establishing a 3D network bridge and reinforcement inside the soil matrix, resulting in a stronger, more homogenous, and stiffer structure. The cation exchange and binding forces generated in the soil matrix improve soil integrity, decreasing consolidation by as much as 30%. The incorporation of liquid bio-enzymes into the soil matrix enhances the cohesion, binding, and bio-coating or bio-encapsulation of soil particles and reduces the swelling and shrinkage of soil by as much as 45%. Moreover, bio-clogging of soil particles reduces soil matrix permeability by as much as 30 percent due to the filling of inter-particle gaps. Due to the interlocking and binding of soil particles, bio-enzymes' role in reducing soil erosion rises to 75% as curing time increases. In this respect, interlocking and binding of soil particles can result in a 252% improvement in CBR values. Also, cation exchange and binding of soil particles reduce liquefaction potential.

Biopolymer stabilizers, most notably Xanthan gum, can interact favorably with soil particles at optimal concentrations (0.4–2%). When a biopolymer solution is introduced to soils, the dissolved particles begin to enter the pores and saturate the surface of the soil. The wetting process is expedited by the presence of silica and cation exchange on the soil surface and the water solubility of biopolymers (Fig. 17). When the biopolymer comes into contact with the soil, it coats the soil particles with a strong film, forming the necessary bridges to initiate the bonding process and resulting in greater density and lower liquid and plastic limits. The dehydration process of the biopolymer matrix improves bonding strength by bringing the particles closer together and compressing the pore spaces by allowing water to evaporate during the curing period. Shorter connecting chains are more resistant to external loads, thereby enhancing geotechnical performance during the drying process. A higher dosage of biopolymer (up to its optimal quantity) leads to better bonding between soil particles and shear, compressive, and tensile strengths up to 250%. The mechanical improvement of cohesive and granular soils is a result of biochemical bonding and interlocking forces created by various hydrogen bonding, electrostatic interactions, hydrogen bonding, cation, and ionic connections, or van der Waals bonds. By the interaction of biopolymers with the adsorbed water layer, the thickness of water around the soil
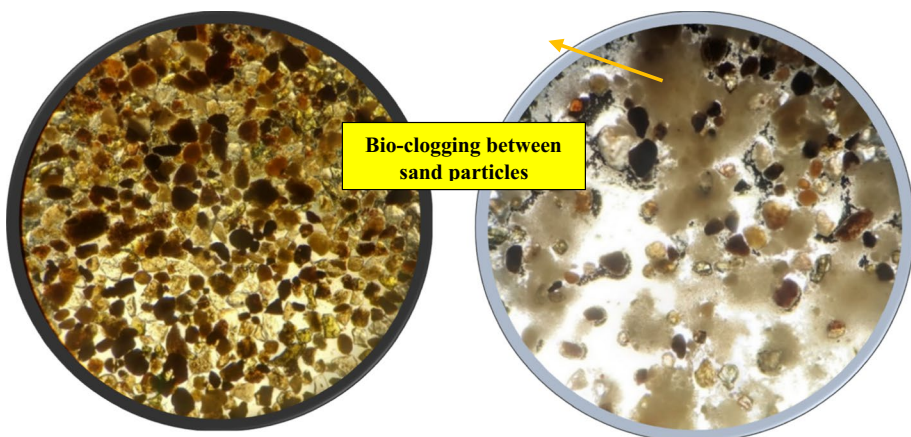

Fig. 17 Bio-clogging of soil particles and their connection in soil matrix with biopolymer

particles decreases, leading to a reduction in inter-particle space, an increase in soil density, and an increase in bio-clogging. These factors play a key role in reducing permeability and swelling by up to 40%. Depending on the dosage and concentration, according to the microstructural features of coarse and fine-grained soil, the incorporation of bio-polymer substances can dramatically increase the surface roughness and diminish the liquefaction potential.

Changes in the physical and chemical properties of bio-enzyme and biopolymer solutions and microorganisms surrounding soil particles or inhabiting the space between particles are generally responsible for alterations in soil characteristics. As presented in Table 5, it is possible to extract a specific trend for describing the effects of these types of stabilizers on soil geotechnical properties.

9 Advantages and major challenges of bio-based compounds in soil matrix

Stabilizing soil with native and non-native bacteria includes some benefits and drawbacks. The geographical distribution of bio stabilizers is one of the most significant benefit of these additives, which may be obtained and used around the world easily (Liu et al., 2018). Numerous researchers have considered the abundance of bacteria in nature and soil masses as one of the benefits of using bacteria to stabilize soil to improve its mechanical and dynamic properties. However, using this technique has specific limitations. Table 6 outlines the benefits and limitations associated with the application of this technique (Almajed et al., 2021; DeJong et al. 2014a; Osinubi et al., 2020).

Studying bio-enzymes demonstrates the significant enhancement of the soil's behavior and stability mechanism during the stabilization process. Same as other stabilization techniques, this method has advantages and disadvantages (or limitations). This biomaterial has advantages such as saving time, energy, and financial resources and reducing carbon emissions from conventional stabilizers that contribute to environmental pollution**.** On the other hand, there are some limitations to using them, as shown in Table 7 (Mekonnen et al., 2020; Rajoria & Kaur, 2014).

Biopolymers are also abundant in nature, owing to the biochemical nature of biological macromolecules. These molecules are made up of a large number of small, similar subunits that are covalently linked to form a long chain. They are biodegradable and have no adverse environmental impact. Therefore, they are suitable for enhancing the soil's properties. In addition, unlike the MICP method, fine-grained soils are used, and microorganisms are not required to stabilize the soil and ensure soil persistence, as shown in Table 8 (Jang, 2020; Soldo et al., 2020).

10 Economic and environmental feasibility of bio stabilizers

Typically, a life cycle analysis (LCA) is performed to assess the sustainability, cost, and performance of a product or process, including the reuse of materials throughout its life cycle. Conventional stabilizers, such as lime, cement, and fly ash can be up to 3 times more costly than bio-microbes, bio-enzymes, and bio-polymers in some regions and are much more expensive when they must be delivered over long distances at construction sites due to their volumes (Lee et al., 2019)**.** Bio-enzymes and bio-polymers, on the other hand, are

Table 5 Influence of plant-based fibers on different soils

Type of bio-stabilizers	Type of soil	Recommended dosage	Positive impacts
Microorganism	Cohesive soils (clayey silt, sandy clay, silty clay, clay, and organic clay)	0.2–0.75 M	Bio-microorgansims, especially S. pasteurii and Bacillus sphaericus, can improve compressive, tensile, and shear strength (cohesion and friction) by as much as 50% to 350%. They can also increase elastic modulus through bio-cementation. On the other hand, they can reduce soil deformation, settlement, shrinkage, and swelling of expansive soils by up to 90% via the binding of soil particles. They can also reduce the hydraulic conductivity by up to 95% via bio-clogging
	Granular soils (gravel, sand, and silt)	0.5–2 M	
Bio-enzyme	Cohesive soils (clayey silt, sandy clay, silty clay, clay, and organic clay)	0.1–1 ml per kg of soil	Bioenzymes expedite the biochemical reactions on the soil's surface through bio-coating. Thus, they increase the soil's density and decrease its capacity to retain water. TerraZyme boosts compressive, tensile, and shear strength (cohesion and friction) by as much as 40% to 270% electrostatics forces. Renolith can reduce the liquefaction, settlement, shrinkage, and swelling of soils due to the interlocking and cohesion forces by up to 70%
	Granular soils (gravel, sand, and silt)	0.5–3 ml per kg of soil	
Bio-polymer	Cohesive soils (clayey silt, sandy clay, silty clay, clay, and organic clay)	0.2–2%	Due to aggregation conglomeration between soil particles through the ionic exchange, electrostatics forces and the formation of hydrogel biopolymers, especially Xanthan Gum and Guar Gum, can enhance CBR, compressive, tensile, and shear strength (cohesion and friction) by as much as 30% to 252%. Moreover, biopolymers can reduce soil compressibility, settlement, erosion, shrinkage, and swelling by up to 40% due to creating a 3D reinforcement structure
	Granular soils (gravel, sand, and silt)	0.5–4%	

typically sold as concentrated liquids that are diluted with water on the building project and are then either sprayed on the soil before compaction or pressure-injected into deeper soil layers. As a result, it is possible to carry materials at an affordable cost (Table 9). The price for producing bio-materials depends on the bio-compound process. In the past three decades, the price of 0.5% bio-microbes, bio-enzymes, and bio-polymers such as xanthan gum for soil treatment has decreased from $290–$19 (Chang et al., 2016b; Mujah et al., 2017). Although the vast majority of bio-polymers and bio-enzymes are now relatively expensive, the real cost of their usage in geotechnical engineering depends greatly on the chosen bio-compounds. Moreover, the current market prices of most prospective bio-enzymes and bio-polymers are for high-quality food-based industry with extremely high purity, resulting in much higher production costs. This purity is not necessary for soil improvement applications; therefore, it is reasonable to anticipate that the price of bio-based stabilizers will

Table 6 Benefits and limitations of using microbes for soil stabilization

Benefits	Drawbacks and limitations
Bacteria can be found almost everywhere, from harsh to friendly environments so needs less cost for incorporation	Potential risks to the ecological balance must be properly assessed by the release of undesirable $NH4^+$ and other undesired byproducts, ammonia, and hydrogen sulfite
Bacteria can alter their environment, generating calcite precipitation with an enzyme such as urease, causing calcite crystals to form on the surface of grains, forming cementation links between particles, lowering soil permeability, and increasing compressive strength	Microbial grouting increases the pH of groundwater to highly alkaline levels and, thus, can cause serious environmental problems and contribute to ecosystem disturbance
Since the biochemical reaction occurs at room temperature, it requires minimal extra energy	Insufficient performance in fine soils with small pores, especially stiff clays, impedes bacterial migration to other layers of soil and non-uniform injection of microbes
Applying bacteria in soil on a small scale is an economical and sustainable process	The transport, cultivation, and fixation performance of bacteria is not consistent
Bacteria can survive in unfavorable conditions. Also, some of them are spore-forming organisms at low to high acidity or salinity, allowing them to withstand harsh conditions such as high pressures of several hundred bars and temperatures ranging from below freezing to above the boiling point of water	Difficulties with field performance prediction and ensuring appropriate design where $CaCO_3$ precipitation behavior differs from in situ chemical conditions and the presence of natural bacteria and organic substances

Table 7 Benefits and limitations of using bio-enzymes for soil stabilization

Benefits	Drawbacks and limitations
Cost-effective and faster than conventional soil stabilization methods	Inadequate awareness among engineers and a lack of consistent data
Bio-enzymes are often sold as concentrated solutions that are diluted with water on-site and then applied to the soil before compaction or pressure injected to treat deeper soil layers	Inadequate information provided by manufacturers can cause certain limitations in their applications
Bio-enzymes are non-toxic, environmentally friendly, and organic technology	Bio-enzymes are sensitive to changes in temperature and pH
Applying bio-enzyme for soil stabilization does not need to use any foreign stabilizing materials	They need fine aggregates to function better

Table 8 Benefits and limitations of using biopolymers for soil stabilization

Benefits	Drawbacks and limitations
Biopolymers are abundant in nature	Lack of standards in laboratory approaches
Biodegradable and with no negative effects on the environment	Biopolymers are defined by their biodegradability, which means their efficiency may deteriorate over time
Covers vast range of soils	High-water vapor permeability
Compared to MICP, biopolymer does not require microorganism cultivation in the *soil*	Susceptibility to the presence of water

Table 9 Cost comparison of conventional stabilizers and bio-based materials

Materials		Cost (USD/Ton)	References
Cement Lime Bitumen	Conventional stabilizers	from 70 to 128 USD/ton from 20 to 78 USD/ton from 460 to 1100 USD/ton	(Archibong et al., 2020; Firoozi et al., 2017; Jha & Sivapullaiah, 2020; Lakhan-pal & Chopra, 2018)
Bio microbe Bio enzyme Biopolymer	Sustainable stabilizers	from 18 to 70 USD/ton from 38 to 96 USD/ton from 170 to 1900 USD/ton	(Aamir et al., 2019; Chang et al., 2016b; Mekonnen et al., 2020; Mujah et al., 2017; Rajoria & Kaur, 2014)

be cheaper by up to 40% when they are produced exclusively to enhance soil properties (Mekonnen et al., 2020; Rajoria & Kaur, 2014). In addition, due to the devastating environmental risks posed by the release of carbon dioxide, the quantity of CO_2 emitted during bio-compound production is examined and compared to the CO_2 emitted during cement and lime production processes. Earlier research found that 8 kg of CO_2 was emitted during the production of 1 ton of bio-based compounds, while CO_2 emissions from lime and cement manufacture were estimated to be up to 150 kg/ton (Mekonnen et al., 2020; Rajoria & Kaur, 2014).

11 Future research work

Applying bio-compounds and biomaterials for soil stabilization constitutes an interdisciplinary science of geotechnical engineering, microbiology, ecology, chemical engineering, and biological engineering. Although some studies in the mentioned areas have generated substantial data and interpretations, significant gaps still remain. To the best of the authors' knowledge, no extensive and comprehensive study has been conducted to determine the optimal process for using microbes, bio-enzymes, and biopolymers in terms of cost and related implementation factors. Most studies on the influence of biomaterials have been conducted in the laboratory at the micro level (particle contacts particle) and the macro level (e.g., soil columns), and the optimization of these materials has been established at these levels. Thus, studies with larger scales and studies on the behavior and performance of these biomaterials in complex natural environments and implementation are required. Advanced research is also needed to develop in-situ implementation methods, design criteria, and quality control guidelines to ensure the durability and reliability of microbes, bio-enzymes, and biopolymers in adapting to real-world conditions. For large-scale bio mineralization engineering applications, lowering technology costs remains a critical factor. Changes in bacterial and microbial growth, bioenzyme, and biopolymer activity are difficult to measure and precisely control. Therefore, it is necessary to develop empirical and numerical models of these parameters in various soil environments to simulate the formation of contact between soil particles. Examining the durability of these materials in natural environments is one aspect of future research that should be carefully considered. Manufacturers and researchers must conduct additional research and collaborate for a more precise evaluation of the components of commercial bio-enzymes.

12 Conclusion

This study was conducted to address a knowledge gap about the type and quantity of environmentally sustainable bio-stabilizers, and to determine their efficiency in enhancing the mechanical properties of soil. This paper examined approximately 200 articles about biomaterial applications in geotechnical engineering, published from 1995 to 2023. According to the findings, the number of articles published in this field has increased dramatically in the last seven years. The behavior of bio-additives in all types of soils was explored by combining a fundamental evolutionary perspective with the analysis of prior studies. In addition, the behavior of bio-stabilizers and their properties, mechanisms, effectiveness, and interaction with soil particles at macro- and micro-scales were investigated.

Recent findings indicate that the biochemical performance of various bio-methods for soil stabilization in the environmental geotechnical process comprises four distinct concepts: bio-cementation, bio-clogging processes for bio-microbes, and bio-coating and bio-encapsulation processes for bio-enzymes and bio-polymers. The findings demonstrated that the most significant functional and biochemical mechanisms of bio-additives are linked to higher compressive and shear resistance due to bio-cementation and void reduction. Studying the behavior and mechanism of bio-enzyme- and biopolymer-stabilized soil revealed that the thickness of double water layers, viscosity, and dosage of bio-stabilizers greatly affect their adherence and hydrogel formation on soil particles. On the other hand, the capacity of ion exchange plays a crucial role in bio-microbes. A comparison of the studies demonstrated that microorganisms are the most prevalent in granular soils, and that S. pasteurii and *B.* sphaericus have superior efficiency in bio-cementing coarse-grained soils. In addition, the period of microbe infusion and higher cultivation process strengthens the soil up to 250%. In contrast, TerraZyme bio-enzyme, xanthan gum, and guar gum bio-polymers can increase the adhesion, friction, and roughness of fine-grained soils by 300%. The link between mechanical characteristics and bio-stabilizers' concentration is not always linear. In other words, the optimal stabilizer concentration exists in any soil increased with any biomaterial. Thus, a higher dosage of biomaterials does not ensure the soil's high resistance. It was also found that when water is scarce, as in drought conditions, bio-polymers and bio-enzymes can form direct hydrogen bonds with clay particles or indirect ion interactions with these particles in the presence of intermediary ions such as alkaline or alkaline earth metal ions. Direct and indirect bonding in the soil generates a solid bio-clay matrix, which considerably improves soil adhesion. Due to the increased interaction between soil particles and bio-stabilizers, the appropriate mixing of coarse particles, clay particles, and bio-materials significantly affects the development of bridge gaps between particles. The most important criteria for appropriate types and concentrations of bio-stabilizers were found to rely on the type of soil, its biochemical properties, and its moisture content. However, due to the unique non-linear, time- and moisture-dependent nature of geo-technical materials, it is recommended that sensitivity analyses be performed to identify the most influential parameters for a desired output under given conditions. In addition, macro- and micro-investigations demonstrated that enhancements are the result of increased bonding and interlocking forces in the soil matrix. Furthermore, the incorporation of bio-stabilizers to dry, semi-dry, and expansive soils is beneficial for reducing permeability, swelling, and soil erosion. The maximum biochemical activity was observed in the first 5 to 10 days of processing, and the optimal temperature for enhancing the performance of soil behavior was found to be 20–50 °C.

This innovative research provides a method for identifying the optimal type and dosage of bio-additives depending on the soil type and stabilization target for use in efficient and effective geotechnical designs. The findings can also significantly lower the expenditure of research, laboratory work, and deployment of biological soil amendments.

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