



Review article

Review of bio-enzyme for soil improvement

Yuhao Li^a, Xiangwei Fang^{a,b,*}, Chunni Shen^c, Wenchen Jiang^d, Sheng Huang^d, Guoliang Ma^e^a School of Civil Engineering, Chongqing University, Chongqing 400045, China^b Innovation Center for Biogenic Construction Technology, Chongqing University, Chongqing 400045, China^c School of Civil Engineering and Architecture, Chongqing University of Science and Technology, Chongqing 401331, China^d Shanghai Municipal Engineering Design Institute (Group) Co., Ltd., Shanghai 200092, China^e Department of Civil and Environmental Engineering, The Hong Kong Polytechnic University, Hong Kong, China

ARTICLE INFO

Keywords:

Bio-enzyme
Soil stabilization
Curing mechanism
Engineering mechanical properties
Engineering application

ABSTRACT

Traditional soil stabilizers, such as cement and lime, typically entail substantial energy consumption and environmental pollution. In contrast, bio-enzyme has emerged as a promising alternative, aligning with the imperatives of sustainable development, cost-effectiveness, and environmental friendliness. Bio-enzymes are primarily one or more protein molecules that catalyzes chemical reactions in the soil to form a cementing bond that stabilizes the soil structure and reduces the soil's affinity for water. Currently, a plethora of studies on bio-enzyme have been conducted by scholars worldwide, yet there remains a notable absence of the systematic organization and comprehensive review of these findings. This study offers a thorough examination of bio-enzyme technology, encompassing its biochemical properties, mechanisms, the engineering properties of stabilized soil, bio-enzymatic composites, and its engineering applications. And current trends and future prospects of bio-enzyme are also scrutinized. This forward-looking study indicates that bio-enzyme functions through mechanisms such as cation exchange, specific binding, and surfactants, among others to diminish the electric double layer thickness and hydrophilicity of soil, consequently enhancing engineering properties of soil. And the improvement performance can be influenced by various factors, including soil properties, enzyme dosage, specificity, and sample preparation, etc. It is also noted that the composites of bio-enzyme with conventional stabilizers tend to enhance improvement performance more efficiently. The engineering applications of bio-enzyme have demonstrated its superiority over traditional stabilizers in soil improvement. However, the performance of treated soils with available bio-enzyme remains limited, highlighting the necessity for extracting novel bio-enzyme from plants/animals and determining its mechanisms and engineering mechanical properties. It is also essential to develop more bio-enzymatic composites and conduct application in-situ to develop relevant standards and application guidelines.

1. Introduction

The natural properties of soils vary significantly, and the majority might not be directly utilized as engineering construction materials owing to their poor engineering characteristics. For instance, the unstable structure of soil leads to two major types of engineering issues: geological problems such as foundation settlement, collapse, landslides, and mudslides; and environmental problems like desertification, soil erosion, and vegetation degradation. Consequently, enhancing soil properties becomes imperative. Soil stabilization is the prevailing method utilized to improve its performance (Huang et al., 2021).

The history of soil stabilization technology traces back over 5000 years, with both the Romans and Chinese employing diverse methods for soil solidification in constructing buildings and roads (Aamir et al., 2019; Shalchian & Arabani, 2022). Over time, this technology has evolved into a discipline that integrates theories from structural mechanics, soil chemistry, material chemistry, and colloid chemistry. Depending on the principles of soil stabilization, it can be categorized into physical, chemical, and biological methods. The physical methods, which have been utilized for an extensive period, include techniques such as compaction, dynamic consolidation, and others. Chemical stabilization is another commonly employed method, where inorganic materials like cement, lime, and industrial by-products are added to the

* Corresponding author.

E-mail address: fangxiangwei1975@163.com (X. Fang).¹ ORCID: 0000-0001-9392-6045

soil to enhance its properties. Additionally, chemical methods can be combined with physical methods to further augment the stabilization effect (Onyejekwe & Ghataora, 2015). Indeed, these approaches to soil improvement may present notable limitations owing to their adverse environmental repercussions (Arabani & Shalchian, 2023). Cement and lime, as predominant stabilizers, typify this paradigm, with their production entailing considerable energy consumption and heightened carbon emissions, thereby constituting a significant climate challenge. Moreover, prolonged reliance on these traditional stabilizers can precipitate the depletion of natural resources (Shalchian & Arabani, 2022). Consequently, a pressing imperative emerges for the adoption of materials congruent with the principles of sustainable development and possessing eco-friendly attributes to supplant conventional soil stabilizers.

Recently, biological methods have emerged as the promising alternative to traditional approaches in soil improvement strategies due to their integration of engineering practices with ecological principles. This approach involves the design and construction of systems that incorporate living plant materials as integral structural components (Verma et al., 2021). It offers benefits not only from an engineering standpoint but also from ecological and environmental perspectives (Choi et al., 2016). Biological methods primarily comprise microbial/enzymatically induced carbonate precipitation (MICP/EICP) and bio-enzyme. MICP/EICP involves the decomposition of urea by urease into carbonate ions, which subsequently interact with metal ions in the environment, leading to the formation of precipitated carbonate minerals. These minerals could improve soil properties through filling, covering, and cementation effects within the microstructure (Lim et al., 2020; Wang et al., 2018a). However, as reported in the literatures (Liu et al., 2020; Wang et al., 2020, 2023a), MICP/EICP could necessitate multiple treatment cycles, which may escalate both the time and economic expenditures involved in practice. While bio-enzyme technology presents a relatively short period of treatment process (Sen & Singh, 2015; Saini & Vaishnav, 2015; Tingle et al., 2007), it may also need a long curing age. In general, these biotechnologies are ease of construction, as sometimes maintenance and curing purposes can be accomplished by directly spraying bio-solutions on the soil surface.

The exploration of bio-enzyme technology stemmed from the observations of termites and ants in Latin America, Africa, and Asia utilizing saliva to construct robust soil structures. These structures, reaching several meters in height, exhibit remarkable durability, enduring the challenges posed by tropical rainy seasons. Motivated by this phenomenon, certain researchers have endeavored to leverage termite dust as a soil stabilizer to improve soil (Samuel et al., 2016). Multiple studies have demonstrated the enzyme-rich composition of ant saliva and its significant influence on soil stability (Wood, 1988; Garniersillam et al., 1988, 1989; Lopezhernandez et al., 1989), as validated through experiments. Since the 1980s, developed countries have intensified their research endeavors concerning bio-enzymes and developed various products such as Conaidsuper, Terrazyme, Permazyme, Roadbond and Endurazyme (Ravi et al., 2009; Puneet & Sunnet, 2014). These bio-enzymes, commonly derived from plant fermentation, comprise a natural, non-toxic, non-flammable, and non-corrosive liquid enzyme formulation (Sen & Singh, 2015; Saini & Vaishnav, 2015). Upon introduction in a dissolved state into the soil, bio-enzymes have been shown to augment soil strength (Ren et al., 2024) and diminish permeability. Importantly, road bases compacted using bio-enzyme demonstrate cost advantages over traditional ones while maintaining comparable performance indicators (Bajpai, 2014; Rajorjal & Kaur, 2014).

Review of extant literature reveals that enzymes can enhance the physical and mechanical characteristics of soil, but certain research findings suggest that the improvement may be unsubstantial or even counterproductive. This underscores an inadequate comprehension of the physicochemical effects, mechanisms, and adaptability of bio-enzymes to different soil types, as reported in the literature (Jang, 2020;

Ramdas et al., 2021). Furthermore, soil manifests intricate mechanical and dynamic attributes influenced by myriad factors, including soil physicochemical properties, biological composition, natural enclosure state, and environmental conditions (Arabani & Shalchian, 2023). Consequently, the biochemical reactions, mechanisms, and adaptability of bio-enzymes for solidifying diverse soils exhibit non-uniformity, yielding distinct solidification effects across soil types. Hence, a comprehensive examination of bio-enzyme theory and application becomes imperative to catalyze continued innovation, progress, and utilization of bio-enzymes in geotechnical engineering and allied domains.

This study furnishes a thorough review of theories, mechanisms, and performance pertaining to soil improvement through bio-enzyme, with the objective of delineating strategies for its application in geotechnical engineering aligned with sustainability and environmental considerations across diverse engineering practices. To achieve this, the biochemical traits and attributes of bio-enzyme are scrutinized, succeeded by an examination of soil improvement mechanisms facilitated by bio-enzyme. Base on this, the study delves into the impacts of bio-enzyme on soil stabilization, mainly focusing on the engineering properties of enzyme-modified soil. Following this, the present landscape of research concerning the amalgamation of biological enzymes with other stabilizers for soil improvement is encapsulated, culminating in an overview of bio-enzyme application in field scenarios. Ultimately, the main challenges and potential research directions for bio-enzyme are discussed.

2. The substances of bio-enzyme

Bio-enzyme exhibits intricate biochemical compositions. While certain manufacturers endorse the utilization of their enzymes in experimental investigations, many express reservations regarding chemical analysis, fearing potential infringement upon their proprietary rights to product formulas. The fact that some of the biochemical components of bio-enzymes are hard to determine by laboratory testing is due to their proprietary chemical composition. Velasquez et al. (2005) argued that the inability of a bio-enzyme to catalyze the degradation of certain compounds does not necessarily indicate the absence of active enzymes in the sample. This is because bio-enzymes are highly purified solutions containing specific enzymes or enzyme groups, which can be challenging to test due to their selectivity and potential for specific reactions.

It has been shown that distinct bio-enzymes may exhibit comparable compositions. For instance, Permazyme comprises a diverse of enzymes, proprietary ingredients, and unspecified organic materials. Similarly, Eko enzyme is a multi-enzyme product incorporating lipase, amylase, and protease as its active constituents, and is made by the extraction of water and proteins from fermented plants (Pooni et al., 2021). The typical composition of Eko enzyme comprises 20% water, 20% nonionic surfactant, and 60% of the base ingredient ferment, which includes 30% water (Renjith et al., 2020). Terrazyme, on the other hand, comprises ethoxylated alcohols, fermented vegetable extracts, and nonionic surfactants (Khan et al., 2015), such as the Terrazyme is molasses extract (Eujine et al., 2017a). For Earthzyme, the composition includes fermented extracts of plant polysaccharides, potassium chloride, magnesium sulfate, lactic acid, and nonionic surfactants (Chandler et al., 2017; Mgangira, 2009). Further laboratory studies indicate that both Permazyme and Terrazyme contain proteins and nonionic surfactants (AbouKhadra et al., 2018). Velasquez et al. (2005) proposed that proteins in bio-enzymes demonstrate properties akin to surfactants, displaying enhanced efficacy in lowering water surface tension compared to conventional surfactants like SDS. Furthermore, bio-enzymes typically harbor notably elevated levels of potassium (K) and moderate to high concentrations of calcium (Ca), magnesium (Mg), and sodium (Na). Table 1 shows the main composition of different bio-enzymes.

Overall, the composition of bio-enzyme is intricate and can be difficult to ascertain through laboratory testing. The current consensus

Table 1
The composition of different enzymes.

Bio-enzymes	Main compositions	Sources
Permazyme	Fermented vegetable extract, potassium chloride, and nonionic surfactants.	(Mgangira, 2009; AbouKhadra et al., 2018)
Terrazyme	Ethoxylated alcohols, fermented vegetable extracts, and nonionic surfactants.	(Sen & Singh, 2015; Saini & Vaishnava, 2015; AbouKhadra et al., 2018)
Eko enzyme	Multi-enzyme product and fermented vegetable extracts.	(Pooni et al., 2021; Renjith et al., 2020; Kushwaha et al., 2018; Pooni et al., 2019)
Earthzyme	Fermented vegetable extract, potassium chloride, magnesium sulfate, lactic acid, and nonionic surfactants.	(Chandler et al., 2017; Mgangira, 2009)

suggests that bio-enzyme originates from plant fermentation/animal extraction and typically comprises surfactants, proteins, and some proprietary ingredients (Pooni et al., 2021; Mekonnen et al., 2020).

3. Principles of bio-enzyme

Bio-enzyme displays specificity in catalyzing biochemical reactions, and the reaction system is complex. Understanding precisely how and under what conditions bio-enzymes function remains elusive (Ramdas et al., 2021). Study into the mechanism of bio-enzyme is still exploratory, leading to a lack of comprehensive understanding. Presently, three primary mechanisms can be applied to stabilize soil using bio-enzyme:

(1) Cation exchange mechanism

The cation exchange mechanism involves enzymes being adsorbed onto clay lattice sites, with cations subsequently released as exchangeable ions. This process resembles cation exchange, leading to a reduction in

the thickness of the electric double layer of soil, thereby decreasing the penetration of moisture into the clay lattice. For instance, Pooni et al. (2021) proposed that bio-enzyme was adsorbed onto clay minerals, inducing relaxation of the mineral lattice. The relaxation led to inner-layer expansion and subsequent moisture retention. Consequently, there was a reduction in the absorption of moisture within the soil structure, lowering its affinity for water and mitigating the impact of moisture on the soil. This process fosters soil particle aggregation (as shown in Fig. 1A and B). AbouKhadra et al. (2018) posited that enzymes might supply strong and soluble cations, displacing weaker clay cations to remove water from the clay structure. The moisture loss facilitates enhanced arrangement of clay particles, along with a decrease in particle size and plasticity, resulting in higher density and enduring structural alterations (Rauch et al., 2002). Ganapathy et al. (2016) proposed that bio-enzyme decreased the charge of hydrogen ions within adsorbed water molecules, leading to a weakening or disappearance of their bond with clay particles and a reduction in the thickness of the adsorbed water film surrounding these particles.

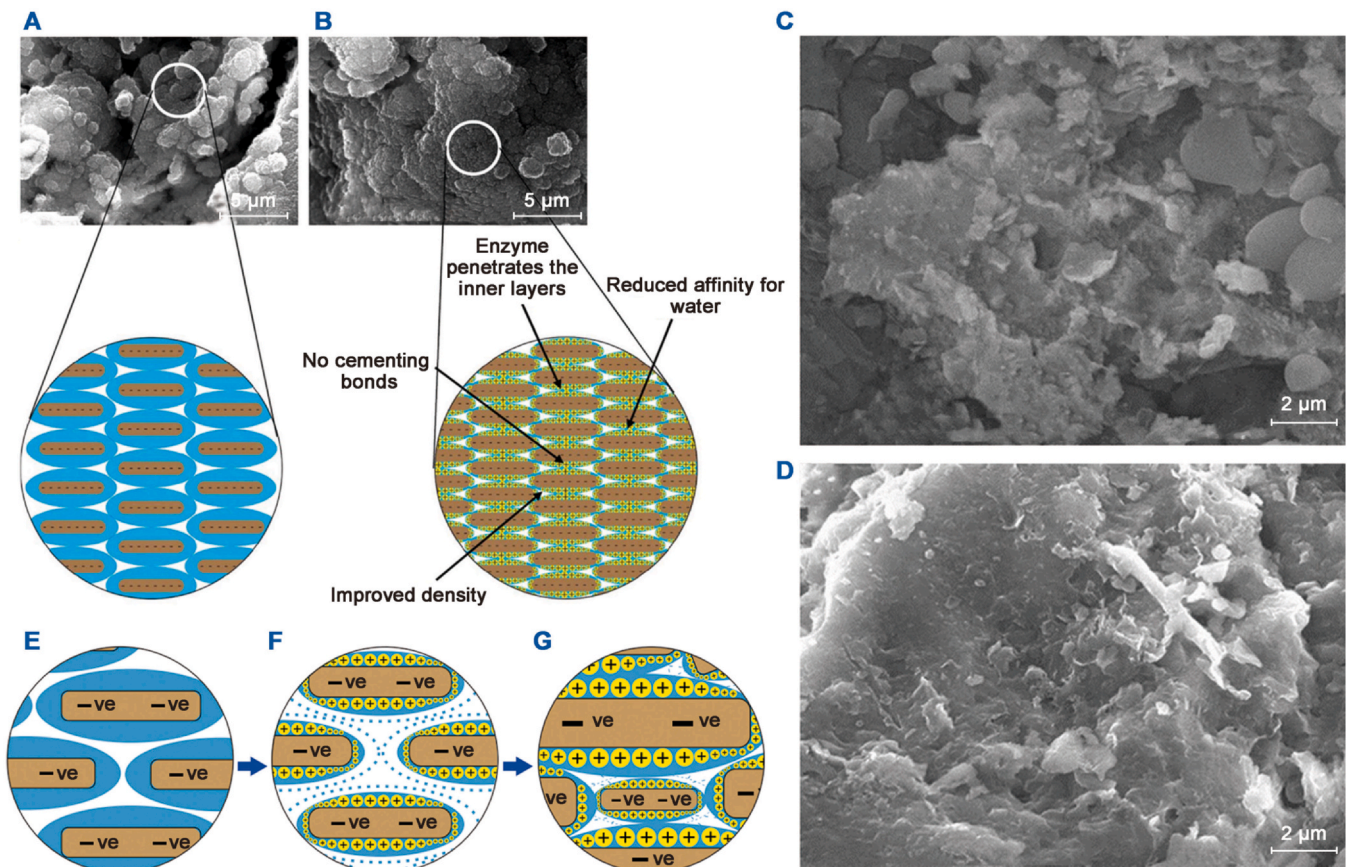


Fig. 1. Schematic illustration of the enzyme stabilization mechanism on expansive soil (Pooni et al., 2021):(A) Untreated soil, (B) Stabilization soil; SEM image of untreated and treated soil with bio-enzyme (Ganapathy et al., 2016): (C) Untreated soil, (D) Treated soil; Enzyme stabilization mechanism (Renjith et al., 2020): (E) Natural clay particle with high affinity for water, (F) Organic encapsulation decreasing the double layer of water, (G) Stable clay particles.

(2) Specific binding mechanism

The specificity binding mechanism refers to bio-enzyme binding specifically to organic molecules surrounding clay particles, enabling positively charged organic molecules to adsorb and envelop soil particles. Simultaneously, these organic molecules diffuse within the double layer and engage in ion exchange (similar to cation exchange), neutralizing the surface charge of clay and reducing the affinity for water (Khan et al., 2015; Chandler et al., 2017; Thomas et al., 2018). This mechanism was proposed by Scholen (1995), who elucidated that during the enzyme treatment process, a specific substance engaged in an exchange reaction with the clay structure. This specific substance originated from the combination of enzymes with organic molecules. Subsequently, the electric double layer was disrupted, leading to the expulsion of water and aggregation of soil particles, further promoting granulation for cementation. Correspondingly, enzyme can adhere to both the inner and outer regions of clay minerals to impede water absorption. Velasquez et al. (2005) supported the perspective of Scholen et al., indicating that certain enzymes can adhere to the interior of clay structures and interact with various metal cations within the clay matrix. Moreover, these enzymes can be transported alongside the electrolyte in the soil, stimulating the activity of various bacteria and releasing hydrogen ions, thus creating a significant pH gradient on the surface of soil particles, greatly enhancing soil structure. Simultaneously, in the presence of enzymes, numerous organic molecules combine to form intermediate products. When these intermediates exchange cations with the clay structure, they alter the original soil structure, leading to sealing effects that weaken the ability of soil particles to adsorb water and swell. Consequently, this results in denser soil and increased resistance to permeability. Numerous researchers have reported similar mechanisms (Tingle et al., 2007; Puneet & Sunnet, 2014). Furthermore, Chandler et al. (2017) conducted two one-year-long capillary rise soil column experiments with a height of 1 m each, providing robust evidence that enzymes reduce the affinity of soil for water.

(3) Surfactant mechanism

The surfactant mechanism whereby the surfactant within bio-enzyme participating in reactions to reduce the surface tension of solution, allowing soil particles to bind more tightly together. Velasquez et al. (2005) performed enzyme activity tests and analyzed protein content in commercial bio-enzymes. The results showed high concentrations of proteins but a deficiency in active enzymes. Nevertheless, quantitative surface tension tests and qualitative observations indicated that the performance of these bio-enzymes resembled surfactants. They exhibited greater efficacy in reducing water surface tension compared to conventional surfactants like Sodium Dodecyl Sulfate (SDS). This characteristic may be effective in improving soils. AbouKhadra et al. (2018) suggested that wetting agents (surfactants) presented within enzymes facilitated the penetration of enzymatic active ingredients, while enzymes improved cohesion, bringing soil particles closer together and increasing their density. Thomas et al. (2018) proposed that bio-enzymes, functioning as biological surfactants, can inhibit water ingress. Additionally, the influence of dielectric constant on double layer thickness could be considered, as the presence of alcohols in enzymes may decrease the thickness of the electric double layer.

There are some other mechanisms existing as well. For example, bio-enzyme may catalyze the reaction between clay and organic cations directly, thereby accelerating cation exchange rates and reducing the thickness of the electric double layer, without involvement in the formation of encapsulating structures (Sen & Singh, 2015). Additionally, other studies have demonstrated the potential production of calcium silicate hydrate in particle voids in the presence of water, leading to tighter aggregation of soil particles (as shown in Fig. 1C and D) and increasing stability (Ganapathy et al., 2016).

Based on the various perspectives on the mechanisms of bio-enzymes, it can be summarized that there are primarily three mechanisms: cation exchange, specific binding, and surfactant. The first two mechanisms work by reducing the net negative charge on soil particles and decreasing the thickness of the electric double layer through enzymatically catalyzing reactions, thus promoting compaction of the soil structure. The third mechanism, surfactant action, reduces surface tension of soil particles, thereby lowering capillary suction and the thickness of the water absorption layer. All the mechanisms collectively decrease the affinity of soil for water, reduce the thickness of the electric double layer, and facilitate soil compaction, ultimately achieving soil stabilization. Overall, although these mechanisms are functionally similar, there are differences in the way they perform their roles: they function through the adsorption of the enzyme by the clay lattice, the specific binding of the enzyme to organic molecules surrounding clay particles, and the reduction of surface tension by surfactants, respectively. These mechanisms of bio-enzyme could be illustrated in the Fig. 1(E–G).

4. Engineering properties of bio-enzyme stabilized soils

4.1. Physical properties

Bio-enzymes have demonstrated the capacity to enhance the physical properties of soils, for example, reduce the liquid-plastic limit, plasticity index (PI), and optimum moisture content (OMC), while increase the maximum dry density (MDD), etc. Summarizing the results of representative studies on bio-enzyme stabilized soils, as depicted in Fig. 2(A–E), it becomes evident that the improvement effect in the physical properties of stabilized soil is influenced by enzyme dosage and soil characteristics. This improvement can range from slight to significant, and in some cases, it may even lead to counterproductive outcomes, such as a decrease in MDD.

Indeed, the dosage of bio-enzyme plays a crucial role in influencing the physical properties of stabilized soils. Studies have shown that at low enzyme dosages, there may be minimal improvement in the compaction properties (MDD and OMC) (Sen & Singh, 2015; Pooni et al., 2021; Khan et al., 2015; Mgangira, 2009; Rauch et al., 2002; Dandin & Hiremath, 2014; Yilmaz et al., 2009; Panchal et al., 2017). Khan et al. (2015) argued that the inconsistency and inhomogeneity of soil encountered in laboratory could also be the reasons behind this. As the enzyme dosage increases, higher dosage of enzymes can effectively reduce the electric double layer thickness, thus enhancing the effectiveness of soil improvement (Shaka & Rakaraddi, 2016). In various studies, the impact of enzyme dosage on soil stabilization has been examined. For instance, at a dosage of 200 mL/2 m³, enzyme-stabilized soil exhibited reductions in liquid limit (27.77%), PI (31.06%), and OMC (17.64%), while an increment in MDD (5.03%) (Saini & Vaishnava, 2015). Subsequently, increasing the enzyme dosage to 200 mL/0.75 m³ resulted in a notable decrease in consistency limit (33%–50%), a 22.62% decrease in OMC, while a 7.5% increase in MDD (Tiwareti et al., 2021). Moreover, at a dosage of 200 mL/0.25 m³, the liquid limit, plastic limit, and PI of treated soil were reduced by 20.02%, 9.49%, and 26.05%, respectively (Usha et al., 2018). These results underscore the efficiency of increasing enzyme dosage for soil improvement. However, it is apparent that excessively high dosage may diminish the enhancement effect, indicating the existence of an optimal enzyme dosage. Several studies suggest that an enzyme dosage of 200 mL/0.75 m³ represents the optimal level for soil stabilization (Shaka & Rakaraddi, 2016; Tiwareti et al., 2021). Nonetheless, it is important to recognize that the optimal dosage of enzymes can vary across studies due to the specificity of enzymes and variations in soil properties (Saini & Vaishnava, 2015). Generally, the improvement effect of soils tends to increase first and then decrease with increasing enzyme dosage (Kushwaha et al., 2018). However, soils characterized by high plasticity may necessitate higher enzyme dosage to reach the threshold required for effective soil

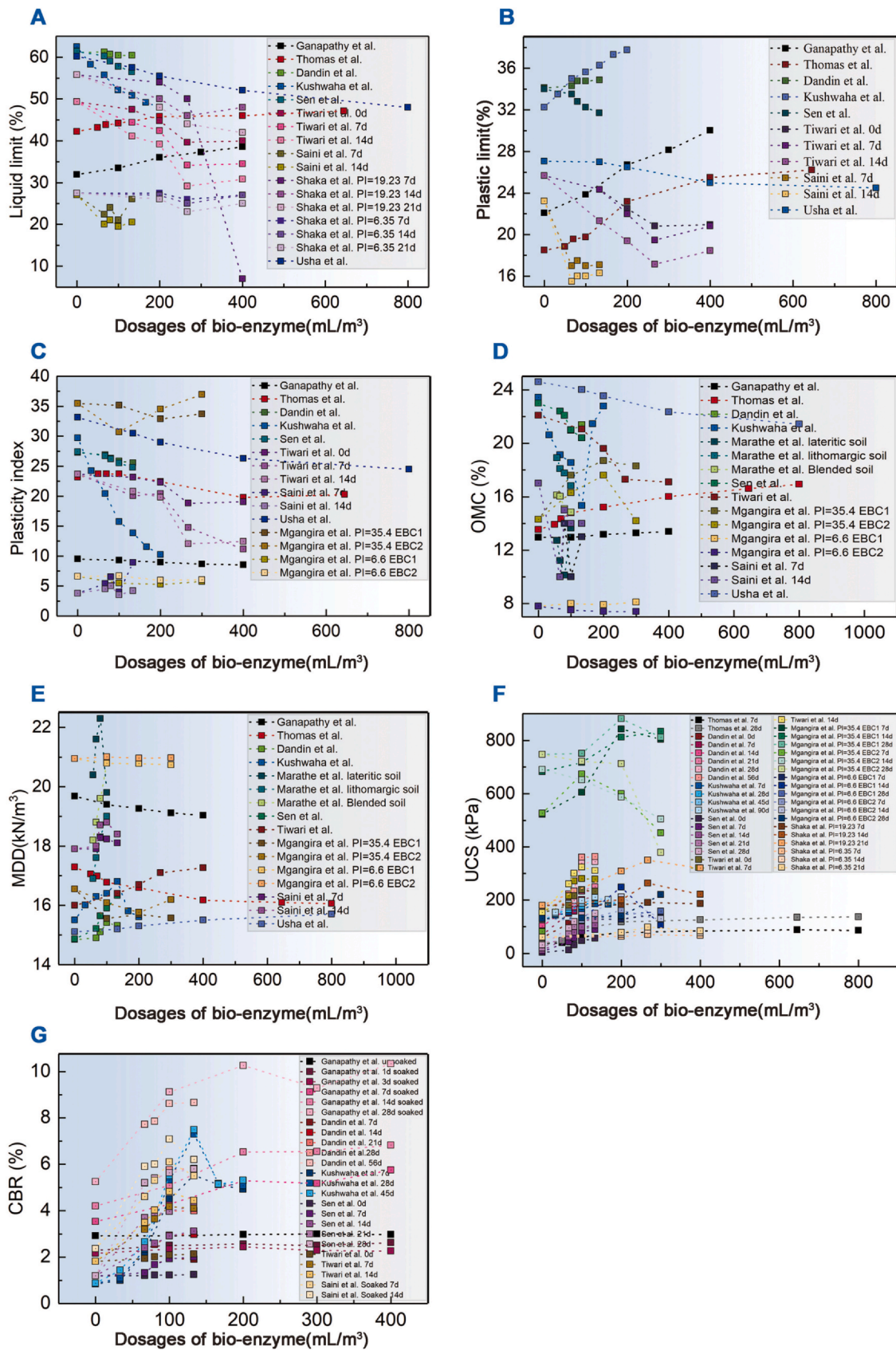


Fig. 2. Effect of enzyme dosages on the physical properties of soils: (A) Liquid limit, (B) Plastic limit, (C) Plasticity index (PI), (D) Optimum moisture content (OMC), (E) Maximum dry density (MDD); Effect of enzyme dosages on the mechanical properties of soils: (F) UCS, (G) CBR.

improvement, otherwise the MDD keeps increasing and the OMC keeps decreasing until reaching the optimal enzyme dosage (Marathe & Shankar, 2023).

Apart from enzyme dosage, the inherent characteristics of soil and the specificity of enzymes also play pivotal roles in soil improvement. Bio-enzymes are typically more effective in modifying highly plastic soils (Chandler et al., 2017; Bergmann, 2000) but show reduced efficacy in soils with lower plasticity (Mgangira, 2009). Moreover, a higher clay content in soil enhances the effectiveness of enzyme application, with soils containing at least 10% clay being suitable for bio-enzyme treatment (Tiwari et al., 2021). For soils with higher content of cohesionless particles, the improvement of the consistency limit is even ineffective (Ravi et al., 2009). Furthermore, certain studies have reported negative modification results, such as an increase in the Atterberg limit (Chaurasia et al., 2021) and OMC for enzyme stabilized soils (Ganapathy et al., 2016), as well as a decrease in dry density (Mgangira, 2009; Thomas et al., 2018; Yilmaz et al., 2009), which can be attributed not only to the enzyme specificity and the soil nature to be treated, etc., but also possibly to the specimen preparation and curing methods (Chandler et al., 2017).

In summary, the outcomes of studies on enzyme-treated soil vary widely, ranging from minimal to significant improvement, and even to contrary effects. These variations are attributed to factors such as enzyme dosage, soil properties, enzyme specificity, etc. Consequently, it is crucial to approach claims or results from enzyme manufacturers with caution, as independent laboratory testing is necessary to verify their efficacy (Taha et al., 2013).

4.2. Mechanical properties

The bio-enzyme treatment has shown potential to enhance the mechanical properties of soil (Puneet & Sunnet, 2014; Divya & Asha, 2023), such as increasing the unconfined compressive strength (UCS) and California bearing ratio (CBR), etc. Summarizing the details of representative studies on the initial soil properties/sample preparation methods and mechanical properties of bio-enzyme stabilized soils, as shown in Fig. 2(F–G) and Table 2, respectively. It can be found that the improvement of mechanical properties of stabilized soil also varies from minor to substantial, and the improvement performance is dependent on factors like water content, specimen preparation methods, soil properties, enzyme specificity and dosage, etc.

Although bio-enzymes might not lead to significantly improvements in the physical properties of soils (Section 4.1), the organic cations in enzymes can interact with the clay and flocculate the soil particles. This interaction, combined with the potential production of calcium silicate hydrate, contributes to making soil particles more compact, thereby enhancing the hardness and integrity of the soil (Pooni et al., 2019). Therefore, the modification effect of enzymes can substantially increase the UCS and CBR of soils (Ravi et al., 2009; Puneet & Sunnet, 2014; Ganapathy et al., 2016; Yilmaz et al., 2009; Panchal et al., 2017; Chaurasia et al., 2021). Some studies have even reported quite impressive improvement effects, with mechanical strength improvements in enzyme stabilized soils reaching up to 400% or more (Ravi et al., 2009; Venkatasubramanian & Dhinakaran, 2011). For example, after 4 weeks of curing with a Terrazeme dosage of 1.5 m³/200 mL, the CBR of treated black cotton soil increased by 387% (Sen & Singh, 2015); after 4 weeks of curing with a Terrazeme dosage of 1.0 m³/200 mL, the CBR of treated black cotton soil increased by 400% while the UCS increased by 500% (Dandin & Hiremath, 2014).

However, the significant improvement in the mechanical properties of enzyme stabilized soil mentioned in the aforementioned studies may be attributed to moisture loss (Chandler et al., 2017; Pooni et al., 2019), given that the moisture content during sample preparation and testing was not documented in these studies. When moisture content is controlled, as in sealed conditions, the mechanical properties of enzyme stabilized soil show either no improvement or only slight improvement (Khan et al., 2015). In contrast, soil samples cured under air-drying conditions show at least a 10% increase in strength gain (Peng et al.,

2011). Rauch et al. (2002) also reported that the water content of the sample was as critical as the enzyme in influencing the strength.

It is noteworthy that certain studies have reported a slight improvement in the mechanical strength of enzyme stabilized soil, which may be correlated with the sample preparation protocol. After the incorporation of enzymes into the soil, the compaction properties of the soil are optimized (with decreasing OMC and increasing MDD), and tests should be conducted according to these optimized properties to observe substantial improvement. This is because changes in OMC/MDD determine the efficacy of the enzyme (Renjith et al., 2020). Pooni et al. (2021) found that under the natural properties of the soil (raw OMC and MDD), the strength variation of the stabilized samples could be negligible or even showed a decrease in mechanical strength (a 3.5% reduction in CBR). However, when enzyme treatment was performed under optimized soil properties (stabilized OMC and MDD), both CBR and UCS increased by 58% and 28%, respectively. Other studies have also reported significant enhancements in mechanical strength when enzyme treatment was carried out under optimized soil properties (Marathe & Shankar, 2023). Furthermore, the maintenance method during sample preparation also influences mechanical strength. It is commonly found that the CBR for soaked samples is lower than that of unsoaked samples (Saini & Vaishnav, 2015; Kushwaha et al., 2018; Ganapathy et al., 2016).

It is pertinent to highlight that variations in soil classification, plasticity, and clay content could also affect the strength of bio-enzyme stabilized soil due to the fact that enzyme catalyzes very specific chemical reactions that are highly soil specific (Mgangira, 2009), and therefore the properties and behaviors presented in established studies may differ when enzyme is applied to different soils within identical conditions (Shaka & Rakaraddi, 2016; Marathe & Shankar, 2023; Eujine et al., 2017b; Choudalakis & Gotsis, 2011). As mentioned before, bio-enzymes are more favorable for modifying soils with high plasticity or high clay content (Chandler et al., 2017; Bergmann, 2000). To achieve optimal mechanical performance of enzyme modified soils, it is recommended that the soil contains 12%–24% clay, accompanied by a plasticity index in the range of 8%–35% (Kestler, 2009). Bergmann (2000) also found that a minimum of 2% clay content was necessary for proper stabilization of soils, and excellent performance could be obtained with 10%–15% clay content. Therefore, it is imperative to underscore that the clay content and plasticity index of the soil should fall within the recommended range for enzyme applications to enhance the strength properties of soil (Kushwaha et al., 2018).

Furthermore, the enzyme dosages also influence the mechanical strength of cured soils, with an optimal enzyme dosage being essential (Saini & Vaishnav, 2015; Kushwaha et al., 2018; Tiwari et al., 2021; Chaurasia et al., 2021). Excessive enzyme dosage generally tends to diminish the improvement efficacy as soil-enzyme interaction decreases while bio-biotic contact increases (Arabani & Shalchian, 2023). High enzyme concentrations also reduce the cohesive aggregation of the soil, making it an unworkable and fragile material, as well as being detrimental to the homogenization of the soil and water in the medium (Renjith et al., 2020). Meanwhile, high dosage even leads to the reduction in the mechanical strength, e.g., a reduction in the UCS of stabilized soils by about 14% at high enzyme dosage relative to the optimal one (Shaka & Rakaraddi, 2016).

In brief, bio-enzymes could enhance soil's mechanical strength, and the performance is subject to various factors, including water content, sample preparation scheme, soil properties, enzyme specificity and dosage, etc. However, there remains contention regarding how soil properties influence the mechanical strength of enzyme stabilized soils, and necessitate further research into both enzyme mechanisms and the structure of their resultant products.

5. Bio-enzymatic composites

New techniques or materials for soil stabilization are crucial for geotechnical engineering (Anagnostopoulos, 2015). The current studies

Table 2
The initial soil properties and sample preparation methods.

Initial soil properties					Sample preparation conditions (for UCS)	Maintenance methods (for UCS)	Sources
Optimum moisture content (%)	Max dry density (g/cm ³)	Liquid limit (%)	Plastic limit (%)	Plasticity index			
23	1.48	61.4	34	27.4	OMC	Keep in desiccator	(Sen & Singh, 2015)
17	17.9	27	23.21	3.79	/	/	(Saini & Vaishnava, 2015)
13.5	1.93	35	25	10	/	Keep in desiccator	(Ravi et al., 2009)
22.9	1.62	48	18	30	MDD	Keep in a sealed airtight container	(Pooni et al., 2021)
17	1.79	29	20	9	/	Air dried/ Oven dried	(Renjith et al., 2020)
32	17.5	79	48	31	/	Keep in desiccator	(Eujine et al., 2017a)
9.7	1.92	/	/	/	/	/	(AbouKhadra et al., 2018)
11.6	1.82						
14.4	1.74	36	25.3	10.7			
17.8	1.76	38	25.2	12.8			
18.5	1.74	32	22.8	9.2			
13	19.7	32	22.2	9.8	/	/	(Ganapathy et al., 2016)
/	/	85	37	47	Density:1.51 g/cm ³ Water content: 28%	Air dried	(Chandler et al., 2017)
13.5	1.72	42.25	18.6	23.65	/	Stored at 95%–100% relative humidity at 25 °C	(Thomas et al., 2018)
14.3	1.65	/	/	35	/	Stored at 100% relative humidity at 25 °C	(Mgangira, 2009)
23.1	14.8	61.4	34	27.4	/	Keep in desiccator	(Dandin & Hiremath, 2014)
/	/	35	16	19	/	Air dried	(Yilmaz et al., 2009)
20	1.608	55.8	36.57	19.23	/	Keep in desiccator	(Shaka & Rakaraddi, 2016)
22	1.432	70.5	37.9	32.6			
24	1.432	84.35	48.61	35.74			
14	1.854	27.5	21.15	5.35			
25	15.1	60.26	27.07	33.13	/	/	(Usha et al., 2018)
20.54	1.57	62.5	31.13	31.37	/	/	(Kushwaha et al., 2018)
13.9	1.92	49.2	27.2	22	OMC, MDD	Keep in desiccator	(Marathe & Shankar, 2023)
19.62	1.63	59.3	29.7	29.6			
16.2	1.81	54.5	27.9	26.6			
22.5	1.825	53.33	29.2	24.13	/	Air dried	(Chaurasia et al., 2021)
28	1.52	60.9	31.4	29.5			
22.9	1.62	48	18	30	OMC	Keep in a sealed airtight container	(Pooni et al., 2019)
28.5	1.4	91	33	58	OMC	Keep in desiccator	(Thomas & Rangaswamy, 2019)

on bio-enzyme soil stabilization primarily focuses on the use of bio-enzyme in isolation, resulting in suffering from longer maintenance age and poorer physical and mechanical properties. Therefore, it is recommended that combining bio-enzyme with other materials to obtain the desirable properties of soils (Ramdas et al., 2021). For MICP/ECIP technology, there are substantial favorable studies on the combination of MICP/ECIP and fibres (Wang et al., 2018b; Iamchaturapatr et al., 2022; He et al., 2023) and polymeric compounds (Almajed et al., 2020; Annadurai, 2024; Baig et al., 2024; Miao et al., 2020; Sun et al., 2021, 2022; Wang et al., 2023b, 2023c; Pandey, 2018; Refaei et al., 2020). For bio-enzymes (Terrazyme, Permazyme and others), while there have also been endeavors to combine them with other soil improvement materials, such as cement or lime, aiming to enhance soil plasticity, strength, and other geotechnical properties to a greater extent (Kushwaha et al., 2018; Ganapathy et al., 2016), there are fewer studies.

It is reported that when bio-enzymes are combined with gypsum for soil stabilization, Terrazyme could accelerate the chemical reaction between gypsum and soil, promote the cementation of soil particles, and reduce the hydrophilicity of soil by thinning the bonded water film and reduce the expansion of soil, therefore effectively increasing the UCS, CBR and MDD of the soil (Usha et al., 2018). Bio-enzyme can also collaborate with nanoparticles and cement to stabilize the soil. The resulting composite densifies the particles, decreases pore volume, and enhances bonding between the cemented particles. Consequently, this amplifies stress dissipation, energy absorption, and improves the shear modulus and damping ratio of the soil (Thomas & Rangaswamy, 2020).

In addition, when bio-enzymatic composite lime and sugarcane ash to reinforce soils, the enzyme acts as an ionic surfactant that changes the hydrophilicity of the clay and lime to hydrophobicity. Simultaneously, the enzyme catalyzes the reaction between clay and water, producing calcium silicate hydrate, thus substantially enhancing the soil properties (Chitragar et al., 2019). Moreover, Eujine et al. (2017a, 2017b, 2017c) conducted an extensive study on soil improvement using bio-enzymatic composite lime and found that: (a) the enzyme lime accelerated the improvement efficiency and promoted the cementation of fine particles; (b) the enzyme lime reduced soil swelling, dust formation, and lime consumption; and (c) the enzyme lime could utilize local soils, reduce construction costs and times, and minimize the need for other raw materials. Eujine et al. (2017a) concluded that in the presence of the bio-enzyme (Rhodasurf R(OC₂H₄)OH), the lime undergoes a further reaction with calcium aluminate and calcium aluminosilicate, leading to replace the original aluminum cation with a calcium cation to form stronger compounds. The mechanism of the enzyme lime is shown in Fig. 3A.

Besides, other studies have also demonstrated the effectiveness and efficiency of bio-enzymatic composites for soil improvement, and these results have been confirmed by the microscopic tests, such as FT-IR (as shown in Fig. 3B), XRD, and SEM (Thomas & Rangaswamy, 2019; Jairaj et al., 2019).

In summary, the use of bio-enzymes in combination with other curing materials not only significantly improves the effectiveness and efficiency of soil improvement, but also reduces the negative impact on the environment.

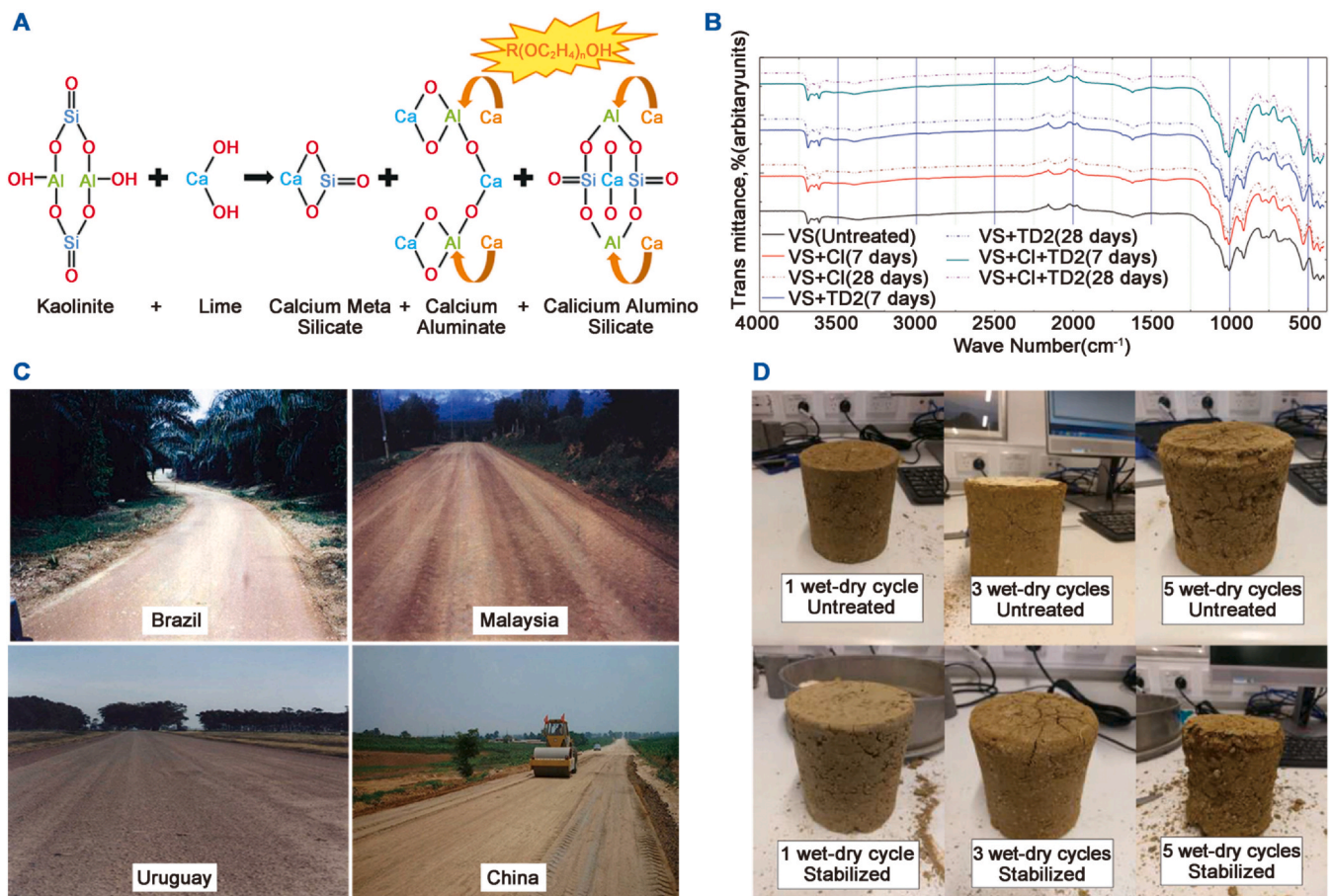


Fig. 3. (A) Schematic representation of enzymatic lime stabilization mechanism (Eujine et al., 2017a); (B) FTIR patterns of cement-treated, and cement and enzyme treated specimens (Thomas and Rangaswamy, 2019); (C) The in-situ application of bio-enzymes in multiple countries (Chen, 2007); (D) Soil damage of untreated and stabilized soil samples (Pooni et al., 2019).

6. Application of bio-enzyme in road engineering

Studies on bio-enzyme has shown that it helps to form a dense, hard, waterproof soil layer, providing an ideal soil surface for paving roads and controlling dust or erosion (Renjith et al., 2020; Mekonnen et al., 2020; Parik & Patra, 2023), and bio-enzyme stabilized pavements have a lower cost (Bajpai, 2014). Consequently, the main application of enzymes in engineering is currently in road engineering. Since the 1970s, enzymes have been successfully applied in road and highway engineering in over forty counties (Velasquez et al., 2005). As shown in Table 3 and Fig. 3C, enzymes have been successfully used for pavement stabilization in several countries and regions across Europe, the United States, Africa, South America, and South Asia. These engineering applications demonstrate that bio-enzyme can be employed to improve a variety of soil, including sandy clay, silty clay, plastic clay, and non-plastic clay.

In Paraguay, to test and compare the improvement performance of various enzymes, a comprehensive study on the soil stabilizer in secondary unsurfaced roads was funded by the World Bank in 2000. This study involved field trials of nine soil stabilizers, including various enzymes and inorganic stabilizers. The findings indicated that enzyme stabilized pavements performed the best, with TerraZyme showing the most effective results. This outcome provided technical support for the promotion of bio-stabilization technology in South America. In USA, Pennsylvania applied TerraZyme to a road section and conducted a comparative analysis after the road had undergone four freeze-thaw cycles. The findings showed that the enzyme treated road was not subjected to the common pavement distresses, such as rutting and potholes, and that no further required post-maintenance. This

Table 3

Regions and countries applying bio-enzymes in engineering (Chen, 2007).

Regions	Countries
Africa	Congo, Gabon, Kenya, Tanzania, Uganda
Asia	Azerbaijan, Bangladesh, China, India, Indonesia, Japan, Korea, Malaysia, Philippines, Thailand, Myanmar
Americas	Argentina, Bolivia, Brazil, Canada, Chile, Colombia, Ecuador, Paraguay, Peru, Uruguay, USA
Europe	Netherlands, Spain, Poland, Portugal, Turkey, Romania

significantly reduced the costs associated with manual upkeep (Sedgwick, 2003). In Malaysia, the Palm Oil Research Institute of Malaysia (PORIM) concluded after three years of research that compared to untreated soil sections, the maintenance costs of enzyme treated sections were reduced by a total of 75%, encompassing both interim and subsequent maintenance. More importantly, there was no need for major repairs on these sections, and common pavement distresses were virtually eliminated, demonstrating the remarkable effect of TerraZyme in improving road surfaces. In India, numerous experimental roads have been constructed using TerraZyme, such as those in Kerala, Puducherry, Trichy, and Tamil Nadu (Bajpai, 2014), all of which have demonstrated the effectiveness of enzyme. Many roads in Bangalore have also been stabilized with the enzyme, gaining a threefold increase in construction speed and a 25%–30% reduction in costs. Rajorial & Kaur (2014) employed Terrazyme as a soil stabilizer in the public works department in Maharashtra and showed that enzyme reduces the cost of road construction by approximately 18%–26% and has greater strength.

Other studies also confirmed the superior mechanical properties and lower cost of bio-enzyme cured pavements (Ravi et al., 2009; Moloisane & Visser, 2014).

In conclusion, bio-enzyme modified pavements may be more effective than traditional stabilizers, potentially reducing common pavement distresses (rutting, potholes, etc.) and the cost of subsequent maintenance, and improving construction efficiency. However, the effect of soil properties on the effectiveness of bio-enzyme should be checked in the laboratory before field application. It is also worth noting that the complex and costly production of bio-enzyme could still result in greater limitations in practical engineering applications (Lahalih & Ahmed, 1998).

7. Recommendations for future studies

In recent years, bio-enzyme has garnered widespread attention and application due to its notable environmental and economic advantages. Studies on bio-enzymatic composites and engineering applications are continually emerging. However, several significant challenges persist in the investigation of bio-enzymes. Firstly, there are remarkable discrepancies among existing studies, resulting in a lack of consistent conclusions. Additionally, comprehensive and integrated studies aimed at determining the optimal processes for bio-enzyme treatment are lacking. Furthermore, there is a notable absence of relevant standards and quality control guidelines for bio-enzyme technology. Consequently, the current pressing issues and potential future research directions are delineated below:

- (1) Further extraction and development of novel bio-enzyme are required. The utilization of bio-enzyme represents one of the key strategies for environmentally benign energy and material-saving biochemical processes today (Jang, 2020). Therefore, it is crucial to identify more novel enzymes (such as amylases, arylsulfatases, β -glucosidases, etc (Das & Varma, 2010).) from plant fermentation/animal extraction and to elucidate their reaction patterns in various soil geochemical processes. Additional, detailed and transparent descriptions of enzyme production processes and compositions are also essential to enable further innovation and application of bio-enzyme.
- (2) The mechanisms and engineering mechanical properties of bio-enzymes in soil stabilization require thorough investigation. The mechanism of bio-enzyme is highly complex, and the improvement effect could be influenced by multiple factors. However, the analysis of biochemical reactions, microstructure, and pore structure in the existing studies remains limited; current micro-testing methods struggle to accurately describe and measure the solidification extent; most current studies primarily focus on the effects of enzyme dosage and maintenance age; enzyme stabilized soil exhibits poor resistance to freeze-thaw and dry-wet cycling (Dandin & Hiremath, 2014), with enzymes playing a limited role in mitigating soil degradation (Fig. 3D). Meanwhile, the magnitude of the improvement brought about by enzymes on the erosion resistance of a soil seems to be soil specific (Shafii et al., 2019). Therefore, future research should not only encompass more systematic studies on the mechanisms, microstructures, and deterioration properties of enzymes, but also delve into the relationships between the materials, structures, and performance of bio-enzymes. And it is important to establish relevant mathematical models and optimize the mix proportions and preparation processes to guide the development of bio-enzyme-based soil solidification materials with excellent performance that align with the needs of engineering practice.
- (3) Research and development efforts should focus on novel bio-enzymatic composite. Bio-enzymatic composites typically lead to more significant improvements in soil engineering mechanical properties (Eujine et al., 2017b; Thomas & Rangaswamy, 2020; Chitragar et al., 2019), but reports in this topic are scarce and the existing

macro-micro analyses are superficial. Hence, there is a necessity for the continued advancement of composites consisting of bio-enzyme and traditional stabilizers, along with thorough research on the macro-micro properties of cured soils. And further exploration is needed to elucidate the interaction mechanisms between bio-enzymes, curing agent components, additives, and soil particles, as well as the quantitative relationships among them to further optimize performance of cured soil and reduce cost.

- (4) More practical experience is still lacking in the engineering implementation of bio-enzyme. Bio-enzyme holds potential for enhancing various soils encountered in engineering, yet their application in civil engineering remains relatively nascent overall.

And some engineering applications of bio-enzymes for road surface treatment serve merely as demonstrations. Therefore, to truly expand the application of bio-enzyme on a large scale, a systematic and in-depth study of the long-term durability of bio-enzyme treated road under the coupled effects of structural loads and environmental factors is necessary. Moreover, establishing corresponding structural design theories and methods is also crucial, and it is imperative to develop unified technical specifications and performance testing standards as soon as possible.

8. Conclusion

The application of bio-enzyme in environmental geotechnics has shown great potential, yet there remains a distinct lack of the systematic organization of available findings. This work is an attempt to review the development of bio-enzyme technology, and the key points are summarized as follows:

- (1) Bio-enzymes represent an emerging class of natural, organic, and biodegradable soil improvement materials derived from plant fermentation/animal extraction. They typically comprise surfactants, proteins, and other proprietary components, and are devoid of negative environmental impacts. By virtue of mechanisms such as cation exchange, specific binding, and surfactants, bio-enzymes could diminish the net negative charge on soil particle surfaces, thereby reducing both the electric double layer thickness and water affinity to contribute to soil enhancement.
- (2) Bio-enzymes could modify the engineering properties of soil, generally yielding favorable effects. They could reduce the liquid-plastic limit and the OMC, while enhance the MDD, UCS, and CBR. However, the improvement performance depends on the soil properties, enzyme dosage, specificity, and sample preparation, among other factors.
- (3) Bio-enzymes could be synergized with other stabilizers for soil improvement, and these bio-enzymatic composites might more considerably improve the physical and mechanical properties as well as other geotechnical characteristics of soils, and enhance the modification efficiency.
- (4) The engineering applications of bio-enzyme have demonstrated its capacity to mitigate common pavement diseases, decrease maintenance expenses, and enhance construction efficiency.

CRedit authorship contribution statement

Yuhao Li: Writing – original draft, Validation, Formal analysis, Data curation. **Xiangwei Fang:** Writing – review & editing, Validation, Supervision, Project administration, Funding acquisition, Conceptualization. **Chunni Shen:** Validation, Supervision, Conceptualization. **Wenchen Jiang:** Writing – review & editing, Investigation. **Sheng Huang:** Writing – review & editing, Investigation. **Guoliang Ma:** Writing – review & editing, Validation.

Declaration of Competing Interest

Wenchen Jiang and Sheng Huang are currently employed by Shanghai Municipal Engineering Design Institute (Group) Co., Ltd. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by the Fundamental Research Funds for the Central Universities (No. 2023CDJKYJH105), Chongqing Talent Innovation and Entrepreneurship Demonstration Team Projects (No. cstc2024ycjh-bgzxm0012), Scientific Research Project of Shanghai Municipal Engineering Design Institute (Group) Co., Ltd (No. K2023K124A), and Chongqing Construction Science and Technology Plan Project (No. City Science-2024-2-2).

References

- Huang, J., Kogbara, R. B., Hariharan, N., Masad, E. A., & Little, D. N. (2021). A state-of-the-art review of polymers used in soil stabilization. *Construction and Building Materials*, 305, Article 124685. <https://doi.org/10.1016/j.conbuildmat.2021.124685>
- Aamir, M., Mahmood, Z., Nisar, A., Farid, A., & Khan, T. A. (2019). Performance evaluation of sustainable soil stabilization process using waste materials. *Processes*, 7(6), 378. <https://doi.org/10.3390/pr7060378>
- Shalchian, M. M., & Arabani, M. (2022). A Review of soil reinforcement with planetary fibers. *Journal of Soil Science and Plant Nutrition*, 22(4), 4496–4532. <https://doi.org/10.1007/s42729-022-01052-y>
- Onyejekwe, S., & Ghataora, G. S. (2015). Soil stabilization using proprietary liquid chemical stabilizers: Sulphonated oil and a polymer. *Bulletin of Engineering Geology and the Environment*, 74(2), 651–665. <https://doi.org/10.1007/s10064-014-0667-8>
- Arabani, M., & Shalchian, M. M. (2022). A review of the use of bio-based substances in soil stabilization. *Environment, development and sustainability*, 1–53. <https://doi.org/10.1007/s10668-023-03241-w>
- Verma, H., Ray, A., Rai, R., Gupta, T., & Mehta, N. (2021). Ground improvement using chemical methods: A review. *Heliyon*, 7(7), Article e07678. <https://doi.org/10.1016/j.heliyon.2021.e07678>
- Choi, S. G., Wang, K., & Chu, J. (2016). Properties of biocemented, fiber reinforced sand. *Construction and building materials*, 120, 623–629. <https://doi.org/10.1680/jgrim.19.00023>
- Lim, A., Atmaja, P. C., & Rustiani, S. (2020). Bio-mediated soil improvement of loose sand with fungus. *Journal of Rock Mechanics and Geotechnical Engineering*, 12(1), 180–187. <https://doi.org/10.1016/j.jrmge.2019.09.004>
- Wang, Y., Soga, K., Dejong, J. T., & Kabla, A. J. (2018a). A microfluidic chip and its use in characterising the particle-scale behaviour of Microbial-Induced Carbonate Precipitation (MICP). *Géotechnique*, 69(12), 1086–1094. <https://doi.org/10.1680/jgeot.18.p.031>
- Wang, Z., Zhang, N., Jin, Y., Li, Q., & Xu, J. (2020). Application of microbially induced calcium carbonate precipitation (MICP) in sand embankments for scouring/erosion control. *Marine Georesources and Geotechnology*, 39(12), 1459–1471. <https://doi.org/10.1080/1064119X.2020.1850949>
- Liu, J. L., Hou, T. S., Luo, Y. S., & Cui, Y. X. (2020). Experimental study on unconsolidated undrained shear strength characteristics of synthetic cotton fiber reinforced soil. *Geotechnical and Geological Engineering*, 38(2), 1773–1783. <https://doi.org/10.1007/s10706-019-01129-z>
- Wang, Y., Konstantinou, C., Tang, S., & Chen, H. (2023a). Applications of microbial-induced carbonate precipitation: A state-of-the-art review. *Biogeotechnics* Article 100008. <https://doi.org/10.1016/j.bjgtech.2023.100008>
- Sen, J., & Singh, J. (2015). Stabilization of black cotton soil using bio-enzyme for a highway material. *International Journal of Innovative Research in Science, Engineering and Technology*, 4(12), 12453–12459. <https://doi.org/10.15680/IJRSET.2015.0411146>
- Saini, V., & Vaishnav, P. (2015). Soil stabilization by using terrazyme. *International Journal of Advances in Engineering Technology*, 8(4), 566–573.
- Tingle, J., Newman, J., Larson, S., Weiss, C. A., & Rushing, J. F. (2007). Stabilization mechanisms of nontraditional additives. *Transportation Research Record Journal of the Transportation Research Board*, 1989, 59–67. <https://doi.org/10.3141/1989-49>
- Samuel, A., Fidelis, O., & Umoh, U. (2016). Potentials of processed termite as a stabilizing agent in clay soil. *Journal of Mechanical and Civil Engineering*, 13(04), 40–50. <https://doi.org/10.9790/1684-1304014050>
- Wood, T. G. (1988). Termites and the soil environment. *Biology and Fertility of Soils*, 6(03), 228–236. <https://doi.org/10.1007/bf00260819>
- Garniersillam, E., Toutain, F., Villemin, G., & Renoux, J. (1988). Transformation of vegetable organic material under the action of the termite *Macrotermes mulleri* (sjosstedt) and its symbiotic fungus. *Canadian Journal of Microbiology*, 34(11), 1247–1255. <https://doi.org/10.1139/m88-219>
- Garniersillam, E., Renoux, J., & Toutain, F. (1989). The humic complexes of the termites *Thoracotermes macrothorax* (soil-feeder) and *Macrotermes mulleri* (fungus-grower). *Soil Biology and Biochemistry*, 21(04), 499–505. [https://doi.org/10.1016/0038-0717\(89\)90121-1](https://doi.org/10.1016/0038-0717(89)90121-1)
- Lopezherandez, D., Nino, M., Nannipieri, P., & Fardeau, J. C. (1989). Phosphatase activity in *Nasutitermes-ephrate* termite nests. *Biology and Fertility of Soils*, 7(02), 134–137. <https://doi.org/10.1007/BF00292571>
- Ravi, S. A. U., Kumar, R. H., & Ramrsha, M. I. (2009). Bio-enzyme stabilized lateritic soil as a highway materia. *Journal of the Indian Roads Congress*, 70(2), 143–151.
- Puneet, A., & Sunnet, K. (2014). Effect of bio-enzyme stabilization on unconfined compressive strength of expansive soil. *International Journal of Research in Engineering and Technology*, 03(5), 30–33. <https://doi.org/10.15623/ijret.2014.0305007>
- Ren, H. X., Wen, C. P., & Chen, X. (2024). Research on the dynamic elastic modulus and damping ratio of silty soil improved by bioenzyme. *Geotechnical and Geological Engineering*, 42(2), 1505–1518. <https://doi.org/10.1007/s10706-023-02632-0>
- Bajpai, P. (2014). Non-conventional soil stabilization techniques the way forward to an aggregate free pavement and a Cost effective method of road construction. *International Journal of Scientific Engineering Research*, 5(6), 1063–1066.
- Rajorial, V., & Kaur, S. (2014). A review on stabilization of soil using bio-enzyme. *International Journal of Research in Engineering and Technology*, 3(1), 75–78. <https://doi.org/10.15623/ijret.2014.0301011>
- Jang, J. (2020). A review of the application of biopolymers on geotechnical engineering and the strengthening mechanisms between typical biopolymers and soils. *Advances in Materials Science and Engineering*, 2020, Article 1465709. <https://doi.org/10.1155/2020/1465709>
- Ramdas, V. M., Mandree, P., Mgangira, M., Mukaratirwa, S., Lalloo, R., & Ramchuran, S. (2021). Review of current and future bio-based stabilisation products (enzymatic and polymeric) for road construction materials. *Transportation Geotechnics*, 27, Article 100458. <https://doi.org/10.1016/j.trgeo.2020.100458>
- Pooni, J. S., Robert, D. J., Gunasekara, C., Giustozzi, F., & Setunge, S. (2021). Mechanism of enzyme stabilization for expansive soils using mechanical and microstructural investigation. *International Journal of Geomechanics*, 21(10), Article 04021191. [https://doi.org/10.1061/\(ASCE\)GM.1943-5622.0002164](https://doi.org/10.1061/(ASCE)GM.1943-5622.0002164)
- Renjith, R., Robert, D. J., Gunasekara, C., Setunge, S., & O'Donnell, B. (2020). Optimization of enzyme-based soil stabilization. *Journal of materials in civil engineering*, 32(5), 1–12. [https://doi.org/10.1061/\(ASCE\)MT.1943-5533.0003124](https://doi.org/10.1061/(ASCE)MT.1943-5533.0003124)
- Khan, T. A., Taha, M. R., & Wang, H. (2015). Effect of three bioenzymes on compaction, consistency limits, and strength characteristics of a sedimentary residual soil. *Advances in materials science and engineering*, 2015, 1–9. <https://doi.org/10.1155/2015/798965>
- Eujine, G. N., Chandrakaran, S., & Sankar, N. (2017a). Accelerated subgrade stabilization using enzymatic lime technique. *Journal of Materials in Civil Engineering*, 29(9), Article 04017085. <https://ascelibrary.org/doi/abs/10.1061/%28ASCE%29MT.1943-5533.0001923%28>
- Chandler, N., Palson, J., & Burns, T. (2017). Capillary rise experiment to assess effectiveness of an enzyme soil stabilizer. *Canadian geotechnical journal*, 54(10), 1509–1517. <https://doi.org/10.1139/cgj-2016-0511>
- Mgangira, M. B. (2009). Evaluation of the effects of enzyme-based liquid chemical stabilizers on subgrade soils. *28th Southern African Transport Conference*, 192–198. <https://www.researchgate.net/publication/30511903>
- AbouKhadra, A., Zidan, A. F., Gaber, Y., & Villalobos, F. (2018). Experimental evaluation of strength characteristics of different Egyptian soils using enzymatic stabilizers. *Cogent Engineering*, 5(1), 1517577. <https://doi.org/10.1080/23311916.2018.1517577>
- Kushwaha, S. S., Kishan, D., & Dindorkar, N. (2018). Stabilization of expansive soil using eko soil enzyme for highway embankment. *Materials Today: Proceedings*, 5(9), 19667–19679. <https://doi.org/10.1016/j.matpr.2018.06.329>
- Pooni, J., Giustozzi, F., Robert, D., Setunge, S., & O'Donnell, B. (2019). Durability of enzyme stabilized expansive soil in road pavements subjected to moisture degradation. *Transportation Geotechnics*, 21, Article 100255. <https://doi.org/10.1016/j.trgeo.2019.100255>
- Mekonnen, E., Kebede, A., Tafesse, T., & Tafesse, M. (2020). Application of microbial bioenzymes in soil stabilization. *International journal of microbiology*, 2020, Article 1725482. <https://doi.org/10.1155/2020/1725482>
- Rauch, A. F., Harmon, J. S., Katz, L. E., & Liljestrand, H. M. (2002). Measured effects of liquid soil stabilizers on engineering properties of clay. *Transportation Research Record*, 1787(1), 33–41. <https://doi.org/10.3141/1787-04>
- Ganapathy, G. P., Gobinath, R., Akinwumi, I. I., Kovendiran, S., Thangaraj, M., Lokesh, N., et al. (2016). Bioenzymatic stabilization of a soil with poor engineering properties. *International Journal of Civil Engineering*, 15(3), 401–409. <https://doi.org/10.1007/s40999-016-0056-8>
- Thomas, A., Tripathi, R. K., & Yadu, L. K. (2018). A laboratory investigation of soil stabilization using enzyme and alkali-activated ground granulated blast-furnace slag. *Arabian Journal for Science and Engineering*, 43(10), 5193–5202. <https://doi.org/10.1007/s13369-017-3033-x>
- Scholen D.E. (1995). Stabilizer mechanisms in nonstandard stabilizers. Proceedings of the Sixth International Conference Low-Volume Roads, II, 252–260, Minneapolis, MN, USA, June 1995.
- Dandin S., Hiremath S. (2014). A study on some geotechnical properties of bio-enzyme stabilized expansive soil. Proceedings of Indian Geotechnical Conference IGC-2014 December 18-20, Kakinada, India. <https://www.researchgate.net/publication/343685537>
- Yilmaz, Y., Gungor, A. G., Avsa, C., Tutumluer, E., & Al-Qadi, I. (2009). Stabilization of clays using liquid enzymes. *Bearing Capacity of Roads, Railways and Airfields: Proceedings of the 8th International Conference: Urbana-Champaign*, 65–69. <https://doi.org/10.1201/9780203865286.ch8>
- Panchal, S., Khan, M. M., & Sharma, A. (2017). Stabilization of soil using bio-enzyme. *International Journal of civil engineering and technology*, 8(1), 234–237. <http://www.iaeme.com/IJCIET/issues.asp?JType=IJCIET&VType=8&IType=1>.

- Shaka, P., & Rakaraddi, P. G. (2016). Experimental study on the effect of bio-enzyme stabilization on black cotton soils and red soil. *International Journal of Innovative Research in Science, Engineering and Technology*, 5(08), 15378–15386. <https://doi.org/10.15680/IJRSET.2016.0508149>
- Tiwari, A., Sharma, J. K., & Garg, V. (2021). *Stabilization of expansive soil using Terrazyme*. Singapore: Springer113–125. <https://doi.org/10.1007/978-981-33-6444-8>
- Usha, P., Shalini, S., & Shivani, C. (2018). Improvement of strength characteristics of bio enzyme terrazyme treated expansive soil by gyosum as an additive. *International Journal of Advance Research and Innovative Ideas in Education*, 4(02), 1005–1012.
- Marathe, S., & Shankar, A. U. R. (2023). Investigations on bio-enzyme stabilized pavement subgrades of lateritic, lithomargic and blended soils. *International Journal of Pavement Research and Technology*, 16(1), 15–25. <https://doi.org/10.1007/s42947-021-00107-0>
- Bergmann. (2000). Soil Stabilizers on Universally Accessible Trails. USDA Services of Forest, San Dimas Development and Technology center.
- Chaurasia V.S., Pandey P.P., Mishra A.V., Gupta S.S., Pawar A.U. (2021). Stabilization of soil using terrazyme for road construction. Proceedings of the Indian Geotechnical Conference 2019: IGC-2019 vol III. Springer Singapore, 2021: 671-683. <https://doi.org/10.1007/978-981-33-6444-8>.
- Taha, M. R., Khan, T. A., Jawad, I. T., & Firoozi, A. (2013). Recent experimental studies in soil stabilization with bio-enzymes-A review. *Electronic Journal of Geotechnical Engineering*, 18, 3881–3894.
- Divya, V., & Asha, M. N. (2023). Microstructural studies on enzyme-modified lateritic subgrade. *International Journal of Pavement Research and Technology*, 16(2), 356–369. <https://doi.org/10.1007/S42947-021-00136-9>
- Thomas, A. G., & Rangaswamy, B. K. (2019). Strength behavior of enzymatic cement treated clay. *International Journal of Geotechnical Engineering*, 15(3), 259–272. <https://doi.org/10.1080/19386362.2019.1622854>
- Velasquez, R., Marasteanu, M. O., Hozalski, R., & Clyne, T. (2005). *Preliminary Laboratory Investigation of Enzyme Solutions as a Soil Stabilizer*. Minneapolis, USA: University of Minnesota.
- Venkatasubramanian, C., & Dhinakaran, G. (2011). Effect of bio-enzymatic soil stabilization on unconfined compressive strength and california bearing ratio. *Journal of Engineering and Applied Sciences*, 6(05), 295–298. <https://doi.org/10.3923/jeasci.2011.295.298>
- Peng, H., Su, H., Zhang, X., & Wang, J. (2011). An experimental comparison of compressive strengths of soils stabilized with enzyme and ground quicklime. *International Conference of Green Building Materials and Energy-saving Construction*, 280, 9–12. <https://doi.org/10.4028/www.scientific.net/AMR.280.9>
- Eujine, G. N., Chandrakaran, S., & Sankar, N. (2017b). Influence of enzymatic lime on clay mineral behavior. *Arabian Journal of Geosciences*, 10(20), 1–8. <https://doi.org/10.1007/s12517-017-3238-z>
- Choudalakis, G., & Gotsis, A. D. (2011). Permeability of polymer/clay nanocomposites. A review. *European Polymer Journal*, 1312(4), 967–984. <https://doi.org/10.1557/opl.2011.482>
- Kestler M.A. (2009). Stabilization selection guide for aggregate and native-surfaced low roads. United States Department of Agriculture, Washington, DC.
- Anagnostopoulos, C. A. (2015). Strength properties of an epoxy resin and cement-stabilized silty clay soil. *Appl Clay Science*, 114, 517–529. <https://doi.org/10.1016/j.clay.2015.07.007>
- Wang, X., Tao, J., Bao, R., Tran, T., & Tucker-Kulesza, S. (2018b). Surficial soil stabilization against water-induced erosion using polymer-modified microbially induced carbonate precipitation. *Journal of Materials in Civil Engineering*, 30(10), Article 04018267. [https://doi.org/10.1061/\(ASCE\)JMT.1943-5533.0002490](https://doi.org/10.1061/(ASCE)JMT.1943-5533.0002490)
- Iamchaturapatr, J., Piriyaikul, K., & Petcherdchoo, A. (2022). Characteristics of sandy soil treated using EICP-based urease enzymatic acceleration method and natural hemp fibers. *Case Studies in Construction Materials*, 16, Article e00871. <https://doi.org/10.1016/j.cscm.2022.e00871>
- He, J., Huang, A., Ji, J., Qu, S., & Hang, L. (2023). Enzyme induced carbonate precipitation with fibers for the improvement of clay soil slopes against rainfall and surface runoff erosions. *Transportation Geotechnics*, 42, Article 101074. <https://doi.org/10.1016/j.trgeo.2023.101074>
- Pandey G. (2018). Feasibility study of water based/polymer modified EICP for soil improvement involving recycled glass aggregate. Master's thesis, University of Akron.
- Almajed, A., Lemboye, K., Arab, M. G., & Alnuaim, A. (2020). Mitigating wind erosion of sand using biopolymer-assisted EICP technique. *Soils and Foundations*, 60(2), 356–371. <https://doi.org/10.1016/j.sandf.2020.02.011>
- Miao, L., Wu, L., Sun, X., Li, X., & Zhang, J. (2020). Method for solidifying desert sands with enzyme-catalysed mineralization. *Land Degradation Development*, 31(11), 1317–1324. <https://doi.org/10.1002/ldr.3499>
- Sun, X., Miao, L., Yuan, J., Wang, H., & Wu, L. (2021). Application of enzymatic calcification for dust control and rainfall erosion resistance improvement. *Science of Total Environment*, 759, Article 143468. <https://doi.org/10.1016/j.scitotenv.2020.143468>
- Sun, X., Miao, L., Wang, H., Chen, R., & Wu, L. (2022). Bio-cementation for the mitigation of surface erosion in loess slopes based on simulation experiment. *Journal of Soil and Sediments*, 22(6), 1804–1818. <https://doi.org/10.1007/s11368-022-03190-3>
- Wang, H., Miao, L., Sun, X., Wu, L., Fan, G., & Zhang, J. (2023b). The use of N-(N-butyl)-thiophosphoric triamide to improve the efficiency of enzyme induced carbonate precipitation at high temperature. *Acta Geotechnica*, 18(9), 5063–5087. <https://doi.org/10.1007/s11440-023-01864-x>
- Wang, Y., Sun, X., Miao, L., Wang, H., Wu, L., Shi, W., & Kawasaki, S. (2023c). State-of-the-art review of soil erosion control by MICP and EICP techniques: Problems, applications, and prospects. *Science of the Total Environment*, 912, Article 169016. <https://doi.org/10.1016/j.scitotenv.2023.169016>
- Annadurai, R. (2024). Synergistic effect of polyvinyl acetate (PVA) and enzyme-induced carbonate precipitation (EICP) on the mechanical properties of natural sands. *Case Studies in Construction Materials*, 20, Article e03323. <https://doi.org/10.1016/j.cscm.2024.e03323>
- Baig, A. R., Alarif, S. A., Murtaza, M., Kamal, M. S., Mahmoud, M., AlAhmari, M. M., & Humam, A. (2024). Assessing the viability of different bio-polymers and synthetic-copolymers with modified enzyme-induced carbonate precipitation solutions for sand consolidation applications. *Journal of Petroleum Exploration and Production Technology*, 1–17. <https://doi.org/10.1007/s13202-024-01862-z>
- Thomas, G., & Rangaswamy, K. (2020). Dynamic soil properties of nanoparticles and bioenzyme treated soft clay. *Soil Dynamics and Earthquake Engineering*, 137, Article 106324. <https://doi.org/10.1016/j.soildyn.2020.106324>
- Chitragar, S. F., Shivayogimath, C. B., & Mulangi, R. H. (2019). Study on strength and volume change behavior of expansive soil using non-traditional (bio-enzyme) and traditional (lime and bagasse ash) stabilizers. *Lecture Notes in Civil Engineering*, 29(2019), 587–594. https://doi.org/10.1007/978-981-13-6713-7_46
- Eujine, G. N., Chandrakaran, S., & Sankar, N. (2017c). The engineering behaviour of enzymatic lime stabilised soils. *Proceedings of the Institution Civil Engineers: Ground improvement*, 170(1), 1–11. <https://doi.org/10.1680/jgrim.16.00014>
- Jairaj, C., Prathap, K. M. T., & Muralidhara, H. (2019). Shear strength of bc-soil admixed with lime and bio-enzyme. *Materials Science Forum*, 969, 327–334. <https://doi.org/10.4028/www.scientific.net/MSF.969.327>
- Parik, P., & Patra, N. R. (2023). Applicability of clay soil stabilized with red mud, bioenzyme, and red mud-bioenzyme as a subgrade material in pavement. *Journal of Hazardous, Toxic and Radioactive Waste*, 27(2), Article 04023003. <https://doi.org/10.1061/JHTRBP.HZENG-1182>
- Refai, M., Arab, M.G., & Omar, M. (2020). Sandy soil improvement through biopolymer assisted EICP. Geo-Congress 2020: Foundations, Soil Improvement, and Erosion (GSP 315), 315, 612–619. <https://doi.org/10.1061/9780784482780.060>
- Chen, X. L. (2007). *Research on application of TerraZyme soil stabilization technology in rural road*. China: Hunan University.
- Sedgwick J. (2003). *Soil Stabilization Technology for Superior Roads*. New York: NATURALPIUS, Inc, 2003, 10-13.
- Moloisane, R. J., & Visser, A. T. (2014). Evaluation of the strength behaviour of unpaved road material treated with electrochemical-based non-traditional soil stabilisation additives. *Journal of the South African Institution of Civil Engineering*, 56(1), 28–39. [https://doi.org/10.1061/\(ASCE\)HY.1943-7900.0000834](https://doi.org/10.1061/(ASCE)HY.1943-7900.0000834)
- Lahalih, S. M., & Ahmed, N. (1998). Effect of new soil stabilizers on the compressive strength of dune sand. *Construction and Building Materials*, 12(6-7), 321–328. [https://doi.org/10.1016/s0950-0618\(98\)00024-5](https://doi.org/10.1016/s0950-0618(98)00024-5)
- Das, S. K., & Varma, A. (2010). Role of enzymes in maintaining soil health. *Soil Biology*, 22, 25–42. https://doi.org/10.1007/978-3-642-14225-3_2
- Shafii, I., Shidlovskaya, A., & Briaud, J. L. (2019). Investigation into the effect of enzymes on the erodibility of a low-plasticity silt and a silty sand by EFA testing. *Journal of Geotechnical Geoenvironmental Engineering*, 145(3), [https://doi.org/10.1061/\(ASCE\)GT.1943-5606.0002019](https://doi.org/10.1061/(ASCE)GT.1943-5606.0002019)