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Enhancement of Engineering Mechanical Properties and Soil Stabilization Using Novel Bio-enzymatic Composites

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ABSTRACT

This review paper discusses biomimetic bio-enzymatic composites as eco-friendly, innovative solutions for soil stabilization and geotechnical engineering. Enzymes from microorganisms, plants, and organic waste are attractive for their sustainability and catalytic properties. Microbial enzymes such as urease and phosphatase from species like Sporosarcinapasteurii, Bacillus, and Pseudomonas improve soil strength by mineralizing organic matter and binding particles. Plant-derived enzymes, including amylases, cellulases, pectinases, proteases, peroxidases, and polyphenol oxidases, promote soil aggregation and organic matter transformation. These enzymes are integrated into engineered composites that mimic natural processes, combining soil with natural polymers, mineral additives like nano-silica and clays, and industrial by-products such as fly ash and GGBS. Such composites enhance soil structure, compaction, durability, and load capacity through enzyme-driven bonding and microbial mineralization. Recent advances include bio-enzyme clay mineral and bio-enzyme nanomaterial systems that improve catalytic stability via enzyme immobilization. Green synthesis methods using plant capping agents are also explored for eco-friendly hybrid systems, where antioxidative enzymes facilitate oxidative coupling, creating cross-linked organic–mineral networks that strengthen bonding and enhance erosion resistance. Biomimetic bio-enzymatic composites thus offer a sustainable alternative to conventional soil stabilizers, minimizing environmental impact, enhancing engineering performance, and supporting green, resilient infrastructure. This review covers sources, mechanisms, material design strategies, emerging applications, current challenges, and future research directions for large-scale use.

Keywords: *biomimetic, bio-enzymatic composites, soil stabilization, geotechnical engineering, sustainable alternative, soil stabilizers.*

Introduction

1.1 Bio-enzymatic: An alternative to conventional stabilizers

The bio-enzymatic system involves stabilization (strengthening) methods that use enzymes (biological catalysts) of biological origin, primarily from microbial (small organisms such as bacteria or fungi) or plant sources, to induce physicochemical (physical and chemical) and structural changes in materials such as soil. The term "novel bio-enzymatic" refers to innovations beyond traditional enzyme-based products and established ideas, including new enzyme systems, enzymes derived from unconventional, sustainable sources such as agro-industrial waste, and formulations with enhanced catalytic activity (greater ability to speed up reactions). Innovation may also come from the first-time application of bio-enzymatic systems to specific soil types, geotechnical conditions (different earth material challenges), or engineering environments, as well as from novel production methods or previously unreported stabilization mechanisms. Essentially, these approaches represent newly developed or uniquely applied enzyme-based strategies to improve soil stabilization (making soil more suitable for construction) [1]. These novel bio-enzymes are new materials that use enzymes from biological sources to induce beneficial changes or that employ specially designed enzymatic systems to sustainably enhance material properties. They serve as eco-friendly alternatives to conventional (chemical) stabilizers.

1.2 Bio-enzymes and their natural biological sources

microbial sources include bacteria such as *Sporosarcinapasteurii*, Bacillus species, and Pseudomonas, produced enzymes such as urease and phosphatase. Plant-based bio-enzymes are produced in various tissues such as seeds, roots, leaves, fruits, and latex, and are relevant to soil stabilization, organic matter transformation, and environmental applications. These enzymes participate in metabolic processes, cell wall remodeling, defense responses, and stress adaptation in plants [2-3]. Reported plant-derived enzymes include amylases (from germinating seeds), cellulases and hemicellulases (from roots, stems, and decaying biomass), pectinases (from fruits), and proteases (from papaya and pineapple). Peroxidases, polyphenol oxidases, and laccase-like enzymes are also common and involved in lignin modification and phenolic compound transformation.

Some bio-enzymes are produced by fermenting organic materials such as fruit and vegetable residues from agricultural and kitchen waste [4]. Common wastes include orange and lemon peels, banana and pineapple peels, papaya waste, and vegetable scraps like cabbage and spinach leaves.

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These materials ferment with specific microorganisms, along with jaggery (or sugar) and water, to create enzyme-rich solutions. Such fruit waste-based bio-enzymatic solutions act as natural bio-stimulants, promoting plant growth and enhancing soil fertility, and are widely used for soil improvement. Mixed kitchen waste fermentation yields multi-enzyme solutions relevant to agriculture, wastewater treatment, and eco-friendly soil stabilization, and these solutions are effective at degrading pollutants, reducing odor, and replacing harmful cleaning chemicals. Their use extends to composting, waste management, and bioremediation, highlighting their cost-effective, sustainable nature. Diverse sources thus support the development of eco-friendly bio-enzymatic systems for engineering and environmental applications. Recent studies also note the role of plant root exudate-associated enzymes in soil aggregation, organic matter decomposition, and rhizosphere stabilization [5].

1.3 Biomimetic Bio-Enzymatic Composites Inspired by Rhizosphere Processes

The Biomimetic Bio-Enzymatic Composites system is an advanced research platform. In this system, engineered composite materials mimic natural biological processes [6]. Enzyme-based substances are incorporated to enhance the soil's aggregation, sustainability, performance, and functionality. These mimic natural mechanisms such as microbial mineralization and enzyme-catalyzed bonding of the soil (Table 1).

Table 1: List of Biomimetic Bio-Enzymatic Composites, their features, and applications

S.No.	Engineered Composite Materials			
	Name	Examples	Composition	Key features and Applications
1	Cementitious Composites	Fly ash, blended concrete of Ground Granulated Blast Furnace Slag (GGBFS)	Cement: fly ash: slag ratio (supplementary materials)	Reduced carbon footprint Improved durability Soil stabilization and provide concrete structures
2	Fiber Reinforced Composites (FRC)	Glass-fiber reinforced polymer (GFRP) Carbon-fiber reinforced polymer (CFRP)	Polymer matrix: reinforcing fibers ratio	Provides high-strength and structural strengthening in Construction aerospace work
3	Polymer Matrix Composites	Composites of epoxy: nano-clay material	Polymer resin: nano-fillers ratio	Enhanced mechanical and barrier properties, use in Coatings, packaging, and construction-related works
4	Bio-Enzymatic Composites	Composition of Soil and organic waste-bio-enzyme with lime or fly ash	Composition of Soil and organic waste-bio-enzyme with lime or fly ash	Eco-friendly alternative to cement, Enzyme-treated soil systems promote soil stabilization, Improved compaction Reduced permeability. Use in road construction
5	Nano-Composites	Nano-silica reinforced cement	Ratio of Matrix: nanoparticles	High-performance concrete material. Increased strength and microstructural refinement
6	Hybrid Bio-Nano Composites	Ratio of Bio-enzyme: nano-clay particles: soil	Ratio of Bio-enzyme: nano-clay particles: soil	Advanced geotechnical engineering system having very high catalytic interactions. It showed biomimetic action, providing multi-scale strengthening

This integrated nutrient management system is essential for the sustainable growth and health of the microbial rhizosphere, supporting long-term soil fertility and agricultural productivity. The bio-enzymatic composites, which act as bioorganic fertilizers, control the rhizosphere microbiome by improving the growth of beneficial bacteria (such as Sphingomonas, Gemmatimonas, and Flavobacterium) and reducing the abundance of harmful organisms. Along with bio-enzymatic composites, nanoparticle composites (bio-SiNPs) are also being used in the Biomimetic Bio-Enzymatic Composites system. These nanoparticle composites modulate the rhizosphere microbial community and improve the tolerance of plants to heavy metals (Cd, Zn, Arsenic). Researchers are using these composites as rhizo-ligands/surfactants or materials that mimic natural interactions with plant roots and their exudates. These rhizo-ligand surfactants form stable organo-mineral associations that improve water retention and reduce mucilage swelling. It acts as a "gluing agent" that stabilizes soil aggregates, reducing erosion and enhancing structure, and makes thicker, stable rhizo-sheaths, on which soil is attached to roots. Overall, such systems play an important role in the formation of stable organo-mineral associations and provide long-term soil and rhizospheric stability.

2. Novel Bio-Enzymatic Composites and their Innate Role in Soil Stabilization and Enhancement of Engineering Mechanical Properties

Along with an integrated nutrient management system for sustainable growth, agricultural productivity, and increased soil fertility, these bio-enzymes also influence the engineering properties of soil and improve soil strength through enzyme-catalytic biochemical reactions that drive biomineralization. This reaction promotes cation exchange, reduces the thickness of the electric double layer, and lowers surface tension, thereby improving the binding of soil particles.

As a result, these composites significantly enhance compaction, shear strength, and durability while reducing permeability and swelling, particularly in expansive soils. From a commercial point of view, the microbial bio-enzymes initially reported are Terrazyme, Permazyme, Renolith, Fujibeton, etc., obtained from bacteria such as *Sporosarcinapasteurii, Bacillus paramycoides, Pseudomonas*, and *Citrobacter sedlakii*. Now these days along with these commercial bioenzymes, some natural bioenzymes like amylases cellulases, hemicellulases, pectinases, proteases, urease, phosphatase, peroxidases (POD), polyphenol oxidases (PPO), laccase-like enzymes etc. have been extensively extracted from in plants/ plant roots/ stems, germinating seeds of cereals like barley, wheat, and rice, fruit tissues of citrus, apple, banana, and papaya along with common microbial sources. Although plant-derived enzymes are generally less stable and harder to extract in high yields than microbial enzymes, their biodegradability, low toxicity, and compatibility with natural soil systems make them attractive for sustainable and eco-friendly applications. Consequently, plant-based bio-enzymes used directly, extracted, or integrated into bio-enzymatic composites, represent a promising complementary approach to microbial enzymes for green soil stabilization and sustainable geotechnical engineering.

Novel bio-enzymatic composites represent an advanced class of soil stabilization systems in which bio-enzymes are integrated with additional functional materials to achieve enhanced engineering performance. These composites are multi-component formulations where enzymes are combined with natural polymers (such as polysaccharides, lignin, or plant gums), mineral additives (including clay modifiers, nano-silica, or industrial by-products like fly ash), organic binders, microbial metabolites, or fiber and biopolymer reinforcements. The term composite signifies that the stabilization effect arises not solely from enzymatic activity but from synergistic interactions between enzymes and accompanying materials, leading to improved interparticle bonding, soil structure modification, and mechanical strength. The designation novel indicates newly developed or previously unreported enzyme–additive combinations, innovative interaction mechanisms, or optimized formulations that offer superior soil stabilization performance compared with conventional bio-enzymatic systems. In essence, novel bio-enzymatic composites are newly engineered, multi-material, enzyme-based systems designed to improve the efficiency and durability of soil stabilization. Novel bio-enzymatic composites stabilize soil properties by using enzymes derived from plant extracts or microorganisms and are considered alternatives to conventional stabilizers such as lime and cement. Advanced Novel bio-enzymatic composite techniques include Enzyme-Induced Carbonate Precipitation (EICP) and Microbial-Induced Carbonate Precipitation (MICP) methods, which improve soil strength through biomineralization by forming calcium carbonate bonds. Recently developed novel bio-enzymatic composites and hydrogel-assisted EICP using polyacrylic acid enhance soil stabilization efficiency and minimize the formation of harmful byproducts. These biopolymers help form gel-like matrices that increase soil particle cohesion, decrease permeability, and improve soil parameters such as Dry Density, Moisture Content, and over-swelling properties [7-8]. Due to the non-toxic, biodegradable nature and lower carbon emissions of such bio-enzymatic composites, their demand has increased sharply, and they are widely used for subgrade stabilization in road construction, especially in villages and rural areas. Future research focuses on developing custom-made, multifunctional bio-enzymatic systems appropriate for diverse soil conditions and on establishing standardized strategies and guidelines for their large-scale application.

Bio-enzymes - Clay Interactions: Soil Strengtheners and Engineering Performance

Bio-enzyme clay mineral composites represent an emerging and sustainable approach for soil stabilization in geotechnical engineering. In such systems, naturally derived bio-enzymes (i.e., organic catalysts obtained from microbial fermentation, plant extracts, or organic waste-based bioprocesses) interact with clay minerals such as montmorillonite, kaolinite, and bentonite to modify their physicochemical behavior and enhance engineering performance. Montmorillonite, kaolinite, and bentonite are naturally occurring components of soil and are present in varying proportions. Kaolinite is commonly found in normal, well-drained, and highly weathered soils and exhibits relatively stable behavior. In contrast, montmorillonite is typically present in clayey soils that undergo significant swelling and shrinkage [9]. Bentonite is a clay-rich soil predominantly composed of montmorillonite and is well known for its high swelling capacity. From a physicochemical perspective, these clay minerals differ significantly in their cation exchange behavior. Montmorillonite possesses a highly negative surface charge and a very high cation exchange capacity (CEC), enabling strong interactions with exchangeable cations such as Na^+ and Ca^{2+}, leading to pronounced swelling, especially under sodium-rich (saline) conditions. Kaolinite, on the other hand, has a low surface charge and a low CEC, resulting in limited cation interaction, low swelling, and greater stability. In contrast, bentonite, due to its high montmorillonite content, exhibits a very high negative charge and CEC, leading to strong cation interactions, extreme swelling, and high-water retention capacity. Clay minerals generally possess negatively charged surfaces; therefore, they predominantly interact with cations rather than anions. Consequently, they exhibit very low anion exchange capacity (AEC) and tend to repel anions such as Cl^- and SO_4^{2-}. These mineralogical characteristics govern key soil properties, including plasticity, water retention, and mechanical strength. Typical soil is a mixture of sand, silt, and clay (with these minerals present in varying amounts), whereas bentonite is a clay-dominant soil. Such clay-rich soils, particularly under saline conditions with high sodium content, become highly problematic due to increased swelling, dispersion, and instability. Therefore, stabilization with bio-enzymes is essential for improving soil performance and durability. Montmorillonite-rich soils exhibit strong interaction with bio-enzymes due to their high surface area and cation exchange capacity, leading to a significant reduction in swelling potential. In comparison, kaolinite shows relatively moderate improvement due to its lower surface reactivity. Bentonite, being highly expansive, demonstrates pronounced enhancement upon bio-enzyme treatment. Experimental studies consistently report reductions in the liquid limit and plasticity index, along with improved compaction characteristics, including increased maximum dry density and reduced optimum moisture content. Additionally, strength parameters, including unconfined compressive strength (UCS) and California Bearing Ratio (CBR), are significantly enhanced, indicating improved load-bearing capacity. The underlying mechanism involves bio-enzyme-induced modification of the adsorbed water layer and alteration of cation exchange behavior at the clay surface. Bio-enzymes reduce the thickness of the diffuse double layer and decrease the polarity of water molecules, thereby minimizing repulsive forces between clay particles. This promotes flocculation and aggregation, resulting in a denser and more stable soil matrix. Furthermore, bio-enzymes facilitate cation exchange, replacing weakly bound ions with stronger ones and enhancing interparticle bonding. The formation of stable organic–mineral complexes further improves soil structure and durability. Overall, bio-enzyme–clay mineral composites offer an eco-friendly, cost-effective, and efficient alternative to conventional chemical stabilizers, with significant potential for sustainable infrastructure development. In geotechnical engineering practice, bio-enzymes are applied either in laboratory-developed forms or as commercially available formulations. These naturally derived catalytic systems, produced through microbial or plant-based processes, are used to improve soil strength, reduce plasticity, and enhance compaction behavior. In field applications, they are commonly supplied as standardized liquid formulations such as TerraZyme and Perma-Zyme. These products are manufactured through controlled fermentation processes and are applied by mixing with soil and water during compaction [10]. Their action involves promoting cation exchange, reducing the diffuse double-layer thickness, and facilitating particle aggregation, which collectively improve load-bearing capacity, reduce moisture sensitivity, and enhance the durability of treated soils, particularly in clay-rich and expansive conditions.

2.2 Bio-enzymes Cementitious By-Product Composites: Soil Interaction Mechanisms and Civil Engineering Applications

Recent research is focused on how cementitious by-products like fly ash, ground granulated blast furnace slag (GGBS), and lime will be used to make a bio-enzymatic composite complex, which would be innovative and have a sustainable approach in soil stabilization and construction in material and civil engineering work, and useful to increase the engineering performance and environmental sustainability. This research approach focuses on how the system can utilize naturally derived enzymes that play key roles in soil interactions and the formation of cementitious by-product composites, which are important in the construction and maintenance of civil engineering works. Recent trend of research (2025-26) is mainly focused on hybrid bio-mediated systems (like: MICP (microbially induced carbonate precipitation), EICP(enzymatically induced carbonate precipitation), nano-modified binders, and low-carbon supplementary cementitious materials) in enzyme-assisted sustainable geotechnical engineering applications in conservation strategies for "Bio cleaning of heritage materials and structures [7].

2.3 Plant/ Microbial Derived Bio-enzymes Cementitious By-Product Composites and Their Applications in Civil and Geotechnical Engineering

From an application point of view, the details mentioned below are some important natural microbial and plant-derived enzymes that are quite important in enzyme–cementitious by-product composites:

2.3.1 Urease is a broadly dispersed enzyme obtained from microbial and plant sources, and it plays a crucial role in sustainable civil engineering applications. *Sporosarcinapasteurii, Bacillus subtilis,* and *Proteus vulgaris* are the most commonly used urease-producing bacteria. Jack bean (*Canavalia ensiformis*) and soybean are also well-studied plant-derived sources of urease, but they are limited to laboratory-scale use due to their lower stability. It is a nickel-dependent metalloenzyme that catalyzes the hydrolysis of urea to ammonia and carbon dioxide ($CO(NH_2)_2 + H_2O\quad 2NH_3 + CO_2$). It interacts with urea and calcium ions, and helps to induce precipitation of calcium carbonate (CaCO3) using the 'Microbially induced calcite precipitation (MICP) approach'. In this reaction, urease catalyzes the hydrolysis of urea, producing carbonate ions that react with calcium ions to form $CaCO_3$, which has wide utility in civil and geotechnical engineering due to its ability to bind to soil particles and fill pore spaces. This bio-enzyme-MICP approach may be use for Soil stabilization (foundations and subgrade improvement, in embankments specially in especially in sandy and silty soils areas via increasing shear strength, stiffness, and load-bearing capacity); Reduction of permeability via filling vacuums and pore channels, significantly reduced hydraulic conductivity (this principal may use in canal linings, earthen dams, and reservoirs to minimize water loss through seepage control); Stabilizing loose slopes and preventing landslides (i.e. Slope stabilization); Crack remediation in concrete (bio-cementation) where Urease-MICP approach use to precipitate $CaCO_3$ within cracks in concrete and effectively sealing them and enhancing the durability via self-healing concrete concept; this also form a thin $CaCO_3$ crust on the surface having loose particles (like unpaved roads), reduced dust emission and improving surface durability [11]. This is also used to improve flood-prone areas, where urease-induced mineralization decreases permeability and increases soil strength [12-13]. It improves resistance to water infiltration, erosion, and structural weakening during flooding conditions. From a mechanistic point of view, we can say that Urease catalyzes the hydrolysis of urea, producing carbonate ions (CO_3^{2-}), which react with Ca^{2+} ions to form $CaCO_3$, which acts as a binding or precipitating agent. They provide strength and densify the soil matrix. Overall, the urease-driven bio-mineralization process provides a cost-effective and eco-friendly alternative with wide applicability in sustainable civil engineering practices [14-15].

2.3.2 Protease, another important naturally derived bio-enzyme or biocatalyst, is typically obtained from plant extracts [e. g. papain (latex of papaya) and bromelain (latex of pineapple)], some selected species of microorganisms e.g. Bacillus, Aspergillus, and Pseudomonas via controlled microbial fermentation process, and from the fermented agricultural residues such as molasses, sugarcane waste, fruit peels, and oilseed cakes (i.e. organic waste materials), play a significant role in modifying soil behavior when incorporated into fly ash, slag, lime-based systems or other related cementitious by-product composites. It influences soil structure and interparticle bonding primarily through biochemical interactions with soil organic matter and clay-associated proteins, thereby enhancing soil sustainability for geotechnical applications [16]. During the enzyme-soil interaction mechanism, proteases catalyze the hydrolysis of proteinaceous materials into simpler amino acids and peptides, present in soil organic matter and on clay mineral surfaces, which leads to adsorbed organic coatings that increase water affinity and reduce particle bonding, decrease in diffuse double layer thickness, enhanced particle aggregation and flocculation, and finally improved interaction between soil particles and cementitious binders [17-18]. This enzymatic alteration makes a more favorable environment for pozzolanic reactions in cementitious by-products, refining the strength and durability of stabilized soils and cement-based composites/ or cementitious gels as C-A-H (Calcium Aluminate Hydrate: A secondary hydration product formed from alumina-rich materials, provide early strength and expands resistance to chemical attack) or C-S-H (Calcium Silicate Hydrate: The primary binding gel forms a dense, glue-like matrix that binds soil particles or aggregates together, and provide strength to cementitious systems). This formation occurred through hydration and pozzolanic reactions involving calcium, silica, and alumina sources.

$Ca(OH)_2+SiO_2+H_2O \rightarrow C\text{-}S\text{-}H$ (Calcium hydroxide from the hydration reaction of lime or cement; reacts with silica (SiO_2) present in fly ash/ soil/ or slag, Produces C-S-H gel, which fills voids and binds particles)

$Ca(OH)_2+Al_2O_3+H_2O \rightarrow C\text{-}A\text{-}H$ (Calcium hydroxide reacts with alumina (Al_2O_3) of the clay minerals particles or by-product of industry and forms C-A-H gel, contributing to stiffness and early strength.

All such reactions improve the strength and stiffness of weak and subgrade-type soils, enhance particle bonding, and reduce plasticity. This leads to better load-bearing capacity, which is useful in rural roads/road construction/embankments. It also controls soil erosion and lowers permeability by promoting the aggregation of soil particles and reducing their micropore size. which is beneficial for canal linings, landfill liners, and slope stabilization. It is also considered an eco-friendly ground improvement strategy, offering a sustainable alternative to chemical stabilizers. It reduced the high cement content required, thus lowering emissions of carbon and dust particles in infrastructure projects [19].

Hence, the integration of protease-based bio-enzymes serves as a biocatalytic modifier to support sustainable, cost-effective, and environmentally friendly geotechnical engineering practices in infrastructure development projects.

2.3.3 Amylase, From the conventional engineering point of view and strict research perspectives, concrete construction materials mainly used in civil work are cement, sand, aggregates (gravel), lime, fly ash, or ground granulated blast furnace slag (GGBS). These materials are entirely inorganic and composed mainly of calcium, silica, and alumina oxides, with no polysaccharide or organic substrate. The use of organic matter is strictly undesirable because it adversely affects strength and durability, as per standard specifications (e.g., IS codes). Consequently, amylase has no direct role in concrete production and does not participate in cementitious civil engineering work. Thus, no direct chemical or catalytic interaction is present between amylase-based bio-enzyme functions, as biocatalytic modifiers in civil/geotechnical engineering practices, and these functions do not influence hydration or pozzolanic reactions [20-21].

But comprehensive research data showed that in some geotechnical engineering projects, amylase may play an indirect, conditional role, particularly where soils contain a significant amount of organic matter. In such cases, amylase-based bio-enzyme reduced the organic coatings around clay particles by hydrolyzing the polysaccharides present in soil organic matter. It acts as a pre-treatment agent. This process may expose mineral surfaces and facilitate improved interaction between soil and stabilizers (e.g., lime or fly ashes), leading to better compaction, reduced plasticity, and enhanced strength. Examples of such applications have been reported in rural road construction over clayey and organic-rich subgrades, commonly found near agricultural lands and hut settlements. In such environments, natural organic matter and microbial polysaccharides form coatings on soil particles, increasing moisture retention and plasticity, and reducing bearing capacity. Amylase, if applied, may hydrolyze these compounds, leading to slight modification of soil fabric, improved particle rearrangement, and enhanced compaction behavior. This can indirectly improve the California Bearing Ration (CBR)and reduce swelling when combined with conventional stabilizers. However, this approach remains experimental and case-dependent, not a standardized engineering solution [22]. Thus far, it is important to highlight that this mechanism is not well established or widely validated, and there is no strong, standardized evidence supporting the use of pure amylase for cementitious soil stabilization. Additionally, amylase is used more consistently in the "conservation of heritage structures and monuments," where amylase-based bio-enzyme is used in controlled bio-cleaning processes to degrade and remove polysaccharide residues from traditional organic additives such as plant extracts, gums, and jaggery after microbial growth. Generally, microbial growth on monuments or heritage structures leads to biodeterioration via biochemical and physical mechanisms. Initially, microbial strains (such as bacteria, fungi, algae, and lichens) form colonies and secrete polysaccharide-rich extracellular polymeric substances (EPS) similar to starch. It forms a biofilm-like sheet that acts as an adhesive matrix, trapping moisture, dust, and pollutants and creating a favorable microenvironment for microbial activity [23]. Retained moisture promotes salt crystallization, resulting in microcracking and weakening of the walls or substrates of heritage sites. Further, organic materials (such as jaggery, plant extracts, and natural gums) were used in traditional construction, serving as good nutrient sources for microbial proliferation and growth. Different strains of microorganisms produce organic and inorganic acids (such as oxalic acid, citric acid, and sulfuric acid) that react with minerals (such as calcium carbonate), causing dissolution. Additionally, microbial haustoria and hyphal structures (fungi and lichens) penetrated into pores or walls, resulting in mechanical disruption and granular disintegration. Thus, we conclude that amylase is an important bioenzyme that plays dual roles. Under natural conditions, microorganisms producing amylases accelerate biodeterioration by degrading complex polysaccharide molecules into simple sugars, increasing nutrient availability and accelerating microbial growth. It also increased the secretion of polysaccharides, lipids, proteins, and nucleic acids, which are part of EPS (extracellular polymeric substances), and led to comparatively thick biofilm formation, indirectly supporting surface adhesion and colonization of microorganisms on the walls of monuments and causing acid-mediated mineral dissolution. On the other hand, under controlled conditions, it acts as a selective, eco-friendly bio-enzyme that can clean organic coatings and help conserve the structural integrity of our Indian heritage.

2.3.4 : these bio-enzymes, extracted from the plant and used to stabilize soil durability and strength, reduce permeability through soil-enzyme and mineral-enzyme complexes. For example, in *Carica papaya*, the enzyme papain interacts with the proteinaceous part of organic matter in soil and clay particles, breaking them into simpler peptides and thereby reducing plasticity and improving soil cohesion tension, which is the foundation of soil stabilization in embankment areas of rivers, canals, etc. From *Ananas comosus*, Bromelain, an important enzyme, is isolated; it forms a complex with organic colloids and promotes the flocculation of fine clay particles, enhancing soil particle aggregation and making it suitable for slope stabilization. Peroxidase,(catalytic-antioxidative bio-enzymes) extracted from leaves of *Vitex negundo* (nirgundi), *Moringa oleifera*, and Raphanus sativus, catalyzed the oxidative-coupling reaction and formed a cross-linked organic mineral complex that can increase the particle binding capability and improve river/canal banks linings, finally control erosion. Polyphenoloxidase (PPO) again showed antioxidative property, extracted from *Vitex negundo* (nirgundi), *Azadirachta indica,* and *Camellia sinensis*. It reduces permeability and improves performance in flood-prone areas by promoting the polymerization of phenolic compounds. It also helps form stable humic-like complexes with clay minerals, thereby controlling soil erosion in the embankment areas of rivers and canals [24-25]. Overall, plant-derived bio-enzymes significantly enhance the aggregation of soil particles, improve load-bearing capacity, and make highly effective embankments of water reservoir systems. They improve canal/ river linings and stabilize the slopes of rivers/canals, and control water infiltration and erosion in flood-prone areas where these processes are critical [26-27].

Hence, enzyme–cementitious by-product composite approaches are the latest technology for sustainable, low-carbon-emission studies, prior to widespread adoption in civil engineering practice.

However, the role of bio-enzymes (such as amylase, cellulase, phosphatase, urease, etc.) is substrate-specific, and they are not involved in the hydration or pozzolanic reactions of inorganic cementitious materials. Instead, their application is being explored in organic-rich, clay-dominated soils, where soil organic matter (polysaccharides and cellulose residues) is enzymatically degraded, altering surface charge properties and promoting improved particle rearrangement. This treatment enhances the interaction between soil particles, improving the compaction behavior and mechanical strength of the soil fabric [28-29].

2.4 Bio-enzyme–Nano-Mineral Composites in Sustainable Geotechnical Engineering

In recent advances in bio-nanotechnological research, different types of nanomaterials, such as nanoparticles, nanoporous tubes, and nanofibers, have been discovered as 'novel-carrier molecules' that play an important role in enzyme immobilization and strengthening. Additionally, a no-biocatalytic system [11]. Additionally, it overcomes key limitations of the free enzymatic system, such as poor stability, low recyclability, and limited operational lifespan. Research data showed that immobilization onto nanocarriers (i.e., nano-immobilized enzyme systems) provides a strategic pathway to improve soil stabilization and act as functional modifiers rather than direct binders. Direct application of bio-enzymes in soil systems remains constrained; thus, their immobilization onto nanocarriers offers a strategic pathway to enhance their performance and applicability [30]. soil stabilization, plant substrate-derived capping agents under “Green Synthesis” are currently emphasized, with developments focused on designing hybrid bio-enzyme systems in combination with nanominerals, such as nano-silica, nano-clays, and other reactive mineral additives. They enhance soil stabilization efficiency through synergistic interactions. Such enzymes initially alter the organic and surface chemistry of the soil particles, and then the nanocomposite of hybrid bio-enzymes improves the density, porosity, pore-filling capacity, physicochemical bonding, and packing of the soil matrix. All this synthesis of hybrid composites can be achieved through mountable, cost-effective methods [31-32]. Despite promising advancements, the utilization and application of bio-enzyme hybrid nano-mineral composites in soil stabilization remain in their early developmental stages. Challenges remain to be solved, and the optimization of immobilization techniques is in the research pipeline to ensure and retain native enzymatic activity and long-term durability, and to establish standardized, application-based protocols for performance evaluation.

Conclusion and future perspectives

The stabilization of soil with bio-enzyme composites is an environmentally friendly, revolutionary technique that is becoming popular worldwide. It leads to greater improvements in the mechanical properties of soil. The use of bio-enzymes is one of the key strategies for environmentally benign, energy- and material-saving biochemical processes today. Recently, reports have stated that bio-enzymes such as Perma-Zyme, Terra-Zyme, Rrenolith, Fujibeton, etc. are available for soil stabilization and are quite effective and cost-effective. These bio-enzymes, when mixed with soil, alter the soil's engineering properties. Further efficiency of such bio-enzymes depends on field conditions, soil type, and the dose amount. Bio-enzymes increase the compressive strength, flexibility, and durability of soil, and reduce the crack formation. Although research data have shown that bio-enzymes have potential in soil engineering, their application in civil engineering remains relatively nascent overall. More practical experience is still lacking in mechanisms and engineering the mechanical properties of bio-enzymes. Further standardized protocols for the extraction and development of novel bio-enzymes have not yet been formulated. Therefore, it is crucial to identify novel plant- or microbial-based enzymes (such as amylases, arylsulfatases, β-glucosidases, etc.) using standardized bioprocesses or fermentation-based formulations, and to elucidate their reaction patterns across various soil geochemical processes to further innovate and apply bio-enzymes in Civil and Geotechnical Engineering.

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