

A Review of Biochemical Processes and Techniques for Soil Stabilization and Resilience

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Abstract

Biochemical, chemical, and mechanical, techniques have been employed to enhance soil resilience for decades. While the use of mechanical techniques requires transporting huge amounts of soil materials, the cement used in chemical techniques may lead to increase atmospheric carbon dioxide. Numerous studies indicate that biochemical techniques may be less expensive, cost effective, and environmentally friendly. Biopolymers and enzymes derived from microorganisms have been suggested as biological enhancers in strengthening and fortifying soils used for earthen structures. Lime and other treatment techniques used as biobased materials have been shown to be less effective for stabilizing soils. Here, we review biochemical processes and techniques involved in the interactions of soil enzymes, microorganisms, microbial extracellular polymeric substances, and other biopolymers with soil particles, and the challenges and strategies of their use as biobased materials for stabilizing soils. This review provides their impacts on various soil properties and the growth potentials of agricultural crops.

Keywords

Biochemical, Earthen Structures, Soil Resilience, Biopolymers, Soil Enzymes, Agriculture, Microorganisms, Extracellular Polymeric Substances

1. Introduction

Earthen structures are structures constructed largely from soils. An ideal soil consists of 45% minerals (sand, silt, clay), 5% organic materials (microorganisms, plants, and animals), 25% air and 25% water [1] [2] impacting soil structures and stability. Mechanical, chemical, and biological techniques have been employed to enhance or improve soil stability and resilience. Mechanically stabilizing soils involve soil compaction, vibration, anchors and geosynthetics, while

chemically involve the use of cementitious binders like cement, lime, fly ash, and hydrophilic gels [3]. Mechanical techniques require heavy machinery and the transport of large quantities of soils. The most common chemical stabilizing agents used in chemical techniques are cement and lime, however; cement production is accompanied by huge quantities of carbon dioxide emissions to the atmosphere. It is estimated that the production of a single ton of cement emits approximately 0.95 ton of CO₂ [4]. Less expensive and environmentally friendly options include using such biochemical techniques as enzymes, microorganisms, and biopolymers and mixing soils with biobased materials and evaluating their effects on soil engineering properties that include standard proctor test, California bearing ratio, Atterberg limits, Unconfined compressive resilience, and Consistency limits etc. [5] [6] [7] [8].

The method used to amend soils depends on the desired outcome. When used for engineering or geotechnical purposes the amendments are mixed with the bulk soil to obtain a homogenous mixture then compacted to obtain the desired soil strength. In amending agricultural soils emphasis is on minimum soil disturbance for successful crop production. Generally, less soil disturbance can lead to lower potential for soil erosion. Soil stabilization is primarily influenced by pH, clay mineral, and soil organic matter content. These factors affect the net charge and the bonding ability of the soil particles. The negatively charged ions on soil organic matter (SOM) bind metal cations that will act like bridges to bind negatively charged soil particles together. Studies have shown that certain distinct components of SOM rather than the total pool, are more important in affecting soil stability [9]. However, large amounts of SOM can decrease soil pH; hence affecting its reaction with the additives. Soils with high amounts of sesquioxides contain reduced amounts of negative charges on clay particles. Different soil stabilization materials require varying amounts of moisture to produce the desired results. Temperature affects the reactions between the soil particles and the soil binders, and eventually the curing process. The curing time may affect the soil strength. If soil containing high amounts of sulfides or sulfates is amended with CaCO₃-containing binders, the sulfuric acid formed will attack and destroy the stabilized soil. Generally, stabilized soil is a composite material obtained from the combination and optimization of properties of constituent materials [10].

Understanding the biochemical processes involved in the use of biobased materials to improve soil stability, resilience, and load-bearing capacity is very important. In this review, we highlight the processes and challenges involved in the use of microorganisms, isolated enzymes, microbial excretory-secretory products (extracellular polymeric substances), and other biopolymers to stabilize soils, and suggest strategies to obtain better outcomes.

2. Application of Microorganisms in Stabilizing Soils

Microbial geotechnology is the use of microorganisms and/or their derivatives e.g., metabolic products to alter such soil engineering properties as soil stability,

durability, resilience, and stiffness. The impacts of soil microorganisms and enzymes in improving soil engineering properties have been explained based on the concepts of bioaggregation, bioclogging, and biocementation [11] [12]. Soil bioaggregation is considered the association of soil particles to form more stable units based on the application of biochemically derived materials. Soil bioclogging is the filling of pore spaces in soils by microbial biomass and microbial processes [13]. The cell walls of most microorganisms, particularly bacteria known to produce extracellular polymeric substances (EPS) that bind soil particles, are negatively charged, and bind cations on clay minerals surfaces, thus acting as bridges in binding soil particles [14]. The microbial biomass and exopolysaccharide sheath (EPS) block the pathway of water in the pore spaces thus reducing soil porosity and hydraulic conductivity [15]. When bacteria are mixed with soil, biological clogging is thought to occur in three phases namely: 1) development of biofilms that cover the grain surface, 2) microbial growth and development of microcolonies or plugs, and 3) formation of a bulk biomass at the macroscopic scale [13]. Selection of microorganisms to stabilize soils is based on their ability to enzymatically produce cementitious compounds, extracellular polymeric substances (EPS), and the tendency of the cell walls binding soil particles.

2.1. Microbially-Induced Precipitation of Soil Particle-Binding Materials

Soil biocementation is the microbially induced precipitations of soil particle-binding materials to improve soil resilience, durability, and stiffness. The production of soil binding compounds, particularly, calcium carbonate, calcium phosphate and calcium sulphate are mediated by microbial enzymes. Microbially induced calcium carbonate precipitation (MICCP) is a biogeochemical process that induces calcium carbonate to precipitate within the soil matrix. The processes may involve ureolysis [8] [16], nitrate reduction [17] [18], cyanobacterial carbonate mineralization [19], sulfate reduction [20] [21], methane oxidation [22], and ammonification of amino acids [23] (Table 1).

MICCP by ureolysis is a popular technique to produce cementitious materials to stabilize soils, however; its reactivities generate toxic ammonia, and ammonium [8]. Using such chelating agents as EDTA, dimethylglyoxime, oxalate to manage soils limits the applications of this technique to improve soil resilience because these chelating agents do inhibit urease activity by binding to Ni^{2+} ions at its active sites [24]. Microbially induced calcium carbonate precipitation (MICCP) by nitrate reduction has potential applications in civil engineering because it promotes more CaCO_3 precipitations per mole of reagents than ureolysis [16] and does not require exogenous organic nitrogen [25]. Although, denitrification requires very low nitrate (0.080 mM) concentrations [26], using this technique to enhance soil resilience is limited by the fact that denitrification is inhibited by high nitrate concentrations (>25 mM) due to N_2O reductase inhibition by NO_3^- [27]. The optimum pH for most denitrifying bacteria strains is between

Table 1. Microbially induced calcium carbonate precipitation (MICCP) reactions.

Microbial process	Enzymatic reaction	Soil stabilizing microorganisms
MICCP by ureolysis	$\text{CO}(\text{NH}_2)_2 + 2\text{H}_2\text{O} \xrightarrow{\text{Urease}} \text{NH}_4^+ + \text{CO}_3^{2-}$ $\text{CaCl}_2 \rightarrow \text{Ca}^{2+} + 2\text{Cl}^-$ $\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3 (\text{s})$	<i>Sporosarcina pasteurii</i> (<i>Bacillus pasteurii</i>) [8] [16]
MICCP by nitrate reduction	$\text{NO}_3^- \xrightarrow{\text{Nitrate reductase}} \text{NO}_2^- \xrightarrow{\text{Nitrite reductase}} \text{NO}$ $\xrightarrow{\text{Nitric oxide reductase}} \text{N}_2\text{O} \xrightarrow{\text{Nitrous reductase}} \text{N}_2$ $2.6\text{H}^+ (\text{aq}) + 1.6\text{NO}_3^- (\text{aq}) + \text{CH}_3\text{COO}^- (\text{aq})$ $\rightarrow 0.8\text{N}_2 (\text{g}) + 2\text{CO}_2 (\text{g}) + 2.8\text{H}_2\text{O} (\text{l})$	<i>Pseudomonas denitrificans</i> . [17] [18]
MICCP by cyanobacterial carbonate mineralization	$\text{CO}_2 (\text{aq}) + \text{H}_2\text{O} \xrightarrow{\text{Carbonic anhydrase}} \text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$ $2\text{HCO}_3^- + \text{Ca}^{2+} \rightarrow \text{CH}_2\text{O} + \text{CaCO}_3 + \text{O}_2$	<i>Synechococcus</i> and <i>Prochlorococcus</i> [19]
MICCP by sulfate reduction	$\text{SO}_4^{2-} + 2\text{CH}_2\text{O} + \text{OH}^- \xrightarrow{\text{sulfate reductase}} \text{HS}^- + 2\text{HCO}_3^- + 2\text{H}_2\text{O}$ $\text{Ca}^{2+} + \text{CO}_3^{2-} \rightarrow \text{CaCO}_3$	<i>D. bizertensis</i> [20] [21]
MICCP by methane oxidation	$\text{CH}_4 + \text{SO}_4^{2-} \xrightarrow{\text{MMO}} \text{HCO}_3^- + \text{HS}^- + \text{H}_2\text{O}$ $\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}$	<i>Methylococcus capsulatus</i> [22]
MICCP by ammonification of amino acids	$\text{RCH}(\text{NH}_2)\text{-COOH} + \text{H}_2\text{O} \rightarrow \text{RCOOH} + \text{CO}_2 + \text{NH}_4^+$ $\text{C}_4\text{H}_8\text{N}_2\text{O}_3 + \text{H}_2\text{O} \xrightarrow{\text{Asparaginase}} \text{C}_4\text{H}_6\text{NO}_4^- + \text{NH}_4^+$ <small>Asparagine Aspartate</small> $\text{C}_4\text{H}_6\text{NO}_4^- + \text{H}_2\text{O} \rightarrow \text{C}_3\text{H}_7\text{NO}_2 + \text{HCO}_3^-$ $\text{Ca}^{2+} + 2\text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{CO}_2 + \text{H}_2\text{O}$	<i>Bacillus megaterium</i> [23]

MMO = Methane Monooxygenase.

7 and 8; outside this range, toxic intermediates (NO_2^- and N_2O) accumulate [28]. Generally, the MICCP technique requires supplementing the soil mixture with CaCl_2 to precipitate CaCO_3 . Calcium chloride retards soil drying when subjected to accelerated drying [29]. While calcium is crucial for plant growth it makes plants less susceptible to diseases and pests. However, high chloride concentrations may influence toxicity problems in crops and reduce yields [30] [31].

2.1.1. Microbially Induced Calcium Phosphate Precipitation

Soil phosphatase enzymes are produced by bacteria, fungi and plant roots and cleave a phosphate group from organic P forms into assimilable inorganic phosphate. In an experiment, in which phytase-active yeast *Arxula adenivorans* was introduced into a column filled with quartz particles and amended with calcium phytate, calcium-phosphate crystals were observed as cementation materials between the sand particles [32]. Phytase released from yeast cells will catalyze the dephosphorylation of phytate and the released inorganic phosphate combines with Ca^{2+} to form calcium phosphate precipitate between the quartz particles.



The soil stabilizing effects of calcium phosphate is due to the ease with which phosphate binds to siliceous clays, humic material, and iron and aluminum oxides. The insoluble $\text{Ca}_3(\text{PO}_4)_2$ formed may quickly harden and not easily translocated within the soil medium. It has been suggested that the use of short-lived biodegradable chelating agents such as ethylenediamine-N-N'-disuccinic acid (EDDS) as an alternative to EDTA which temporarily binds Ca^{2+} and prevents the formation of $\text{Ca}_3(\text{PO}_4)_2$ may provide a solution to hardpan formation in soils [33]. As the chelating agent degrades over a relatively short time, Ca^{2+} can remain soluble and mobile, while the PO_4^{3-} remains bioavailable to form $\text{Ca}_3(\text{PO}_4)_2$ during the curing period.

2.1.2. Microbially Induced Silicate Precipitation

Bacterial cell walls are usually negatively charged, largely because of carboxyl groups. At low pH, positive charges can be introduced on bacterial cell walls by protonation of amino residues and proton motive forces in the membranes of respiring cells [34]. *Bacillus subtilis* is a gram-positive bacterium with a high metal binding capacity because its cell walls contain the highly electronegative polymers. During metabolism, a membrane-induced proton motive force continuously pumps protons into the cell walls fabric and reduces the negative charges of living bacterial cells. Also, at low pH, amine groups from either the D-alanines of teichoic acid or the diaminopimelic acid from peptide portion of peptidoglycan and the amino sugars of glycan of *B. subtilis* bacterial cell walls acquire positive charges which can bind silicate ions through ionic interactions [35]. It is possible to introduce positive charges on *B. subtilis* cell walls by incubating the bacteria with ethylenediamine [36]. The carboxyl groups attached to bacterial cells react with ethylenediamine to produce positively charged ammonium residues.



The positively charged cell walls of *B. subtilis* have been shown to bind silicate anions to precipitate silica with materials such as pozzolan cement [37] which can be used to improve soil stability and resilience. Results have shown that crystalline silicate precipitates are more stable than CaCO_3 at low pH conditions [38]. *B. subtilis* has great potential in enhancing earthen structures because it precipitates both CaSiO_3 and CaCO_3 [36] [39] and produces extracellular polymeric substances [40]. However, mineral formation is not controlled by bacteria, because the bacterial cells simply provide a nucleation surface on which the silicates precipitate [41].

2.1.3. Microbially-Induced Calcium Sulfate Precipitation

Arylsulfatase in soils catalyzes the biochemical conversion of organic sulfur to inorganic sulfate [42]. The sulfate ions react with Ca^{2+} ions adsorbed on the clay mineral surfaces to form calcium sulfate which binds the clay particles together. Studies have shown that when calcium sulfate is added to lime and pozzolan, higher values of unconfined compressive resilience (UCS) of clayey soils are

formed. The valor of $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ on stabilized clayey soils depends on the $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ contents, curing time, soil types and mineralogical compositions of the stabilized soils [43]. The use of microbially-induced calcium sulfate precipitation to strengthen earthen structures may be limited by the fact that large amounts of CaSO_4 in soils may promote the proliferation of sulfate reducing bacteria. Sulfate-reducing bacteria produces hydrogen sulfide and is a major cause of corrosions of underground storage tanks and iron pipes [44] [45].

2.2. Extracellular Polymeric Substances (EPS) and Soil Stabilization

Extracellular polymeric substances (EPS) are mixtures of organic substances synthesized and secreted or excreted by microorganisms. It has been shown that EPS are mainly of polysaccharidic nature and contain proteins, lipids, and nucleic acids that enable resident microbes to survive in their environment [46]. Extracellular polymeric substances in a variety of bacteria provide protection against dehydration, phage-induced lysis, phagocytosis, and antibody recognition. They are constituents of biofilms and are thought to improve the biofilm communities' ability to scavenge both water and nutrients from the environment [47]. Extracellular polymeric substances have been employed to stabilize soils to reduce water and wind erosions [48] [49]. Extracellular polymeric substances have great potentials in practical applications in enhancing earthen structures because they can be readily produced in the laboratory and have been shown to be effective in stabilizing soils and reducing soil erosion at very low (0.01% - 0.5%) concentrations [50]. The added advantages of using microbial ESP in soil stabilization include being produced in large quantities by culturing the appropriate bacteria within a short period; providing a carbon source for soil microorganisms; easily liquified and mixed with soils to bind positively charged heavy metals due to their net anionic nature.

3. Enzymes and Soil Stabilization

Soils are considered a system of humus and minerals containing immobilized enzymes and occluded microbial cells [51]. The use of cell-free enzymes rather than whole microorganisms in biocementation to enhance earthen structures lowers the risk of spreading pathogens and altering microbial diversities in the environment [8]. Also, using bacteria to stabilize soils requires specific handling to control the bacterial cultures. The high number of substances used might hamper the microorganisms in hydrolyzing the substrates thus limiting their effectiveness in soils [52]. Desired soil binding products can be obtained by mixing pure enzymes with specific substrates. Isolated enzymes using urease, arylsulfatase, and phosphatase enzymes have been used to induce the precipitation of CaCO_3 , CaSO_4 , $\text{Ca}_3(\text{PO}_4)_2$ binders respectively. However, using cell-free enzymes is limited by the fact that most enzymes function best under optimum temperature and pH conditions which vary for different soils and environments. Patented commercial enzymes have been used to stabilize soils and for road con-

structions [5]. Some commercial enzymes used to improve soil engineering properties include: TerraZyme, Permzyme, DZ-1X, EarthZyme, Eko Soil, Renolith and Fujibeton [6] [53] [54] [55].

It is challenging to know the definite compositions of these enzymes and their modes of action because they are composed of mixtures of proprietary ingredients. They are reported to be non-toxic, eco-friendly, biodegradable, and biological in origin. Two mechanisms of bioenzyme for soil stabilizations have been reported [56]. Bioenzymes boost the engineering characteristics of soils through cationic exchange [57]. The enzyme-based soil stabilizations of some commercial enzymes are through organic encapsulation [5]. Positively charged enzyme molecules encapsulate the negatively charged organic molecules attached to negatively charged clay particles, producing a covering effect on the clay particles, which blocks further water absorption and loss in density [58].

An evaluation of the effects of bioenzymes in enhancing soil resilience is to compare the geotechnical properties of treated and untreated soils. In a study using the Eko soil for constructing an unpaved road, the CBR test results showed that it significantly increased soil resilience [5]; however, when applied to an expansive clay, it decreased the plasticity and increased the soil resilience. As reported the increase was not sufficient to make the soil easily used as a sub-base or base course material [55]. Results of CBR and UCS tests showed that Terrazyme and Permazine significantly increased the resilience of fine soils [6] and the rate of improvement was proportional to the clay contents in the soils. Soils treated with Terrazyme showed a significant improvement in consistency limits, standard proctor test, UCS and CBR [7]. When DZ-IX, EarthZyme and Terrazyme were applied to a clay-loam soil, the three enzymes did not produce any chemical change, nor improvement in Atterberg limits, compaction, and unconfined compression tests, but they prevented moisture absorption in bringing the soil particles closer [54].

4. Biopolymers and Soil Stabilization

Biopolymers are natural polymers derived from microorganisms. Most common biopolymers found in soils include polysaccharides (most abundant), proteins, polynucleotides (e.g., RNA and DNA), and phospholipids [2]. Biopolymers have been used in recent years to improve the engineering properties of soils, particularly for dust control, soil enhancement and erosion control [59]. Biopolymers for soil stabilization are selected based on their surface charge, structure, flexibility, and sustainability. Soil clay particles and most biopolymers are negatively charged but bind together through ionic bridges, bonded with metal cations absorbed on the clay colloids. A cationic biopolymer will form strong electrostatic bonding with the negatively charged soil particles, thereby increasing soil resilience. Very few biopolymers possess intrinsic cationic charges, e.g., chitosan (a polysaccharide) and proteins, at low pH are positively charged. Cationic groups (e.g., cationic cellulose, cyclodextrins and dextrans) have been introduced to

some neutral or negatively charged biopolymers to increase their binding force with soil particles. Cationic cellulose derivatives have been synthesized by reacting trimethyl ammonium substituted epoxide with a hydroxyethyl cellulose backbone [60]. The introduction of positive charges on biopolymers does not only increase the binding resilience of soil particles but it also increases biopolymers solubility in water. Hydrophobic groups on biopolymers will improve the hydraulic conductivities of soils while hydrophilic groups will provide strong attachments to soil particles. Some studies have shown that soil aggregate stability is improved and maintained with time by hydrophobic than by hydrophilic organic materials [61]. However, the studies further suggest that long-lasting soil aggregate stability can be achieved by amending soils with hydrophobic and hydrophilic organic materials. Carboxyl, hydroxyl, and amino groups associated with biopolymers can form H-bonds with organic colloids adsorbed on clay particles or directly with the hydroxyl groups on clay mineral surfaces. This is enhanced by ion-dipole interactions, van der Waals forces, and ionic bridges in the presence of intermediate ions such as alkaline earth metal ions in the soil [62] [63].

Because several organic compounds are not soluble in water, a challenge using biopolymers to stabilize soils is obtaining a uniform biopolymer-soil mixture, and this may lead to decreased reactivity and lower effectiveness. A biopolymer needs to be flexible to act as grouting material between the soil particles. Gellan gum is flexible but high temperatures are required to melt it. Applying biopolymers to increase soil resilience must be sustainable, abundant, and inexpensive. Therefore, the choice of industrial waste products and nonfood portions of plants are critical for selecting biopolymers. Lignin-based biopolymers are a priority choice because they are obtained as by-products of wood and the paper industry. Chitosan and cellulose are abundant and have been extensively used to improve soil resilience in mitigating soil erosion [64]. Most of the studies on biopolymers as soil additives to improve soil resilience use air-dried soils. Air-dried soils do have lower biological activities compared to field-moist soils. Liquid biopolymers are preferable to solids; however, numerous biopolymers are not readily soluble in water and need solubilization. Heat might be employed to transform them from solid to liquid. In a study [65] on the effects of two thermo-gelation biopolymers (gellan gum and guar gum) on enhancing soils, the biopolymers were heated to melt. But before adding the hot biopolymer solutions to the soil, the soil was heated above 60°C and the mixing process performed at 90°C [65]. Such high temperatures affect the interactions of added biopolymers with those in the native soil. The results showed that the soil compressive resilience was higher with thermal than non-thermal gelation. The positive effects of heat may be to facilitate the penetration of the gel into soils before solidifying, rather than due to increase in biological interactions. In a study on the effects of a polysaccharide gum on soil aggregate stability, gum increased the aggregate stability when organic matter was removed than in the soil with organic matter [66].

Studies have shown that soils with the same texture but collected from various locations respond differently to similar biopolymer amendments. When a bio-fuel co-product (BCP) containing 5% lignin was added to two different sandy silty soils with clay, the unconfined compressive resilience (UCS) increased for a particular soil type and decreased for another, as curing time increased [67]. When the amount of gellan gum was increased from 1% to 3% the compressive resilience of a clayey soil more than doubled [68]. However, the unconfined compressive resilience (UCS) of a lignosulfonate-treated sandy silt soil increased significantly as the percentage of lignosulfonate increased from 0% to 2%. However, UCS decreased slightly when the percentage of lignosulfonate exceeded 2%, indicating that the optimum percentage of lignosulfonate for this soil was approximately 2% [69]. Biopolymers that effectively bind soil particles together and increase soil resilience, must be resistant to biodegradation. Earthen structures amended with biopolymers must withstand any load bearing capacity and erosion for a considerable period. Biopolymers which serve as nutritional sources for soil microbes are not economically suitable for use to stabilize soils. Lignin is resistant to biodegradation, hence lignin-based biopolymers will be suitable for stabilizing soils. Biopolymers applied to soils may have important consequences on some soil biogeochemical and physical properties and plant nutrient mineralization. An evaluation of the use of biopolymers in stabilizing soils should include assessing their effects on soil productivity indices, plant growth and development.

5. Conclusion

Soil stabilization using mechanical, chemical, or biochemical methods is a very important factor to consider in altering agricultural soil structures and geotechnical soil properties including their shear strength, compressibility, permeability, and plasticity. Mechanical methods involve mixing two or more soil types and applying mechanical energy through compaction and densification to obtain the desired specifications. Chemical methods involve mixing of soil with cement, lime, fly ash, geotextiles, fabrics, and soil binders like CaCl_2 to increase soil strength. Although mechanical and chemical methods have been shown to greatly increase and improve soil strength, they involve the transporting huge quantities of materials. Biochemical methods involve the use of microorganisms, isolated enzymes, microbial excretory-secretory products, and other biopolymers from microorganisms and plants. Microbial extracellular polymeric substances (ESP) and other biopolymers may have greater potential for wider applications because they may be cheaper and more environmentally friendly. A proper understanding of the biochemical processes involved in the interaction of microorganisms, enzymes, and biopolymers with soil particles will enable selecting the appropriate biomaterials for applications.

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Data Availability

No data, models or codes were generated for this review. Data included in the review were obtained through the DOI links of the relevant publications and authors appropriately cited or credited in the text and reference section.

Conflicts of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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