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Transforming Soil Stability: A Review on Harnessing Plant Cell Compounds and Microbial Products for Modifying Cation Exchange Capacity

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ABSTRACT

Soil stabilization is a very important method of science and engineering for improving the properties of soil. This paper aims to stabilize expansive black cotton soil through a biological approach involving plant extracts, plant waste materials, and microorganisms. While chemical methods exist, i.e., lime stabilization, geotextiles, etc., they are not economically feasible for large-scale applications. The primary issue with black cotton soil is due to the presence of montmorillonite clay mineral, which makes it unsuitable for the construction of roads and airfields. The cation exchange capacity (CEC) can be defined as the ability of soil to absorb and exchange positively charged ions; thus, if free positively charged ions are not available, the soil will not exchange them with others. The CEC of the soil is diminished, and ultimately, the soil is stabilized to some extent. This paper explores the preparation of plant extract, which contains a high number of anions, and directly inoculates it with soil, which nullifies the positive charge of the soil and diminishes the CEC. The use of cellulose and lignin-degrading microorganisms as an energy source and other minerals that are needed for their growth will be utilized from the soil to reduce CEC, i.e., Mg required for DNA replication and Ca required for their growth and maintenance. Another approach to diminishing the CEC is to use the microorganisms that produce EPS, which require Ca and Mg as adhesions for the formation of biofilm, i.e., *Pseudomonas aeruginosa, Bacillus subtilis,* and *Escherichia coli*. The use of microorganisms that have specific enzymes is also used in the diminishing soil CEC, i.e., by using ureolytic enzyme-producing bacteria like *Sporosarcina pasteurii*, *Bacillus paramycoides, Citrobacter sedlakii,* and *Enterobacter bugadensis.*

INTRODUCTION

Some of the partially saturated clayey soils are very sensitive to variations in water content and show excessive volume changes; such soils are classified as expansive soils. It can also be known as swell soil or black cotton sail. Many stabilization techniques are in practice for improving expansive soils in which the characteristics of the soils are altered or the problematic soils are removed and replaced, which can be used alone or in conjunction with specific design alternatives. These types of soil are spread mostly across interior Gujarat, Maharashtra, Karnataka, and Madhya Pradesh.

Black clays, tropical black earth, or black cotton are known to be potentially expansive soils that are "black" or "greyish black" or, in their eroded phase, "greyish white" heavy loam or clay (usually 50%), with a predominant clay mineral of the smectite group, rich in alkali earth elements, and the horizons sometimes contain calcium carbonate or magnesium oxide concretions as shown in Fig.1. Many other terms have been applied locally, such as "regur" soils in India, "margalitic" soils in Indonesia, "black turf" in Africa, and "tirs" in Morocco. Although there are several names, the term "black cotton soil" is adopted in this paper because of its extensive use in India. The term "black cotton" is believed to have originated in India, where the locations of these soils favor cotton growth (Gidigasu & Gawu 2013).

The main characteristics of black cotton soils, among others, are:

1. Black or darkish gray to brown color.
2. High content of expansive clay mineral montmorillonite.
3. Possess the tendency to shrink and swell with changes in moisture conditions.
4. Exhibits heave and crack as geo-environmental phenomena.

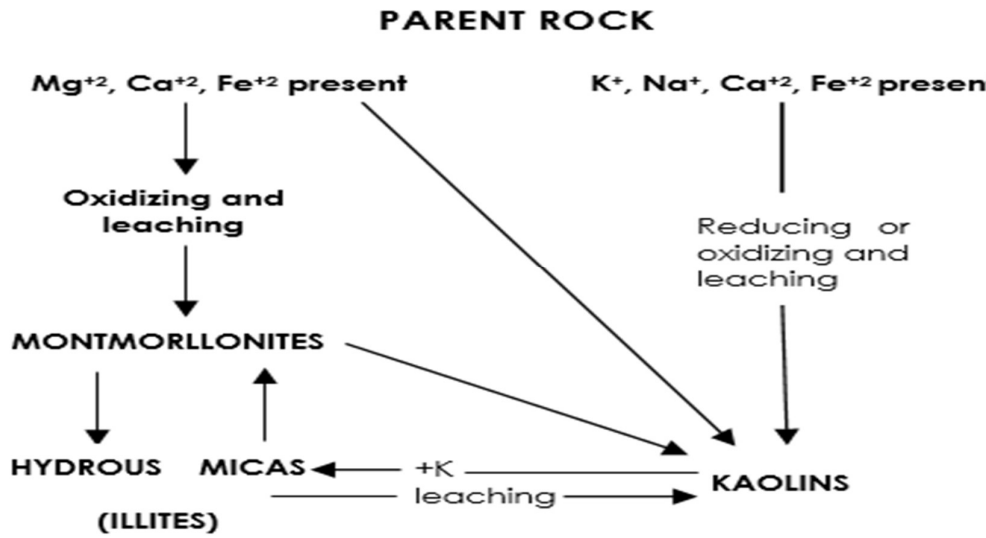

Fig. 1: The environment of formation of illite, kaolinite and montmorillonite (Frederickson 1952).

Problems Associated with Black Cotton Soil

Major issues with several tropical countries, particularly in Africa and India, are their black cotton soils. Because they are frequently expansive due to the presence of substantial percentages of expansive clay minerals, such as montmorillonite, they are poor materials by temperate zone standards and challenging to employ for road and airfield construction. When these soils come into touch with water, they swell and shrink. Because the soil deposits are typically thick, it is impossible to avoid or bypass them when building engineering projects. The seasonal volume variation (i.e., swell and shrinkage) of these soils has been documented to have caused discomfort to many roads and light-building foundations (Chen 1988). According to reports, these soils cause annual damages and repairs to earth buildings and infrastructure, totaling billions of dollars (Gidigasu & Gawu 2013).

Soils made of black cotton absorb a lot of water, expand, get mushy, and lose their strength. These soils have a propensity to heave in damp conditions and are readily compressible when damp. During the heat, black cotton soils lose volume and start to break. When dry, they exhibit severe hardness and fractures. They are not good earth construction materials or foundation soils because of these characteristics. Since the subgrade and embankment act as pavement foundations, they have a significant impact on the stability and performance of the pavements. In places with black cotton soil, a thorough understanding of the characteristics of the soil is necessary for the development of a good and long-lasting road network. Adopting appropriate construction techniques and sophisticated design approaches is necessary for such soils (Nadgouda & Hegde 2010).

CATION EXCHANGE CAPACITY

The cation exchange capacity of CEC is the total amount of exchangeable cations that a soil can absorb. Positively charged ions (Na^+, Ca^{+2}, and K^+) that are present on or close to negatively charged soil particles make up the majority of these exchangeable cations. These are easily interchangeable cations that can be swapped out for other positively charged ions, as seen in Fig. 2. The plants can also easily access them. Terrestrial ecosystems cannot sustain plant development without cation exchange, which is a vital life-supporting activity that works in tandem with photosynthesis.

Cation exchange capacity is measured by one of several standard methods where all adsorbed cations in soil are replaced by a common ion (such as NH_4^+), and then the amount of adsorbed common ion is determined. The standard way to describe soil CEC is as one of two numerically identical sets of units: $cmolc.kg^{-1}$ (centimoles of charge per kilogram of dry soil) or $meq.100g^{-1}$ (milliequivalents of charge per 100 g of dry soil).

The exchangeable cations and cation exchange capacity (CEC) of soils can be measured using a variety of techniques. Some employ buffered solutions, including barium chloride, sodium acetate, or ammonium acetate. For the others, unbuffered salts like ammonium chloride or barium chloride are used to carry out the exchange process. CEC values can be calculated for a given reagent by counting the amount of a fixed cation (index cation) or by adding up the exchanged cations. Numerous methods are proposed to measure the cation exchange capacity (CEC) and exchangeable cations in soils. Some of them are the ammonium acetate method at pH 7, the barium chloride compulsive exchange method,

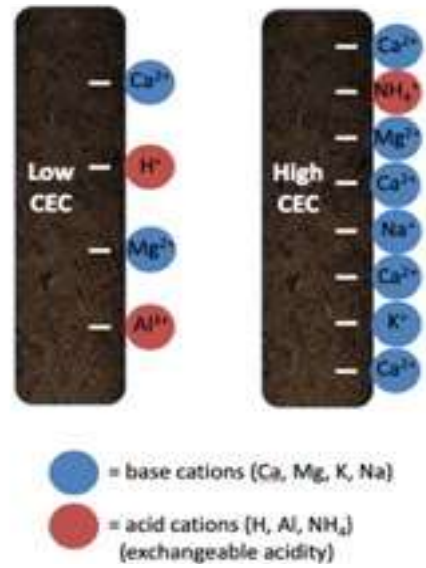

Fig. 2: Cation exchange capacity of soil.

the methylene blue test (spot test and turbidimetric test), the cobalt-III hexamine chloride method, and the silver thiourea (AGTU) method. The methylene blue test is a quicker, easier, and more affordable procedure than other methods.

Methylene Blue Test for Measurement of CEC

The Methylene blue test was created in France to identify the clay content of granular material and assess its viability for use in the production of concrete. The chemical formula for methylene blue powder is $C_{16}H_{18}N_3SCl$, and it reacts with water like a cationic dye. Chloride ions in methylene solution exchange places with cations in clay minerals when combined with soil solution, where they are adsorbed on the surface of clay minerals. The amount of adsorbed methylene solution changes according to the number of clay minerals and clay type, cation exchange capacity, and specific surface area (Turkoz & Tosun 2011). The methylene blue test is a widely used test procedure (as shown in Fig. 3) since it is relatively simple to perform and doesn't require any specialized equipment. The methylene blue adsorption test is a dependable and uncomplicated technique for determining whether clay minerals are present in soils and what their characteristics are. In actual practice, two test methods-the "turbidimetric" approach and the "spot method"-have been employed. A titration procedure that is simplified is the spot method.

By confirming how much methylene blue is needed to cover the whole surface area of the clay particles in the soil, the test allows one to determine the ion adsorption capacity of the soil. This test method is based on titration created by a chemical reaction between free methylene blue cations acquired by dissolving methylene blue in water and replaceable clay cations. The greatest potential for cation exchange is possessed by clay particles with the largest specific surface area and the highest negative electrical charge. The particular surface area and electrical charge of the clay particle both influence the adsorption (Turkoz & Tosun 2011). It is advised to collect soil test samples for ANFOR standard analysis, taking into account the soil's clay content; 30 to 60 g should be taken from clayey or overly clayey soils, and 60 to 120 g from less clayey soils. 500 milliliters of distilled water are used to dissolve soil samples collected at this ratio.

The soil sample solution is then supplemented with 5 milliliters of methylene blue solution, which is made at a concentration of 10 g/L. One drop of the mixed solution is applied to the filter paper one minute later. When the dye around the aggregate dye area creates a second, lighter-colored blue halo and remains stable for five minutes, the test is considered to have ended (Turkoz & Tosun 2011).

In the end, it is said that (Chiappone et al. 2004), the ANFOR standard defined test method yields results that represent the entire material and should be used in heterogeneous samples, while the ASTM standard test method is appropriate to use for homogenous, fine-grained material; in other words, only verifying the clay content. The most popular technique used by all researchers to determine the CEC of soil is the methylene blue spot test.

PLANT EXTRACTION METHODS

Extraction is the process of separating a variety of plant metabolites that are used medicinally, including alkaloids,

glycosides, phenolics, terpenoids, and flavonoids, utilizing established techniques and selected solvents. Separating the soluble plant metabolites from the insoluble cellular marc is the goal of all solvent extraction techniques. The following are popular methods for extracting plant material: Certain plants offer significant benefits when utilized as a simple and affordable method for extracting plant material. Phytonic procedures are one of them, and they have a lot of benefits and a broad variety of applications over other techniques. The selection of the desired solvent and extraction method is quite a difficult task.

Maceration, Infusion, Percolation and Decoction

According to Azwanida (2015), the maceration extraction method is utilized to extract bioactive compounds from plants as well as in the wine-making process. Plant materials (coarse or powdered) were macerated by soaking them in a solvent in a stoppered container and letting them stand at room temperature for at least three days while stirring often. Pressing, straining, and filtering were the next steps in the process. Conventional methods involve the transfer of heat by convection and conduction, with the solvent chosen following the target compound for extraction. Similar to maceration, infusion, and decoction involve immersing the ingredients in either boiling or cold water (Azwanida 2015). Decoction often produces more oil-soluble chemicals than maceration and infusion, and it is only appropriate for extracting heat-stable compounds from hard plant materials like roots and barks (Azwanida 2015).

Hot Continuous Extraction (Soxhlet)

Using this technique, coarsely powdered crude plant material is put into the soxhlet apparatus's chamber in a porous bag or "thimble" composed of sturdy filter paper. Vapors from the heated solvent in the flask condense in the condenser. By dripping into the thimble holding the raw plant material, the condensed extractant facilitates extraction by contact. Until a drop of solvent from the siphon tube evaporates without leaving any trace, the procedure is ongoing. Notably, this process saves a significant amount of time, energy, and money by enabling the extraction of vast amounts of plant material with a far smaller amount of solvent.

Accelerated Solvent Extraction (ASE)

When compared to maceration and Soxhlet extraction techniques, accelerated solvent extraction is a more effective solvent extraction procedure. Compared to maceration and Soxhlet extraction techniques, this method uses the least amount of solvent (Gomes et al. 2017). To keep the sample from aggregating and clogging the system tubes, the ASE uses inert packing material, like sand, packed inside stainless-steel containers (Barros et al. 2013). According to Gomes et al. (2017), the extraction process takes less than an hour to finish, and the technique regulates temperature and pressure for every single sample.

Microwave-Assisted Extraction (MAE)

This extraction method uses microwave energy to enhance the partition of analytes from the sample matrix into the solvent (Zhao et al. 2012). As a result of interactions between microwave radiation and the dipoles of polar and polarizable materials, such as solvent, a plant sample heats up close to the material's surface, and conduction transfers heat. Dipole rotation of the molecules caused by microwave electromagnetics breaks hydrogen bonding, boosts the movement of dissolved ions, and improves

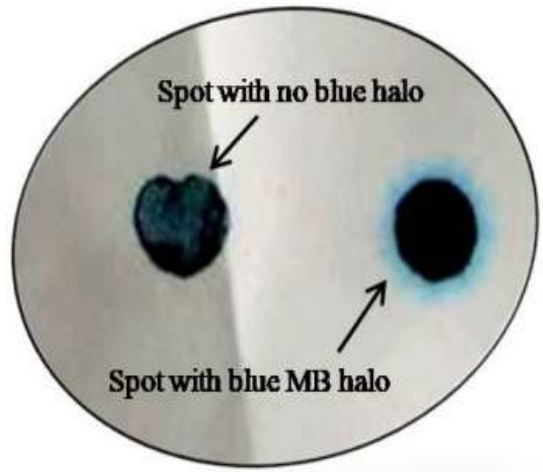

Fig. 3: Methylene blue spot test result capacity.

solvent penetration into the matrix (Kaufmann & Christen 2002).

Ultrasound Extraction (Sonication)

The extraction method known as ultrasound-assisted extraction (UAE) is noted for using low temperatures, shortening extraction periods, and increasing extraction yield. With the use of ultrasonic frequencies between 20 and 2000 kHz, this extraction technique increases cavitation and permeability of the cell wall. Although it works well for some uses, including the extraction of rauwolfia roots, high prices prevent it from being widely used. One significant disadvantage is that ultrasonic radiation (over 20 kHz) can occasionally have a detrimental effect on the active ingredients in medicinal plants, resulting in the production of free radicals and unfavorable molecular changes.

Supercritical Fluid Extraction (SFE)

The method of separating one component (the extractant) from another (the matrix) by employing supercritical fluids as the extracting solvent is known as supercritical fluid extraction or SFE. SFE is an alternate technique for preparing samples that aims to increase sample throughput while using less organic solvent. Temperature, pressure, sample volume, analyte collection, modifier addition, flow and pressure control, and restrictors are examples of critical parameters. Because of its abundance, affordability, safety, and favorable physical characteristics, CO_2 is the recommended extraction solvent. However, the use of organic solvents is required due to their polarity limits. Because argon is inexpensive and inert, it has been investigated as a possible substitute recently. Significant capital investment requirements impede the practical deployment of SFE, notwithstanding its benefits in extracting different chemicals.

Enzyme-Assisted Extraction (EAE)

Enzymes, known as biocatalysts, are obtained from fruits, vegetables, animal organs, and microbes (bacteria and fungi). The fundamentals of enzyme-assisted extraction (EAE) entail a catalytic hydrolysis reaction that releases intracellular components into the extraction medium by disrupting the plant cell wall under ideal experimental conditions. Disentangling phytochemicals from the polysaccharide-lignin network supported by hydrogen bonding and hydrophobic interactions like van der Wall forces can be difficult in some plants. The solvent extraction technique does not make the phytochemicals in their matrices accessible; instead, they stay scattered in the cell cytoplasm. By dissolving cellular walls, these enzymes are added during extraction to increase phytochemical output.

Moreover, these enzymes hydrolyze lipid bodies and other carbohydrates, like cellulose. Particular enzymes, including amylase, pectinase, and cellulase, are employed. Two prominent techniques that involve enzymes during extraction are enzyme-assisted aqueous extraction (EAAE) and enzyme-assisted cold pressing (EACP). While the first technique has been used largely to extract oils from various seeds, the latter has been utilized to hydrolyze the plant's seed cellular wall (Yi et al. 2009). By pre-treating the plant material with particular enzymes, the bound phytochemicals in such samples are efficiently released at high yields (Yi et al. 2009).

Phytonic Process

Advanced Phytonics Limited has developed a patented "phytonics process" using hydrofluorocarbon-134a as a non-flammable, non-toxic, and ozone-friendly solvent. This process allows for the selective extraction of specific phytoconstituents, resulting in low residual solvent levels. The closed-loop processing plant ensures solvent recycling, minimal energy consumption, and no environmental emissions, making it a sustainable and efficient extraction technique.

Advantages of the Process

The phytonics process is a gentle, cool, and environmentally friendly method that does not damage products by high temperatures. It does not require vacuum stripping, and the process is conducted at a neutral pH, preventing acid hydrolysis damage or oxidation. The technique offers a variety of operating conditions and end products, is less threatening to the environment, requires minimal electrical energy, releases no harmful emissions, and produces innocuous waste products.

The solvents used are non-flammable, toxic, and ozone-depleting.

Applications

Phytonic procedures are used in a wide range of businesses, including the food, essential oil, and taste sectors, as well as biotechnology for the synthesis of antibiotics and herbal drugs. Notably, it is used in the manufacturing of phytopharmaceuticals, pharmacologically active intermediates, antimicrobial extracts, and premium pharmaceutical-grade extracts. Its versatility goes beyond these fields and includes the extraction from a variety of plant materials of essential oils, oleoresins, natural food colors, tastes, and aromatic oils. The method is also used to refine crude products that come from other extraction methods; it guarantees that only pure chemicals free of waxes and

impurities are extracted, and it makes it easier to remove biocides from contaminated biomass.

Parameters Governing the Selection of an Appropriate Extraction Method (Fig. 4)

1. Verification of plant material authenticity, including careful removal of extraneous material.
2. Using the appropriate plant portion and documenting information for quality control, such as plant age, collection time, season, and location.
3. Customizing the drying environment to the chemical makeup of the plant components, preferring techniques such as hot or cold airflow.
4. Specification of grinding procedures, avoiding techniques creating heat.
5. Plant material powder is sieved to ensure consistent particle size.
6. Consideration of the nature of constituents, such as selecting a non-polar solvent for non-polar medicinal constituents and utilizing acceptable extraction procedures based on thermo ability.
7. Implementation of measures for constituents sensitive to organic solvents.
8. Standardization of extraction duration to provide comprehensiveness while avoiding extraction of undesired components
9. Determining and managing the water or extraction solvent quality.
10. Adoption of concentration and drying techniques, such as lyophilization and reduced pressure drying, that guarantee the stability and safety of active ingredients.
11. Consideration of extractor design and construction material.
12. Recording analytical metrics, like TLC and HPLC fingerprints, to keep an eye on the quality of various extract batches.

MICROBIAL PROCESSING OF PLANT MATERIALS

Microorganisms are essential to the effective recycling of agricultural wastes because they help to bioconvert agricultural residues into compost, which has many advantages. In addition to improving soil fertility, this procedure also improves soil health, which lowers ecological hazards, increases agricultural output, increases soil biodiversity, and creates a better environment. One particularly good solution for handling the large amounts of agro-waste produced worldwide is composting. Crop leftovers are a major but underutilized source of renewable biomass produced in large numbers in the context of agriculture. The amount of crop waste in India alone is estimated to be around 620 million tons. Remarkably, 50% of all residues generated are attributed to three key crops: oilseed, wheat, and rice. On average, these residues contain 1.5% potassium oxide (K_2O), 0.2% phosphorus pentoxide (P_2O_5), and 0.5% nitrogen (N).

With half of the agricultural residues being used for fuel and cow feed, the remaining residue has a significant amount of nutrients left in it; 6.5 million tons of NPK (nitrogen, phosphorous, and potassium) can be produced annually. This makes up 30 percent of India's overall

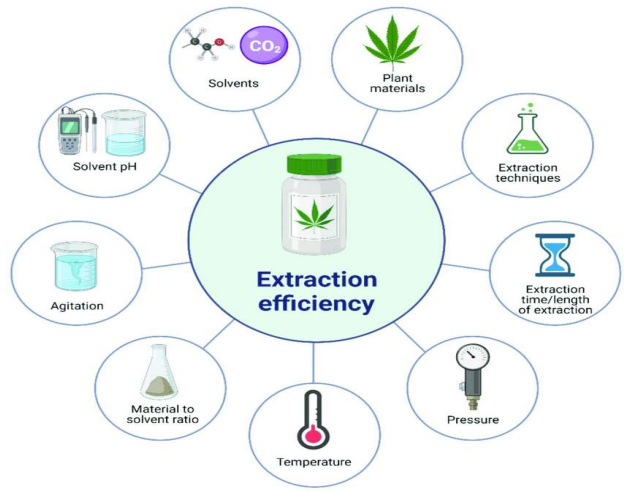

Fig. 4: Factors affecting on extraction of plant material.

NPK consumption. Composting agricultural leftovers is a feasible and cost-effective technique that is made possible by lignolytic, lignocellulolytic, and cellulolytic bacteria. It efficiently recycles cellulosic, lignocellulosic, and lignin waste, supporting nutrient management and sustainable farming methods.

Lignin Degradation

The most common naturally occurring aromatic heteropolymer and one of the fundamental components of lignocellulosic biomass is lignin. As part of the secondary cell wall of plants, lignin (15–30%), cellulose (30–50%), and hemicellulose (15–30%) work together to maintain the integrity of the cellulose/hemicellulose/pectin matrix (Boerjan et al. 2003). Nearly all forms of lignin are composed of three basic monolignols, or phenol derivatives: p-coumaryl alcohol (M1H), coniferyl alcohol (M1G), and sinapyl alcohol (M1S). Lignin is a complex, highly branching, three-dimensional phenolic structure. In the polymer, every monolignol yields p-hydroxyphenyl, guaiacyl, and syringyl subunits (Calvo & Dobado 2010). Lignin has a variable molecular mass due to random cross-linking by polymerization of phenolic groups, which results from radical coupling processes between phenolic radicals (Boerjan et al. 2003).

Lignin comprises functional groups such as phenol hydroxyl, methoxyl, carboxyl groups, and alcohol hydroxyl, which impact the reactivity of lignin (Christopher et al. 2014). Lignin is synthesized by combining monolignol units through peroxidase-mediated dehydrogenation, in which the structures of linked basic units are created and linked to one another by aryl ether linkages with β-aryl ether and aryl-glycerol as well as C–C bonds. The biggest proportion is β-O-4 aryl ether bonds, about 50–70%, followed by β-β, β-5, 5-5, and 5-O-4 bonds (Calvo & Dobado 2010). Among the bonds that are hardest to break are carbon-carbon ones (Christopher et al. 2014). Through interactions with other polymers found in the cell wall, such as cross-linking proteins and polysaccharides, monolignols form a complex three-dimensional matrix (Calvo & Dobado 2010), giving the tissues and cell walls of all vascular plants strength, rigidity, and resistance to microbial degradation (Fisher & Fong 2014).

Lignin can be categorized into four main categories as shown in Fig. 5 according to the number of basic phenol units such as guaiacyl (G), syringyl (S), and p-hydroxyphenyl(H) in the polymer into type G, type G-S, type H-G-S, and type H-G (Calvo & Dobado 2010). Because lignocellulosic material can replace high-value chemicals made from petroleum derivatives, there is growing interest in it (Asina

Table1: Details of lignin and its application.

Sr. No.	Main Lignin Preparation	Degradation of Lignin	Application	Derived from Monolignol units
1.	Organosolv lignin	Physical	Biofuels	Guaiacyl (G)
2.	Steam-exploded lignin	Microbial 1. Bacterial 2. Fungal	Component for composites & co-polymers	4-hydroxy-phenyl (H)
3.	Lignin sulfonates	Chemical	Dispersant agent for pesticide	Syringyl (S)
4.	Kraft lignin	-	Biner	-
5.	-	-	Heavy metal sequestration	-
6.	-	-	Emulsifier	-
7.	-	-	High-value products	-

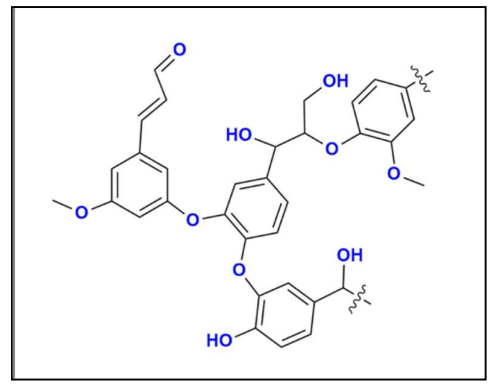

Fig. 5: Chemical structure of lignin.

et al. 2016). It can also be used to produce biofuels (Fisher & Fong 2014), paper (Calvo & Dobado 2010), sorbents, activated carbon, carbon fibers with a very large surface area and pore volume, and bioplastics.

Referring to Table 1, lignin degradation can be carried out chemically and thermally by sophisticated oxidation processes: photocatalytic, pyrolysis, electrochemical, or enzymatically/biologically. Because of its complicated and irregular structure and the absence of conventional repeating covalent connections, lignin is a refractory material that cannot be broken down by most degradation techniques (Yadav et al. 2022). The use of selective ligninolytic enzymes and microorganisms to control lignin biodegradation and prevent the formation of undesirable byproducts makes biological methods of lignin degradation preferable to chemical ones. Biological processes also do not result in yield loss, unlike thermal lignin decomposition.

The lignin-degrading bacteria can be isolated on the MSML (minimal salt medium containing alkaline lignin) medium or LB medium supplemented with guaiacol or tannic acid from a soil sample, and the confirmation of the lignin-degrading bacteria can be done by using methylene blue as an indicator for the MSML medium, while for the LB medium, the lignin-degrading bacteria will give rise to a brown-colored zone surrounding the colony.

Biological Lignin Degradation

Fungi and bacteria do not produce any toxic chemicals; therefore, the biological breakdown or decomposition of lignin is considered a green and environmentally beneficial process (Niu et al. 2021). Hydrolytic enzymes cannot cleave lignin due to the branching three-dimensional structure and C-C and C-O ether bonds (Abdel-Hamid et al. 2013). Moreover, low-potential oxidoreductases, like plant oxidases, which start lignin polymerization, are unable to oxidize the non-phenolic aromatic subunits of lignin. Because of this composition of lignin, bacteria, and fungi have evolved to produce a variety of groups of enzymes with ligninolytic activity (Bugg et al. 2011). The oxidative process of lignin biodegradation necessitates the synthesis of extracellular ligninolytic enzymes, which include dye-decolorizing peroxidase (DyP), lignin peroxidase (LiP), manganese-dependent peroxidase (MnP), versatile peroxidase (VP), and laccases (LaC) (Yadav et al. 2022). The type and structure of lignin are related to the binding affinity of enzymes that break down lignin. The interactions between lignin and amino acids in the enzymes involve the three primary types of non-covalent bonds: hydrogen, hydrophobic, and electrostatic.

According to Fisher & Fong (2014), there is considerable variation in both the lignin's chemical structure and the degradation products produced by the enzymes that break it down. C-C and C-O monomer bonds break during many metabolic transformations, while side chain modifications, hydroxylation, and demethylation also take place. Most modifications take place at the same time (Sanchez 2009). There are two stages to the breakdown of lignin (Bugg et al. 2011).

The breaking of the β-O-4 aryl ether bond in the phenylen unit is the primary process of the first phase of lignin degradation (Niu et al. 2021). The cleavage of the core ring results in the formation of a number of intermediates during the second phase. During the biochemical conversion of lignin, the main intermediates are the aromatic chemicals that are generated, specifically catechol and protocatechuic acid (Abdelaziz et al. 2016). According to Bugg et al. (2011), several catabolic pathways lead to the breakdown of lignin components. These pathways include the breakdown of β-aryl ether by bacteria and fungi, biphenyl by bacteria, diarylpropane by bacteria, phenyl coumarane, and pinoresinol by bacteria, bacterial degradation of ferulic acid, and oxidative cleavage of protocatechuic acid by bacteria.

One of the methods by which biodegradation of lignin happens is composting. The diverse microbial community in the compost pile is active during the composting process, and microorganisms break down organic material to produce compost (humus), carbon dioxide, water, and heat. It is thought that lignin, polysaccharides, and nitrogenous chemicals make up the majority of humus, preventing lignin from fully mineralizing throughout the composting process. Actinomycetes and thermophilic microfungi break down lignin during composting (Grgas et al. 2023). The majority of research on lignin modification and degradation has been done on basidiomycetes (Fisher & Fong 2014). Because they secrete extracellular ligninolytic enzymes, white and brown rotting fungi are crucial in the decomposition of lignocellulosic biomass (Sanchez 2009). Various combinations of enzymes, such as LiP and MnP, MnP and LaC, and LiP and LaC, are produced by distinct white-rot fungi. Fungi that cause brown rot may effectively break down cellulose and hemicellulose but not lignin, at least not completely. Basidiomycetes, an aerobic white-rot fungus, are capable of completely breaking down lignin. Since high yields and productivity are necessary, the industrial application of fungi is restricted due to their demanding growth requirements (Niu et al. 2021). Laccase genes, mostly identified in Firmicutes, Proteobacteria, and Actinobacteria, vary in occurrence across bacterial strains.

Actinomycetes such as *Streptomyces viridosporus T7A*, *Sphingomonas paucimobilis SYK-6, Comamonas, Nocardia*, and *Rhodococcus* are examples of bacteria that break down

lignin. These bacteria can release extracellular peroxidases and break down both lignin and lignocellulose carbohydrates. Noteworthy eubacteria and actinomycetes include *Bacillus, Acinetobacter, Xanthomonas, Micromonospora*, and *Streptomyces,* underlining the vast spectrum of bacteria adept in lignin breakdown. A well-researched lignin-degrading bacteria called *Streptomyces viridosporus T7A* generates extracellular peroxidases that break the -aryl ether bond in lignin to depolymerize it and release low-molecular-weight phenols (Yadav et al.2022). There are also identified bacteria that can break down lignin, like *Rhodococcusjostii RHA1* and *Pseudomonas putida mt-2*. Fungi are superior to bacteria in the breakdown of lignin, while bacteria alter lignin by producing smaller aromatic molecules that are then imported into cells and metabolized.

Cellulose Degradation

The main type of carbohydrate that plants synthesize is cellulose. Therefore, a significant portion of the carbon cycle in the biosphere is represented by the breakdown of cellulosic biomass. For the same reason, biotechnologists' interest in using cellulolytic enzymes to treat cellulose for practical uses has not abated. However, a lack of fundamental understanding of the process prevents cellulose hydrolysis from being improved. Made up of glucose subunits connected by 1,4 linked bonds, cellulose is a linear polymer. Since each glucose residue is 180 degrees rotated in relation to its neighbors, cellobiose is the fundamental repeating unit. The range of chain lengths is 100–14000 residues. Rigid, insoluble microfibrils are formed when an abundance of intra- and intermolecular hydrogen bonds is formed by cellulose chains. The lateral diameter of microfibrils varies from 3–4 nm in higher plants and up to 20 nm in the case of the alga *Valoniarnacrophysa*, whose microfibrils can contain several hundred cellulose chains.

The chains are parallelly aligned and create highly structured crystalline domains with more amorphous, disordered areas strewn in between as shown in Fig. 6. Depending on origin and pre-treatment, the degree of crystallinity of cellulose can vary from 0% for amorphous, acid-swollen cellulose to over 100% for the cellulose isolated from Valoniamacrophysa (Beguin & Aubert 1994). There are various commercially accessible types of pure cellulose, including cotton, filter paper, and Avicel. Usually, these forms are employed to evaluate the effectiveness of whole cellulase systems. However, their physical variability (degree of crystallinity, accessible surface area, pore size) hinders detailed enzymological research. Because of their quick rate of hydrolysis, amorphous forms such as soluble carboxymethyl cellulose (CMC) and acid-swollen cellulose are commonly utilized in tests. The most popular media for testing bacteria that break down cellulose is carboxymethyl cellulose, which is then washed with NaCl after being flooded with Congo red (Beguin & Aubert 1994).

Cellulolytic Microorganisms

One method that cellulases catalyze is cellulose hydrolysis. Cellulose, a nearly limitless carbon, and renewable energy source, is a linear polymer composed of D-glucopyranose units joined by β-(1-4) glycosidic bonding. Since each glucose residue is 180 degrees rotated in relation to its neighbors, cellobiose is the fundamental repeating unit. The range of chain lengths is 100–14000 residues. Rigid, insoluble microfibrils are formed when an abundance of intra- and intermolecular hydrogen bonds are formed by

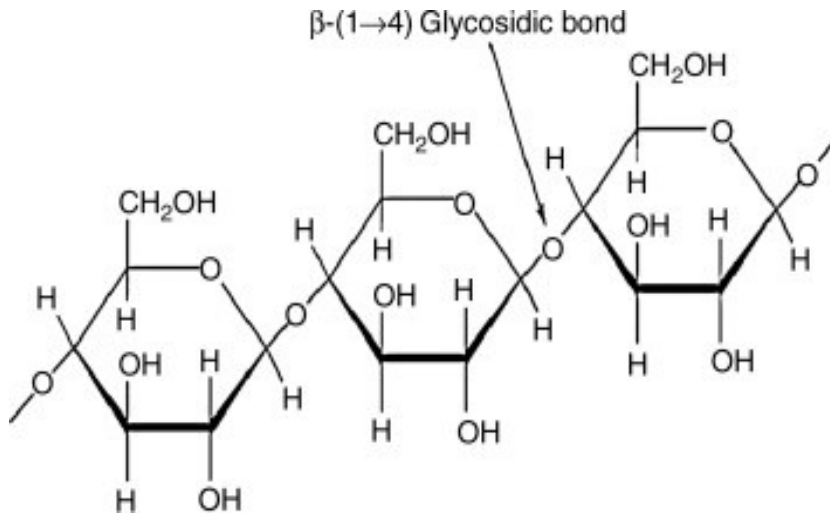

Fig. 6: Chemical structure of cellulose.

cellulose chains. The chains are parallelly aligned and create highly structured crystalline domains with more amorphous, disordered areas strewn in between. Hemicellulose is formed of complex carbohydrate polymers, with xylans and glucomannans as the primary components. Despite the complexity of the transformation processes, cellulose offers the best opportunities for lowering the production costs of many products because it is abundant and may be less expensive than other substrates (Silalertruksa & Gheewala 2020). Hemicellulose and lignin combine to generate cellulose (both crystalline and amorphous), which is limited in its destruction by a compact network structure that is insoluble in water (Faria et al. 2020).

Because of the quick rate of hydrolysis, amorphous forms of cellulose, such as soluble carboxymethyl cellulose (CMC) and acid-swollen cellulose, are commonly utilized in tests. Grown on CMC agar, Reasoner's 2A agar, or LB broth medium, the cellulose-degrading organisms are subsequently screened and verified by flooding congo red onto a plate and then being washed with NaCl as shown in Fig. 7. These organisms are employed to degrade and decompose the plant waste material into simpler forms.

Biological cellulose degradation: Fermentable sugar release requires the physical, chemical, and biological pre-treatment. Because it is environmentally friendly, biological pre-treatment using enzymes and cellulolytic microorganisms is still the best way to deal with this problem. Cellulase is a complete enzyme system comprising endoglucanase and exoglucanases, including cellobiohydrolases and β-glucosidase (Paudel & Qin 2015), which breaks down β-1,4-linkages in cellulose polymer to liberate glucose units. Numerous researchers have documented those fungi, actinomycetes, and both aerobic and anaerobic bacteria may manufacture cellulase enzymes (Shida et al. 2016). These microorganisms have an effective enzyme breakdown system and secrete free or cell surface-bound cellulases.

Among several types of microorganisms, bacteria are the most efficient cellulose degraders because they develop fast and have high cellulase synergistic activity (Bilal & Iqbal 2020). Cellulases have proven to be highly effective in the industrial utilization of lignocellulosic biomass degradation. Numerous industries, including chemicals, food and feed, pulp and paper, textiles, drinks, cars, electronics, and, most significantly, energy, use cellulases in a variety of ways (De Souza & Kawaguti 2021). From a phylogenetic perspective, the aerobic cellulolytic bacterial community found in soil is highly diverse, with members of several phyla such as Actinobacteria, Firmicutes, Bacteroidetes, and Proteobacteria. Prominent taxa include *Streptomyces, Bacillus, Cellulomonas, Cytophaga, Cellvibrio*, and *Pseudomonas*. The taxonomic categories in which cellulolytic microbes are found are incredibly diverse. The majority are eubacteria and fungi, but the rumen has also been found to include anaerobic protozoa that break down cellulose.

Although cellulolytic enzymes are also produced by the avocado fruit and the slime mold *Dictyostelium discoideum* (Blume & Ennis 1991), it is believed that the primary purpose of these enzymes is connected to the development of the corresponding fruits and spores. The most significant aerobic biota where cellulosic wastes build up is the topsoil. Because wood has a high lignin content, it is the component that cellulolytic microbes destroy the slowest. *Phanerochaete chrysosporium*, the fungus that causes white rot, is one of the rare microorganisms that can fully degrade lignin by oxidation. On the other hand, a wider range of organisms, especially Actinomycetes, can cause partial delignification to get access to the cellulose substrate.

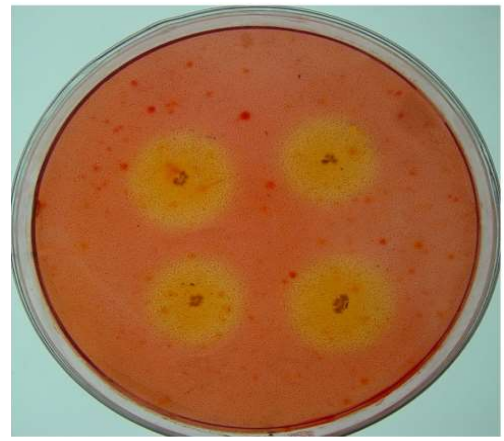

Fig. 7: Screening of cellulolytic organism.

The aerobic, highly cellulolytic Deuteromycete fungus *Trichoderma reesei* is most likely the microbe whose cellulase system has been studied the most. Numerous species of aerobic, cellulolytic soil bacteria have been thoroughly investigated. These include members of the genera *Cellulomonas, Pseudomonas (Cellovibrio), Thermomonospora,* and *Microbiopora*.

The hydrolysis reaction that is catalyzed by glycosidases, such as xylanases and cellulases, is typically thought to operate by an acid-base mechanism that involves two residues. The oxygen of the acidic bond is protonated by the first residue, which also functions as a general acid catalyst. The second residue works as a nucleophile, which either interacts with the oxocarbonium intermediate (for retaining enzymes) or stimulates the production of an OH^- ion from a water molecule (for inverting enzymes). A two-step mechanism involving a double inversion of configuration at the anomeric carbon and the production of an oxocarbonium intermediate is responsible for reactions that result in configuration retention (Sinnott 1990). The lysozyme mechanism serves as the model for this kind of response. Reactions leading to inversion of configuration proceed via a single nucleophilic substitution (Sinnott 1990).

The non-catalytic cellulose-binding domains (CBDs) of numerous cellulolytic enzymes are present. These are typically found at the NH, or COOH, terminus of the enzymes, and glycosylated, Pro/Thr/Ser rich linker sequences frequently divide them from the catalytic domains. Only a small percentage of the putative domains that can be identified by sequence similarity have been shown to exhibit cellulose-binding characteristics. Currently, there is no evidence to refute the latter's functionality; however, expected variations in binding affinity are quantifiable. These cellulolytic bacteria break down plant waste and use the cellulose as a carbon source. They also use the soil to get other cations that are necessary for their growth.

The Role of EPS of Bacteria in CEC Changes

When it comes to how variable charge soils interact with cations and anions, their charge characteristics are crucial. An increase in the surface negative charge or a decrease in the surface positive charge of the soils might be caused by the selective adsorption of anions. Because of their low pH and cation exchange capacity (CEC), variable charge soils are unique in that heavy metal pollution can occur in them. By adding biochar from various sources, several researchers have tried to raise the pH buffering capacity and CEC of variable charge soils (Shi et al. 2017). Low molecular weight organic anions have also been shown to modify the electrokinetic characteristics of variable charge soils; this has a significant impact on the sorption of cations and anions.

Extracellular polymeric substances (EPS) are produced by bacterial cells and coat their cell surfaces. EPS is a diverse biomolecule mostly made up of proteins, lipids, nucleic acids, and polysaccharides. This heterogeneous biomolecule has acidic functional groups that are pH-dependent and progressively become negatively charged as pH increases. According to reports, interactions including electrostatic, covalent, polymer-polymer, and hydrophobic control bacterial adhesion to surfaces, with hydrogen bonding being a significant factor (Hong et al. 2012). *Escherichia coli* cells in the stationary phase exhibited a greater capacity for adhesion compared to those in the mid-exponential phase.

According to Tsuneda et al. (2003), bacterial deposition on surfaces was thought to be promoted by the presence of a comparatively greater amount of EPS on the cell surface. According to other research (Liu et al. 2007), the EPS-deficient strains of *Pseudomonas aeruginosa* had a lower deposition potential on surfaces under comparable conditions, even though there was no discernible difference in their zeta potentials. Additionally, it was observed by Long et al. (2009) that bacterial deposition on silica surfaces was decreased when EPS was removed from bacterial surfaces. It has also been demonstrated that bacterial adherence depends on the surface on which it occurs, both in the presence and absence of extracellular polymer shielding (EPS). In contrast, *Pseudomonas aeruginosa SG81*, which produces EPS, and *Pseudomonas aeruginosa SG81R1*, a strain that does not produce EPS, showed comparable deposition capabilities on surfaces with varying levels of hydrophobicity. These opposing opinions were noted elsewhere as well, and it was believed that some bacterial species secreted uronic acids (mannuronic and glucuronic acids), which prevented the germs from depositing (Liu et al. 2015).

Bacteria have employed EPS secretion as a useful defense against heavy metal toxicity or to control the bioavailability of heavy metals in the environment (Wei et al. 2011). Plant growth-boosting properties have been demonstrated by bacteria such as *P. fluorescens*. According to Cardoso et al. (2018), rhizobacteria can promote plant growth in two ways: either by promoting the adsorption of nutrients like nitrates, phosphates, and important minerals or by controlling the negative impacts of pathogens on plant growth. In the last several years, there has been increased interest in the interactions of bacteria with soil colloids or minerals. Nevertheless, little research and understanding have been done on the part of EPS in bulk soil bacterial motility.

Nkoh et al. (2020) employed four bulk variable charge soils that included three species of *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas fluorescens*, as well

as three Ultisols and three Oxisols. Alfisol, a soil with a constant charge, was added for comparison. The alterations on the surfaces of the bacteria both before and after treatment with cation exchange resin (CER) and the soils following interactions with the bacteria were examined using zeta potential and spectroscopic techniques. The term "adsorption isotherm" refers to the relationship that exists between the amounts of a material adsorbed at a specific temperature and its equilibrium concentration. The Langmuir (Equation 1) isotherm was utilized to examine the bacterial adhesion behaviors on soil surfaces (Nkoh et al. 2020).

$$\frac{C}{Q} = \frac{1}{KQm} + \frac{C}{Qm} \quad \ldots(1)$$

Where C represents the equilibrium concentration of the bacteria, Q is the number of bacterial cells adsorbed per unit mass of soils, Qm is the maximum adsorption capacity, and the constant K is related to the binding capacities of the soils.

With the majority of the $r^2 > 0.8$, the adsorption behavior of the bacteria to varying charge soils fit the Langmuir isotherm well. The Langmuir isotherm was not well-fitted by the adherence of *E. coli* to the Oxisol and Alfisol ($r^2 < 0.2$). Ultisol>Oxisol>Alfisol was the order of adhesion for the soils. In contrast, *B. subtilis* > *P. fluorescens*> *E. coli* was the natural bacteria's order of attachment for Ultisol and Oxisol. The native bacterial attachment order for the Alfisol was *P. fluorescens*> *B. subtilis* > *E. coli*. This is because *E. Coli* cells and soil particles resisted each other more electrostatically than the other bacteria did. The electrostatic repulsion at pH values above their IEP inhibited *E. coli*'s migration to binding sites on soil surfaces, which in turn reduced the bacteria's ability to adhere to soil particles because of the increased quantity of negative charges on the surface of the bacteria (Nkoh et al. 2020).

According to Hong et al. (2012), the oxides of Fe and Al are the primary adsorbents for anions in soils with varying charges. The oxides are said to have a greater affinity for bacterial cells than silicate minerals. These oxides are thought to play a major role in the overall positive charge of variable-charge soils in acidic environments. Compared to the other soils, the Ultisol's higher Fe_2O_3 content significantly improved its ability to adsorb negatively charged bacterial cells. Although Oxisol's amount of free Fe_2O_3 is higher than Ultisol's, it has a reduced ability to cling to these germs. The bacterial cells' ability to adhere to one another was dramatically changed after they were treated with CER to eliminate EPS. The quantity of adherent native cells was substantially ($p < 0.05$) higher for all species of bacteria than the quantity of EPS-free cells adhered to Ultisol, Oxisol, Ultisols, and Jinxian. *P. fluorescens* and *B. subtilis* adhesion, however, displayed an unusual pattern when the constant charge Alfisol was used (Nkoh et al. 2020). *B. subtilis*'s surface functional group concentration increased as a result of CER treatment, which increased the cells' deposition on goethite but decreased it on clay minerals (kaolinite and montmorillonite). Furthermore, Hong et al. (2012) employed the DLVO theory to describe how hydrophobicity, Van der Waals forces, and electrostatic interactions between bacteria and soil particles interact. Furthermore, Tsuneda et al. (2003) showed that electrostatic interaction prevented bacterial cell attachment when the EPS covering bacterial surfaces was tiny. The scientists also noticed that polymeric interaction improved bacterial cell adherence when a comparatively higher quantity of EPS coated the cells.

Bacterial adhesion enhanced cation sorption: The sorption of Mg^{2+} by Ultisol in the presence and absence of *E. coli* was investigated to determine whether bacterial adherence increased the surface negative charge of variable charge soils. With an initial Mg^{2+} concentration, the Ultisol and Ultisol-bacteria composite absorbed more Mg^{2+}. As the initial concentration of *E. coli* introduced rose, so did the sorption of Mg^{2+} by the soil bacterium composite. In contrast, the amount of Mg^{2+} adsorbed by the Ultisol-bacteria composite was substantially larger ($p < 0.05$) than the amount adsorbed by the free Ultisol and *E. coli* individually. The zeta potential tests showed that *E. coli* adherence to the soil surface increased the soil's surface negative charge, making the soil composites more negative than the individual soil systems devoid of *E. coli*. A rise in the soil's ability to retain cations is implied by an increase in the effective negative charge on the soil surface. The concentration of *E. coli* that was deposited on the soil surface was directly correlated with the degree to which *E. coli* adhesion improved the adsorption of Mg^{2+}.

The Use of Bioenzymes for Soil Stabilization

Bioenzymes are naturally occurring liquid enzyme compositions that are non-toxic, non-flammable, and non-corrosive. They are produced from fermented carbohydrates. They are known to boost stability, enable higher soil compaction densities, and enhance the engineering properties of soil. It is expected that bioenzymes will speed up the cationic exchange process to lower the thickness of the adsorbed layer and catalyze the reactions between the clay and large organic cations (Rajoria & Kaur 2014).

Enzymes are used by some soil microbes to maintain environmental equilibrium. They generate particular enzymes that catalyze the interactions between organic cations and clay particles, resulting in the formation of stable soil clods around the roots of the plants (Rajoria & Kaur 2014). Among these microbes are ureolytic bacteria. Numerous taxa of urease-producing bacteria have possibly been researched, including

Lactobacillus, Streptococcus, Arthrobacter, Weissella, Enterococcus, Enterobacter, Citrobacter, Pseudomonas, and *Yersinia* (Alizadeh et al. 2014). *Bacillus, Sporosarcina, Sporolactobacillus, Clostridium, Desulfotomaculum, Lactobacillus, Streptococcus, Arthrobacter, Weissella, Enteroccocus, Enterobacter,* and *Providenciarettgeri* (Phang et al. 2018). These microbes not only produce urease but also a variety of other enzyme groups such as transferase, lyase, aspartase, amidohydrolase, oxido reductase, hydrolase, L. glutaminase, dehydrogenase, acid phosphatase, alkaline phosphatase, arylsulfatase, betaglucosidase, amylase, catalase, alkaline phosphormonoesterase, phosphodiesterase, deaminase, invertase, cellulase, protease, asparaginase, amidase, chitinase, lipase, carbohydrase, phenoloxidase, peroxidase, laccase, lipase, aminopeptidase, and glucoseoxidase.

CONCLUSION

Address the challenges posed by expansive soil's swelling and shrinkage involves three main strategies: chemical, mechanical, and biological stabilization, having the following outcomes:

- Chemical stabilization utilizes lime, cement, and polymers to reduce cation exchange capacity (CEC).
- Mechanical methods include re-molding and compaction, fill replacement, pre-wetting, and sub-drainage. These approaches collectively offer effective solutions to mitigate the detrimental effects of expansive soil.
- Among these methods, we recommended the biological methods, which are economical, feasible, and environment friendly compared to other methods.
- It involves the use of plant extract with different concentrations, which are commonly available at every location, and also the use of microorganisms that produce enzymes and EPS.
- Lignin degradation can be carried out chemically and thermally by advanced oxidation processes: photocatalytic, pyrolysis, electrochemical, or enzymatically/biologically. Since the chemical structure of lignin is highly variable, as well as the enzymes used to degrade lignin, the degradation products also vary.
- During multiple biochemical transformations, C–C and C–O monomer bonds are split, and hydroxylation, demethylation, modification of side chains, and other transformations occur.
- It is generally assumed that the hydrolysis reaction catalyzed by glycosidases, including cellulases and xylanases, proceeds via an acid-base mechanism involving two residues. The first residue acts as a general acid catalyst and protonates the oxygen of the acidic bond.
- The second residue acts as a nucleophile, which either interacts with the oxocarbonium intermediate (for retaining enzymes) or promotes the formation of an OH- ion from a water molecule.
- EPS-producing microbes like *E.coli, Pseudomonas fluorescens,* and *Bacillus subtilis* also require Mg and Ca-like cations as adhesion factors for the production of biofilms.
- The use of ureolytic enzymes will diminish CEC by catalyzing the reaction between organic cation and clay minerals and producing clods of stabilized soil.

Based on the above review done for various aspects of biological stabilization, the application of natural microbes available in nature itself can resolve such engineering issues with the added advantage of ecology, environment, economics, and ease with an application for large project areas.

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