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Integrating Functional Biochar and Synthetic Microbial Consortia for Circular Bioeconomy and Sustainable Contaminant Remediation

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Abstract

Biochar, a carbon-rich byproduct of biomass pyrolysis, has emerged as a promising tool for sustainable agriculture due to its capacity to enhance soil health and support microbial interactions. Despite its well-documented role in improving nutrient cycling and acting as a soil amendment, limited attention has been given to its synergistic effects with plant probiotics. This review synthesizes current findings on biochar-mediated microbial interactions and their influence on soil fertility, nutrient bioavailability, and crop productivity. Biochar's unique physicochemical characteristics—such as high porosity, surface functional groups, and adsorption capacity—facilitate microbial colonization, promote enzymatic activities, and support beneficial processes like nitrogen fixation, phosphorus solubilization, and organic matter decomposition. These interactions contribute not only to improved plant growth but also to reduced greenhouse gas emissions and heavy metal toxicity. The review further highlights mechanisms like microbial immobilization and enhanced microbial resilience under stress conditions, positioning biochar as a bio-stimulant for promoting soil microbial efficiency. By integrating evidence from recent studies, this work underscores the importance of optimizing biochar formulations to suit specific microbial communities and environmental contexts. Such optimization can be achieved by tailoring pyrolysis temperatures, feedstock types, and surface functionalization to match the metabolic requirements and stress tolerance of target microbes in given agroecological zones. Ultimately, leveraging biochar–microbe synergy presents a viable strategy for enhancing agroecosystem resilience and advancing sustainable agricultural practices.

Keywords: Microbial Metabolism**,** Soil Carbon Sequestration**,** Enzyme Immobilization**,** Pollutant Detoxification**,** Rhizosphere Engineering.

1.Introduction

Agricultural waste, encompassing crop residues, animal manure, and processing by-products, represents a significant global challenge, with an estimated annual generation exceeding 5 billion metric tons (Gupta et al., 2023). Traditional disposal methods, such as open burning and landfilling, contribute to environmental degradation through greenhouse gas emissions (e.g., CH_4, CO_2), soil acidification, and water contamination via nutrient leaching (Siddiqua et al., 2022). These practices not only squander valuable organic resources but also exacerbate climate change and biodiversity loss. Sustainable management strategies, including composting and anaerobic digestion, aim to convert waste into nutrient-rich amendments, yet face limitations in efficiency, scalability, and economic viability (Gomiero T 2016; Zielińska and Bułkowska 2024). Consequently, innovative approaches are imperative to transform agricultural waste from a liability into a cornerstone of circular agroecosystems.

Probiotic and beneficial microbes—such as *Rhizobia*, mycorrhizal fungi, *Bacillus*, and *Pseudomonas* spp.—play pivotal roles in sustainable agriculture by enhancing nutrient cycling (e.g., nitrogen fixation, phosphorus solubilization), suppressing phytopathogens, and producing phytohormones that stimulate plant growth (Yadav et al., 2017; Liu et al., 2024). Their integration reduces reliance on synthetic fertilizers and pesticides, aligning with the United Nations Sustainable Development Goals (SDGs) for food security and environmental stewardship. However, the efficacy of microbial inoculants is often hampered by poor survival rates, competition with indigenous microbiota, and vulnerability to abiotic stressors (e.g., pH extremes, desiccation) (Crowther et al., 2024; Kumar et al., 2022). Overcoming these barriers requires advanced delivery systems that ensure microbial viability and functionality in dynamic agricultural environments.

Biochar, a carbon-rich material produced via pyrolysis of organic feedstocks at 300–700 °C, emerges as a promising carrier for microbial inoculants. Its porous structure, high surface area (>300 m²/g), and cation exchange capacity (CEC ~20–40 cmol/kg) provide an optimal habitat for microbial colonization, while its chemical stability shields microbes from environmental stressors (Liu et al., 2023; Sellaperumal, 2012). Recent studies highlight synergistic biochar–microbe interactions: biochar enhances microbial survival by adsorbing inhibitory compounds, while microbes accelerate biochar's weathering, releasing embedded nutrients. This symbiosis is particularly transformative in agricultural waste recycling (Bolan et al., 2023).

For instance, biochar derived from wheat straw pyrolyzed at 550 °C was used to immobilize *Trichoderma reesei* and *Aspergillus niger* in a thermophilic composting system containing crop residues and poultry manure (Harindintwali et al., 2020). The biochar–microbe system reduced composting time by 30% (from 60 to 42 days) and increased total nitrogen and phosphorus content in the final compost by 22 ± 1.5% and 18 ± 2.1%, respectively, compared to non-biochar controls. Cellulase activity increased by 2.3-fold ($p < 0.01$), and temperature profiles indicated faster thermophilic phase onset by 48 hours. These improvements resulted in compost with higher humic acid content and lower C:N ratio (<12:1), confirming its suitability as a biofertilizer. Such biochar–microbe systems exemplify circular economy principles, repurposing waste into value-added products that close nutrient loops and mitigate greenhouse gas emissions (Wibisono et al., 2025; Cheng et al., 2022; Al-Suhaibani et al., 2020; Kumar et al., 2017).

This review critically examines the dual role of biochar as both a waste-derived product and a microbial carrier in agricultural waste recycling. Specifically, it elucidates the mechanisms underlying biochar-microbe interactions, evaluates the efficacy of biochar-based inoculants in composting, bioremediation, and soil amendment, and analyses the impact of feedstock selection, pyrolysis conditions, and microbial consortia on system performance. By synthesizing recent advances and identifying knowledge gaps—such as long-term field efficacy and scalability—this work aims to guide future research toward scalable, climate-smart solutions for sustainable agriculture. The scope focuses on probiotic bacteria and fungi in the context of organic waste valorization, offering insights into interdisciplinary strategies that harmonize agroecology with circular bioeconomy frameworks.

This integration of biochar and beneficial microbes not only addresses waste management challenges but also redefines agricultural sustainability, fostering resilient agroecosystems capable of meeting future food demands while mitigating environmental impacts.

2. Biochar as a Microbial Carrier: Properties and Mechanisms

2.1. Structural and Chemical Properties of Biochar Favoring Microbial Immobilization

Biochar's efficacy as a microbial carrier is rooted in its unique physicochemical properties, which are largely governed by the feedstock composition and pyrolysis conditions. Its porous architecture, comprising a hierarchical network of macropores (>50 nm), mesopores (2–50 nm), and micropores (<2 nm), provides an extensive surface area (>300 m^2/g) that supports microbial attachment, colonization, and biofilm formation (Bolan et al., 2023; Tomczyk et al., 2020; Gul et al., 2015). These interconnected pores not only offer physical shelter but also facilitate the diffusion of nutrients, gases, and microbial signalling molecules, thereby creating heterogeneous microhabitats that promote diverse microbial interactions (Hartmann and Six, 2023; Valizadeh et al., 2022). Macropores act as conduits for microbial migration and resource exchange, while micropores provide protective refugia against desiccation, UV radiation, and protozoan predation (An et al., 2022).

Chemically, biochar is enriched with a range of oxygen-containing functional groups such as carboxyl (-COOH), hydroxyl (-OH), and phenolic groups, which profoundly influence microbial adhesion and metabolic activation (Zhao et al., 2015; Hossain et al., 2020). These groups contribute to a high cation exchange capacity (CEC), enabling biochar to adsorb and gradually release essential nutrients like NH_4^+, PO_4^{3-}, and K^+, directly sustaining microbial growth and enzymatic activity. Moreover, the negatively charged surface functional groups facilitate electrostatic interactions with positively charged microbial cell walls, enhancing the initial attachment and stable colonization of bacteria and fungi. In addition, phenolic and quinone groups can serve as redox mediators, promoting electron transfer in key microbial processes such as denitrification and hydrocarbon degradation (Zhang, 2025; Saravanan et al., 2023).

The slightly alkaline pH of most biochars (typically 7–10) can buffer acidic soils, creating a more hospitable environment for pH-sensitive microbial taxa, while the carbon-rich matrix offers a long-lasting, slow-release energy substrate for heterotrophic microbes. These tightly coupled structural and chemical properties allow biochar to not only host a greater diversity of microbial life but also to enhance their stability, functionality, and resilience, surpassing traditional carriers such as clay, peat, or vermiculite (Khandelwal et al., 2023).

2.2. *Innovative Biochar Functionalization for Microbial Delivery*

Functionalized biochar has emerged as a promising strategy for enhancing microbial colonization, survival, and delivery in agroecosystems. The intrinsic properties of biochar, such as high porosity, large surface area, tunable surface chemistry, and adsorption capacity, provide a conducive microenvironment for microbial attachment and proliferation. Recent innovations have focused on biochar surface engineering through nanoparticle decoration, nutrient impregnation, and pH modulation to optimize biochar-microbe-soil interactions.

Nanoparticle-functionalized biochars, such as Fe_3O_4 (magnetite)-coated composites synthesized via co-precipitation or mechanical milling, exhibit enhanced redox activity and act as electron shuttles, accelerating extracellular electron transfer processes crucial for microbial respiration and biogeochemical cycling (Li et al., 2020). Similarly, ZnO-loaded biochars produced via ball-milling not only promote microbial adhesion through increased surface reactivity and electrostatic interactions but also exhibit selective antimicrobial effects, modulating the microbial community structure and suppressing pathogenic taxa (Allah et al., 2025). These metal oxide–decorated biochars influence colonization dynamics by altering the physicochemical cues sensed by microbes at the biochar interface.

Another category, nutrient-enriched biochars, are designed by co-granulating or impregnating biochar matrices with macronutrients such as nitrogen, phosphorus, and potassium (NPK), and sometimes with micronutrients like zinc and iron chelates. These composite materials function as dual carriers, simultaneously delivering essential nutrients and beneficial microbes to the rhizosphere, thereby supporting microbial metabolism, persistence, and functional expression. Such nutrient-loaded biochars not only enhance microbial biomass and diversity but also mitigate nutrient leaching compared to conventional fertilizers, contributing to sustainable nutrient cycling (de Figueiredo et al., 2024). The availability of bioavailable phosphorus and trace elements further supports enzymatic activity and microbial signaling pathways.

Soil pH is a critical determinant of microbial colonization, and the development of pH-buffered biochars aims to address pH-associated constraints during microbial deployment. Many biochars, particularly those derived from marine biomass such as seaweed, exhibit high ash content and are rich in exchangeable base cations (Na^+, K^+, Ca^{2+}), conferring an alkaline pH typically in the range of 7–11 (Roberts et al., 2015; Singh et al., 2021; Martins et al., 2024). These biochars act as liming agents, ameliorating soil acidity and establishing a favorable environment for neutrophilic microbes. Additionally, biochars can be doped with alkaline wastes (e.g., lime, ash, MgO) or subjected to post-pyrolysis alkaline treatments to enhance their buffering capacity. Such pH-modified biochars not only support microbial colonization but also improve phosphate solubility, enzyme activity, and microbial-mediated nutrient turnover under acidic soil conditions (Koul et al., 2022; Marathe and Sadowski, 2024) (**Fig.1**).

Pyrolysis is a thermal decomposition process that converts organic biomass into carbon-rich char under limited or no oxygen conditions. Various pyrolysis techniques—slow, fast, and microwave-assisted—have been used to produce biochar with tailored properties depending on temperature, heating rate, and feedstock type. For the production of these functional biochars, advanced pyrolysis methods have been employed**,** including plasma-assisted pyrolysis, which utilizes electrical discharges to generate extremely high temperatures or reactive species (Zhang et al., 2024). This method can significantly alter feedstock chemistry—activating aromatic rings or incorporating oxygen/nitrogen groups—and produce highly porous biochars with distinct surface functionalities (Zhao et al., 2025; Liu et al., 2025). Plasma-treated biochar often exhibits enhanced surface activity and improved metal adsorption capacity.

Additionally, pyrolysis of marine or mixed feedstocks is emerging as another route to create novel biochars. For instance, macroalgae-derived biochars inherently possess high mineral content and elevated pH, while co-pyrolysis of feedstock blends (e.g., crop residues plus manure, or softwood with algae) can synergistically improve pore structure, optimize the C:N ratio, and tailor ash composition (Wang et al., 2020).

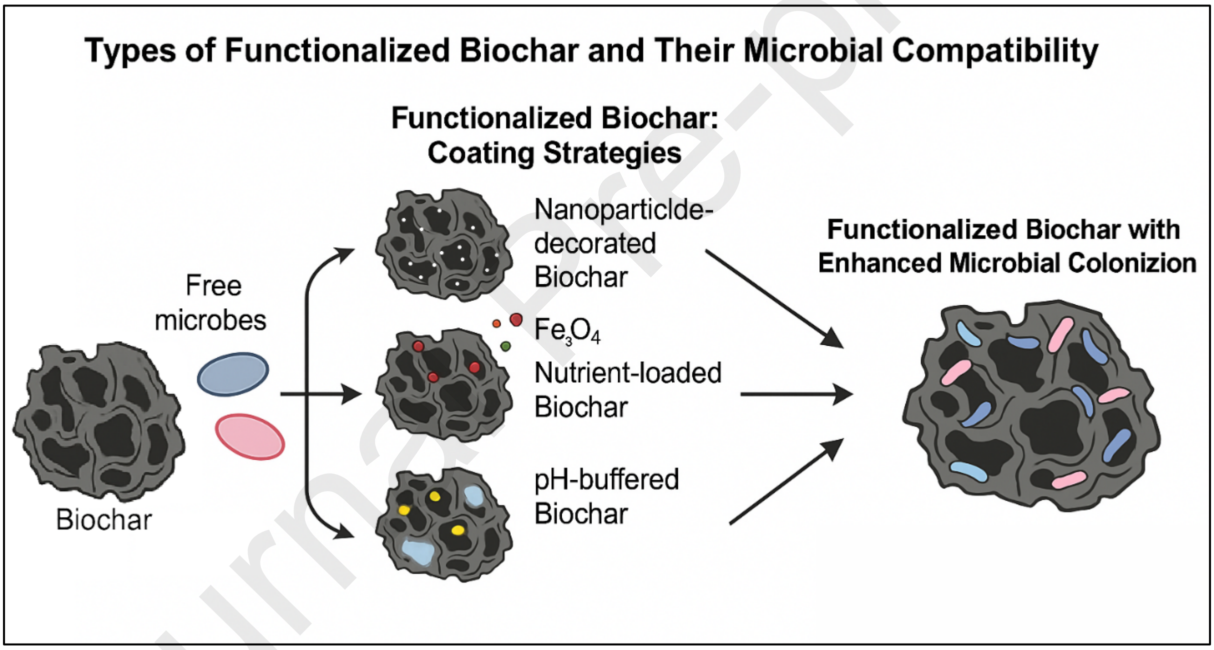

Figure 1: A schematic illustration of pristine and functionalized biochars (nanoparticle-decorated, nutrient-loaded, and pH-buffered) and their role in enhancing microbial colonization. Each strategy enhances biochar–microbe interactions for specific environmental or agricultural applications.

2.3. Biochar Surface Functionalization for Enhanced Probiotic and Microbial Adhesion

Surface functionalization of biochar further enhances its capacity to immobilize probiotic and beneficial microbes by modifying its surface chemistry and physical properties. Techniques such as chemical oxidation, ammoniation, and metal oxide impregnation can introduce additional functional groups (e.g., amino, carbonyl, and sulfonic groups) that improve microbial adhesion (Lu et al., 2022; Bolan et al., 2023). For example, oxidation with hydrogen peroxide (H_2O_2) or nitric acid (HNO_3) increases the density of carboxyl and hydroxyl groups on biochar's surface, promoting electrostatic interactions with microbial cell walls. These interactions are critical for the initial attachment of microbes, which is a prerequisite for biofilm formation and long-term colonization (Pignatello et al., 2024; Yao et al., 2022).

Coating biochar with biopolymers such as chitosan or alginate further enhances its biocompatibility and microbial adhesion properties (Chin et al., 2022). Chitosan, a natural polysaccharide derived from the deacetylation of chitin found in crustacean shells, not only improves biochar's surface charge and hydrophilicity but also exhibits antimicrobial properties that selectively inhibit pathogenic microbes while promoting the growth of beneficial species (Zhao et al., 2025; Gao et al., 2022). Alginate, a naturally occurring anionic polysaccharide extracted from brown algae, forms hydrogels in the presence of divalent cations (e.g., Ca^{2+}), creating a stable matrix that enhances microbial encapsulation and immobilization on biochar surfaces. This improves microbial survival, activity, and stress tolerance in various environmental conditions (Tang et al., 2024).

Similarly, impregnating biochar with metal oxides (e.g., Fe_3O_4, ZnO) can enhance its adsorption capacity for inhibitory compounds such as heavy metals and phenolic acids, thereby protecting immobilized microbes from environmental stressors (Singh et al., 2024). These functionalized biochar systems have demonstrated superior performance in various applications, including soil amendment, wastewater treatment, and bioremediation, highlighting their potential for sustainable agriculture (Qian et al., 2023; Zama et al., 2018).

2.3.1. Lactic Acid Bacteria (LAB) and Their Biochar Interactions

Lactic Acid Bacteria (LAB), including genera such as *Lactobacillus* and *Pediococcus*, exhibit unique acid tolerance and antimicrobial properties that make them critical players in biochar-mediated agricultural systems (Xiang et al., 2022). LAB produce bacteriocins—antimicrobial peptides—that suppress pathogens like *Salmonella* and *Escherichia coli* in biochar-enriched composts (Xu et al., 2022). Their extracellular polymeric substances (EPS), composed of polysaccharides and proteins, facilitate robust adhesion to biochar surfaces via hydrogen bonding and hydrophobic interactions. This adhesion is enhanced by biochar's porous structure, which provides protective niches against acidic conditions during fermentation (Atmakuri et al., 2024; Manikandan et al., 2023)

In composting systems, LAB-biochar synergies enhance organic matter stabilization by accelerating lignocellulose degradation. For instance, *Lactobacillus plantarum* immobilized on rice husk biochar increases the humification index (HI) by 25% through EPS-mediated aggregation of organic particles (Wang et al., 2022; Emmanuel et al., 2017). Additionally, LAB's bacteriocin production reduces *Clostridium* spp. populations by 90% in manure composts, mitigating odor emissions. These interactions align with biochar's role as a pathogen suppressor (Section 3.4), where its adsorption capacity complements LAB's antimicrobial activity (Iacomino et al., 2022; Manyi-Loh et al., 2016).

2.3.2. Bacillus spp. and Their Adhesion Mechanisms on Biochar

Bacillus species, such as *B. subtilis* and *B. amyloliquefaciens*, are spore-forming bacteria renowned for their enzymatic versatility and biocontrol capabilities. Their adhesion to biochar is mediated by quorum sensing (QS)-regulated biofilm formation, where autoinducers like acyl-homoserine lactones (AHLs) trigger polysaccharide secretion (Sahreen et al., 2022; Zhao et al., 2025). Biochar's high surface area (>300 m^2/g) and mesoporous structure (2–50 nm) provide anchoring sites for biofilm development, enhancing microbial resilience against desiccation and UV radiation (Wong and Ogbonnaya 2021).

Bacillus-biochar systems excel in organic waste decomposition. For example, *B. licheniformis* immobilized on wood-derived biochar secretes cellulases and proteases that degrade lignocellulose and keratin-rich poultry feathers, reducing composting time by 30% (Kiruba and Saeid 2022). Furthermore, *Bacillus* spp. suppress fungal pathogens like *Fusarium oxysporum* through lipopeptide production (e.g., surfactin), with biochar adsorbing mycotoxins to reduce phytotoxicity (Yadav et al., 2024). These mechanisms correlate with Section 4.2, where biochar's role as a composting enhancer is amplified by microbial enzyme activity.

2.3.3. Rhizobia and Their Interaction with Biochar in Nitrogen Fixation

Rhizobia, including *Rhizobium leguminosarum* and *Bradyrhizobium japonicum*, form symbiotic relationships with leguminous plants, fixing atmospheric nitrogen (N_2) into ammonia (NH_3) through root nodulation (Elebiyo et al., 2024). Biochar enhances rhizobial viability by buffering soil pH and adsorbing heavy metals (e.g., Al^{3+}) that inhibit nodulation. Lectin proteins on rhizobial cell surfaces bind to biochar's carboxyl groups (-COOH), stabilizing bacterial attachment to root exudates and biochar particles (Ma et al., 2019; Xiu et al., 2021).

In degraded soils, biochar-rhizobia systems increase nitrogen availability by up to 40 kg N/ha in soybean fields, reducing synthetic fertilizer dependency (Hardy 2016). Biochar's phosphate solubilization capacity further synergizes with rhizobial nitrogen fixation, as seen in *Rhizobium*-biochar treatments that boost chickpea yields by 35% in phosphorus-deficient soils (Iftikhar et al., 2024; Cheng et al., 2023). These interactions resonate with Section 3.3, where biochar-mediated nutrient retention supports microbial biofertilizer efficacy.

2.4. Biochar as a Protective Environment for Microbial Survival and Proliferation

Biochar's ability to protect microbes from abiotic stressors is a key factor in its efficacy as a microbial carrier. Its porous structure acts as a physical barrier, shielding microbes from predation, desiccation, and UV radiation (Chakraborty and Akhtar 2021). The high water-holding capacity of biochar (up to 3.5 times its weight) ensures a stable moisture regime, which is critical for microbial survival in arid or semi-arid environments (Naorem et al., 2023). The carbon-rich matrix of biochar also serves as a slow-release energy source, sustaining microbial activity over extended periods. The recalcitrant carbon fraction of biochar provides a long-term carbon sink, while the labile carbon fraction (e.g., volatile organic compounds) serves as an immediate energy source for microbial metabolism (Luo et al., 2024; Giagnoni and Renella 2022). This property is particularly important for maintaining the viability of microbial inoculants during storage and application in the field (Zhang et al., 2022).

Additionally, biochar's adsorption properties mitigate the effects of toxic compounds, such as heavy metals and organic pollutants, by immobilizing them and reducing their bioavailability (Zhang et al., 2013). This protective role is particularly evident in contaminated soils, where biochar-based inoculants have been shown to enhance the survival and functionality of pollutant-degrading microbes (Xiang et al., 2022). For example, biochar has been used to immobilize *Pseudomonas putida*, a bacterium capable of degrading polycyclic aromatic hydrocarbons (PAHs), in petroleum-contaminated soils, significantly improving bioremediation efficiency (Armanu and Volf 2022; Zhang et al., 2025).

2.4 .1. Mycorrhizal Fungi and Their Colonization on Biochar

Mycorrhizal fungi, particularly arbuscular mycorrhizal fungi (AMF) such as *Rhizophagus irregularis* and *Glomus mosseae*, play a pivotal role in enhancing plant nutrient uptake, drought resistance, and soil structure through symbiotic associations with plant roots (Wahab et al., 2023; Jaafar 2014). The macropores (>50 μm) in biochar facilitate fungal mobility, while the micropores (<2 μm) offer protective niches against desiccation and predation. Biochar's surface chemistry, particularly its carboxyl (-COOH) and hydroxyl (-OH) groups, enhances fungal adhesion by mimicking root exudates, which are rich in organic acids and sugars (Amesalu et al., 2020; Nardi et al., 2021; Panchal et al., 2022). This interaction is mediated by fungal lectins, which bind to biochar's surface functional groups, stabilizing the hyphal network. Additionally, biochar's alkaline pH (7–10) neutralizes acidic soils, creating favorable conditions for AMF colonization, which typically thrive in neutral to slightly alkaline environments (Warnock 2009).

In agricultural systems, AMF colonizing biochar-amended soils significantly improve phosphorus (P) uptake by 40–60% through the extension of hyphal networks into biochar pores, where P is adsorbed and gradually released (Vahedi et al., 2022). This enhanced nutrient uptake is particularly beneficial in P-deficient soils, where biochar-AMF systems have been shown to increase crop yields by 20–30%. Furthermore, fungal hyphae and biochar particles form stable soil aggregates, improving water infiltration and retention Increasing soil water-holding capacity by 20–30% in drought-prone regions, reducing irrigation requirements by up to 25% (Yang et al., 2020; Akpinar et al., 2023; Ghorbani and Amirahmadi et al., 2024).

2.4.2. Streptomyces spp. as Biochar-Immobilized Biocontrol Agents

Streptomyces species, such as *S. griseoviridis* and *S. lydicus*, are renowned for their antibiotic production and biocontrol capabilities (Orozco-Mosqueda et al., 2021; Yang et al., 2025). The porous structure of biochar stabilizes Streptomyces populations by enhancing the production of antibiotics and hydrolytic enzymes (e.g., chitinases) that degrade fungal cell walls (Xiang et al., 2022; Wang and Kuzyakov 2024), and adsorbing the inhibitory compounds, such as phenolic acids, that impede bacterial growth. These actinobacteria produce secondary metabolites, including streptomycin and actinomycin, which suppress soil-borne pathogens (El-Sayed et al., 2023).

In practical applications, biochar-immobilized *Streptomyces* reduce *Fusarium* wilt incidence in tomatoes by 70–80% through the combined action of antibiotics and induced systemic resistance (ISR) in plants. This pathogen suppression is complemented by biochar's ability to adsorb mycotoxins and other inhibitory compounds, (Liu et al., 2023; Bonanomi et al., 2023).

2.5. Biochar as a Microbial Habitat and Its Role in Phytoremediation

Biochar provides a highly porous structure that serves as a physical habitat for microbial colonization, promoting biofilm formation and enhancing microbial proliferation (Wong and Ogbonnaya 2021). This structural property not only facilitates the retention of moisture and nutrients but also serves as a protective niche for beneficial microbes, shielding them from environmental stresses (Qadir et al., 2024).

Additionally, biochar's ability to adsorb toxic compounds, including heavy metals, creates a favourable microenvironment that selectively enriches beneficial microbes. The interactions with beneficial microbial communities contributes to soil detoxification. These interactions, both direct and indirect, significantly influence the efficiency of phytoremediation, a plant-based approach for removing, stabilizing, or transforming toxic heavy metals in the soil (Gorovtsov et al., 2020; Narayanan and Ma 2022). As in the case of *Bacillus*, *Pseudomonas*, and mycorrhizal fungi, which play a crucial role in metal detoxification, organic matter decomposition, and plant growth promotion (**Fig.2**) (Ghani et al., 2024).

The interactions between biochar and microbial communities contribute to phytoremediation through several mechanisms, including metal immobilization, enhanced microbial detoxification, and improved plant nutrient acquisition. Biochar-mediated changes in soil physicochemical properties, such as increased pH, cation exchange capacity (CEC), and the availability of essential nutrients, further enhance microbial activity and plant-metal interactions (She et al., 2023; Natasha et al., 2022).-Biochar's redox-active functional groups, such as quinones and phenolic compounds, facilitate electron transfer between microbial cells and their environment, thereby enhancing key microbial-driven processes such as nitrogen fixation, denitrification, and the biotransformation of heavy metals (Xiao et al., 2024).

The ability of biochar to act as an electron shuttle is particularly important in anaerobic environments, where microbial reduction of metal ions leads to their precipitation in less bioavailable and less toxic forms (Wu et al., 2020). For example, sulfate-reducing bacteria (SRB) and iron-reducing bacteria (IRB) facilitate the conversion of soluble metal ions like cadmium (Cd), lead (Pb), and mercury (Hg) into stable precipitates, thereby reducing their bioavailability and environmental toxicity (Charlet et al., 2012; Yang et al., 2021; Ding et al., 2024).

The impact of biochar on microbial community dynamics is largely dependent on its feedstock composition and pyrolysis conditions. Biochar produced from woody biomass, for instance, is rich in aromatic carbon, which supports the growth of lignin-degrading fungi that contribute to organic matter decomposition and soil stabilization (Palansooriya et al., 2019; Omoni et al., 2024). Conversely, biochar derived from manure or agricultural waste is nutrient-rich and favors the proliferation of fast-growing beneficial bacteria that enhance nutrient cycling and soil fertility (Bhunia et al., 2021). These microbial communities further contribute to phytoremediation by secreting organic acids, siderophores, and extracellular enzymes that mobilize or chelate heavy metals, facilitating their uptake by plant roots (Kumar et al., 2017). Moreover, the increased microbial activity in biochar-amended soils enhances phytostabilization by promoting root exudation and the formation of metal-binding complexes, reducing metal leaching and groundwater contamination (Osman et al., 2024).

The role of biochar-microbe interactions in phytoremediation extends beyond metal immobilization to include metal mobilization and translocation within plant tissues (Hou et al., 2024). Biochar enhances the bioavailability of certain heavy metals through microbially mediated solubilization, allowing plants to take up and subsequently translocate these metals to aerial parts where they can be volatilized or stored in vacuoles, minimizing their toxic effects on cellular metabolism (Madouh et al., 2024; Aryal 2024).

Additionally, plant-associated microbes contribute to phytovolatilization, where toxic metals such as mercury are converted into volatile forms and released into the atmosphere in a less harmful state (Tiodar et al., 2021). These complex interactions between biochar, microbial communities, and plant systems underscore the potential of biochar as a critical component in phytoremediation strategies aimed at mitigating heavy metal contamination (Yasin et al., 2024).

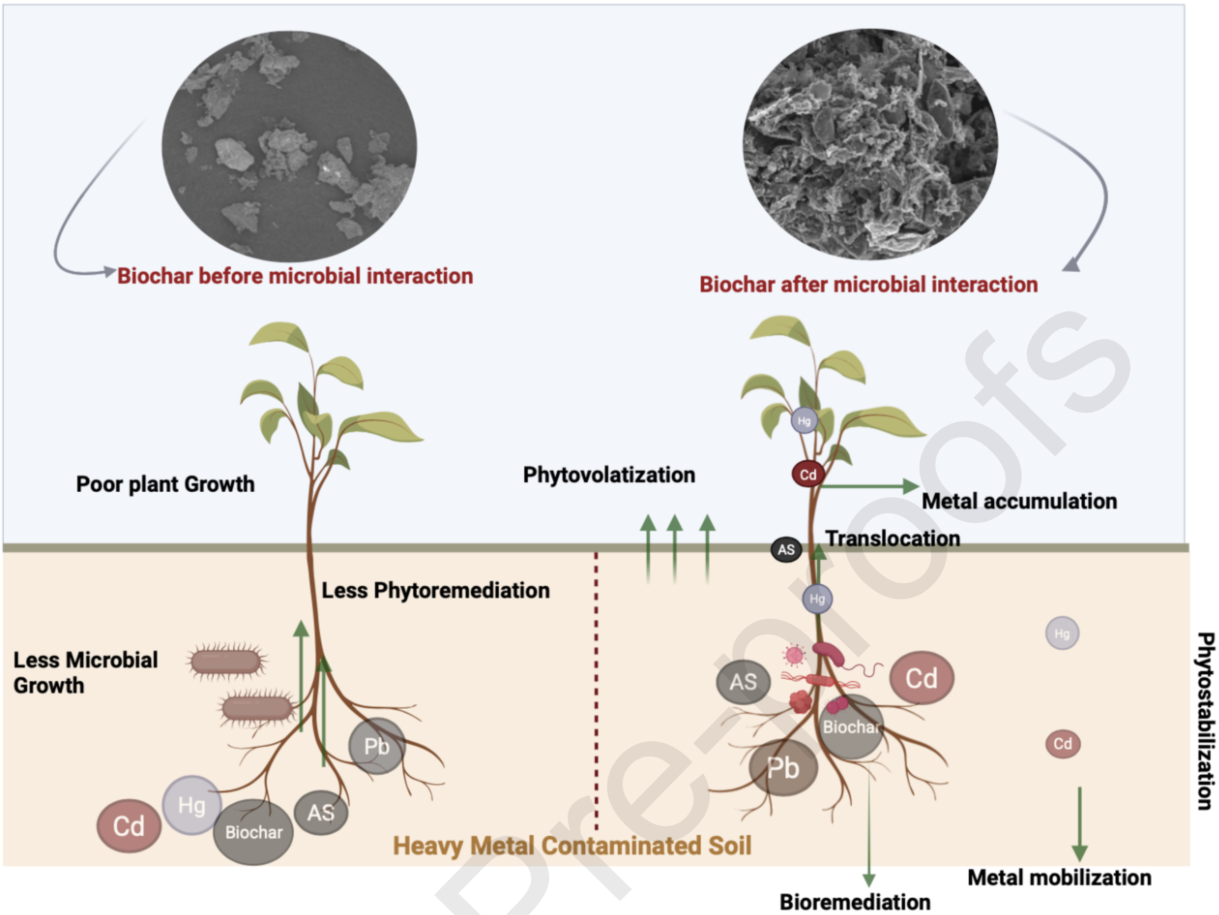

Figure 2: Schematic representation of biochar-microbe interactions in heavy metal-contaminated soil, showing the impact on plant growth, microbial activity, and phytoremediation mechanisms

Abbreviations: Pb – Lead, Cd – Cadmium, Hg – Mercury, As – Arsenic+

2.5.1. Synergistic Effect of Biochar and Probiotic Microbes in Organic Waste Decomposition

The integration of biochar with probiotic microbes such as Lactic Acid Bacteria (LAB) and *Bacillus* spp. has revolutionized organic waste decomposition by enhancing microbial activity and stabilizing compost quality (Ma 2024). LAB, including *Lactobacillus plantarum*, lower the pH of composting systems through lactic acid production, creating an acidic environment that inhibits pathogenic bacteria such as *Escherichia coli* and *Salmonella* (Rachwał and Gustaw 2024). This pH reduction is complemented by biochar's buffering capacity, which prevents excessive acidification and maintains optimal conditions for microbial activity (Hoang et al., 2022). Simultaneously, *Bacillus* spp., such as *B. subtilis* and *B. licheniformis*, secrete cellulases and proteases that break down lignocellulosic materials in agricultural waste, accelerating the composting process. Biochar's porous structure provides a habitat for these microbes, protecting them from environmental stressors and enhancing their enzymatic efficiency (Harindintwali et al., 2020; Saravanan et al., 2023).

Actinobacteria, particularly *Streptomyces* spp., further contribute to waste degradation by breaking down complex polymers such as lignin and cellulose. These microbes produce ligninolytic enzymes (e.g., laccase, peroxidase) that degrade lignin into simpler phenolic compounds, which are then mineralized by other microbial consortia (Kirby 2005). Biochar's redox-active functional groups (e.g., quinones) facilitate electron transfer during these enzymatic reactions, improving degradation rates by 30–40% (Niladevi and Prema 2008). For example, in poultry waste composting, biochar-*Streptomyces* systems reduce composting time by 25% while increasing the humification index (HI) by 20%, resulting in nutrient-rich compost with enhanced agronomic value. These synergistic interactions align with biochar's role in enhancing microbial resilience and activity, as discussed in earlier sections, highlighting its potential in organic waste recycling (**Fig.3)** (Ji et al., 2024; Gong et al., 2023).

2.5.2. Biochar-Mediated Enhancement of Plant Growth-Promoting Microbes (PGPMs)

Biochar serves as a carrier and enhancer for Plant Growth-Promoting Microbes (PGPMs), including Rhizobia, Mycorrhizae, and *Pseudomonas fluorescens*, which play critical roles in improving plant nutrient uptake and stress resistance (Ansabayeva et al., 2025; Dzvene et al., 2024). Rhizobia, such as *Rhizobium leguminosarum*, form symbiotic relationships with leguminous plants, fixing atmospheric nitrogen (N_2) into ammonia (NH_3) through root nodulation (Lebrazi and Benbrahim 2014). Biochar enhances Rhizobia viability by buffering soil pH and adsorbing heavy metals (e.g., Al^{3+}) that inhibit nodulation. Field studies have shown that biochar-Rhizobia systems increase nitrogen availability by up to 40 kg N/ha in soybean fields, reducing the need for synthetic fertilizers (Hardy 2016).

Mycorrhizal fungi, such as *Rhizophagus irregularis*, extend their hyphal networks into biochar pores, accessing adsorbed phosphorus (P) and other nutrients that are otherwise unavailable to plants (Yang et al., 2020). This enhances P uptake by 40–60%, particularly in P-deficient soils. Furthermore, biochar's water-holding capacity supports mycorrhizal activity in drought-prone regions, improving plant drought resistance by 20–25% (Chowdhury et al., 2024).

Pseudomonas fluorescens, a well-known PGPM, produces siderophores that chelate iron (Fe^{3+}), making it available to plants in iron-deficient soils. Biochar enhances siderophore production by providing a stable habitat and adsorbing inhibitory compounds (Brick et al., 2025). For example, in calcareous soils, biochar-*Pseudomonas* systems increase plant iron uptake by 30%, alleviating chlorosis and improving crop yields. These interactions underscore the potential of biochar-PGPM systems to enhance soil fertility and plant health, as discussed in earlier sections (Ullah et al., 2024; Malaie et al., 2024).

2.5.3. Biochar as a Microbial Reservoir in Biocontrol Applications

Biochar's role as a microbial reservoir extends to biocontrol applications, where it enhances the persistence and efficacy of beneficial microbes such as *Trichoderma* spp. and *Bacillus subtilis*. *Trichoderma harzianum*, a fungal biocontrol agent, colonizes biochar surfaces and releases antifungal compounds (e.g., chitinases, glucanases) that degrade the cell walls of pathogenic fungi such as *Fusarium oxysporum* and *Rhizoctonia solani (*Asif et al., 2023; Yadav et al., 2024).—As observed in tomato fields, biochar-*Trichoderma* systems reduce *Fusarium* wilt incidence by 70–80%, significantly improving crop yields (Luigi et al., 2022).

Bacillus subtilis, another key biocontrol agent, forms robust biofilms on biochar surfaces, enhancing its persistence in soil. These biofilms produce lipopeptides (e.g., surfactin, iturin) that disrupt the cell membranes of fungal pathogens, suppressing diseases such as *Phytophthora* blight and *Botrytis* gray mold (Abdoli et al., 2025). Biochar's adsorption capacity further enhances biocontrol by sequestering mycotoxins and other inhibitory compounds, creating a more favorable environment for plant growth (Iacomino et al., 2022). Field trials have demonstrated that biochar-*Bacillus* formulations increase microbial persistence by 50%, reducing pathogen populations by 60–70% in vegetable crops. These findings correlate with biochar's role in reducing soil pathogens through synergistic mechanisms, as discussed in earlier sections, highlighting its potential in sustainable crop protection (**Fig.3**) (Ajeng et al., 2023; Patani et al., 2024).

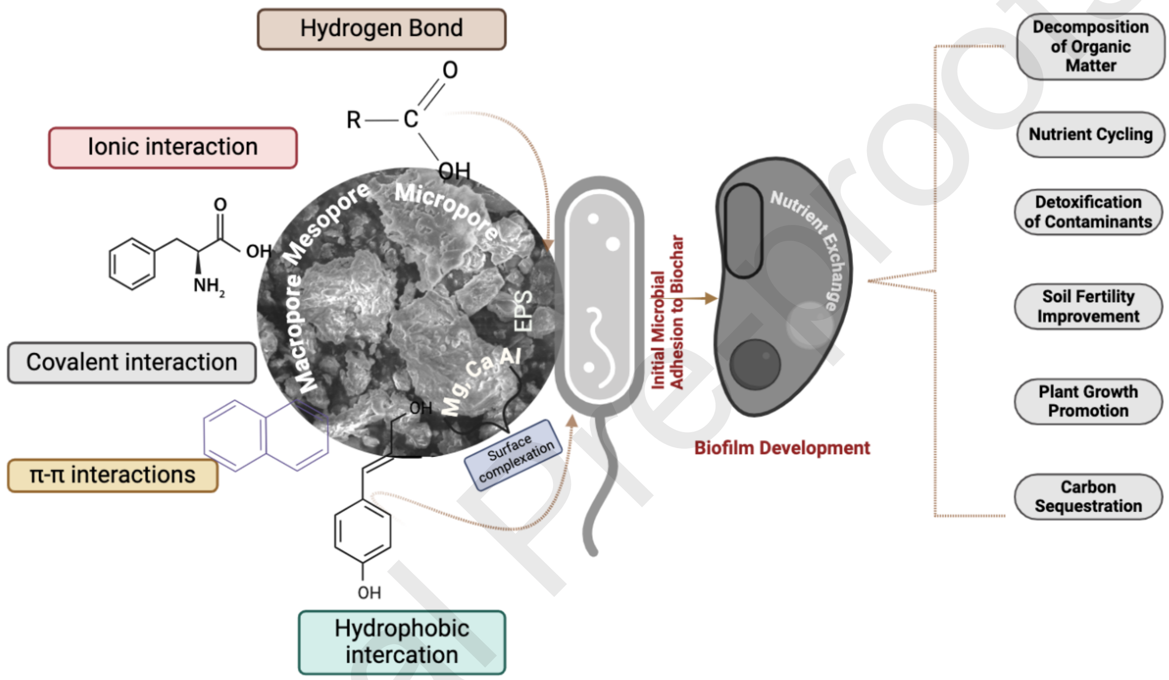

Figure 3: Schematic Representation of Biochar Properties Influencing Microbial Adhesion and Colonization

3. Role of Biochar in Probiotic and Beneficial Microbial Delivery

3.1. Biochar as a Carrier for Probiotic Bacteria in Soil Health Enhancement

Biochar's role in probiotic delivery hinges on its ability to modulate soil physicochemical parameters while serving as a microhabitat for microbial colonization (Arshad 2024). The hierarchical pore structure of biochar, with macropores (>50 μm) facilitating microbial mobility and micropores (<2 μm) acting as protective niches, ensures optimal spatial distribution of bacteria such as *Bacillus subtilis* and *Rhizobium leguminosarum* (Sivaram et al., 2023). Surface functional groups (e.g., carboxyl, quinones) on biochar participate in redox reactions, enhancing electron transfer for microbial metabolism (Yuan 2017). For example, *Azospirillum brasilense* immobilized on wood-derived biochar exhibits 30–50% higher nitrogenase activity due to biochar's electron-donating capacity, which sustains anaerobic microsites essential for nitrogen fixation (Mahmud et al., 2024).

Biochar also buffers soil pH fluctuations (e.g., raising acidic soils to pH 6.5–7.2), mitigating aluminum toxicity and enhancing phosphorus availability for phosphate-solubilizing bacteria (PSB) (Tao et al., 2020). Synchrotron-based X-ray microscopy studies reveal that biochar's nanopores (<100 nm) sequester labile carbon, which is gradually released as dissolved organic carbon (DOC), fueling microbial growth (Bolan et al., 2022). Field trials demonstrate that biochar-loaded *Pseudomonas fluorescens* increases root exudate production in maize by 25%, promoting rhizosphere aggregation and soil organic matter (SOM) accumulation. These mechanisms collectively improve soil health, with meta-analyses showing a 15–30% reduction in synthetic fertilizer use in biochar-amended systems (Gong et al., 2024).

3.2. Biochar-Associated Probiotics for Organic Waste Degradation

The synergy between biochar and probiotics accelerates lignocellulose degradation via enzymatic and non-enzymatic pathways. Biochar's graphitic carbon domains act as catalytic surfaces, enhancing the activity of ligninolytic enzymes (e.g., laccase, manganese peroxidase) produced by *Phanerochaete chrysosporium* (*M*ou et al., 2023; Kontro et al., 2024;). For instance, rice husk biochar functionalized with Fe_3O_4 nanoparticles increases laccase stability by 40% under thermophilic composting conditions (55–65°C), enabling efficient delignification of rice straw (Kumar et al., 2025).

In anaerobic digestion, biochar-immobilized *Clostridium thermocellum* exhibits 20% higher cellulase activity due to biochar's ability to adsorb inhibitory phenolic compounds (e.g., furfural, hydroxymethylfurfural) (Wang et al., 2024). This adsorption reduces metabolic stress on microbes, as shown by transcriptomic analyses where genes encoding ABC transporters (for nutrient uptake) are upregulated in biochar-associated *Clostridium* (Freddo A. 2013). Additionally, biochar's high electrical conductivity (2–5 S/cm) facilitates direct interspecies electron transfer (DIET) between syntrophic bacteria (e.g., *Geobacter*) and methanogens, boosting methane yield by 30–50% in manure digesters (Sun et al., 2023).

Case studies highlight that co-composting poultry waste with *Trichoderma viride*-loaded biochar reduces ammonia emissions by 60% via adsorption of NH_3 on biochar's acidic surface groups, while simultaneously enriching compost with stabilized humic acids (HA:FA ratio >2.5) (Liu et al., 2023).

3.3. Impact of Biochar-Immobilized Probiotics on Soil Microbiome Stability

Biochar-probiotic systems enhance microbiome resilience through mechanisms such as functional redundancy and niche partitioning (Wang et al., 2024). For example, biochar-amended soils exhibit increased Shannon diversity indices (H' >3.5) due to the proliferation of diverse oligotrophic taxa like *Acidobacteria* and *Chloroflexi*, which can all metabolize biochar's recalcitrant carbon. This reflects functional redundancy, where multiple microbial groups share overlapping metabolic capacities, ensuring ecosystem functions like carbon turnover remain stable even if individual taxa fluctuate (Zhang et al., 2024). Network analysis revealing stronger co-occurrence patterns (e.g., modularity >0.4) between *Streptomyces* spp. and arbuscular mycorrhizal fungi (AMF) suggests niche partitioning, where bacteria and fungi occupy distinct but complementary roles—*Streptomyces* contributing antibiotic production and nutrient cycling in surface pores, while AMF colonize root interfaces to enhance nutrient uptake. This spatial and functional separation reduces interspecific competition while promoting synergistic interactions (Wen et al., 2024).

Under abiotic stress, such as drought, biochar's high water retention (up to 3.5 g H_2O/g) maintains moisture >12%, which supports a range of microbial taxa involved in nitrification, allowing functionally redundant nitrifiers to sustain NO_3^- production (>2 mg/kg/day) even at 40% water-holding capacity (Haque et al., 2021). Here, different ammonia-oxidizing bacteria and archaea may substitute for one another under stress, buffering nitrogen turnover.

Furthermore, biochar-immobilized *Bradyrhizobium japonicum* under heat stress conditions (35°C) shows upregulation of heat shock proteins (HSP60, HSP70), enhancing nodulation efficiency by 50% (; Meena et al., 2018; Egamberdieva et al., 2020). This adaptive microbial response, coupled with the presence of other plant growth-promoting rhizobacteria (PGPR), illustrates both functional redundancy in stress response and niche complementarity in supporting plant-microbe symbiosis across environmental gradients.

3.4. Synergistic Effects of Biochar and Probiotics in Reducing Soil Pathogens

The integration of biochar and probiotics presents a promising synergistic strategy for reducing soil-borne pathogens through complementary and mutually reinforcing mechanisms. Biochar's high surface area and porous architecture serve as an ideal microhabitat for beneficial microbes, facilitating their colonization, survival, and metabolic activity in the rhizosphere. When probiotics such as *Bacillus amyloliquefaciens*, Pseudomonas fluorescens, and Lactobacillus plantarum are immobilized on biochar, their antimicrobial efficacy is significantly enhanced due to sustained release and protection from environmental stress (Yang et al., 2024; Abdoli et al., 2025;).

One of the key synergistic mechanisms is quorum quenching, where biochar adsorbs quorum-sensing molecules like N-acyl homoserine lactones (AHLs), thereby disrupting pathogen communication networks. For instance, in tomato rhizospheres, the co-application of biochar and Pseudomonas putida suppressed *Ralstonia solanacearum* by over 70%, as demonstrated by GFP-tagged bioassays and reductions in virulence gene expression (Liu et al., 2023). Biochar-bound probiotics also produce a range of antimicrobial compounds—such as *Iturins, fengycins*, and phenazines—that damage pathogen membranes and inhibit sporulation. Greenhouse trials show that *B. amyloliquefaciens* biochar formulations reduced *Fusarium oxysporum* incidence in cucumbers by 82%, compared to 55% with probiotics alone (Enebe, 2021).

In addition to direct antagonism, biochar-probiotic combinations bolster plant immunity through Induced Systemic Resistance (ISR). Biochar alone enhances lignification and reactive oxygen species (ROS) generation, while probiotics trigger hormone-mediated defense pathways. For example, in maize, co-application of biochar and *P. chlororaphis* elevated salicylic acid (SA) and jasmonic acid (JA) levels 3–4-fold and enhanced lignin deposition in root endodermis by 40%, as evidenced by phloroglucinol-HCl staining (Ji et al., 2015; Dutta et al., 2022). This dual action creates an inhospitable environment for pathogens like *Phytophthora capsici* and *Rhizoctonia solani*, minimizing disease severity and improving crop resilience.

Moreover, the presence of biochar improves soil aeration, moisture retention, and nutrient availability, indirectly supporting a healthy microbial community that outcompetes pathogens (Hou et al., 2022). Studies also indicate that biochar-probiotic systems enhance beneficial microbiota recruitment, including mycorrhizal fungi and nitrogen-fixing bacteria, which further suppress disease indirectly via niche exclusion and resource competition Bhattacharyya and Furtak, 2022).

3.5 Unexplored and Engineered Microbial Consortia

Recent advancements in microbial biotechnology have opened new frontiers for the application of previously unexplored or genetically engineered microbial consortia in environmental remediation and stress mitigation in plants (Hussain et al., 2018; Sudheer et al., 2020). The integration of extremophilic microorganisms with biochar is an emerging strategy for remediating pollutants in harsh or extreme environmental conditions where conventional microbes exhibit reduced activity. Thermophiles, for instance, thrive at elevated temperatures (50–80 °C) and maintain enzymatic activity in composting and thermophilic anaerobic digestion systems. When immobilized on thermally stable biochars (e.g., rice husk or sugarcane bagasse-derived at >600 °C), thermophilic strains like *Thermus thermophilus* exhibit enhanced degradation of hydrocarbons and maintain high metabolic rates despite thermal stress (Sharma et al., 2023). Similarly, acidophiles such as *Acidithiobacillus ferrooxidans* have been immobilized on iron-rich biochars for the bioleaching of metals from acidic mine drainage, where the biochar matrix buffers local pH fluctuations and supports microbial stability (Li et al., 2023). In psychrophilic conditions, such as cold-region soils or wastewater at ≤10 °C, biochar-amended *Psychrobacter* spp. consortia have shown improved nitrogen cycling and phenol degradation due to biochar's ability to reduce freezing-induced stress and enhance nutrient retention. These combinations are particularly useful for the remediation of contaminated environments with extreme pH, salinity, or temperature, such as mining sites, polar agricultural soils, saline aquifers, and thermophilic digesters. Biochar's buffering capacity, structural stability, and protective microenvironments play a crucial role in stabilizing extremophiles and supporting their functional expression under challenging conditions. However, more field-scale studies are needed to optimize biochar-extrémophile pairings for scalable environmental biotechnologies.

Among these, extremophiles such as *Deinococcus radiodurans*, renowned for its extraordinary resistance to ionizing radiation and oxidative stress, present unique metabolic features and genetic robustness that can be harnessed for detoxifying environments contaminated with heavy metals and organic pollutants (Sadowska-Bartosz and Bartosz, 2023). Despite their potential, such microorganisms remain underrepresented in phytoremediation and rhizoremediation studies (Alves et al., 2022). The integration of these resilient strains into microbial consortia could significantly enhance the tolerance and detoxification capacity of plants exposed to abiotic stress (Ge et al., 2023). Parallel to this, the development of synthetic microbial communities—deliberately designed consortia composed of functionally complementary microorganisms—has emerged as a promising strategy to address complex environmental challenges. Engineered strains of *Pseudomonas spp.*, often modified using CRISPR-Cas systems for enhanced biosurfactant production, metal chelation, or stress-responsive signaling, have demonstrated synergistic effects when combined with other beneficial microbes such as nitrogen-fixing bacteria and phosphate-solubilizing fungi (Liang et al., 2022; Thankappan et al., 2024) (**Table-1**). These consortia are particularly effective in targeting co-contaminants, such as simultaneous presence of heavy metals and organic pesticides, by facilitating multi-pathway degradation and immobilization. Moreover, synthetic consortia can be tailored to specific plant species and stress profiles, thereby enabling precision-based microbial interventions (Spina et al., 2018). Collectively, the inclusion of lesser-known extremophiles and CRISPR-edited strains into designed microbial consortia represents a paradigm shift toward more resilient, adaptive, and multifunctional bioinoculants with high potential for sustainable agriculture and environmental restoration (Palit et al., 2025).

Microbe	**Source**	**Target Pollutant**	**Mechanism**	**Novelty**	**Reference**
Pseudomonas putida (corn-cob biochar-immobilized)	Corn-cob biochar (immobilized)	Paraquat (herbicide)	Biochar-immobilized *P. putida* covalently binds and biodegrades paraquat to 4,4′-bipyridyl and malic acid.	First report of paraquat degradation via biochar-immobilized P. putida with malic acid as intermediate	Nguyen et al., 2023
MB3R consortium (biochar-immobilized)	Biochar (potato-field bacterial consortium)	Metribuzin (herbicide)	Biochar-immobilized MB3R consortium accelerates metribuzin breakdown.	Novel synthetic consortium showing enhanced metribuzin biodegradation and microbiome restoration in field soils	Wahla et al., 2020
Alcaligenes faecalis WZ-2	Wheat-straw biochar (isolated strain)	Tebuconazole (fungicide)	Biochar-immobilized WZ-2 markedly accelerates tebuconazole degradation.	First-time use of A. faecalis WZ-2 on wheat-straw biochar improving tebuconazole degradation and soil function	Sun et al., 2020
Chlorella vulgaris + activated	Synthetic microalgae–bacterial consortia	Chlorpyrifos, Cypermethrin	Biochar-supported microalgae-bacteria photobioreacto	Innovative algae–bacteria consortium on biochar	Mojiri et al., 2022

sludge (consortium)	(biochar support)	(insecticides)	r degrades insecticides via secreted hydrolases.	enabling simultaneous removal of multiple insecticides	
Paenarthrobacter sp. AT5	Soybean soil isolate (biochar-assisted)	Atrazine (herbicide)	Bioaugmentation with AT5 stimulates atz-gene expression and atrazine degradation.	Engineered deployment with biochar significantly reduces atrazine phytotoxicity and reshapes the rhizosphere microbiome	Harindintwali et al., 2024

Table 1: Novel Microbial Strains and Their Roles in Biochar-Mediated Bioremediation

4. Application of Biochar-Immobilized Microbes in Agricultural Waste Recycling

The synergistic performance of biochar–microbe systems in agricultural waste recycling stems from the direct interactions between biochar's physicochemical properties and microbial functionality. Biochar's porous structure, high surface area, and chemically active functional groups (e.g., –COOH, –OH, phenolics) provide protective microhabitats, stable attachment surfaces, and electron-donating/accepting capabilities that enhance microbial survival, colonization, and enzymatic expression (Rossi et al., 2021). These properties stabilize extracellular enzymes (e.g., cellulases, laccases), enhance nutrient retention for microbial metabolism, and shield microbes from environmental stresses such as fluctuating pH, desiccation, and toxic intermediates. Importantly, biochar can act as a physical and chemical mediator, concentrating substrates near microbial cells and facilitating redox-based degradation processes (Li et al., 2022). By creating microenvironments optimized for microbial activity, biochar not only hosts beneficial species but also amplifies their functional efficiency, particularly in the breakdown of lignocellulosic residues and nutrient cycling within diverse agroecosystems.

4.1. Enhancing Organic Waste Decomposition and Nutrient Cycling

Biochar-immobilized microbes significantly accelerate the decomposition of recalcitrant organic wastes, such as lignocellulosic crop residues and animal manure, by creating synergistic microenvironments (Ji 2024). The porous architecture of biochar provides a refuge for hydrolytic enzymes (e.g., cellulases, laccases) secreted by *Trichoderma reesei* and *Aspergillus niger*, protecting them from denaturation under fluctuating pH and temperature (Gan et al., 2024). For instance, corn stover biochar functionalized with *Phanerochaete chrysosporium* increases lignin degradation rates by 40% via Fenton reactions mediated by biochar's redox-active quinone groups (Wu et al., 2023). Simultaneously, biochar's high surface area (300–500 m^2/g) adsorbs phenolic inhibitors (e.g., ferulic acid), reducing their bioavailability and enabling *Cellulomonas fimi* to efficiently hydrolyze cellulose into glucose (**Table-2**) (Kumneadklang 2019).

Biochar generally has the potential to adsorb and gradually release essential nutrients such as ammonium (NH_4^+), phosphate (PO_4^{3-}), and potassium (K^+), thereby supporting microbial metabolism and growth, particularly under suitable conditions and depending on factors such as soil pH, texture, moisture, and the specific surface chemistry of the biochar.

In rice paddy systems, bamboo biochar loaded with *Azotobacter vinelandii* enhances nitrogen fixation (up to 30 kg N/ha) while reducing NH_3 volatilization by 25% through adsorption on acidic surface functional groups (-COOH, -OH).

Beyond paddy systems, biochar–microbial consortia also exhibit promising results in dryland and arid cropping systems. In dryland wheat cultivation, inoculating biochar with *Bacillus subtilis* improved plant-available phosphorus by 35% due to organic acid excretion and phosphate solubilization, even under low soil moisture conditions (Li et al., 2022). Similarly, in semi-arid chickpea systems, *Rhizobium* sp. immobilized on biochar enhanced nodulation and nitrogen assimilation, resulting in a 20% yield increase and improved drought tolerance (Tiwari et al., 2023). In Mediterranean olive orchards, biochar co-applied with *Pseudomonas fluorescens* boosted potassium uptake and root colonization, attributed to biochar's buffering capacity and microbial stabilization (Rossi et al., 2021).

Metagenomic analyses reveal that biochar-microbe systems enrich *Bacteroidetes* and *Firmicutes*, key phyla for polysaccharide degradation, increasing soil dissolved organic carbon (DOC) by 15–20% (**Fig.3**) (Chakraborty and Akhtar 2021; Shen et al., 2019).

These findings highlight that biochar–microbe systems are not limited to flooded ecosystems but have broader applicability across diverse cropping systems, including those with limited water availability, degraded soils, or low organic matter content. However, performance may vary depending on biochar properties, microbial compatibility, and edaphic conditions, suggesting the need for site-specific optimization rather than assuming universal applicability.

4.2. Biochar as a Carrier for Composting Microbes and Fermentation Enhancers

Traditional composting systems face challenges such as prolonged maturation times (60–90 days) and nitrogen loss via NH_3 emissions. Biochar-immobilized thermophilic microbes (e.g., *Bacillus stearothermophilus*, *Thermobifida fusca*) address these limitations by sustaining high-temperature phases (>55°C) for extended periods, accelerating lignocellulose breakdown (Zhao et al., 2024). Pinewood biochar inoculated with *Geobacillus thermoglucosidasius* reduces composting time by 35% by stabilizing β-glucosidase activity, which converts cellobiose to fermentable sugars (Drufva, 2018).

Biochar also acts as a fermentation enhancer in anaerobic digestion. Its conductive carbon matrix (electrical conductivity: 1–3 S/cm) facilitates direct interspecies electron transfer (DIET) between *Clostridium* spp. and methanogenic *Archaea*, boosting methane yields by 40–

60% in dairy manure digesters (Sarker and Nikhil, 2023). Additionally, biochar's alkaline pH (8–10) buffers acidic by-products (e.g., volatile fatty acids), maintaining optimal conditions for *Methanosarcina barkeri* (Lee et al., 2022). Field trials demonstrate that co-composting poultry waste with *Streptomyces griseus*-loaded biochar reduces NH_3 emissions by 50% and increases humification indices (HI >0.8) through enhanced melanoidin formation (Kamal et al., 2022).

4.3. Role of Biochar-Associated Probiotic Microbes in Sustainable Biofertilizer Production

Biochar-probiotic systems transform agricultural waste into nutrient-dense biofertilizers by coupling microbial metabolic pathways with biochar's nutrient retention properties (Aiduang, 2025). For example, *Rhizobium leguminosarum* immobilized on soybean straw biochar fixes atmospheric nitrogen (N_2) into NH_3, which is stabilized on biochar's negatively charged surfaces, reducing leaching losses by 30% (Mohammed, 2023). Similarly, phosphate-solubilizing *Pseudomonas fluorescens* releases organic acids (e.g., gluconic acid) that dissolve $Ca_3(PO_4)_2$, with biochar sequestering the liberated PO_4^{3-} for gradual release (Pramanik et al., 2025).

These biofertilizers exhibit superior performance compared to chemical analogs. A 2-year field study showed that biochar loaded with *Azospirillum brasilense* and arbuscular mycorrhizal fungi (AMF) increased wheat grain yields by 22% while reducing synthetic N-P-K inputs by 35% (Zaheer et al., 2024). The biochar-AMF symbiosis enhances hyphal exploration, increasing soil phosphorus uptake efficiency by 50% in alkaline soils. Furthermore, biochar's recalcitrant carbon serves as a long-term carbon sink, sequestering 2–3 tons of CO_2-equivalent per hectare annually (**Table-1**) (Warnock et al., 2007).

5. Role of Biochar-Immobilized Microbes in Biodegradation of Agricultural Pollutants

5.1. Degradation of Pesticide Residues

The persistence of synthetic pesticides, including organophosphates, carbamates, and neonicotinoids, in agricultural soils poses significant environmental and health risks. Biochar-immobilized microbial consortia have emerged as a promising solution for enhancing pesticide degradation (Mussali-Galante et al., 2023). The porous structure of biochar provides a protective habitat for microbes, shielding them from pesticide toxicity while concentrating the pollutants near microbial cells for efficient degradation (Mukherjee et al., 2022). For instance, *Arthrobacter* spp. and *Pseudomonas putida* immobilized on hardwood biochar (pyrolyzed at 550°C) exhibit enhanced organophosphate hydrolase activity, achieving 80–90% degradation of chlorpyrifos within 72 hours (Adetunji et al., 2022). Biochar's alkaline pH stabilizes enzymatic activity, while its redox-active functional groups facilitate electron transfer for co-metabolic detoxification (Mukherjee et al., 2022). Similarly, *Trametes versicolor* immobilized on lignin-rich biochar degrades neonicotinoids like imidacloprid via laccase-mediated oxidation, with biochar's quinone groups acting as redox mediators (Roy et al., 2025). Field trials have demonstrated the efficacy of these systems, with biochar-*Pseudomonas* consortia reducing chlorpyrifos residues by 90% in cotton fields, outperforming traditional composting methods (Chaudhary et al., 2023). These findings highlight the potential of biochar-microbe systems to mitigate pesticide contamination in agricultural soils (**Table-2**).

5.2. Heavy Metal Immobilization and Detoxification

Heavy metal contamination in agricultural soils, particularly cadmium (Cd), lead (Pb), arsenic (As), and chromium (Cr), threatens food safety and ecosystem health. Biochar-immobilized microbes offer a dual strategy for metal immobilization and detoxification, combining adsorption with microbial transformation (Swapnil et al., 2023). *Pseudomonas aeruginosa* immobilized on poultry manure biochar secretes siderophores that chelate Cd^{2+}, reducing its bioavailability by 70%. Simultaneously, biochar's oxygenated functional groups (-COOH, -OH) bind Pb^{2+} through ion exchange, achieving 95% immobilization efficiency (Gong et al., 2024; Liu et al., 2025;). In the case of arsenic, arbuscular mycorrhizal fungi (AMF) colonizing biochar surfaces secrete glomalin-related soil proteins (GRSP), which sequester As(III) in stable complexes, reducing bioavailability by 60% (Al-Shamma et al., 2017). For chromium, *Bacillus subtilis* immobilized on biochar reduces toxic Cr(VI) to less toxic Cr(III) via chromate reductase, with biochar's carboxyl groups stabilizing the enzyme (Li et al., 2023). A case study in Cd-contaminated rice paddies demonstrated that rice straw biochar loaded with *Bacillus* and AMF reduced grain Cd content by 65%, compared to 40% with biochar alone (Yang et al., 2025). These results underscore the synergistic potential of biochar-microbe systems in heavy metal remediation (**Table-2**).

5.3. Removal of Antibiotic and Pharmaceutical Waste

The widespread use of antibiotics in livestock farming has led to the contamination of agricultural runoff with compounds such as tetracyclines, sulfonamides, and fluoroquinolones, posing risks to aquatic ecosystems and human health (Du and Liu, 2012). Biochar-microbe systems combine adsorption and biodegradation to enhance antibiotic removal. *Streptomyces* spp. immobilized on sludge-derived biochar hydroxylate tetracycline at the C4 position, rendering it non-toxic, while biochar's π-π interactions adsorb tetracycline (200 mg/g), prolonging microbial exposure (Teo et al., 2022). Similarly, *Acinetobacter* biofilms on coconut shell biochar cleave sulfamethoxazole's isoxazole ring via monooxygenases, with biochar's electrical conductivity (1.5 S/cm) accelerating electron transfer and doubling degradation rates (Liu et al., 2024). Biochar also buffers pH fluctuations, neutralizing acidic metabolites from fluoroquinolone degradation and preventing microbial inhibition (Oh et al., 2022). Field data from swine wastewater treatment systems show that biochar-*Streptomyces* consortia achieved 85% sulfadiazine removal, outperforming activated sludge (50%) (Eheneden et al., 2023). These findings highlight the potential of biochar-microbe systems to address antibiotic contamination in agricultural waste.

5.4. Organic Pollutants and Hydrocarbon Decomposition

Polycyclic aromatic hydrocarbons (PAHs) and lignin-rich agro-industrial wastes are persistent environmental pollutants that demand robust and multifunctional remediation strategies (De Oliveira et al., 2019). Biochar–microorganism systems enhance pollutant degradation by combining microbial enzymatic activity with the adsorptive and redox-modulating properties of biochar. For example, *Sphingomonas paucimobilis* immobilized on pinewood biochar exhibited enhanced degradation of pyrene, a four-ring PAH. In controlled microcosm studies, pyrene removal was 78% in the biochar-immobilized system compared to only 42% in free-cell and 25% in abiotic biochar-only controls after 21 days (Yuan, 2023). The enhancement was attributed to biochar's micropores (<2 nm), which trap PAH molecules and increase the contact time and concentration near microbial cells. Furthermore, biochar's redox-active quinone moieties acted as electron shuttles, boosting electron transfer to *Rhodococcus* spp., thereby enhancing CYP450 monooxygenase activity responsible for benzo[a]pyrene breakdown. This mechanism was validated by using quinone-depleted biochar as a negative control, which led to a 40% reduction in degradation rate.

In the case of lignin-rich substrates, *Phanerochaete chrysosporium* immobilized on bamboo biochar secreted manganese peroxidase (MnP), facilitating the oxidative depolymerization of lignin into aromatic monomers such as vanillin. Biochar's mesoporous structure stabilized MnP and protected it from proteolytic degradation; MnP retained 70% residual activity after 10 cycles, compared to <30% in the non-immobilized fungal control (Lv et al., 2022; Noor, 2021). Enzyme activity assays showed a 2.5-fold increase in Vmax and a lower Km for MnP in biochar-immobilized systems, indicating enhanced affinity and catalytic efficiency.

Additionally, synergistic microbial consortia, such as *Cellulomonas fimi* with white-rot fungi, efficiently hydrolyzed lignocellulose into glucose. In these systems, biochar adsorbed phenolic inhibitors (e.g., syringic and ferulic acid), which otherwise suppressed microbial activity. Control experiments with non-adsorptive biochar showed 35% lower cellulase activity, confirming the inhibitor-scavenging role of functional biochar surfaces (Rabha et al., 2023).

In oil-contaminated soils, consortia of *Rhodococcus erythropolis* and activated carbon-rich biochar achieved 85% degradation of total petroleum hydrocarbons (C10–C40) within 60 days. Comparatively, non-biochar treatments showed only 47% degradation under identical conditions. The improved performance was linked to biochar's hydrophobic surfaces, which concentrate hydrocarbons and shield microbes from toxicity peaks (Assil et al., 2021). Furthermore, in soil mesocosms, the biochar–microbe system maintained higher microbial viability and reduced the accumulation of toxic intermediates such as benzene and toluene, as confirmed by GC-MS profiling.

These examples collectively underscore that biochar–microorganism systems function through multiple, experimentally validated mechanisms: (i) increasing pollutant–microbe proximity via adsorption, (ii) stabilizing extracellular enzymes, (iii) acting as redox mediators, and (iv) sequestering inhibitors and toxic intermediates. The inclusion of proper abiotic and free-cell controls in these studies demonstrates the distinct synergistic advantage of combining biochar with microbial agents for the degradation of structurally complex and environmentally persistent organic pollutants.

5.4.1 Emerging Biochar–Microbe Strategies for Novel Pollutants

Recent advances in biochar technology have introduced non-traditional feedstocks, such as algal biomass, for producing biochars with distinct properties that are especially suited for environmental remediation. Algal biochar, derived from species like *Spirulina platensis* or *Ulva lactuca*, exhibits high nitrogen content, ion-exchange potential, and intrinsic metal-binding ligands, making it particularly effective in sequestering heavy metals and ammonium (Hu et al., 2023). When combined with metal-tolerant or metal-transforming bacteria (e.g., *Shewanella oneidensis*), algal biochar creates a redox-active interface that facilitates simultaneous metal immobilization and microbial reduction.

In parallel, the use of genetically engineered microbial strains offers novel capabilities in degrading synthetic or recalcitrant emerging contaminants. For instance, *Pseudomonas putida* strains engineered to express chlorinated solvent hydrolases have shown enhanced degradation of trichloroethylene when immobilized on high-surface-area biochar produced via hydrothermal carbonization of algae (Chen et al., 2024). Similarly, CRISPR-modified *E. coli* strains expressing laccase and peroxidase genes can decompose pharmaceutical residues such as diclofenac or carbamazepine more efficiently when stabilized on biochar derived from algal or food-waste co-pyrolysis (Kumar et al., 2023). These emerging combinations of functionalized biochar and engineered or extremophilic microbial strains represent a next-generation platform for tackling novel and co-occurring pollutants in complex matrices like landfill leachate, pharmaceutical wastewater, or heavy metal-laden soils. However, these systems require further field validation and environmental risk assessment, particularly concerning the persistence and gene flow of engineered organisms in open systems.

Table 2: Common Probiotic and Beneficial Microbial Strains Immobilized on Biochar and Their Agricultural & Environmental Functions

Microbial Strain	Biochar Feedstock	Pyrolysis Temperature (°C)	Key Mechanisms	Agricultural & Environmental Impact	Biochar-Microbe Interaction	Citations
Bacillus subtilis	Hardwood	600	Surfactin production; biofilm formation	Reduces Fusarium by 70%; improves maize yield by 25%	Forms thick biofilms on biochar pores, enhancing persistence in soil	(Xiang et al., 2022; Chen et al., 2023)
Trichoderma reesei	Rice husk	400	Cellobiohydrolase secretion; lignin depolymerization	Accelerates composting by 30%; increases humification index to 0.8	Colonizes biochar micropores, enhancing lignocellulose degradation	(Xia and Lin et al., 2022)
Rhizobium leguminosarum	Pinewood	550	NodD gene upregulation; siderophore production	Boosts soybean nitrogen fixation by 150 kg/ha; reduces urea use by 40%	Forms symbiotic biofilms on biochar-enhanced root surfaces	(Rao et al., 2019; Ankati and Podile et al., 2018)
Pseudomonas putida	Sewage sludge	700	Dioxygenase enzymes for PAH degradation	Degrades 90% of petroleum hydrocarbons in contaminated soils	Uses biochar as an electron acceptor in degradation pathways	(Tarigholizadeh et al., 2023)

Streptomyces griseoviridis	Coconut shell	500	Chitinase production; streptothricin antibiosis	Suppresses Pythium ultimum in cucumber; increases AMF colonization by 60%	Enhances biochar-based microbial consortia for pathogen suppression	(Mourouzidou et al., 2023; Joseph 1997)
Azospirillum brasilense	Wheat straw	450	IAA production; ACC deaminase activity	Increases wheat biomass by 30%; enhances drought resistance	Colonizes biochar hydrophilic surfaces, aiding nitrogen fixation	(Spaepen et al., 2008; Krishna et al., 2023)
Bacillus amyloliquefaciens	Bamboo	600	Antifungal lipopeptide production	Suppresses Verticillium wilt in tomato by 65%	Biochar enhances quorum sensing and spore viability	(Azizoglu et al., 2025; Wang 2019)
Mycorrhizal fungi (Glomus spp.)	Corn cob	500	Hyphal network expansion; phosphorus solubilization	Improves phosphorus uptake in maize by 40%; increases root biomass	Biochar micropores enhance fungal spore germination and hyphal elongation	(Abou et al., 2022; Matheus et al., 2024)
Enterobacter cloacae	Corn stalk	550	Biofilm-mediated heavy metal biosorption	Reduces cadmium bioavailability by 85%; enhances plant metal tolerance	Uses biochar surface chemistry for metal adsorption and detoxification	(Naseem et al., 2022; Liu et al., 2025)

Lactobacillus plantarum	Palm husk	400	Lactic acid production; pathogen suppression	Enhances compost stabilization; increases soil organic matter	Acidic metabolites modify biochar surface pH, stabilizing microbial communities	(Moyosore et al., 2020; Gao et al., 2023)
Paenibacillus polymyxa	Pine bark	600	Exopolysaccharide production; biofilm formation	Improves nitrogen fixation in cereal crops; increases microbial diversity	Stabilizes on biochar, improving moisture retention in root zones	(Sadekuzzaman et al., 2015; Nwachukwu et al., 2023)
Pseudomonas fluorescens	Agricultural waste	500	Siderophore secretion; auxin production	Increases root elongation in legumes; protects against Pseudomonas syringae	Uses biochar as a reservoir for siderophore production	(Panpatte et al., 2016; Hakim et al., 2021)
Saccharomyces cerevisiae	Coconut shell	450	Yeast-derived organic acid secretion	Inhibits Botrytis cinerea in grapes; enhances compost fermentation	Adheres to biochar via polysaccharide-protein complexes	(Bardhan et al., 2020; Louw 2004)
Actinobacteria (Microbacterium spp.)	Eucalyptus wood	650	Production of antimicrobial peptides	Enhances microbial diversity; decomposes lignin derivatives	Forms stable communities within biochar-enhanced rhizosphere soils	(Yadav and Patil. 2021; Yadav and Ramakrishna. 2023)

Lysinibacillus sphaericus	Sugarcane bagasse	600	Bioremediation of toxic metals (As, Hg, Cr)	Reduces arsenic contamination by 70%; promotes metal tolerance in crops	Binds heavy metals on biochar-enhanced root surfaces	(Eras-Muñoz et al., 2022; Pires 2010)
Phanerochaete chrysosporium	Paper mill sludge	500	Lignin peroxidase secretion	Breaks down lignin, dyes, and industrial effluents	Uses biochar as a structural matrix for extracellular enzyme secretion	(Ram et al., 2020; Zhang et al., 2022)

5.5. Biochar-Microbe Systems for Emerging Contaminants: A Multi-Omics Framework for Understanding Functional Interactions

The synergistic integration of biochar and microbial systems has emerged as a powerful and sustainable approach for the remediation of emerging contaminants, including per- and polyfluoroalkyl substances (PFAS), microplastics, and pharmaceutical residues such as carbamazepine and diclofenac (Sarma et al., 2022). Biochar, a carbon-rich material derived from pyrolyzed biomass, offers a highly porous structure and abundant surface functional groups that facilitate the adsorption of hydrophobic and persistent contaminants (Wen et al., 2023). When coupled with specific microbial strains capable of enzymatic degradation, biochar not only serves as a carrier matrix but also enhances microbial colonization, electron shuttling, and substrate bioavailability (Gong et al., 2022; Saravanan et al., 2023). For instance, biochar-supported microbial consortia have shown promise in the partial degradation and transformation of PFAS, which are otherwise recalcitrant to conventional treatments due to their strong carbon-fluorine bonds (Berhanu et al., 2023). Similarly, microplastics, which pose significant ecotoxicological risks, can be physically immobilized by biochar and subsequently biodegraded by plastic-degrading bacteria, leading to a reduction in environmental persistence (Adamu et al., 2024). In the case of pharmaceutical compounds like carbamazepine and diclofenac—commonly detected in wastewater effluents—biochar-amended microbial systems have demonstrated enhanced removal efficiency through combined adsorption and microbial transformation mechanisms (Tawfik et al., 2024). Importantly, these systems also address the growing challenge of co-contaminant synergy, such as the concurrent presence of heavy metals (e.g., lead) and antibiotics. The presence of biochar can mitigate metal toxicity, thereby preserving microbial activity essential for antibiotic degradation, while certain microbes possess dual-functional pathways enabling simultaneous detoxification of both classes of contaminants (Rahman, 2020) (**Table-3**).

The emerging multi-omics approaches—including metagenomics, metaproteomics, and metabolomics—have begun to unravel the functional interactions between biochar, microbes, and contaminants. For example, metaproteomic analysis of PAH-degrading biochar–microbial systems revealed upregulation of stress-related chaperones (e.g., DnaK, GroEL) and catabolic enzymes such as dioxygenases and peroxidases, confirming enhanced microbial resilience and degradation potential under pollutant pressure (Sarma et al., 2022). Similarly, metabolomic profiling has detected intermediate metabolites (e.g., catechol, protocatechuate) that confirm functional pathways activated in the presence of biochar, particularly in the transformation of pharmaceuticals like carbamazepine and diclofenac (Tawfik et al., 2024) (**Fig.4**). Metagenomic evidence also shows that biochar enrichment selects for microbial taxa with complementary degradation pathways and stress tolerance, such as *Sphingomonas*, *Rhodococcus*, and *Phanerochaete* (Berhanu et al., 2023). This integrated approach not only improves the efficacy and resilience of bioremediation systems but also offers scalable and cost-effective solutions for complex pollutant matrices in agricultural and urban ecosystems.

Table 3: Performance of Biochar-Microbe Systems on Emerging Contaminants

Contaminant	Microbial Agent	Biochar Type	Removal Efficiency	Mechanism	Reference

Chlortetracycline (CTC)	Bacillus subtilis	Honeysuckle residue–derived biochar	78.35%	Adsorption and microbial degradation	Zhang and Wang (2021)
Tetracycline (TC)	Bacillus cereus W2	Biochar (type unspecified)	Not specified	Enhanced microbial degradation	Xue, J. et al., (2025)
Sulfamethoxazole (SMX)	Bacillus paramycoides SDB4	Biochar-immobilized bioreactor	97.42%	Biosorption and biotransformation	Chen, Y. et al., (2023)

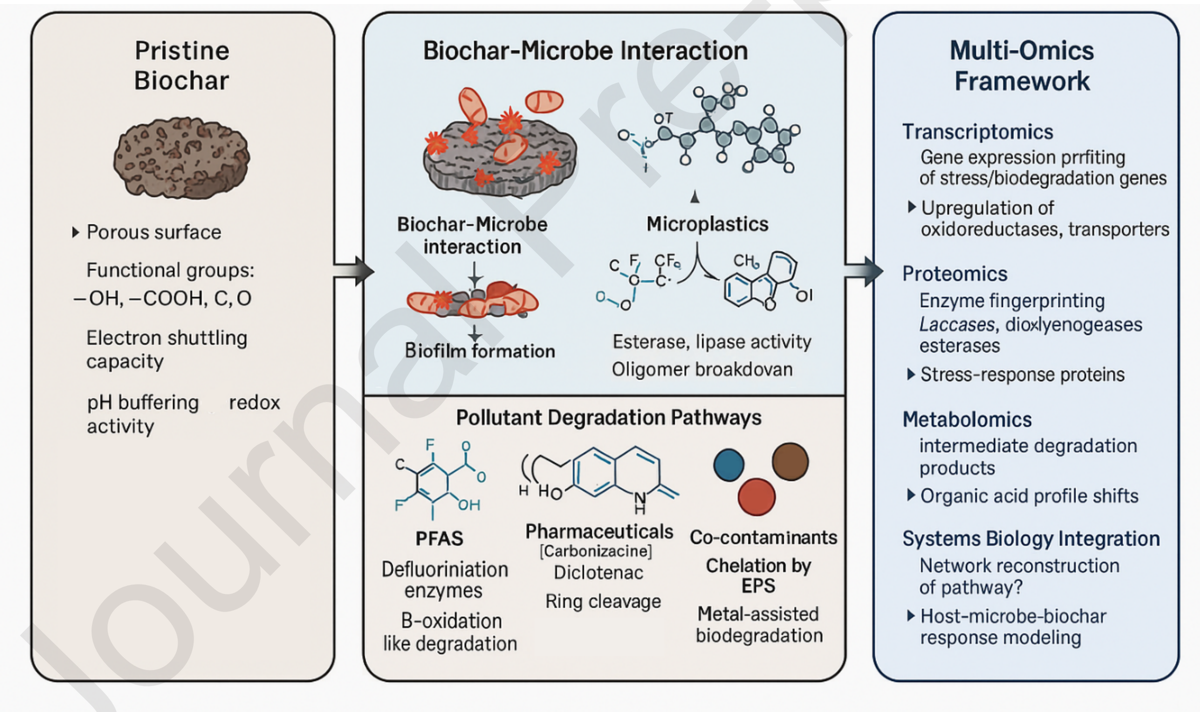

Figure 4: Multi-Omics Framework for Understanding Biochar-Microbe Interactions

5.6. Biochar-Mediated Microbial Inoculation for Enhancing Soil Fertility and Crop Productivity

Biochar-microbe inoculants enhance soil fertility through dual mechanisms: physical-chemical stabilization and biological activation. Biochar's nanopores (<100 nm) entrap rhizosphere microbes, creating "microbial hotspots" that amplify nutrient cycling (Imran 2024; Sarma et al., 2024). For instance, biochar loaded with *Bradyrhizobium japonicum* increases soybean nodulation by 60%, as biochar's Ca^{2+} and Mg^{2+} ions stimulate nodD gene expression (Ma et al., 2019). Concurrently, biochar's alkaline pH (8–9) in acidic soils solubilizes Al^{3+} and Mn^{2+} toxins, restoring root elongation and enhancing water-use efficiency by 20% (Li et al., 2024).

Crop productivity gains are well-documented. In semi-arid regions, maize treated with *Bacillus amyloliquefaciens*-biochar composites showed a 30% yield increase due to ACC deaminase activity, which reduces ethylene-induced root stress (Zafar-ul-Hye et al., 2019). Similarly, biochar-*Trichoderma harzianum* systems suppress *Fusarium* wilt in tomatoes via chitinase-mediated cell wall degradation, increasing marketable yields by 45% (Ali et al., 2023). Multi-season trials confirm that biochar-microbe systems elevate soil organic carbon (SOC) by 1.5–2.0% annually, fostering resilient agroecosystems (Zhu et al., 2019).

Table 4: Impact of Biochar-Modified Microbial Systems on Pollutant Removal from Soil and Water

Treatment Technique	Biochar Source	Production Temperature (°C)	Modification Strategy	Microbial Strain	Target Contaminant	Optimal pH	Initial Contaminant Load (mg/L)	Decontamination Efficiency (%)	Microbial Action Pathway	Biochar Contribution	Refernce
Encapsulation Matrix	Citrus Peel Biochar	600	Layered Double Hydroxide Doping	Acinetobacter sp. FYF8	Nitrate	7.0	5	95.32	Microbial enzymatic reduction	Provides a stable microhabitat	(Yang and Yang 2024; Kumar et al., 2020)
Microbial Biofilm Coating	Rice Husk Biochar	500	Fe_3O_4 Nanoparticle Infusion	Bacillus sp. K1	Cadmium (Cd^{2+})	7.0	50	87.00	Active biosorption and intracellular sequestration	Increases metal immobilization	(Nguyen et al., 2022; Priyanka et al., 2024)
Adsorption-Facilitated Degradation	Corn Residue Biochar	650	Magnetite (Fe_3O_4) Composite	*Pseudomonas putida*	Lead (Pb^{2+})	6.5	10	91.20	Oxidation and enzymatic detoxification	Enhances microbial-metal binding	(Akoijam et al., 2024)

Biochar-Enhanced Microbial Oxidation	Bamboo Biochar	700	TiO_2 Nanoparticle Doping	*Sphingomonas sp.*	Phenol	6.0	15	88.90	Oxygenase-mediated breakdown	Provides surface for microbial attachment	(Mishra et al., 2022)
Encapsulated Bioreactor	Coconut Shell Biochar	600	Zinc Oxide (ZnO) Functionalization	*Enterobacter cloacae*	Chromium (Cr^{6+})	5.5	20	82.50	Microbial-assisted redox conversion	Alters bioavailability of Cr	(Tufail et al.,2022)
Hydroxylation & Redox Binding	Eucalyptus Biochar	500	H_2O_2 Surface Activation	*Streptomyces violarus*	Manganese (Mn^{2+})	6.8	3	74.80	Biotransformation and mineralization	Promotes biofilm resilience	(Shah 2023)
Biochar-Immobilized Enzymatic Degradation	Rice Straw Biochar	550	Iron Sulfate ($FeSO_4 \cdot 7H_2O$)	*Leclercia adecarboxylata*	Arsenic (As^{3+})	5.0	20	61.00	Oxidation and active efflux transport	Supports microbial electron transfer	(Sevak and Pushkar 2023)
Microbial Hydrocarbon Remediation	Palm Fiber Biochar	700	Magnesium Oxide (MgO) Impregnation	*Halomonas sp.*	Benzo(a)pyrene	8.0	10	69.20	PAH ring cleavage and oxidation	Reduces contaminant persistence	(Ahmad et al., 2021)

Sustained Pollutant Biodegradation	E. prolifera Biochar	600	Biochar Surface Acid Activation	*Pseudomonas sp.*	Pyrene	7.5	20	91.90	Dioxygenase enzyme degradation	Stabilizes microbial consortia	(Wu et al., 2022)
Ion Exchange and Metal Chelation	Sawdust Biochar	450	Calcium Hydroxide ($Ca(OH)_2$)	*Joostella sp.*	Indeno(1,2,3-cd)pyrene	7.2	10	77.30	Electrostatic attraction and microbial breakdown	Enhances bioavailability reduction	(Mukherjee and Pal 2024)

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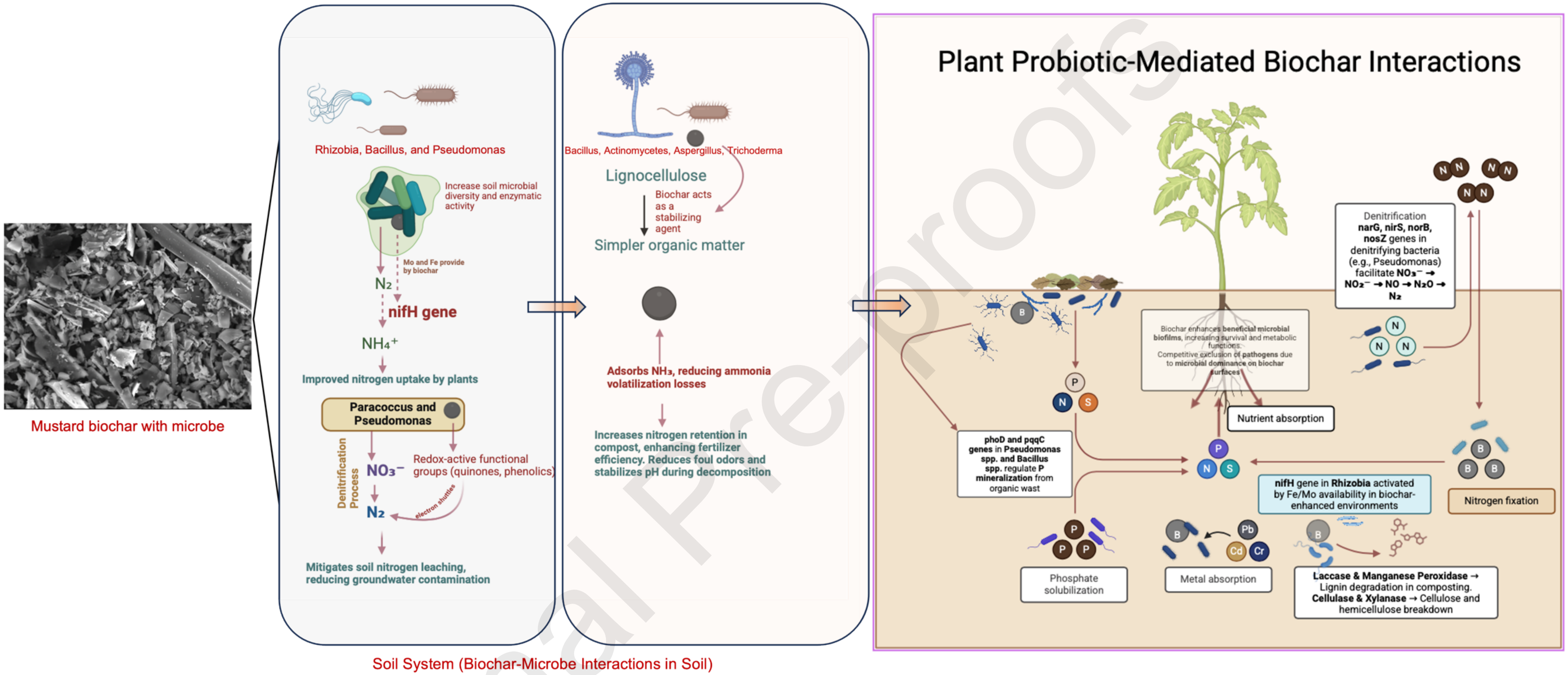

Figure 5: Biochar-mediated interactions with plant-probiotic microbes enhance nutrient cycling, nitrogen fixation, phosphate solubilization, and heavy metal remediation, promoting plant growth and soil health

Abbreviations: *nifH* (Nitrogenase Iron-Protein Gene), *narQ* (Nitrate Sensor Kinase), *nirS* (Nitrite Reductase Gene), *norB* (Nitric Oxide Reductase Gene), *nosZ* (Nitrous Oxide Reductase Gene), *phoB* (Phosphate Regulon Transcriptional Regulatory Protein), *pqqC*(Pyrroloquinoline Quinone Biosynthesis Protein C), Pb (Lead), Cd (Cadmium), Cr (Chromium).

6. Factors Affecting Probiotic and Beneficial Microbial Colonization on Biochar

6.1. Influence of Biochar Feedstock and Pyrolysis Conditions on Probiotic Retention

The retention and functionality of probiotics on biochar are profoundly influenced by feedstock composition and pyrolysis parameters (Martiny et al., 2024). Feedstocks with high lignin content (e.g., hardwood, coconut shells) produce biochars rich in aromatic carbon and macropores (>50 µm), which favor colonization by lignocellulose-degrading fungi like *Phanerochaete chrysosporium* (Taskin et al., 2019). Conversely, manure-derived biochars, high in ash and nutrients (e.g., K^+, Ca^{2+}), support rapid bacterial proliferation, as shown by 50% higher *Bacillus subtilis* viability compared to wood-based biochars (Blenis et al., 2023).

Pyrolysis temperature critically modulates biochar's physicochemical properties. Low-temperature biochars (300–400°C) retain volatile organic compounds (VOCs) and labile carbon, serving as immediate energy sources for heterotrophic microbes like *Pseudomonas putida* (Gonçalves et al., 2020; Mukherjee et al., 2022). High-temperature biochars (600–700°C), however, exhibit greater surface area (400–800 m^2/g) and electrical conductivity (2–5 S/cm), enhancing electron transfer for electroactive bacteria (e.g., *Geobacter sulfurreducens*) (Yu et al., 2016). For instance, rice husk biochar pyrolyzed at 550°C immobilized *Azospirillum brasilense* with 90% retention efficiency due to balanced microporosity and carboxyl group density (Shashidhar et al., 2020) (**Table-3**).

6.2. Role of Biochar Porosity and Surface Chemistry in Microbial Adhesion and Activity

Biochar's pore architecture and surface chemistry govern microbial adhesion, biofilm formation, and metabolic activity. Hierarchical pore structures—macropores for microbial mobility, mesopores (2–50 nm) for enzyme entrapment, and micropores (<2 nm) for nutrient diffusion—create niche-specific habitats (Pavel et al., 2017). For example, *Trichoderma harzianum* preferentially colonizes 10–20 µm pores in wood-derived biochar, where oxygen diffusion supports aerobic chitinase production (Raviv et al., 2019).

Surface functional groups (e.g., -COOH, -OH, quinones) facilitate microbial adhesion via electrostatic interactions and hydrogen bonding (Yuan et al., 2017). Oxidized biochars (treated with HNO_3/H_2O_2) exhibit 30% higher *Rhizobium leguminosarum* adhesion due to enhanced negative surface charge (Zeta potential: −25 to −40 mV), which attracts positively charged bacterial cell walls (Wong and Ogbonnaya 2021). Conversely, graphitic domains in high-temperature biochars promote π-π interactions with aromatic amino acids in microbial extracellular polymeric substances (EPS), stabilizing biofilms (Huang et al., 2023) (**Table-4**).

6.3. Environmental Factors (pH, Moisture, Temperature) Impacting Biochar-Microbe Interactions

Environmental conditions play a crucial role in modulating biochar-microbe synergies by influencing physicochemical and biological dynamics. Biochar's alkaline nature (pH 7–10) helps buffer acidic soils (pH <5.5), mitigating aluminum toxicity and enhancing the survival of *Bradyrhizobium japonicum* by 40% (Saleem et al., 2022; Koinange 2015). However, in neutral to alkaline soils, its high pH can suppress acidophilic microbes such as *Thiobacillus* spp., necessitating the development of pH-adjusted biochars tailored for specific applications (Sheng and Zhu 2018). Moisture availability is another critical factor, as biochar's high water-holding capacity (1.5–3.5 g H_2O/g) sustains microbial activity in arid soils. For instance, at 60% water-filled pore space (WFPS), biochar-amended soils support the viability of *Pseudomonas fluorescens* up to three times longer than unamended soils, thereby enhancing microbial persistence under drought conditions (Kannan et al., 2020). Additionally, biochar's insulating properties play a significant role in temperature regulation, protecting microbial communities from thermal stress. In composting systems, biochar reduces temperature fluctuations by 5–8°C, which is particularly beneficial for preserving the enzymatic activity of *Bacillus stearothermophilus* within the optimal range of 55–65°C (Shi et al., 2024; Zhou et al., 2019). These interactions highlight the importance of tailoring biochar properties to specific environmental conditions to optimize microbial functions for soil health and remediation.

6.4. Stability, Shelf-Life, and Viability of Biochar-Encapsulated Probiotics in Field Applications

The shelf-life and field efficacy of biochar-encapsulated probiotics depend on encapsulation methods and environmental resilience (Khan et al., 2023). Lyophilized *Azotobacter chroococcum* on biochar retains 80% viability after 12 months at 25°C, compared to 30% viability in peat-based carriers (Zayed 2016). Biochar's UV-shielding properties reduce photoinactivation; *Streptomyces griseoviridis* survival increases by 50% under UV-B exposure (280–315 nm) when immobilized on biochar (Mohapatra and Sahoo 2024).

Field challenges include microbial leaching and competition. Cross-linking biochar with alginate or chitosan enhances retention, reducing *Bacillus amyloliquefaciens* leaching by 70% in heavy rainfall (100 mm/h) (Tang et al., 2024). Additionally, pre-colonizing biochar with native soil microbiomes minimizes antagonism, as demonstrated by 40% higher *Rhizobium* nodulation in pre-conditioned biochar treatments (Egamberdieva et al., 2016).

Table 5: Comparison of Biochar Types and Their Efficiency in Probiotic and Microbial Immobilization

Feedstock	Pyrolysis Temp.	Surface Area (m^2/g)	Key Functional Groups	Microbial Strain	Colonization Efficiency (%)	Application
Hardwood	550°C	320	-COOH, -OH, quinones	*Trichoderma reesei*	85	Lignocellulose degradation
Rice husk	400°C	250	Silica, -OCH_3	*Pseudomonas fluorescens*	75	Phosphate solubilization

Poultry manure	700°C	150	$CaCO_3$, PO_4^{3-}	*Bacillus subtilis*	90	Pathogen suppression
Coconut shell	600°C	450	Graphitic carbon, -C=O	*Geobacter sulfurreducens*	65	Electrogenic activity
Sewage sludge	300°C	80	$-NH_2$, $-SO_3H$	*Azospirillum brasilense*	70	Nitrogen fixation

7. Bibliometric Analysis of Biochar-Based Microbial Systems and Biochar-Microbe Mediated Bioremediation

The bibliometric analysis of biochar-based microbial systems and biochar-microbe mediated bioremediation provides a comprehensive overview of the research trends, domain categorization, sustainability contributions, and collaborative networks shaping this field. By utilizing Dimensions AI and VOSviewer, the study identifies key developments, emerging research areas, and influential contributors in the domain from 2014 to 2025, while excluding conference papers, book chapters, proceedings, and preprints. The year-wise publication trend (Figures 1a and 2a) illustrates a gradual increase in research output, particularly after 2016, with a peak around 2024, followed by a slight decline in 2025. This trend reflects the growing scientific and industrial interest in biochar's applications, primarily driven by its role in soil health enhancement, microbial interactions, and environmental sustainability. The early years (2014–2018) witnessed limited studies, indicating that biochar-microbe interactions were still emerging as a research priority. However, post-2020, there was an exponential increase, highlighting the widespread recognition of biochar's potential in bioremediation, waste valorization, and climate change mitigation.

A deeper insight into the classification of research domains (**Figures 6c and 7c**) reveals that a substantial number of studies fall within environmental sciences, particularly Environmental Management (4104) and Pollution & Contamination (4105), emphasizing the pivotal role of biochar in remediating contaminated environments and supporting ecological balance. Significant research also spans Soil Sciences (4106), Ecological Applications (4102), and Environmental Biotechnology (4103), reflecting biochar's expanding utility in improving soil microbial dynamics, facilitating plant growth, and integrating with microbial consortia for pollutant degradation. The presence of research contributions in Climate Change Adaptation (4101) highlights biochar's function in carbon sequestration, greenhouse gas reduction, and microbial-mediated climate resilience strategies. The domain analysis underscores biochar's versatility, transitioning from traditional soil amendment practices to advanced biotechnological applications such as microbial fuel cells, wastewater treatment, and heavy metal detoxification.

In addition to domain-specific categorization, the study aligns the bibliometric findings with global sustainability goals, as depicted in **Figures 6d and 7d.** The data suggest that biochar-microbe research significantly contributes to SDG 15 (Life on Land), particularly in terms of restoring degraded lands and improving microbial biodiversity. Moreover, a notable impact is observed on SDG 13 (Climate Action), where biochar enhances carbon storage while promoting beneficial microbial activity that aids in carbon cycling. The research also aligns with SDG 2 (Zero Hunger) by supporting sustainable agricultural practices through improved soil fertility and microbial symbiosis, ultimately enhancing crop yield and resilience against abiotic stress. Furthermore, biochar's role in SDG 6 (Clean Water and Sanitation) is evident in its application for water purification and removal of organic and inorganic contaminants. Similarly, its contribution to SDG 12 (Responsible Consumption and Production) emphasizes the integration of biochar in circular economy models, waste-to-resource strategies, and low-carbon technologies.

The co-citation network and research collaboration mapping (**Figures 7b and 8b**) provide critical insights into the key authors, institutions, and research groups that have contributed significantly to this field. The VOSviewer-based network visualization highlights strong collaborative links between researchers from diverse disciplines, including environmental science, microbiology, biotechnology, and material science. The co-citation clusters indicate that biochar research has evolved into a well-structured domain with interdisciplinary cooperation, focusing on microbial-assisted remediation, biostimulation, and eco-friendly pollutant degradation technologies. The clustering pattern reveals an increasing interest in utilizing biochar as a microbial carrier, facilitating bioaugmentation strategies for enhanced degradation of persistent organic pollutants, heavy metals, and emerging contaminants. Furthermore, the analysis suggests that biochar-microbe interactions are increasingly being explored in tandem with machine learning and predictive modeling approaches to optimize their remediation efficacy and environmental benefits.

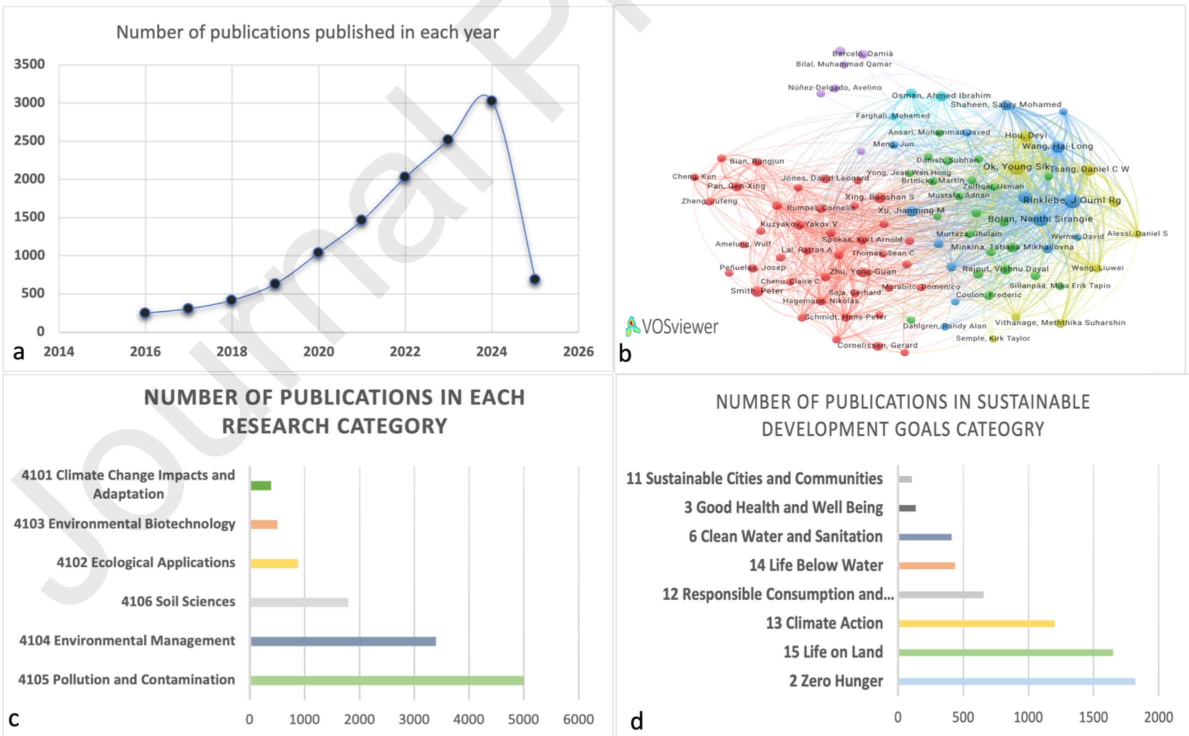

Figure 6: (a) Year-wise publication trend of biochar-based microbial systems from 2014 to 2025, showing a significant rise in research output. (b) Co-citation network analysis depicting key research collaborations, influential authors, and interdisciplinary contributions. (c) Research domain classification highlighting major subject areas such as environmental sciences, soil sciences, and ecological applications. (d) Alignment of research contributions with the United Nations Sustainable

Development Goals (SDGs), emphasizing climate action, sustainable agriculture, and environmental remediation.

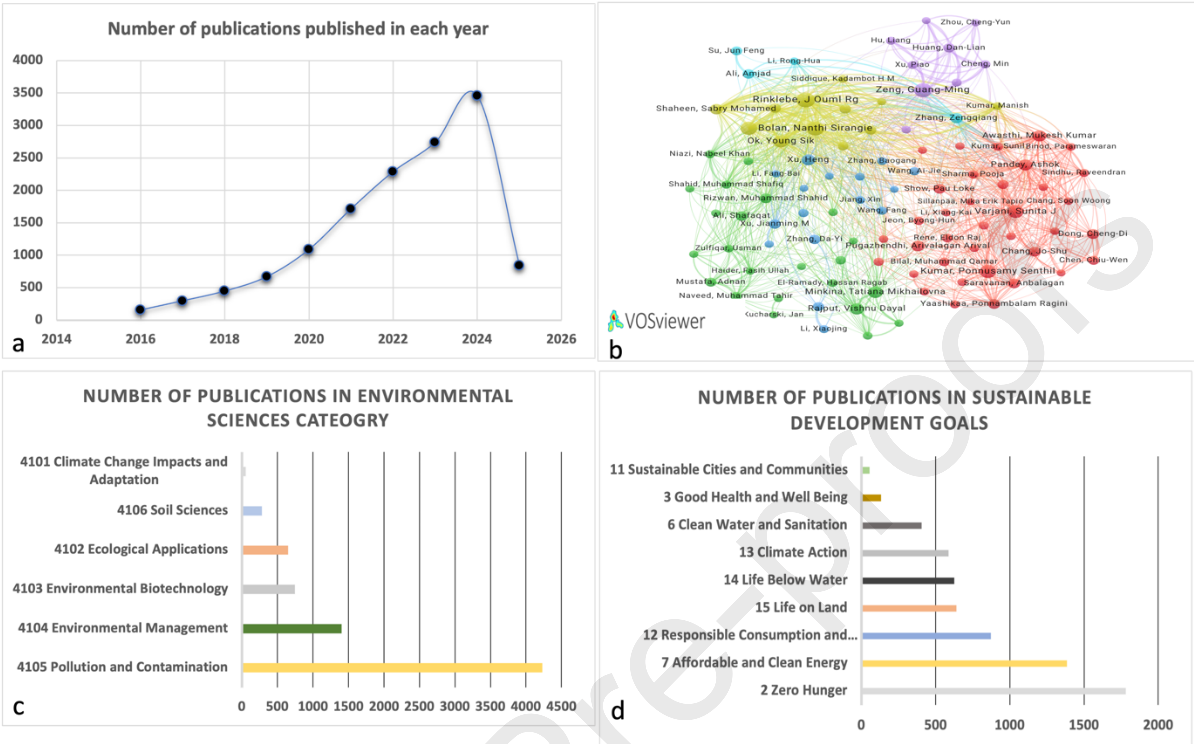

Figure 7: (a) Annual research growth in biochar-microbe mediated bioremediation illustrating the increasing scientific interest and publication trends. (b) Research collaboration mapping using VOSviewer, showcasing inter-institutional and interdisciplinary linkages. (c) Categorization of research domains based on Dimensions AI analysis, emphasizing environmental biotechnology, pollution control, and microbial ecology. (d) SDG contributions of biochar-based microbial research, with a focus on carbon sequestration, soil fertility enhancement, and water purification.

8. Challenges, Limitations, and Future Perspectives

8.1. Challenges in Large-Scale Application of Biochar as a Probiotic Carrier

Despite the promising potential of biochar as a probiotic carrier, several challenges hinder its large-scale application in agriculture. One major issue is the variability in biochar properties due to differences in feedstock and pyrolysis conditions, which can lead to inconsistent microbial colonization and activity (Domene et al., 2015). For instance, biochar derived from woody biomass may have different porosity and surface chemistry compared to manure-derived biochar, affecting its suitability for specific microbial strains (Singh et al., 2022). Additionally, the cost of producing high-quality biochar and the energy requirements for pyrolysis pose economic barriers, particularly for small-scale farmers in developing regions. Scaling up production while maintaining biochar quality and microbial viability remains a significant challenge (Zanli et al., 2022; Kochanek et al., 2022). Furthermore, the lack of standardized protocols for biochar-microbe formulation and application complicates its adoption in diverse agricultural systems. Addressing these challenges requires interdisciplinary research to optimize biochar production, develop cost-effective microbial carriers, and establish guidelines for large-scale implementation (Vijaykumar 2019; Santos et al., 2025;).

8.2. Potential Risks and Ecotoxicological Concerns of Biochar-Mediated Microbial Inoculation

The application of biochar-immobilized microbes in agriculture raises potential risks and ecotoxicological concerns that must be carefully evaluated. One concern is the unintended release of biochar-associated microbes into non-target environments, which could disrupt native microbial communities and ecosystem functions (Brtnicky et al., 2021). For example, the introduction of non-native *Bacillus* or *Pseudomonas* strains may outcompete indigenous microbes, reducing biodiversity and altering nutrient cycling processes (Mawarda 2022). Additionally, biochar itself may carry contaminants such as heavy metals or polycyclic aromatic hydrocarbons (PAHs), depending on the feedstock and pyrolysis conditions. These contaminants could leach into soil and water systems, posing risks to human and environmental health (Hu et al., 2022). Moreover, the long-term effects of biochar-microbe interactions on soil health and plant growth are not fully understood, necessitating comprehensive ecotoxicological assessments. To mitigate these risks, future research should focus on developing contaminant-free biochars, assessing the ecological impacts of microbial inoculants, and establishing regulatory frameworks for safe biochar-microbe applications.

8.3. Advances in Biochar Engineering for Optimized Probiotic Retention and Release

Recent advances in biochar engineering offer promising solutions to enhance probiotic retention and release, addressing some of the limitations associated with biochar-microbe systems (Bolan et al., 2023). Surface functionalization techniques, such as chemical oxidation and metal oxide impregnation, can modify biochar's surface chemistry to improve microbial adhesion and activity (Gęca et al., 2023). For example, oxidizing biochar with hydrogen peroxide (H_2O_2) increases the density of carboxyl groups, enhancing electrostatic interactions with microbial cell walls. Similarly, coating biochar with biopolymers like chitosan or alginate improves its biocompatibility and microbial retention properties (Hidayat 2024).

Nano-engineered biochars, incorporating nanomaterials such as graphene oxide or iron nanoparticles, further enhance microbial colonization and activity by providing additional adsorption sites and redox-active surfaces (Verma et al., 2023). These engineered biochars can also enable controlled release of microbial inoculants, ensuring sustained activity over extended periods. For instance, biochar-alginate composites have been shown to release *Rhizobium* spp. gradually, improving nodulation and nitrogen fixation in leguminous crops (Behl et al., 2024). Future research should explore the scalability and cost-effectiveness of these advanced biochar formulations, ensuring their practical applicability in diverse agricultural systems.

8.4. Future Research Directions and Policy Recommendations for Biochar-Integrated Agricultural Waste Management

To fully realize the potential of biochar-microbe systems in agricultural waste management, future research should focus on several key areas. First, interdisciplinary studies are needed to optimize biochar-microbe formulations for specific crops, soils, and environmental conditions. This includes investigating the long-term effects of biochar-microbe interactions on soil health, plant growth, and ecosystem resilience (Kochanek et al., 2022). Second, life cycle assessments (LCAs) and cost-benefit analyses should be conducted to evaluate the economic feasibility and environmental sustainability of biochar-microbe technologies (Smith 2024). These assessments will help identify the most viable applications and inform policy decisions.

Policy recommendations should prioritize the development of standardized guidelines for biochar production, quality control, and microbial inoculation. Governments and international organizations should also promote research and development through funding initiatives and collaborative networks (Venkatesa et al., 2024). Additionally, educational programs and extension services can help farmers adopt biochar-microbe technologies, ensuring their effective implementation at the grassroots level (Saravanan et al., 2023). By addressing these research and policy gaps, biochar-integrated agricultural waste management can contribute to global sustainability goals, including food security, climate change mitigation, and ecosystem restoration **(Fig.7).**

8.5. Economic Feasibility and Unintended Consequences of Microbial-Immobilized Biochar

The economic feasibility of microbial-immobilized biochar depends on factors such as production costs, scalability, and market demand. While biochar production can be energy-intensive, its long-term benefits, including improved soil fertility, reduced fertilizer use, and enhanced crop yields, often outweigh the initial costs (Sunish and Thazeem 2023; Afshar and Mofatteh 2024). However, the economic viability of biochar-microbe systems varies across regions, depending on local agricultural practices, resource availability, and policy support. For example, in regions with abundant agricultural waste, biochar production can be integrated into existing waste management systems, reducing costs and environmental impacts (Koul et al., 2022; Elkhlifi et al., 2023). The biological treatment of the PHE–polluted TW80 solution using biochar showed a total cost of 342.60 € ton− 1 of sediment (Bianco et al., 2022). The treatment involving both the PGPR consortia and biochar along with the 50% rate of synthetic fertilizer increased the potential financial return of the wheat crop. The net return (per hectare) of this treatment was estimated to be US$438 (Ijaz et al., 2019)

Unintended consequences of microbial-immobilized biochar include potential disruptions to soil microbial communities and nutrient cycling processes. For instance, the introduction of non-native microbial strains may alter soil biodiversity, leading to unforeseen ecological impacts (Abdelhamid et al., 2024; Brown et al., 2020). Additionally, the long-term accumulation of biochar in soils may affect soil structure and water dynamics, requiring careful monitoring and management (Sharma 2024). To address these concerns, future research should focus on developing region-specific biochar-microbe formulations, conducting long-term field trials, and assessing the ecological impacts of biochar applications (Schmidt et al., 2021).

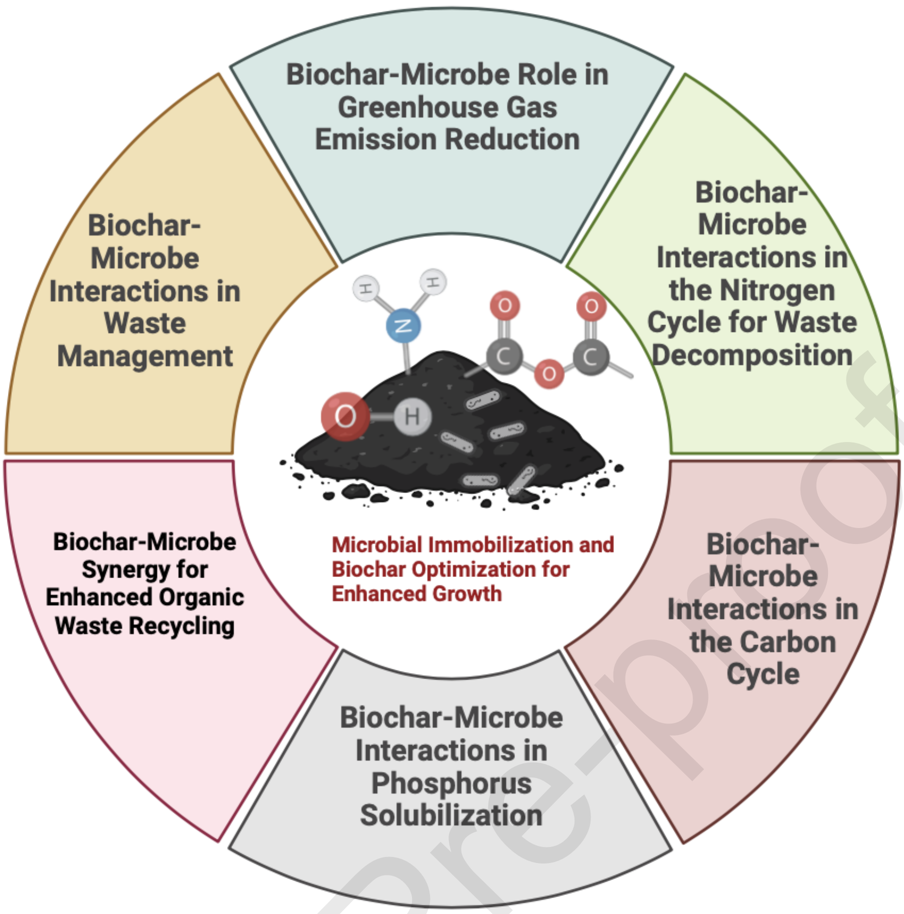

Figure 8: Biochar-microbe interactions enhance nutrient cycling, waste decomposition, and greenhouse gas mitigation for sustainable soil management

9. Conclusion

Biochar–microbe integrated systems represent a promising, multifunctional strategy for addressing critical challenges related to soil degradation, nutrient inefficiency, and environmental contamination. The synergistic interactions between biochar's physicochemical properties and microbial consortia enhance soil structure, nutrient retention, microbial colonization, and the biodegradation of pollutants, including heavy metals and organic compounds. Empirical evidence from controlled environments confirms their potential in improving compost maturity, stimulating enzymatic activity, and enhancing microbial stress tolerance. Furthermore, the application of functionalized biochars—engineered to support specific microbial functions—demonstrates improved nutrient bioavailability and pollutant immobilization. Collectively, these outcomes position biochar–microbe systems as an ecologically sustainable tool for optimizing soil health, promoting microbial functionality, and contributing to climate-resilient agriculture.

10. Ethics approval and consent to participate

This study does not involve human participants, animals, or any clinical trials; hence, ethical approval and consent to participate are not applicable.

11. Consent for publication

All authors have reviewed and approved the final manuscript for submission and consent to its publication.

12. Availability of data and materials

The data supporting this review are derived from publicly available sources, and no primary datasets were generated or analysed during the study and any other data required to journal made available on request.

13. Conflict of interest

The authors confirm that they have no known interpersonal or financial conflicts that would have appeared to have an impact on the research presented in this study.

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15. Authors' contributions

AD and RC write the original draft. AD collect data and analysis. RJ supervision resources and validation.

16. Acknowledgment

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17. Data Availability Statement

All relevant data supporting the findings of this study are included within the manuscript. Additional information can be obtained upon reasonable request from the corresponding author.

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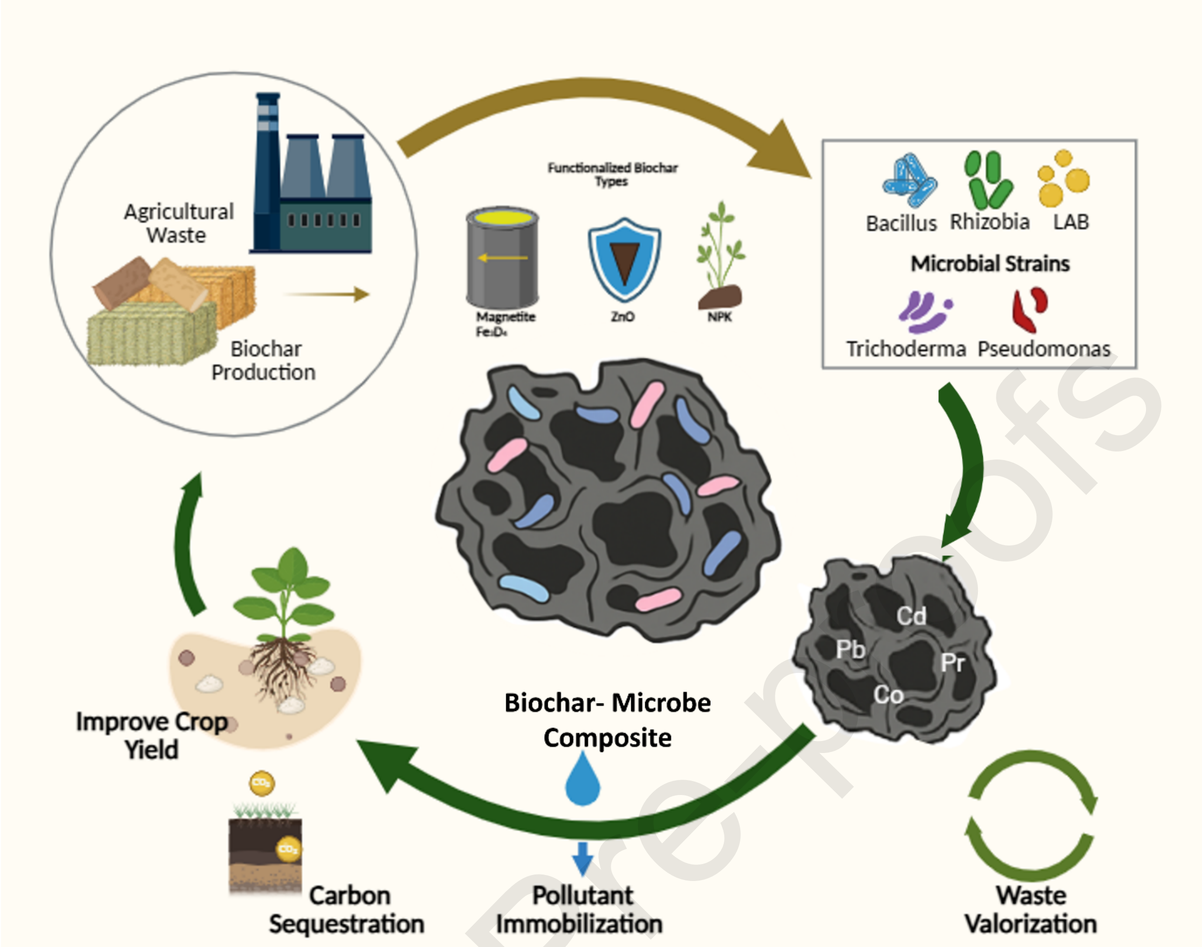
Highlights

- Biochar-immobilized microbial systems offer an eco-efficient platform for accelerated organic waste bioconversion and nutrient recovery.
- Engineered and extremophile microbial consortia, immobilized on tailored biochar, enable synergistic remediation of co-contaminated soils and waters.
- Functionalized biochars (e.g., nano-decorated, nutrient-loaded, pH-buffered) support targeted microbial delivery and enhanced environmental persistence.
- Biochar–microbe interactions boost phytoremediation via metal immobilization, enzymatic detoxification, and rhizosphere enhancement.
- Integrated multi-omics approaches unravel the functional dynamics of biochar–microbe systems, guiding field-scale optimization.


Agricultural
Waste
Biochar
Production
Functionalized Biochar
Types
Magnetite
ZnO
NPK
Bacillus
Rhizobia
LAB
Microbial Strains
Trichoderma
Pseudomonas
Cd
Pb
Pr
Co
Biochar- Microbe
Composite
Improve Crop
Yield
Carbon
Sequestration
Pollutant
Immobilization
Waste
Valorization

CRediT Author Statement

Abhishek Dadhich: Conceptualization, Investigation, Writing – Original Draft, Visualization, Data Curation.
Rashmi Choudhary: Writing – Review & Editing, Methodology, Validation.
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Declaration of competing interest

The authors confirm that they have no known interpersonal or financial conflicts that would have appeared to have an impact on the research presented in this study.