Advances in the remediation of contaminated soils using combined biochar and microorganisms

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Abstract: Heavy metal pollution represents a critical global environmental challenge. Biochar-microbe synergistic remediation (BMS) has emerged as a promising green strategy, enabling simultaneous removal and stabilization of multiple heavy metals through synergistic biochar-microorganism interactions. Experimental results demonstrate its high remediation efficiency: Cr(VI)-reducing bacteria immobilized on Enteromorpha-derived biochar achieved 94.2% conversion of Cr(VI) with approximately 84% total chromium fixation; Pseudomonas-loaded biochar enhanced plant cadmium accumulation by 1.8–2.4 times; and immobilized bacterial communities removed approximately 65% of arsenic via biotransformation and surface co-precipitation. This review systematically elucidates the underlying synergistic mechanisms of BMS, including soil condition improvement, microbial habitat provision, adsorption gradient enhancement, and enzyme activity elevation, while also highlighting its impacts on heavy metal speciation and plant growth. Finally, key future research directions are outlined.

1. Introduction

Soil contamination by heavy metals is a severe environmental crisis[1], threatening food security and human health due to the non-degradable and bioaccumulative nature of these pollutants[2]. While traditional remediation techniques like soil washing or solidification exist, they often suffer from high costs, ecological disruption, or secondary pollution[3].

Biochar, which is produced by the pyrolysis and carbonization of biomass under anaerobic or oxygen-limited conditions, is characterized by a highly aromatic porous structure[4], a vast specific surface area[5], and a wealth of oxygen-containing functional groups[6] (e.g., −COOH, −OH), all of which collectively endow it with an exceptional capability for the adsorption and immobilization of heavy metal ions. However, biochar's remediation mechanism primarily relies on physical adsorption and surface precipitation[7], processes inherently limited by finite and easily saturable adsorption sites, meaning it cannot actively transform the toxic speciation of heavy metals. In contrast, Microorganisms, particularly heavy metal-tolerant strains, have evolved diverse mechanisms to counter metal stress[8], including biosorption, bioaccumulation, bioprecipitation[9], and biotransformation. However, the direct application of microbial inoculants frequently faces significant ecological challenges, notably nutrient competition, antagonism from indigenous microbes, and environmental stress (such as pH and moisture fluctuations). These factors collectively lead to low survival rates and compromised remediation efficiency[10]. Consequently, researchers have shifted focus to couple biochar with bioremediation-functional microorganisms (bacteria or fungi)[11]. Having emerged as an effective and sustainable strategy for soil pollution remediation, the Biochar-Microbial Synergistic (BMS) approach is defined by biochar's dual functionality[12]. Biochar operates beyond merely a passive heavy metal "fixative", it actively serves as both a microbial "habitat" and a "nutrient source." This critical duality, therefore, enables a significant enhancement of the overall remediation effect[13].

This paper intends to systematically review recent progress in BMS joint remediation for heavy metal-contaminated soil. By organizing studies on different soil types and BMS combinations, it will focus on three key aspects: elucidating how biochar promotes microbial survival and activity while microbes enhance biochar’s adsorption capacity, assessing the actual effect of BMS in reducing heavy metal bioavailability and restoring soil fertility, and examining representative case studies to identify practical application challenges. It will conclude with a scientific outlook on future research, such as developing more efficient modified biochar-microbial composites.

2. Synergistic mechanisms of BMS soil remediation

The synergy between biochar and microorganisms constitutes a complex, multi-dimensional mechanism

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involving coupled physical, chemical, and biological processes, as shown in Figure 1. This synergistic effect is not merely the superposition of individual functions, but rather the result of mutual promotion and functional complementarity.

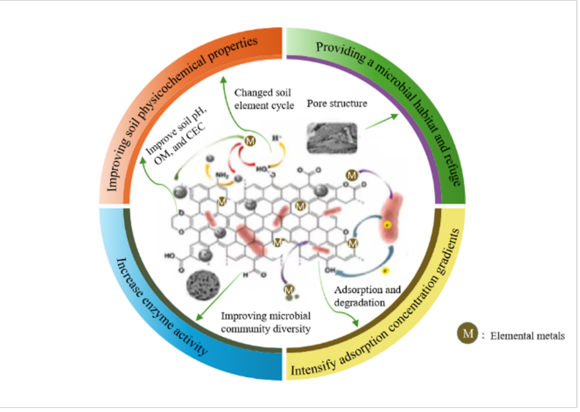

Figure 1 Interaction mechanism between biochar and microorganisms

2.1. Improve soil physicochemical properties

The combined application significantly improves the physicochemical properties of contaminated soil. Most biochars introduce alkalinity via inorganic carbonates (from high-temperature pyrolysis)[9], organic anions (from low-temperature pyrolysis)[13], and surface functional groups[14] (e.g., $-COO-$ and $-O-$). In acidic soils, this alkalinity elevates the soil pH. On one hand, the pH increase directly promotes the precipitation of various heavy metal ions (e.g., Cd^{2+}, Pb^{2+}) as hydroxides or carbonates, reducing their bioavailability[14]. On the other hand, it establishes more conducive growth conditions for microorganisms[15], such as most bacteria, which thrive in neutral environments. Furthermore, biochar's porosity improves soil aeration and water-holding capacity[16]. Bacillus strains can also contribute to alkalinity by producing substances like NH_4^+[14]. Dissolved organic matter may promote heavy metal migration in specific scenarios; however, the large quantity of exchangeable cations and oxidized functional groups present on biochar’s surface contributes to a greater adsorption surface area and higher charge density[17]. This simultaneously enhances soil water-holding capacity; for instance, the biochar-microbial co-treatment increased soil water retention by 17.77% compared to untreated soil[18].Concurrently, the extracellular polymeric substances (EPS) secreted by microorganisms act as cementing agents, promoting soil aggregate formation. This optimizes the soil physical structure, providing a favorable environment for root systems and aerobic microorganisms, thereby promoting the system's ability to co-degrade pollutants[19].

2.2. Providing a microbial habitat and refuge

Due to its highly porous structure, biochar functions as a "refuge" for microbial immobilization[20]. Biochar offers a substantially larger habitable pore volume per unit volume compared to bulk soil. This rich pore structure (ranging from micron to nanometer scale) provides extensive microbial habitats, facilitating microbial colonization within the pores[21]. Living cells adhere to the biochar surface to form biofilms[22]. This colonization allows microorganisms to be transferred into the biochar interior or covered by biochar particles when pollutant concentrations are lethally high, thereby self-protecting[23].

This mechanism confirms biochar's role in shielding microorganisms from external lethal factors, effectively mitigating protozoan predation and extreme environmental stress (such as drought and pH shifts)[24], which significantly enhances their survival rate and biomass in the soil[25]. Immobilized cells maintained an 84.16% viability after ten cycles of reuse and storage stability testing, while free cells dropped to 8.75% after the sixth cycle[26]. Moreover, the soluble organic carbon on the biochar surface and mineral elements (K, P, Ca, etc.) in its ash serve as nutrient sources that accelerate microbial growth and metabolism[27]. Finally, the porous structure reduces soil bulk density, regulating permeability and water content, which improves nutrient utilization efficiency by the resident microorganisms. Chen et al.[22] found that when Enteromorpha prolifera biochar combined with Bacillus cereus whx-1 was used to treat contaminated soil, the organic carbon content increased from 0.76% to 3.13%, and the soil bulk density decreased from 1.07 g/cm^3 to 0.97 g/cm^3, which could significantly improve the soil structure and increase soil nutrients compared with the single bacterial treatment.

2.3. Intensify adsorption concentration gradients

Biochar, endowed with strong adsorption capacity by its unique physicochemical properties—such as a vast specific surface area, abundant porous structure, and numerous surface functional groups (-COOH, -OH)—can rapidly "capture" free heavy metal ions (like Cd^{2+}, Pb^{2+}, and Cr^{6+}) from the soil solution and enrich them on its surface. This process not only reduces the concentration of mobile heavy metals in the soil matrix but also creates a localized "high-concentration heavy metal microenvironment" on the biochar surface. Consequently, microorganisms that colonize the biochar surface (including heavy metal-tolerant bacteria and fungi) gain direct access to this "high-concentration" substrate of heavy metals. Compared to the traditional scenario where microbes must "search" for scattered low-concentration pollutants in the soil— a process that consumes substantial energy and limits metabolic efficiency—this enriched substrate eliminates such energy waste. It allows microbes to directly carry out biotransformation (reducing toxic Cr^{6+} to less toxic Cr^{3+}) or bioaccumulation of the surface-enriched metals, significantly enhancing the overall efficiency of heavy metal remediation.

One piece of research indicated that with the elevation of Cd concentration (ranging from 0 to 250mg/L), the overall biosorption capacity showed a trend of initial increase followed by a decrease. Nevertheless, immobilized cells retained high efficiency under elevated

Cd concentrations (approximately 80−130mg/g)[20]. The observation suggests that microbial activities related to pollutant degradation and transformation can lead to the desorption of biochar carriers, thereby freeing up previously occupied adsorption sites and reestablishing the biochar's adsorption capacity. Microbial activity effectively "empties" biochar adsorption sites, enabling its "regeneration" to adsorb new heavy metals from the soil solution. This cyclical "adsorption-conversion-readsorption" process dramatically improves the durability and efficiency of the remediation system.

2.4. Increase soil enzyme activity

Soil enzyme activity serves as a vital indicator of soil health and microbial function. The biochar-microbial co-application significantly elevates the activity of soil enzymes. These enzymes facilitate the decomposition of soil organic matter, providing rich nutrient substrates for microbial growth.

Biochar also plays a protective role by adsorbing heavy metal ions or phenolic compounds that inhibit enzyme activity, thereby alleviating enzyme suppression. Furthermore, the biochar surface acts as a carrier for enzyme immobilization, which enhances enzyme stability. For instance, in a study evaluating the combined use of metal-immobilizing bacteria (BHC) and biochar, urease activity in the rhizosphere of the treated group increased by 115%−156%[21]. In pot experiments where bacillus subtilis SL-13 was applied to biochar, Tao et al. reported similar findings using bacillus subtilis SL-13 on biochar (MBFS) in pot experiments, noting that invertase and catalase activities in the MBF group were higher than in the untreated control. Soil enzymes are central to global nutrient cycling (C, N, P, S) [22], impacting microbial proliferation and plant growth. The increase in bacterial population consequently affects metabolic activity, influencing the overall activity of key enzymes like catalase and invertase.

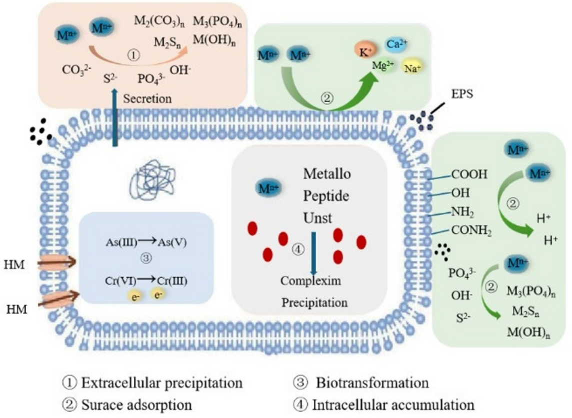

Figure 2. Mechanisms of heavy metal detoxification in microorganisms

Microbial metabolism involves the secretion of organic acids and extracellular enzymes that progressively "etch" the biochar surface, oxidizing its aromatic structure and exposing more oxygen-containing functional groups[15] (e.g., −COOH, −OH), as shown in Figure 2. The introduction of these new functional groups significantly enhances the Cation Exchange Capacity (CEC) and surface negative charge of the biochar, thereby boosting its adsorption capacity for heavy metal cations. Microorganisms also increase biochar pore volume and specific surface area by decomposing unstable carbon moieties (such as aliphatic carbon)[21], generating additional binding sites for heavy metals. Additionally, the biofilm formed by secreted EPS is an efficient biosorbent itself[22], with its rich functional groups capable of complexing substantial quantities of heavy metal ions.

3. Remediation outcomes of combined biochar-microbial application

3.1. Impact on heavy metal speciation and stability

The fundamental objective of heavy metal remediation is not merely to decrease the total concentration but critically, to reduce the heavy metals' bioavailability. Classified into water-soluble, exchangeable, carbonate-bound, iron-manganese oxide-bound, organic-bound, and residual forms, the chemical speciations of heavy metals play a decisive role in their mobility and inherent toxicity. Water-soluble and exchangeable forms exhibit the highest bioavailability, while the residual form represents the most physicochemically stable state. Biochar primarily achieves physical and chemical fixation by converting water-soluble and exchangeable heavy metals into carbonate-bound or organic-bound forms through adsorption, pH-induced precipitation, and electrostatic attraction. Microorganisms, however, enable a deeper, more profound transformation. For instance, Sulfate-Reducing Bacteria reduce sulfate to S^{2-} , which subsequently complexes with Cd^{2+}, Pb^{2+}, and other ions to form extremely stable, low-solubility metal sulfides (the residual state)[23].

Under combined application, heavy metals initially adsorbed by biochar are further transformed into the more stable residual fraction by microbial activity. Extensive research data consistently demonstrate that the exchangeable content of heavy metals in the combined treatment group is significantly lower than in the single biochar treatment, while the residual content is significantly higher. Moreover, the persistent free radicals in biochar can serve as catalysts and electron carriers, enhancing electron transfer between microbial cells and heavy metal ions[24], thereby promoting valence state transformations. The combined treatment of swine manure biochar (PMB) and Phosphate-Solubilizing Bacteria (PSB) increased the apparent rate constant from $0.025\ s^{-1}$ to $0.036\ s^{-1}$, suggesting that the PMB's good conductivity accelerates the PSB's extracellular electron transfer rate, which is beneficial for enhancing the adsorption and mineralization kinetics of Pb and Cd[25]. Biochar also mitigates direct heavy metal toxicity to microbial proliferation by effectively adsorbing and fixing the metals in the soil[26]. For example, biochar surface functional groups can lower the Zn concentration in the cellular surface microenvironment, reducing metal accumulation within the immobilized bacterial cells. This robust evidence confirms that combined remediation achieves a more thorough and permanent "lock-in" of heavy metals.

3.2. Impact on plant growth and phytostabilization

In polluted soils, heavy metal toxicity and poor soil fertility represent the two principal factors constraining plant growth. The biochar-microbial co-application significantly mitigates heavy metal toxicity and reduces plant uptake (especially in non-hyperaccumulator species) by decreasing heavy metal bioavailability in the soil. This enables plant survival and enhanced growth. Studies indicate that the change in heavy metal content is often less pronounced in the roots than in the aboveground tissues of plants grown in treated soil. Biochar's high surface area, by boosting soil microbial activity, may reduce heavy metal translocation to aerial parts, explaining the normalization of plant leaf length and dry weight. In a study focusing on highly polluted soil, the combined treatment was highly effective, biochar alone reduced heavy metal content by 1.44-3.78 fold, the bacterial treatment by 1.34-2.32 fold, and the total reduction from the combined amendment reached 1.94-3.55 fold[26].

Biochar intrinsically supplies available nutrients. Furthermore, microorganisms, particularly Plant growth-promoting rhizobacteria (PGPR), further stimulate plant growth via nitrogen fixation[27], phosphorus and potassium solubilization, and the secretion of phytohormones. Biochar provides a stable habitat for PGPR, ensuring consistent growth promotion in the rhizosphere. Consequently, the combined biochar-microbial treatment typically yields the greatest improvement in plant biomass[28], which is critical for achieving successful "green" vegetation restoration in contaminated sites. In a model experiment involving Cr (VI)-salt contaminated soil, applying 5% biochar combined with two bacteria (Bacillus cereus and Pseudomonas japonicus) achieved the best results in reducing plant toxicity. Specifically, root length, stem length, fresh biomass, and dry biomass increased by 135%, 168%, 370%, and 390%, respectively, compared to the contaminated control. Similarly, introducing the PGPR Neorhizobium huautlense and biochar into Pb and Cd-polluted farmland led to a significant reduction in pollutant content in the edible parts of Chinese cabbage and radish, correlating with a sharp decrease in mobile heavy metals in the rhizosphere[29]. Another study highlighted that adding the highly Hg-recovering Pseudomonas (II) strain with biochar improved process efficiency and accelerated Hg removal from the soil[30]. Recent findings emphasize the positive interaction between biochar and both native and exotic soil bacteria, confirming that the combined approach (using Bacillus subtilis strains immobilized on biochar) is more effective than using biochar alone in reducing metal bioavailability and toxicity to the soil microbial community[31].

3.3. Application of BMS in heavy metal remediation

BMS technology leverages the combined action of biochar carriers and functional microorganisms to simultaneously remove and stabilize multiple heavy metals in contaminated soils. This approach is particularly effective for complex co-contamination of metals such as chromium, cadmium, and arsenic. The core mechanism integrates the superior adsorption capacity of biochar with the transformative functions of specialized microbes, offering a synergistic and sustainable remediation strategy.

Specific applications demonstrate this synergy through distinct mechanisms. In chromium remediation, biochar derived from *Enteromorpha prolifera*, used to immobilize Cr(VI)-reducing bacteria, provides a habitat and contributes persistent free radicals for redox reactions[32]. The immobilized bacteria efficiently reduce toxic, soluble Cr(VI) to less toxic, immobile Cr(III) via enzymatic reduction and extracellular electron transfer, achieving a 94.2% conversion rate and approximately 84% total chromium fixation. For cadmium, biochar-loaded *Pseudomonas* sp. NT-2 first adsorbs and enriches Cd^{2+} ions[33]. The microbes then facilitate stable precipitation into cadmium carbonate and phosphate through the secretion of extracellular polymers and ions, reducing bioavailability and enhancing plant accumulation for phytoextraction by 1.8–2.4 times[34]. In arsenic contamination, immobilized consortia or *Bacillus* XZM remove arsenic through precipitation and ion exchange. Microorganisms alter arsenic speciation via oxidation or reduction[35], while biochar immobilizes it through surface complexation and coprecipitation with metal oxides, achieving about 65% removal and enhancing soil enzyme activity[36].

In summary, BMS composite technology effectively merges physico-chemical adsorption with biological transformation, presenting a powerful and environmentally sound solution for multi-metal pollution. Future advances in strain screening and material modification are expected to further broaden its engineering applications and efficacy.

4. Conclusion

This review systematically examines the synergistic mechanisms and practical applications of BMS in heavy metal remediation. The BMS synergy operates through multiple interconnected pathways where biochar serves as both a protective habitat for microorganisms and an effective adsorbent that creates localized metal concentration gradients. Concurrently, microorganisms perform critical functions including biosorption, enzymatic reduction, and bioprecipitation, transforming toxic metal species into more stable and less bioavailable forms. This synergistic interaction significantly enhances metal immobilization efficiency and promotes soil ecological restoration, as evidenced by successful remediation cases involving chromium, cadmium, and arsenic contamination.

Despite the demonstrated potential of BMS technology, its practical implementation faces several challenges. Future research should prioritize elucidating the underlying molecular mechanisms using advanced omics technologies such as metagenomics and transcriptomics. Optimization of key application parameters—including biochar dosage, pyrolysis temperature, and material type—is essential to maximize remediation efficiency while minimizing potential environmental impacts. Furthermore, developing robust predictive models capable of evaluating system performance across diverse soil types and contamination scenarios will be crucial for advancing targeted field applications of this promising remediation strategy.

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