

## Microbial-Plant-Biochar System for the Removal of Pollutants from Effluents and Contaminated Soil

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Industrial activities release wastewater containing heavy metals and synthetic dyes that remain in the environment for a longer period. These pollutants disrupt ecosystems, pose risks to human health, and continue to accumulate if they are not treated properly. Many conventional remediation methods are costly and often generate secondary waste, which limits their practical use. As a result, researchers are increasingly exploring sustainable and environmentally friendly alternatives. This review discusses an integrated-microbial-biochar system as a promising approach for wastewater and soil remediation. Biochar produced from materials such as sewage sludge, oil-field drilling mud, and agricultural residues offers a highly porous and chemically active surface that can effectively bind heavy metals (Cd, Cu, and Zn) as well as a wide range of industrial dyes. In addition, microbial strains such as *Acinetobacter* *sp.* and *Bacillus subtilis* play an important role in degrading organic pollutants, restoring enzymatic activity and contaminated soils, and improving nutrient cycling. Recent developments, including biochar-microbe beads and composite bioreactor systems, have shown better performance than biochar or microbes used alone. These combined systems enhance microbial survival, reduce toxicity, and significantly improve pollutant removal efficiency. By summarizing recent findings on pyrolysis conditions, microbial immobilization techniques, and pollutant removal behavior, this review highlights the potential of hybrid remediation strategies. Emerging modifications, such as magnetic chitosan-modified biochar, are also discussed as future directions to further strengthen integrated remediation systems.

**Keywords:** Sludge, Microbial-Biochar System, Pyrolysis, Immobilization, Bioreactor System



## Introduction:

The industrial revolution generated substantial economic benefits; however, these advancements also imposed significant environmental burdens. Large volumes of wastewater are discharged daily from textile tanneries, petrochemical industries, metal finishing operations, and mining activities, introducing a wide range of pollutants into natural ecosystems. These types of effluents contain heavy metals (e.g., Cd, Pb, Zn, and Cr) and dyes that resist chemical and biological breakdown. They interfere in ecological processes, limit biodiversity, and impact food security when accumulated in soil and water. In seawater, dyes can block sunlight from entering and reduce gas exchange for microorganisms, and weaken the food chain base. In soil, metals suppress the activity of biological agents, disrupt nutrient cycling, and damage plant health. Techniques such as membrane filtration, chemical precipitation, and advanced oxidation are widely employed for wastewater treatment; however, they are not considered sustainable. Most of these methods require high energy or chemical inputs and produce substantial amounts of secondary sludge that must be safely disposed of. Consequently, researchers have increasingly shifted their focus towards developing treatment materials and technologies that are both cost-effective and environmentally friendly. One such development is biochar, which is made through low-oxygen conditions and by heating biomass such as crop residues, wood waste, and sewage sludge. The whole process in which high temperature and anoxic conditions are provided for thermal decomposition of organic matter to solid-char called pyrolysis. Depending upon the type of feedstock and temperature during pyrolysis, biochar develops reactive surface groups, pores, and mineral compounds that help biochar to bind with heavy metals and dye molecules. Thus, these properties make biochar a good choice in the remediation process for treating waste in soil and water. Recently, researchers used different techniques, such as combining biochar with microorganisms, including *Bacillus*, *Acinetobacter*, and *Pseudomonas*, to check their performance and behavior in contaminant removal. These microorganisms can convert toxic metals into less harmful components by breaking their complex structures in the process of bioremediation.

The survival of microorganisms in industrial effluents is also detrimental because of high pH, salinity, and toxic concentration [1]. However, biochar provides a protective surface for bacterial attachment, making this combination effective enough to tackle industrial waste. The porous structure of biochar helps to trap pollutants near microbial cells, that give better access for the breakdown of contaminants. This interaction of biological agents and biochar is viewed as more than a simple physical attachment. Several studies also help us in the indication of biochar's influence on microbial communication. Microorganisms play a role in electron transfer and stabilization of the local environment; they enhance pollutant removal efficacy. Because of these benefits, the microbial-biochar system is now considered a more competent method than the single one.[2]. Research on microbial biochar remediation has largely been conducted under controlled laboratory conditions, which limits understanding of its performance in complex real-world environments. Uncertainty remains regarding the long-term stability of immobilized metals, particularly under changing pH and redox conditions. In addition, the lack of standardized performance metrics and cost assessments makes it difficult to compare these systems with conventional treatment methods. Addressing these gaps through field-scale studies, integrated microbial monitoring, and long-term stability assessment is essential for practical applications. The main aim of this review is to explore how these two systems work, which factors influence their activity, and their comparison with traditional treatment approaches. We examined recent findings that correlate with biochar production conditions, microbial immobilization, pollutant behavior, and their application in the real world.[3]. This article highlights the strengths and the challenges faced in remediation strategies. That's provide motivation to study and understand by experiment to check whether

these hybrid methods can be adapted and scaled-up for industrial needs while remaining cost-effective and environmentally sustainable.

### Contaminants and Biochar System:

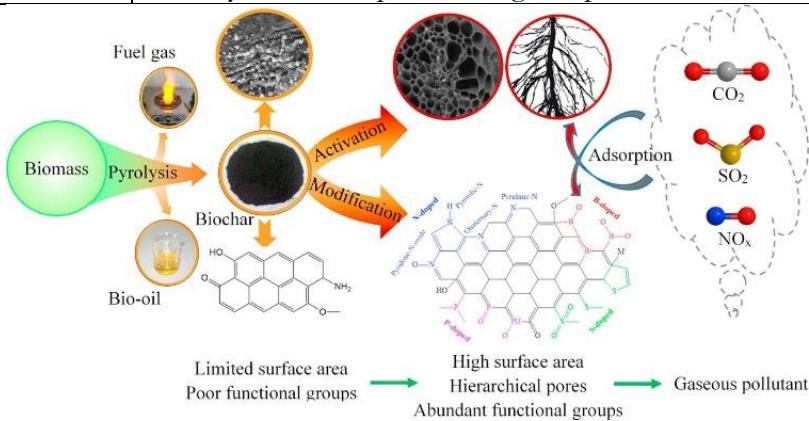
Industrial effluents typically do not contain a single toxin; rather, they comprise a complex mixture of heavy metals such as cadmium, zinc, lead, mercury, and hexavalent chromium (Cr VI) [4]. The industrial wastewater often contains not only heavy metals but also organic dyes, including azo dyes and members of the reactive and basic dye families. Treating such complex mixtures is challenging because each contaminant behaves according to its own chemical properties. Some heavy metals may not degrade at all, while dyes typically break down very slowly and can generate toxic byproducts. In this case, to address all these pollutants, biochar provides a platform to mitigate the problem with flexibility. It is a form of charcoal that is modified and intended for organic uses as in soil. It is a lightweight, black, porous material composed primarily of carbon and mineral ash (Figure 1). Its performance is strongly influenced by characteristics such as pore size distribution, mineral content, and surface functional groups [5]. These properties can be controlled through the conditions used during production. For example, pyrolysis temperature can create more aromatic carbons and a larger surface area, which indirectly helps in the adsorption of complex dyes. Chemical activation, such as treatment with KOH, can increase micro porosity and oxygen-containing groups that bind metals strongly [6].

Biochar that is made from agriculture, sewage sludge residues, or algal biomass carries a different composition. The mineral composition of biochar plays a critical role in influencing both metal binding and nutrient release. Consequently, the origin of the feedstock is equally important, as biochar produced from different biomass sources exhibits varying mineral profiles. This is particularly relevant because the types and the proportions of pollutants discharged from tanneries, electroplating units, mining operations, and textile industries differ significantly. Researchers, therefore, often tailor biochar production and modification techniques to match the specific contaminants they aim to remove. Overall, the combined ability of biochar to immobilize heavy metals and adsorb complex dyes makes it a strong candidate for treating industrial effluents, especially when used in conjunction with microorganisms.

**Table 1.** The following table shows the integrated composition of Biochar

Component Category	Description of Biochar Composition
Carbon (C)	55–90%; predominantly in aromatic and polyromantic structures formed during pyrolysis, providing stability and adsorption affinity [5].
Hydrogen (H)	1–5%; present in residual aliphatic and volatile compounds [7].
Oxygen (O)	5–30%; forms functional groups such as hydroxyl, carbonyl, carboxyl, and phenolic groups, influencing sorption and microbial interactions [8].
Nitrogen (N)	0.1–3%; derived from proteinaceous biomass residues or microbial sources; contributes to nutrient capacity and surface activity.
Ash/Minerals	2–20%; contains Ca, Mg, K, Na, Fe, Si, Al, and P; responsible for alkalinity, buffering capacity, and metal binding [9].
Surface Functional Groups	Carboxyl ( $-COOH$ ), hydroxyl ( $-OH$ ), carbonyl ( $C=O$ ), phenolic, lactonic; facilitate metal complexation, dye adsorption, and microbial adhesion [10].
Mineralogical Phases	Carbonates ( $CaCO_3$ , $K_2CO_3$ ), silicates ( $SiO_2$ ), aluminosilicates, phosphates (Ca–P complexes), metal oxides ( $Fe_2O_3$ , $MgO$ ).

Aromatic Structures	Highly condensed aromatic rings formed during pyrolysis are responsible for hydrophobicity, electron shuttling, and long-term stability [11]
Porosity-Related Composition	Micro-, meso-, and macropore structures composed mainly of carbon matrices; porosity increases with pyrolysis temperature.
Volatile Matter	Low proportion; decreases with higher pyrolysis temperature; affects hydrophobicity and adsorption behavior. [12]
Fixed Carbon	A major fraction contributing to structural rigidity, chemical stability, and adsorption of organic pollutants.



**Figure 1.** Biochar formation and modification processes illustrating improved pore structure and adsorption of gaseous contaminants [13]

### Biochar Production Properties:

The potential of biochar remediation is mainly governed by its production parameters rather than its intrinsic properties [14]. Proper control over thermal conditions, activation procedures, and feedstock selection enables the development of biochar tailored to target specific contaminant matrices.

### Temperature Effects:

Pyrolysis, defined as the thermal decomposition of biomass in the absence of oxygen, converts complex organic material into simpler carbon-rich substances. Pyrolysis temperature strongly influences structural stability, surface reactivity, and porosity of resulting biochar. [15]. Lower temperatures between 300-500°C help preserve oxygen-rich functional groups that are important for binding and removing heavy metals. In contrast, temperatures above 600°C speed up carbonization, increasing both aromatic structure and surface area. These higher temperatures also create hydrophobic  $\pi$  domains that are especially effective for capturing aromatic dyes through electron donor- acceptor interactions [16].

### Activation Methods:

Physical and chemical activation techniques markedly improve the adsorption efficiency of untreated biochar [17]. In physical activation, agents such as steam or CO<sub>2</sub> are introduced to widen and unclog pore networks, resulting in a pronounced increase in available surface area for contaminant uptake. Whereas the chemical activation involves the administration of acids, bases like KOH, or certain oxidizing agents like H<sub>3</sub>PO<sub>4</sub> to functionalize the carbon lattice via micro porosity.

### Surface Chemistry and Functional Groups:

Biochar efficiency is greatly dependent on its surface chemistry. Molecules like hydroxyl and carboxyl that contain oxygen-rich functional groups have a huge influence on metal immobilization through ion exchange [18]. Similarly, the aromatic and graphitic domains play their roles as a structural basis for the removal of organic contaminants by promoting the dye adsorption through  $\pi$ - $\pi$  interaction mechanisms.

**Characterization Techniques:**

Characterization is essential for understanding the physicochemical properties and evaluating the suitability of biochar for environmental applications such as pollutant adsorption and soil remediation. The following table provides a detailed view of the standard methodologies used to analytically examine the remediation potential of biochar.

**Table 2.** Principle Analytical Techniques for Biochar Characterization

Technique	Primary Output and Relevance	Application in Remediation Research
<b>Brunauer-Emmett-Teller Surface Area (BET)</b>	Specific surface area, pore volume, and pore size distribution [19]	Used to predict sorption efficiency for contaminants and to select optimal biochar feedstock or activation conditions [19]
<b>Scanning Electron Microscopy (SEM)</b>	High resolution surface morphology and pore topology [20]	Confirms porous network development and textural changes after activation or modification [20]
<b>Fourier Transform Infrared Spectroscopy (FTIR)</b>	Identification of functional groups (-COOH, -OH, -NH <sub>2</sub> , etc.)	Tracks chemical transformations during pyrolysis and verifies surface interactions post adsorption.
<b>X-Ray Diffraction (XRD)</b>	Crystallinity and mineral phases [20]	<b>Bulk characterization:</b> Detects inorganic precipitates, assesses potential metal immobilization, and monitors structural changes [20]
<b>X-Ray Photoelectron Spectroscopy (XPS)</b>	Surface elemental composition and oxidation states	<b>Surface characterization:</b> Elucidates binding mechanisms, redox changes, and interactions between biochar and contaminants

**Feedstock Selection Criteria:**

In any process being regulated, the quality of the final product is actually defined by the precursor molecules used to carry out the process. Feedstocks ranging from agricultural residues to sludge are responsible for the accurate depiction of ash content, mineral load, and heteroatom doping. These factors mainly affect the chemical adsorption pathways and remediation mechanisms along with the microbial colonization stability [21].

**Bacterial-Loaded Biochar:**

Bacterial-enriched biochar functions as a combined system that connects active microbial cells with the porous carbon framework. Building this setup greatly improves heavy metal immobilization by merging microbial metabolic activity with physical and chemical adsorption. Research shows that this hybrid strategy surpasses the natural phenomena supported by biofilm stability and the production of extracellular polymeric substances (EPS) under various environmental conditions and stress factors consistently observed [22].

**Mechanisms of Bacterial Immobilization:**

The remediation mechanism begins with the physical and chemical attachment of microorganisms to the biochar surface. Biochars with high surface area such as those derived from corn cobs, are often preferred because they provide an ideal substrate for microbial colonization [23]. Bacteria lodge within micropores or mesopores leads to a reduction in the effective hydraulic retention time, ultimately establishing the localized redox microenvironment. Once the attachment is facilitated, bacteria begin to proliferate and form dense biofilms. The EPS secretion is necessary to chelate ions and carry out the metal precipitation for the effective removal of contaminants within biogenic layers [24].

## Microbial Agents and Functional Diversity:

Bacterial taxa are mostly selected based on their specific enzymatic and resistance properties [25]. *Pseudomonas putida* has proved to be a reliable approach in carrying out azo dye biodegradation and hexavalent chromium ion reduction. In acidic environments, *Acinetobacter* sp., when coupled with grapefruit-peel biochar, shows effective removal of heavy metals like Mn, Fe, Zn, and Cu. *Bacillus subtilis* and *Escherichia coli* are known to carry out cadmium biodegradability in soil samples, along with a credible improvement in nutrient cycling and plant growth.

## Synergistic Remediation Pathways:

When bacteria are combined with biochar, a remarkable synergistic effect emerges, significantly enhancing the overall remediation process [26]. Biochar acts as a multifunctional platform, supporting microbial stability and viability while simultaneously helping to regulate the conditions necessary for effective pollutant removal. This synergic effect is more likely to be involved in the complete degradation of reactive dyes and chromium reduction in continuous flow systems.

The immobilization of metals occurs through four primary mechanisms:

**Adsorption:** Surface functional groups such as iron oxides are known to provide high-affinity sorption sites [27].

**Precipitation:** Metals are converted into insoluble hydroxides or oxide forms through the conduct of both biotic and abiotic pathways [28].

**Complexation:** Carboxyl, hydroxyl, and amino groups present on both biomass and the carbon surface are responsible for metal ions sequestration.[29]

**Microbial Reduction:** Bacteria proceed with the reduction of toxic compounds, such as converting Cr (VI) to Cr (III), to facilitate the subsequent precipitation onto the matrix.[30]

## Performance Metrics and Operational Factors:

Quantitative studies have revealed that co-immobilized biochar beads are exceeding chromium removal rates at 99%, whereas the iron-oxide biochar nanocomposites can accomplish full lead uptake in batch test [31]. In acidic mine waters, bioreactors are highly effective in removing heavy metals such as manganese (Mn), iron (Fe), and zinc(Zn) by facilitating their conversion into surface-bound precipitates. Moreover, the efficiency of these processes is highly influenced by the environmental variables. Solution pH mostly affects the metal speciation and surface charge, while contact time and influent concentration allow breakthrough in the curves. Physicochemical properties of biochar, such as porosity and functional group density, modulate overall kinetic performance.

## Advantages over Conventional Sorbents:

Biochar enriched with bacteria is highly preferred over conventional biochar remediation methods. The presence of microbial agents not only accelerates the biotransformation of organic contaminants but also enhances the overall efficiency and stability of the remediation process [26]. The result is the ability to manage complex and multi-pollutant effluents more effectively and accurately than biochar alone.

## Industrial Sludge Biochar:

Industrial or sludge-derived biochar employs the thermochemical conversion of industrial and municipal wastewater sludge. The process implies the formation of porous and surface-functionalized solids whose characteristics are defined by the sludge composition and pyrolysis conditions [32].

## Physicochemical Properties:

Sludge- derived biochar is known to have high mineral (ash) content along with a surface rich in oxygenated functional groups (e.g., hydroxyl or carboxyl groups), which are actually the binding sites for the contaminants [33]. The porosity and surface area of biochar are strongly influenced by the conditions of pyrolysis. While higher pyrolysis temperature

generally increases surface area, it can simultaneously reduce the abundance of surface functional groups. On the industrial scale, sludge biochar causes the release of nutrients, e.g., K and certain metals, depending on pH, temperature, and solid-liquid ratio [34].

#### **Mechanism of Contaminants Removal:**

It also follows the synergistic mechanisms to support the contaminant removal.

**Ion Exchange:** Exchangeable cations (e.g., Ca +2 and Mg+2) are replaced by certain metal ions (e.g., Pb+2 and Cd+2) [35].

**Surface Complexation:** Stable complexes are formed as a result of the binding of metal ions to the oxygenated functional groups on the biochar surface [36].

**Precipitation:** Mineral-driven precipitation is promoted by factors like mineral-rich biochar (with Ca and P). Mineral-phosphate precipitates are generally formed on the inner side of char.

**Diffusion and Film Effects:** The release or uptake of nutrient ions is mainly controlled by diffusion through particle surfaces and liquid-film resistance. Ligand exchange and electrostatic attraction also play a fundamental role in phosphate binding [37].

#### **Performance Parameters:**

Corn-cob biochar is produced at 400°C, this high temperature executes substantial surface area along with micro porosity. This confirms that textural properties are often maintained by the controlled temperature regulations [38]. The same biochar reduces the acid-soluble fraction of heavy metals (Cr, Mn, Zn, and Cu) by large percentages, leading to effective immobilization. In a soil experiment, sludge-derived biochar has proved to be a significant and potential source for the removal of toxic elements (PTEs) in corn [39].

#### **Limitations, Risk, and Optimization Strategies:**

As industrial sludge biochar contains high mineral content, there's always a possibility of leaching out if not properly managed. The addition of metal dopants can improve the adsorption performance. Mostly zero-valent iron is used for the optimization as it introduces more active sites, increases co-precipitation, and also enhances ion-exchange capacity [40]. The coordination between sludge-derived biochar and Nano-zero valent iron also regulates the effective removal of nutrients through ligand exchange and precipitation [41].

#### **Applications of Industrial Sludge Biochar:**

It's effective as a soil amendment to reduce the mobility of heavy metals and decrease their uptake by plants, which eventually supports the remediation of contaminated soils [42]. It introduces long-term quality in polluted soils and improves the crop yield by reducing the uptake of toxic heavy metals. In wastewater treatment, activated sludge biochar is applied to remove emerging pollutants such as carbamazepine and 17  $\alpha$ - ethinyl estradiol (EE2) [43]. It also acts as a slow-release fertilizer carrier by retaining nitrogen, phosphorus, and bio-stimulants from sludge-based nutrient streams while improving the nutrient cycling efficiency.[44]

**Table 3.** The following table shows the comparison between Bacterial-loaded Biochar and Industrial Sludge Biochar

Aspects	Bacterial-Loaded Biochar	Industrial- Sludge Biochar
<b>Functional Basis</b>	A hybrid system that combines biochar sorption with active microbial metabolism [45].	Mineral-rich and pyrolysis-based sludge with high physicochemical sorption capacity [46].
<b>Physicochemical Requirements</b>	High surface area and micro-porosity for microbial colonization [47]	High ash/mineral content with oxygenated surface groups [48].
<b>Mechanisms</b>	EPS-derived metal chelation, microbial enzymatic reduction, and biofilm-associated precipitation [49].	Ion exchange, mineral-driven precipitation, surface complexation, Diffusion-controlled uptake [50].

<b>Target Contaminants</b>	Organic pollutants (dyes, pharmaceuticals) and redox-active metals (Cr, Mn, Fe) [51]	Heavy metals (Pb, Cd, Zn, Cu), PTEs in soils, emerging contaminants [52]
<b>Synergistic Interactions</b>	Biochar protects microbes from toxins, enhances colonization, and concentrates pollutants with biofilms [53]	Mineral fractions synergize with the carbon matrix for co-precipitation and immobilization mechanism [54]
<b>Operational Advantages</b>	Supports the self-regenerating biodegradation process, effective for dynamic wastewater systems [55]	Low-cost scaling, consistent metal retention, enhances soil fertility, stable and long-term effects [56]
<b>System Stability</b>	Biological stability is dependent on environmental stress; the biofilms may be disrupted [57]	High structure stability, i.e., mineral phases ensure long-term immobilization [58]
<b>Limitations</b>	Safe handling of microbes and environmental safety concerns. Microbial activity may decline due to adverse conditions [59]	Concentrated contaminants in biochar, possible leaching of heavy metals, and limited organic pollutant breakdown.
<b>Optimization Strategies</b>	Metal-resistant strains can be used, incorporative redox-active biochar types is efficient [60]	Metal dopants (e.g., nZVI) can be used, pyrolysis temperature can be regulated [61]

### Pyrolysis Parameters and Property Changes:

#### Structural Evolution caused by Temperature:

The carbon alignment is primarily caused by temperature. The biomass holds onto various surface-bound oxygenated groups that supply acidity and increase affinity for pollutants, which are polar at normal heating levels [62]. When the temperature elevates, the volatile fractions evaporate, leading to the formation of condensed aromatic layers. This advancement decreases the O/C and H/C ratios, enriches with the density of carbon, and produces a strong, robust graphite-like matrix. High temperatures also lead to the entry of the closed channels and expansion of pores, giving surfaces that are more approachable for adsorption and catalytic engagement [63].

#### Effects of Heating Levels:

The low and high level of heat determines the internal structure. The slow heating causes stable pores and a uniform internal structure as gases depart uniformly [64]. Rapid internal component evaporation caused by accelerated heating expands the material and creates high surface area char with complicated pore geometry. In other words, the final char is compact and highly functionalized or highly porous and adsorption-oriented, depending upon how the heating is provided

#### Impact Caused by Residence Time:

The level to which the maturity of the structure is increased depends upon the time period of exposure. If the period of residence time is longer, then it leads to deeper carbonization, which enables the carbon domains with robust crystallinity [65]. On the other hand, the shorter residence time maintains labile groups that can take part in complexation, redox processes, and ion exchanges. The balance between the aromaticity and chemical activity is maintained by the residence time [66].

#### Feedstock Chemistry Influence:

Four factors, the mineral content, pH, aromatic fraction, and functional group distribution of biochar, are strongly impacted by the origin of biomass [67]. A strong aromatic substructure is produced by remnants rich in lignin. Char enriched with inorganic phases is produced by feed stocks with high ash content. Microalgae, agricultural wastes, and woody

materials each lead to the production of char with unique adsorption behaviors due to differences in composition [68].

### Activation Treatments Impacts:

The internal landscape of biochar is expanded by the activation methods named as thermal or chemical. Dense networks of micro and mesopores are created by techniques using steam, carbon dioxide, or different activating agents. The wastewater purification systems' ultimate boosting performance is caused by the previous changes, which increase interaction between pollutants and active sites [69].

**Table 4.** Temperature-Property Relationships of Biochar

Temperature Range	Main Properties	Surface Groups	Removal Behavior	Notes (Refs)
300–400 °C	Low carbonization; low surface area; high O/C ratio	–COOH, –OH, phenolic	Strong heavy-metal binding by ion exchange and complexation	Best for Cd, Pb, Cu removal [16][19]
400–500 °C	Moderate carbonization; rising porosity	Mixed oxygenated & aromatic groups	Good metal removal and moderate dye adsorption	Balanced performance [15][18]
500–600 °C	High surface area; aromatic structure; low volatiles	Graphitic carbon dominates	Efficient adsorption of aromatic dyes via $\pi$ – $\pi$ interactions	Textile dye treatment [17][70]
600–700 °C	Highly carbonized; stable graphite-like matrix	A few functional groups	Metals stabilized into residual fractions	Long-term Cr, Pb, Cd immobilization [71][72]
≥700 °C (modified)	Mineral-rich, magnetic, or metal-doped biochar	Fe-oxides, aluminosilicates	Near-complete removal of labile metal fractions	Magnetic & sludge biochar systems [73][74]

### Methods of Eliminating Contaminants:

Biochar microbe systems purify effluents through a complex interplay of biological transformations and abiotic interactions. Instead of operating separately, the two parts combine their strengths to create a remediation platform that is both extremely effective and ecologically friendly.

### Physiochemical Interactions on Biochar Surfaces:

As a dynamic sorbent, biochar facilitates a variety of removal paths. By capturing dissolved contaminants, the network of micro-, macro-, and mesopores immobilizes molecules in small areas. Labile groups such as hydroxyl, carbonyl, and aromatic moieties engage contaminants through processes including complex formation, hydrogen bonding, and ligand exchange. Because of its pH dependent surface charge, biochar is attracted to positively charged metals, dye ions, and other charged impurities by electrostatic forces. When pH is changed, the biochar surface charge also changes, allowing for electrostatic attraction of positively charged metals, dye ions, and other charged impurities. Metals are immobilized as insoluble phases by additional mechanisms like precipitation, co-precipitation, and ion exchange, which are facilitated by inorganic components embedded in biochar [69]. Through  $\pi$ – $\pi$  stacking planar molecules, including various dyes to improve stability and retention, combine with various biochar's aromatic domains [75].

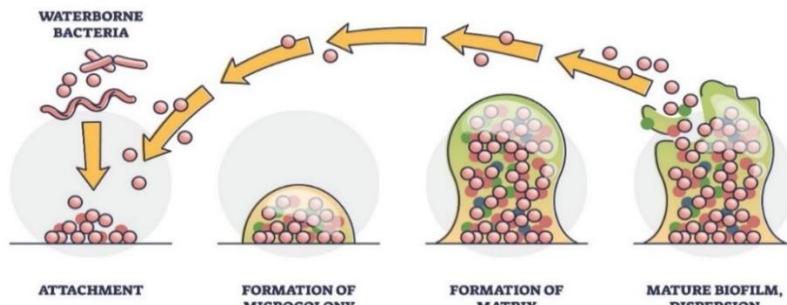
## Microbial Degradation and Transformations:

Microorganisms release hydrolytic, oxidative enzymes that break down organic contaminants, break down dye complexes, and decrease reactive species to much less reactive forms by actively breaking down and converting pollutants. Microbial communities that are living complement biochar. Intracellular sequestration, transmembrane pumping, surface complexation, and valence state alteration are among the few tactics used by microbial cells. These processes result in the immobilization, stabilization, or transformation of dangerous ions into less reactive species.

## Cooperative Relationships at the Biochar- Microbe Interface:

The microenvironment, which is suited to remediation in a unique way, is produced by the joining of biochar and microbes. The surface of biochar is rough or uneven, which acts as a perfect habitat for microbial attachment, allowing dense, resilient biofilms to form [70]. Polymeric materials with a wealth of functional groups that can bind metals, concentrate organic pollutants, and lead to enzyme activity are released by biofilms [76]. The oxidation reduction zones are generated by microbes while in the same way biochar increases electron shuttling. Biochar reduces the bioavailability of dangerous substances while enabling the microbes to reproduce and establish the metabolic activity even in bad industrial effluents [77].

## BIOFILM



**Figure 2.** This figure explains the stages of how biofilm forms from the community of small bacteria. In the first stage, the bacteria attaches on surface and after multiplication, clusters are formed. Bacteria form a protective coat around themselves. After maturation, they disperse, and some of them find a new place for attachment.

## Performance of Integrated Systems:

When all these processes are combined, including adsorption, precipitation, enzymatic cleavage, reductive detoxification, and biofilm-mediated processing, it leads to the elimination of complicated dyes, heavy metals, pollutants, and bacteria-killing medicines [78]. By combining biochar, microbial systems provide a potent and long-lasting solution for sophisticated wastewater treatment by combining structural stability with biological stability.

## Heavy Metal Speciation Shifts and Quantitative Outcomes:

The shift of heavy metals from bioavailable pools to thermodynamically stable fractions is made easier by pyrolysis [79]. While thermal severity is the primary driver of this transformation, feedstock heterogeneity and metal chemistry influence particular results, according to experimental data obtained from sequential extraction frameworks.

Lowering of labile fractions, the decrease in exchangeable and acid-soluble metal fractions, is one of the most persistent trends seen. Studies show that these bioavailable pools are often reduced to negligible levels as pyrolysis temperatures reach 700°C, especially in designed magnetic biochar [71].

The way the intermediate pools behave: The fate of oxidizable and reducible fractions, on the other hand, varies greatly [73]. Fe/ Mn oxide-related reducible pools may enlarge as a result of oxide formation or shrink if metal chlorides cause mobilization. The persistence of

oxidizable fractions in the char, which are frequently associated with organic materials or sulfides, is indicative of the retention of organic binding sites within the carbon lattice [80].

**Dominance of regaining forms:** The residual portion, the most stable silicate or mineral-bound pool, is where metals are driven more and more at higher heat ranges. A crucial strategy for reducing long-term environmental danger, the crystallization of mineral phases, is significantly correlated with this shift.

**Pd, Cd, and Cr Quantitative stabilization:** Studies of co-pyrolysis at 650°C show different stabilization hierarchies. For example, chromium shows remarkable immobilization, with almost 86.7% partitioning into the residual portion [81]. Lead shows reduced leaching potential, with its stable portions accounting for about 68.2% of the total mass, while cadmium undergoes a larger shift towards stable form, reaching nearly 79.3%.

**Variability in Zn, Cu, and Ni behavior:** Several metals display complex, condition-dependent patterns. Zinc, for example, can become more mobile in a high chloride environment due to the volatility of metal chlorides. Copper tends to associate mainly with oxidizable and residual fractions, whereas Ni shows its strongest stabilization in oily sludge systems at moderate temperatures of around 400°C [82].

**Influence of matrix composition:** Ultimately, the chemical setting during the pyrolysis dictates speciation outcomes. Elevated chloride levels can lower char pH and briefly increase metal mobility [83]. In contrast, mineral additives such as zeolite or kaolin act as effective stabilizers. They help in converting easily released metals into more secure, mineral-bound forms.

### **Sequential Extraction, Risk Metrics, and Stabilization:**

#### **Assessment of Immobilization and Risk:**

The quantification of heavy metal immobilization and residual environmental responsibility depends on a suite of sequential extraction procedures, leaching assays, and ecological indices [84]. Reliable interpretation of these metrics necessitates orienting the analytical method with a specific research objective while appreciating the inherent dependent variations.

#### **Sequential Extraction Procedures and Methods Notes:**

The BCR three-step sequential extraction scheme is the emerging protocol utilized to describe operational fractions in biochar obtained from sewage, textile, and wet sludge [85]. To assess temperature-dependent speciation changes, this standardized method is used to categorize metals into acid-soluble (labile), reducible, oxidizable, and residual pools [86]. While the reviewed collection lacked specific data derived from the Tessier method because the included studies did not employ the Tessier sequential extraction method, limiting the cross-method comparisons and interpretations of metal speciation. The modified protocols, such as toxicity characteristics leaching procedures (TCLP) and DTPA extraction, are used to evaluate leachability and plant available fractions, respectively.

#### **Risk Assessment Metrics and Reported Outcomes:**

As the evidence provided by the reduced Risk assessment code (RAC), pyrolysis generally alleviates environmental hazards. The overall environmental threat can be minimized by the magnetic biochar produced at 700°C, which exhibited the lowest RAC for the majority of heavy metals [87]. Similarly, co-pyrolysis significantly lowered the potential ecological risk index to low risks, a metric further improved by the addition of kaolin or zeolite additives. In the context of bioavailability, acid-soluble fractions are diminished by thermal treatment, with some studies reporting near complete removal of the labile pools at high temperature. Leaching assays validate the minimized mobility for Cr, Ni, and Pb, although Zn and Cu occasionally display increased leachability under optimal thermal conditions.

**Stabilization Mechanisms Evidenced:**

The immobilization of heavy metals is driven by multiple physicochemical pathways. During the co-pyrolysis, the crystallization of stable inorganic phases, especially aluminosilicates, increases partitioning into the residual fraction [88]. Recently, leachability has been reduced by physical encapsulation within mineral crystals and the developing carbonaceous matrix. Surface complexation also plays an important role where ferric hydroxide colloids and nascent iron oxides search metals from exchangeable pools [89]. While phosphate precipitation is a theoretically viable mechanism, direct experimental evidence quantifying its preeminence in these specific sludge biochar remains limited.

**Application Potential and Limitations:**

Biochar formed at elevated temperature, i.e., 500 °C or co-pyrolyzed with mineral additives, explains the substantial potential of lowered metal bioavailability for soil application [90]. However, agricultural utilization faces constraints related to the incomplete stabilization of Zn and Cu and the potential mobilization risks analogous to chloride-rich feedstocks. Furthermore, while increased residual fraction recommends the mid-term stability, the recent literature lacks definitive multi-decadal field data and optimal regulatory compliance thresholds across diverse domains.

**Research Gaps and Future Directions:****Field Validation and Environmental Complexity:**

Most documented value rates are obtained from the controlled environments that do not account for variable hydrology and complex effluent matrices available in real-world scenarios. There is a distinct storage of systematic pilot studies capable of validating performance under continuously changing environmental conditions.

**Long-Term Stability and Remobilization:**

The stability of immobilized metals remains a primary concern. Temporal studies tracing metal fractionation are limited, leaving significant uncertainty regarding the potential remobilization of contaminants in response to natural shifts in soil redox potential or pH over extended periods [91].

**Ecological and Biosafety Risks:**

The introduction of non-native microbial strains for bioremediation presents unexplored risks. The potential for horizontal gene transfer and the subsequent displacement or disruption of native soil microbiomes requires rigorous risk assessment before application can be sanctioned [92].

**Standardized and Economic Modeling:**

In this field, the major challenge faced by researchers is that there are no proper guidelines for reporting performances, such as how metal absorbs, the time the process takes, etc. [93]. There are instructions, but they are not consistent and widely accepted. Studies report indicators and compare the results in their own way [94]. Without this information, accurately judging how biological systems stack against traditional methods is very challenging.

**Strategic Recommendations:**

On a literature review basis, microbial-biochar carries strong potential, but several areas require attention for these methods to be applied widely. From the available findings, the following recommendations can help in future research and development.

**From Laboratory to Pilot-Scale Systems:**

Nearly all review articles and studies rely on small scale trial and batch setups. While these experiments provide a base, fully capturing the variability in industrial waste is not fully possible [95]. The sample from the soil includes fluctuating pH, temperature changes, and unexpected chemicals. For this reason, medium-scale reactors such as continuous-flow columns should be tested. These systems allow researchers to observe how these microbes, biochar stability, and contaminants removal change over time.

## Chemical Measurement with Microbial Monitoring:

Most of the experiments focus on biochar chemical characteristics, performance like adsorption capacity, changes in metal and pH buffering [96]. However, the microbial components have equal importance in the remediation process. For this purpose, microbes' structure should be monitored by using molecular tools (e.g., 16S rRNA sequencing or enzymatic activity assays) [97]. This can explain why certain microbes-biochar combinations work better than others. In the selection of these strains, these interactions are very helpful.

## Development of Targeted Biochar Modifications:

Studies focus most on surface area and porosity, but not all industries require the same type of biochar. For example, Chromium-rich effluents can benefit from magnetic modified biochar, while textile dyes may be effectively removed by using chitosan-coated biochar. Researchers should develop different designs that can fulfill different sectors' needs for the removal of unique contaminants. At the same time, the modifications cost must be kept in mind [98].

## Evaluate the Behavior of Immobilized Metals:

In the literature, there is uncertain information about the metals' attachment to biochar, whether they are stable or not in changing conditions of soil and water. Researchers who put their months or years of studies on metal fractionation, check whether the remobilization occurs due to moisture or redox reactions [99]. This is important to determine whether the treatments are safe for the environment or not.

## Conclusion:

Industrial wastewater consists of a complex mixture of pollutants, mainly heavy metals and synthetic dyes. Due to these heavy metals and synthetic dyes, soil health, aquatic ecosystems, and human well-being are at risk. Conventional treatment approaches can be used for the removal of contaminants, but their use is limited because of high operational costs and the production of secondary waste. This review highlights how biochar offers a pathway that can be both adaptable and cost-effective. Biochar effectiveness comes through pyrolysis conditions and selection of feedstock, and the way its structure and chemistry can work. The relationship of biochar with microbes is important because it helps in the degradation of metals and dyes. Microbes alone are capable of converting metals, but their survival becomes impossible in the harsh conditions of industries. Biochar itself enhances the stability and performance of microbes by providing support, a compatible pH, and temperature. Despite the challenges, this technology holds a promising position in integrated biological and engineered methods. This technique can make it possible to create remediation strategies that can be both environmentally friendly and effective to use in the real world.

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