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Review article

Functionalized magnetite-biochar with live and dead bacteria for adsorption-biosorption of highly toxic metals: Cd, Hg, and Pb

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ABSTRACT

Environmental pollution by heavy metals such as cadmium (Cd), mercury (Hg), and lead (Pb) poses severe risks to ecological and human health. Conventional remediation technologies often fall short in efficacy and sustainability. This study explores a novel hybrid system combining functionalized magnetite-biochar with live and dead bacteria for enhanced adsorption-biosorption of these contaminants from wastewater. The synergy of magnetite-biochar and bacterial biomass exploits the high adsorption capacity of the composite and the unique biosorptive abilities of bacteria, offering a dual mechanism for metal removal. The composite's effectiveness was assessed through comparative studies, demonstrating superior removal efficiencies and operational advantages over traditional methods. Key findings include the composite's ability to function effectively across a broad range of environmental conditions and its potential for regeneration and reuse, highlighting its suitability for scalable applications. This research not only presents a viable alternative to existing wastewater treatment technologies but also aligns with sustainable practices by minimizing environmental impact and reducing treatment costs. The promising results suggest significant potential for the practical deployment of this technology in mitigating heavy metal pollution, urging further development towards commercialization and industrial use. The integration of such innovative materials could revolutionize wastewater treatment strategies and contribute to global sustainability efforts in pollution control.

1. Introduction

Cadmium (Cd), mercury (Hg), and lead (Pb) are highly toxic elements that pose significant risks to the environment and human health (Fig. 1). These elements are released through various human activities, including metallurgical processes, chemical industries, battery production, electroplating, waste incineration, artisanal small-scale gold mining, cement production, and the manufacture of paints, pigments, ceramics, glass, and electronic waste. Such activities Pb to the accumulation of these metals in soil [1], water [2], and air [3], resulting in widespread contamination. Their presence in natural environments has been linked to detrimental effects on wildlife. For example, studies in Italy found elevated levels of these metals in Little Owls, with concentrations of 170 ppb for Cd, 297 ppb for chromium, and 312 ppb for Pb in liver and brain tissues [4]. Similarly, marine life in the Adriatic Sea is affected, with contamination detected in Anchovy (20.2 μg/kg of Cd, 82.9 μg/kg of chromium, and 45.9 μg/kg of Pb), Red Mullet, and Mackerel, highlighting the potential for bioaccumulation in aquatic ecosystems [5].

Further evidence of contamination is found in industrialized regions such as Guiyu, China, known for electronic waste recycling. Pb levels in placental samples ranged from 6.51 to 3465.16 ng/g, with a median of 301.43 ng/g in Guiyu compared to 165.82 ng/g in control areas, although Cd and chromium levels showed no significant differences [6].

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Surface water contamination has also been reported in the Bahía Blanca Estuary, Argentina, with levels of chromium (0.89–5.83 μg/L), Cd (0.18–2.48 μg/L), and Pb (0.38–7.53 μg/L) [7]. Human exposure to these metals, especially in vulnerable populations like children, raises further concerns. In Germany, average blood Pb levels were reported at 9.47 μg/L, with Cd and mercury levels also detected in blood and urine samples [8].

High levels of Pb exposure in children have been linked to kidney function reductions, while Cd and mercury correlate with increased urinary protein excretion, indicating kidney stress [9]. Kidney Function Decline (USA): Additionally, studies in the USA have shown that high exposure to Cd and Pb increases the risk of kidney dysfunction [10]. In South Korea, a doubling of blood Cd levels increased the risk of metabolic syndrome by 23 % among men, with those in the highest exposure group facing even higher risks [11]. Global studies on breast milk have revealed Pb levels as high as 1515 μg/L in highly polluted regions, while Cd levels are generally below 2 μg/L, and mercury levels are elevated in areas with high fish consumption [12].

Despite the known dangers, conventional methods to mitigate these contaminants, primarily physical and chemical techniques, are often inefficient, leaving harmful residues and further environmental damage. Recent critiques of these methods point to the urgent need for more sustainable and effective solutions [13]. Biochar and its modification reported as promising materials for treating heavy metals [14]. In this context, the combination of physical and biological methods emerges as a promising alternative. Magnetite-biochar composites, for instance, have shown exceptional efficacy in removing toxic elements from wastewater due to their high adsorption capacity and rapid kinetics. Similarly, biosorption processes utilizing both life and dead bacteria, such as Bacillus cereus and Bacillus sphaericus, have demonstrated significant potential in removing chromium, achieving removal rates as high as 99 % and 44.5 % respectively [15,16]

This study introduces an innovative approach by reviewing the effectiveness of Functionalized Magnetite-Biochar combined with Live and dead bacteria for the removal of Cd, Hg, and Pb from wastewater. Combining magnetite-biochar and live and dead bacteria can improve the synergetic mechanisms from magnetite-biochar and bacteria, functionality, sustainability and adaptability and application flexibility. This review provides crucial insights into the development of a hybrid adsorption-biosorption methodology, potentially setting a new standard in wastewater treatment technologies. Remarkably, this study is the first to explore such a composite in the context of Cd, Hg, and Pb removal, addressing a significant gap in current environmental research. The novelty lies in leveraging both the high efficiency of magnetite-biochar and the natural detoxifying capabilities of bacteria, thus providing a comprehensive and environmentally friendly solution to a pressing global issue.

2. Method

To ensure a comprehensive understanding of the current landscape in the use of magnetite-biochar composites for heavy metal removal, an extensive literature search was conducted. This search spanned several databases including PubMed, Scopus, Web of Science, and Google Scholar. Additionally, manual searches of references from relevant articles and reviews were performed to capture all pertinent studies. Search terms were carefully chosen to encompass a variety of related topics, including "magnetite-biochar composites," "biosorption," "heavy metal removal," and specific mentions of metals like Cd, Hg, and Pb alongside bacterial agents such as "*Bacillus cereus*" and "*Bacillus sphaericus."*

2.1. Inclusion criteria and data extraction

The criteria for inclusion required that articles focus explicitly on the synthesis and application of magnetite-biochar composites, utilizing either live or dead bacteria for the biosorption of toxic metals in wastewater treatments. Only peer-reviewed journal articles published in

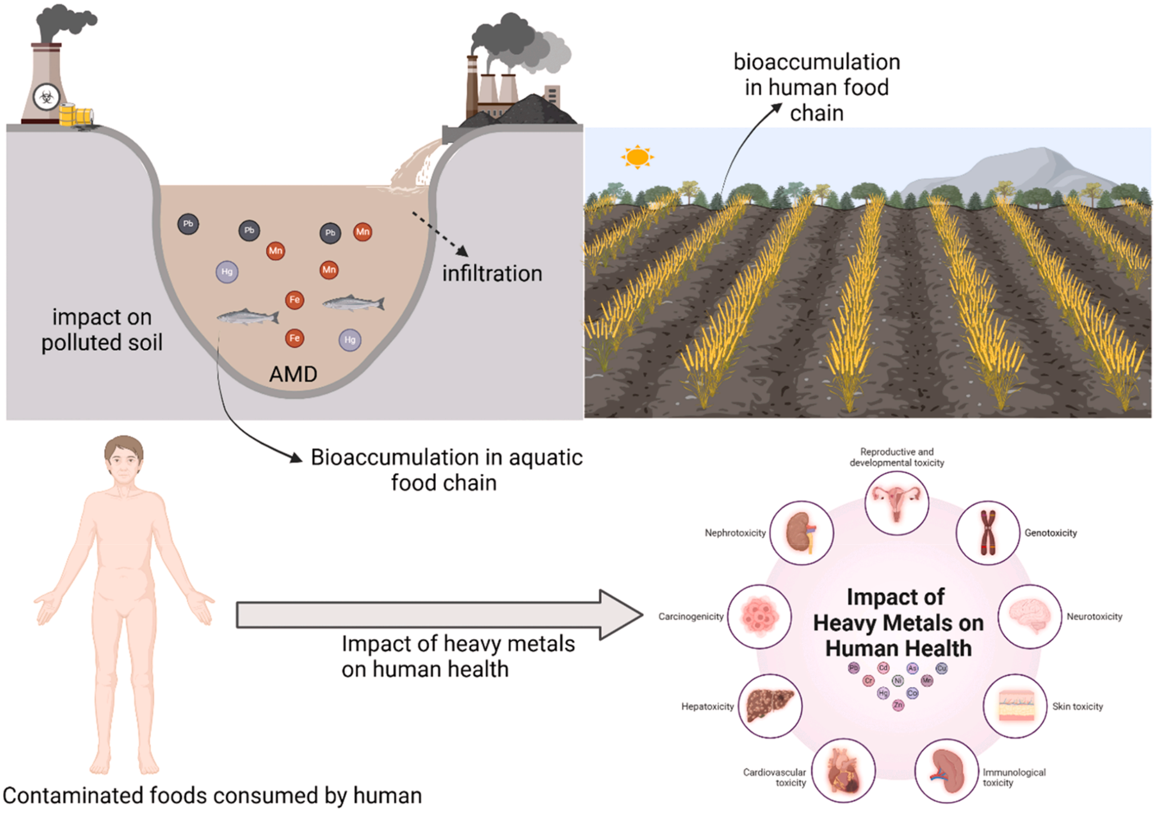

Fig. 1. Pathways of heavy metal entry into the human body and associated health risks.

English were considered. Exclusion criteria ruled out non-English articles, conference abstracts without full texts, and studies not directly related to biosorption in wastewater treatment. From each included study, key data were extracted, including the nature of biosorbents, experimental conditions, types of metals treated, concentrations, removal efficiencies, and specifics of bacterial usage.

2.2. Data synthesis and critical appraisal

Following data extraction, a thematic analysis was performed to organize findings into categories such as synthesis methods, biosorption mechanisms, and real-world applicability. This analysis also helped in identifying research gaps and setting the direction for future studies. A comparative analysis was then employed to scrutinize the efficacy of different biosorbents across various conditions, enhancing the understanding of their performance and environmental suitability. To ensure the credibility and reliability of the conclusions drawn, the methodological quality of each study was assessed, focusing on experimental design, analytical precision, and result reproducibility. Sensitivity analyses were conducted where data permitted to assess the robustness of findings across different biosorbent materials and bacterial species.

This methodology section provides a detailed narrative of the

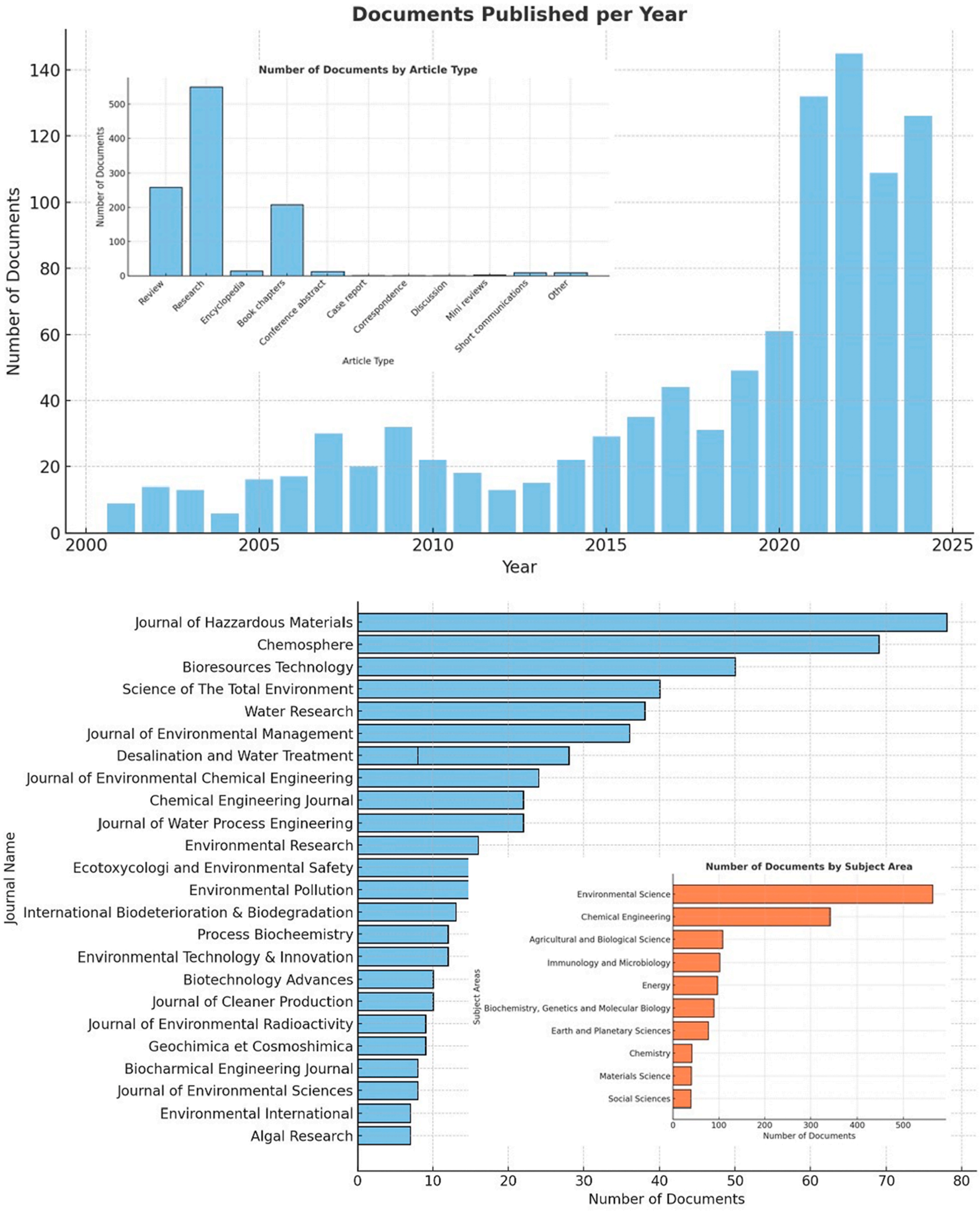

Fig. 2. Trends and distribution of published documents related to the study topic based on the scopus database according to the annual publication trends, distribution of documents by article type, categorization of documents by subject area, and top contributing journals ranked by the number of documents.

systematic approach adopted to review and synthesize the available literature on magnetite-biochar composites enhanced with bacteria for effective heavy metal removal from wastewater. It emphasizes the rigor of the selection process and the analytical methods used to ensure that the conclusions drawn are based on solid and reproducible evidence.

3. Result and discussion

This section presents a detailed analysis of the adsorption-biosorption performance of functionalized magnetite-biochar combined with bacterial biomass for the removal of toxic metals. The discussion explores various aspects, including the comparative effectiveness of this hybrid system versus conventional methods, performance under different environmental conditions, and adsorption dynamics modelled through isotherms and kinetics. Additionally, the mechanisms underlying metal removal and the life cycle impact of this approach are examined to assess its potential for sustainable, large-scale applications.

3.1. Recent trends in published documents

Scopus database showed the published documents with keywords “Adsorption Biosorption using Life and dead Bacteria” are 1066 documents. The trends depicted in Fig. 2 highlight the growing importance and interdisciplinary nature of research in this domain. Fig. 1 shows a significant upward trend in the number of publications over time, with a notable surge after 2015. This reflects an increased awareness of the role of adsorption biosorption in addressing environmental pollution, particularly for removing heavy metals and other contaminants. The peak in recent years, such as 2022–2024, suggests heightened research interest driven by advancements in bioremediation technologies and stricter environmental regulations. In contrast, earlier years (2000–2010) exhibit sporadic interest, likely due to the nascent stage of this research field.

Fig. 1 also emphasizes the dominance of research articles, followed by review articles, indicating a strong experimental foundation supported by comprehensive literature overviews. The smaller contributions from book chapters, conference abstracts, and other types of publications demonstrate that dissemination is primarily academic and research-focused. The limited number of case reports and mini-reviews suggests that while there are practical applications, the field’s focus remains largely on theory and experimentation.

The figure highlights the interdisciplinary nature of this research, with environmental science and chemical engineering leading as the primary subject areas. This dominance reflects the focus on practical applications for wastewater treatment and pollution control. The presence of agricultural and biological sciences, along with biochemistry, genetics, and molecular biology, indicates significant exploration of bacterial mechanisms and their biosorption capabilities. Fields such as social sciences and materials science have minimal representation, suggesting that societal or material innovation aspects are not the central focus of this research.

Fig. 1 also identifies key journals contributing to the dissemination of knowledge in this field. Journals such as *Journal of Hazardous Materials* and *Chemosphere* are the most prominent, showcasing their role in advancing this technology. Other journals like *Bioresources Technology* and *Science of The Total Environment* further underscore the emphasis on sustainability and environmental solutions. The variety of journals featured in the chart highlights the multidisciplinary nature of this research and its broad applicability across different scientific domains. Overall, this figure illustrates the dynamic evolution of adsorption biosorption research using life and dead bacteria. The growing number of publications, diverse subject areas, and wide range of journals underscore the field’s significance in addressing global environmental challenges. Future research should focus on scaling up these technologies for industrial applications, improving cost-effectiveness, and fostering interdisciplinary collaborations to maximize impact.

3.2. Adsorption-biosorption using live and dead bacteria vs other methods

The evaluation of wastewater treatment methods has gained significant attention due to rising pollution levels and the need for sustainable solutions. Biosorption, which utilizes biological materials for the adsorption of contaminants, has emerged as a promising alternative to traditional physical and chemical methods. Commonly, the wastewater treatment classified as biological, physical and chemical treatment (Fig. 3). A previous study showed the biochar prepared by pyrolysis or hydrothermal and can modified using chemical, physical and biological modification to create a unique characteristics including pore filling, surface complexation, ion pair, n-π interaction, $\pi-\pi$ interaction, hydrogen bonding, hydrophobic and electrostatic interaction [17] that reported highly efficient for organic dyes [18].

This section compares adsorption-biosorption with conventional treatment methods, focusing on their performance in terms of removal efficiency, cost-effectiveness, and environmental impact. Notably, biosorption offers an adaptable and cost-efficient solution with reduced secondary pollution, making it a valuable approach in addressing wastewater contaminants such as Cd, Hg, and Pb [19-21].

The synthesis of waste material-based nanocomposites for AMD treatment presents both opportunities and challenges. The several methods of biochar production showed in Fig. 4. One promising approach involves the AMD sludge-derived Al-Fe_3O_4 nanocomposites have achieved a tetracycline degradation efficiency of up to 93.9 % within 60 minutes, with a recyclability of over four adsorption-desorption cycles [22]. Further studies have demonstrated that magnetite nanoparticles synthesized from AMD achieved complete removal of Al, Mg, and Mn, and over 90 % removal of Fe, Ni, and Zn during treatment, with settling time significantly influencing the removal of Mg, Ca, and Na [23]. Similarly, the synthesis of ZnS/TiO_2 nanocomposites from bioremediated AMD effluents has proven feasible, integrating biological sulfate reduction processes with nanoparticle production [24].

Innovative methods have also explored the production of valuable minerals such as goethite, hematite, and magnetite from AMD treatment residues. These efforts integrate advanced processes like sequential precipitation and reverse osmosis, achieving efficient recovery and valorization of minerals for industrial applications [25]. Additionally, nanocomposites synthesized using mining waste and AMD have demonstrated performance comparable to those produced from pure chemicals, further highlighting their potential in environmental applications [26].

In other side, biosorption processes using both life and dead microbial biomass as well as other organic materials, demonstrate competitive or even superior removal efficiency relative to traditional methods. Unlike physical adsorption techniques, which rely primarily on surface interactions, biosorption engages complex chemical mechanisms including ion exchange, complexation, and microprecipitation. The biosorption process is influenced by various parameters such as pH, temperature, and pollutant concentration, allowing it to be tailored to specific wastewater compositions [27,20,28].

For example, a novel composite biosorbent utilizing Bacillus cereus demonstrated significant efficacy in heavy metal remediation, achieving a maximum removal efficiency of 92.13 % for Pb(II) ions when combined with activated carbon [29]. Similarly, enhanced Cd removal has been reported with Bacillus cereus immobilized on magnetic biochar. Fig. 5 showed the preparation of materials, this study also reported the maximum biosorption capacity reached 93.02 mg/g for Cd. This innovative approach underlines the enhanced capabilities of magnetic biochar when used in conjunction with microbial agents [30]. Furthermore, the integration of Bacillus sp. K1 into magnetic biochar composites has been shown to increase Cd removal by 230 %, illustrating a significant improvement over the use of raw magnetic biochar alone [31]. This

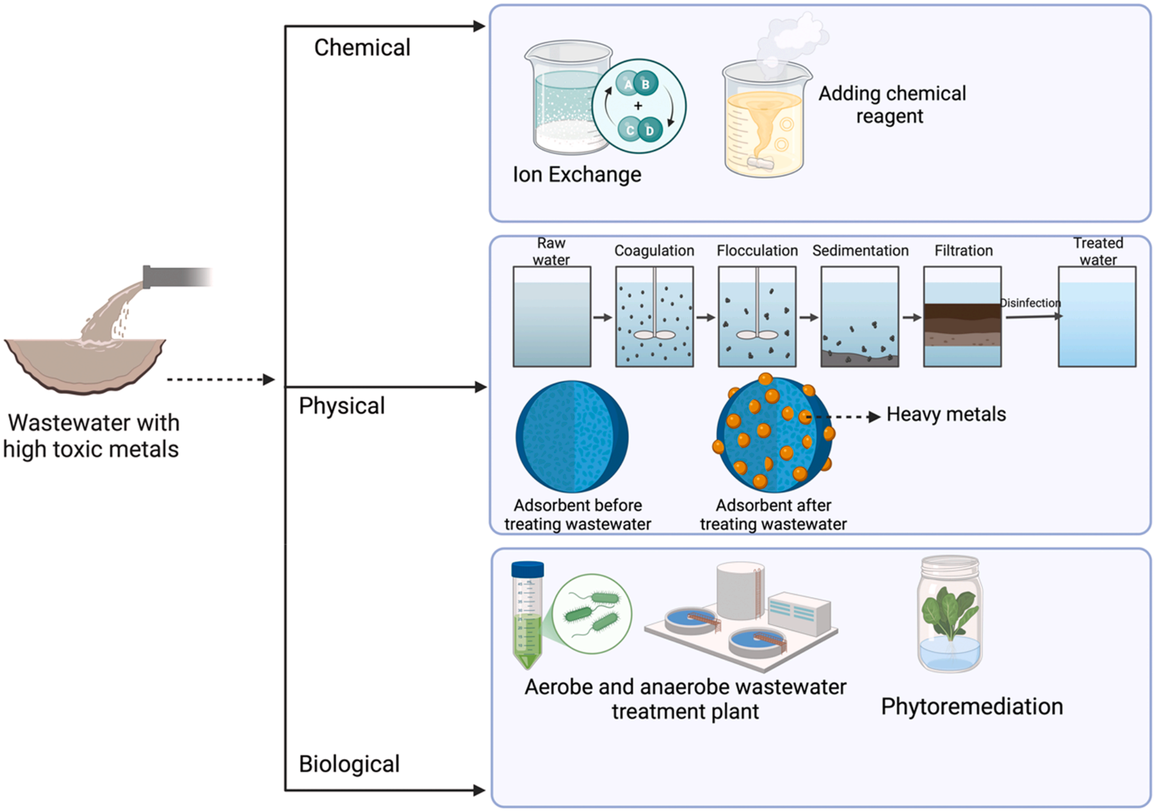

Fig. 3. Schematic representation of various methods for treating wastewater containing high levels of toxic metals including chemical, physical and biological methods.

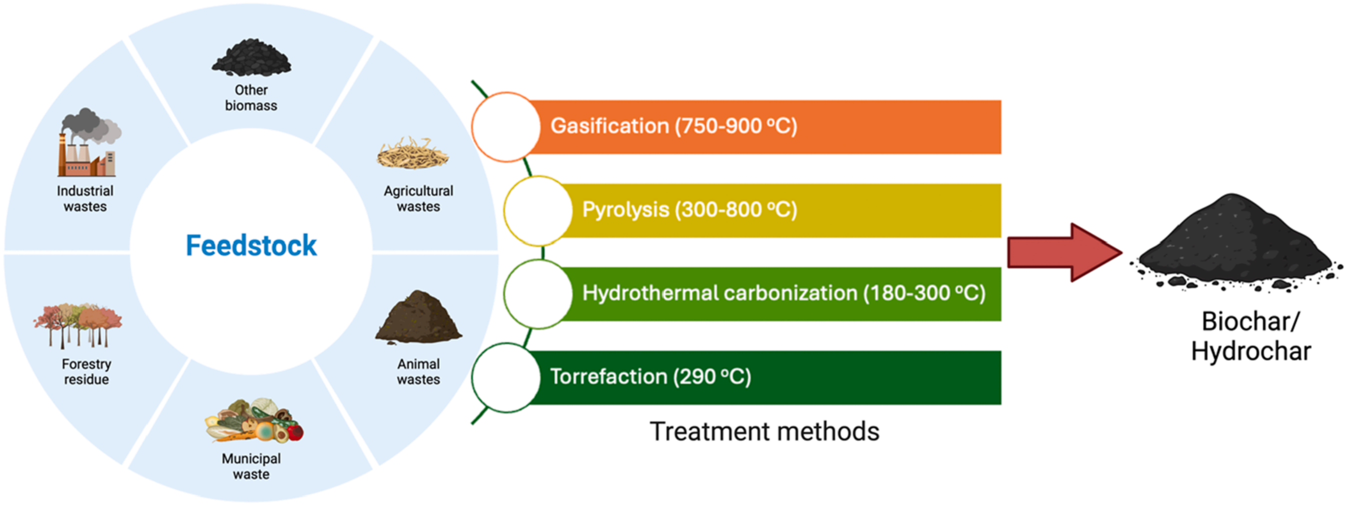

Fig. 4. Several methods for biochar and hydrochar synthesis.

synergy between magnetic biochar and microbial technology offers a potent solution for the removal of not only Cd but also arsenic, highlighting its broad applicability in contaminant removal strategies.

In comparison, conventional methods such as chemical precipitation often struggle with variable efficiencies, especially when treating mixed-metal solutions, making biosorption a flexible solution. Biosorption models, such as Langmuir and Freundlich isotherms, are commonly applied to assess the capacity and behaviour of biosorbents. These models aid in optimizing the process, and biosorption kinetics frequently align with pseudo-second-order models, indicative of chemical interactions that enhance heavy metal capture [32-34]. Traditional techniques lack this flexibility, often yielding lower efficiency in capturing specific metal ions due to high variability in the aqueous chemistry of pollutants [35,36]. Table 1

Cost-effectiveness is a crucial determinant in the choice of wastewater treatment methods. Conventional methods like ion exchange and chemical precipitation involve significant operational costs, primarily due to the ongoing need for chemical reagents, high energy input, and complex maintenance. These methods also require substantial infrastructure investment, especially in industrial-scale applications, leading to high capital costs [55,56]. By contrast, biosorption leverages low-cost biosorbents that can be derived from agricultural or microbial biomass,

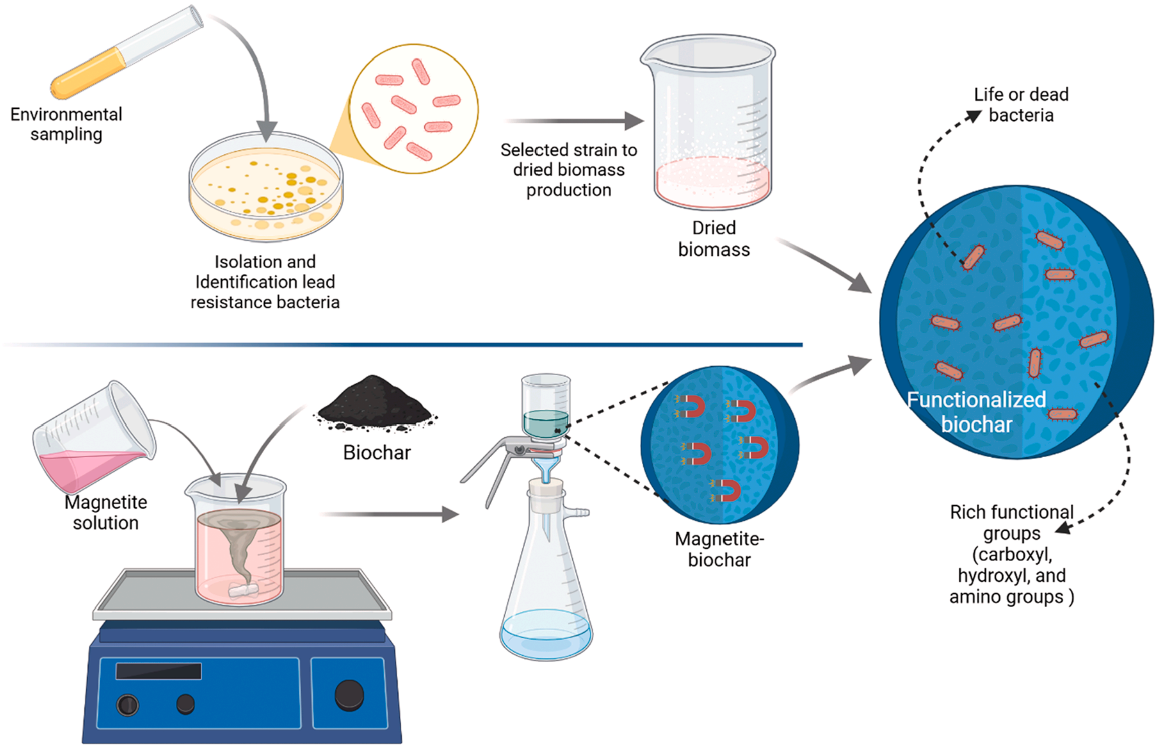

Fig. 5. Preparation of functionalized magnetite-biochar with live and dead bacteria.

substantially reducing expenses associated with treatment. Materials such as spent coffee grounds, rice husk, and fungal biomass are not only inexpensive but also highly effective in metal adsorption [21,36]. Additionally, biosorbents can be regenerated through relatively simple methods, allowing their reuse across multiple cycles without significant efficiency loss. For instance, studies indicate that biosorbents like functionalized biochar can undergo multiple adsorption-desorption cycles with minimal decline in performance, thus enhancing the long-term cost-efficiency of biosorption [30,57]. This material is also classified as a sustainable and low-cost adsorbent that is easy to regenerate [58]. This regenerative capability of biosorption materials contrasts with traditional methods, which often generate a sludge byproduct requiring additional disposal costs, further burdening the treatment budget.

The environmental impact of wastewater treatment techniques is increasingly under scrutiny as global efforts toward sustainable resource management intensify. Conventional methods, while effective, are associated with significant environmental drawbacks, including the production of hazardous secondary waste. For example, chemical precipitation and ion exchange generate solid residues laden with concentrated pollutants, which require proper handling and disposal to prevent secondary contamination [59]. These disposal challenges are minimized in biosorption processes, which utilize naturally derived or waste materials, reducing the likelihood of hazardous waste generation. In particular, biochar-based biosorbents not only reduce metal ions in wastewater but also contribute to carbon sequestration, given that biochar itself is a carbon-rich product [21,60]. Furthermore, biosorption aligns well with circular economy principles by repurposing agricultural waste and other organic materials, thereby addressing both waste minimization and resource recovery objectives. This dual benefit places biosorption at an advantage over conventional approaches, especially in industries looking to lower their ecological footprint [27,57,19].

Lastly, while biosorption’s effectiveness and environmental benefits are well established, optimizing its performance under different environmental and operational conditions remains a critical focus. The adsorption capacity and stability of biosorbents can be highly variable, influenced by factors such as pH, metal concentration, and contact time. Studies on bacterial biosorbents demonstrate that optimizing these parameters significantly enhances metal removal efficiencies. For instance, certain bacterial strains, when immobilized on supports or enhanced with functional groups, show heightened adsorption stability and resilience across pH and temperature gradients [20,28,60]. Immobilization not only increases the mechanical stability of biosorbents but also allows for easier handling and potential reusability, essential factors for practical wastewater treatment applications [30,61]. Table 2 showed the comparison performance and mechanism of highly toxic metals removal using adsorption-biosorption using bacteria vs other materials.

Conventional systems, which often require precisely controlled conditions, lack this adaptability, making biosorption a more flexible solution for diverse wastewater environments. In conclusion, the comparative analysis of adsorption-biosorption with conventional treatment methods underscores the advantages of biosorption in terms of removal efficiency, cost savings, and environmental sustainability. These findings, supported by ongoing research in optimizing biosorbent materials and operational conditions, suggest that biosorption has significant potential as a scalable, eco-friendly technology for the treatment of heavy-metal-laden wastewater.

3.3. Adsorption-biosorption using live and dead bacteria in various conditions

The examination of adsorption and biosorption rates using live and dead bacteria under varying environmental conditions is central to advancing bioremediation strategies for heavy metal removal from wastewater. The interaction between bacteria and heavy metals is significantly influenced by factors such as pH, temperature, and metal ion concentration, alongside the structural composition of the bacterial cell wall and the physiological state of the bacteria. These elements collectively determine the overall efficiency and mechanism of metal uptake, highlighting the complexities of biosorption as a dynamic process that can be harnessed for environmental applications [76,77]. This section explores the distinct roles of life and dead bacterial cells in metal binding and adsorption-biosorption rates across varied environmental

Table 1
Removal percentage of adsorption-biosorption and other methods.

Methods	Performance	References
polyampholyte (Poly(EGDE-MAA−2MI))	182 mg/g for Cd(II) and 202 mg/g for Pb(II). Removal percentage: 72 % for Cd(II) and 62 % for Pb(II)	Copello et al., [37]
CSG-BiPO4/FePO4	Removal percentage of 96.24 % for Cd(II), and 95.55 % for Pb(II)	Kheirandish et al., [38]
Biochar	Pb removal efficiency up to 89.03 % and Cd removal up to 75.84 %	Wu et al., [39]
Titanate nanotubes	Adsorption capacities of 520.83 mg/g for Pb and 238.61 mg/g for Cd. More than 80 % of Pb and 85 % of Cd desorbed	Xiong et al., [40]
Multi-metal binding biosorbent	Removal capacities of 31.73 mg/g for Cd and 76.25 mg/g for Pb	Abdolali et al., [41]
MgO nanoparticles	Removal efficiencies of 2294 mg/g for Cd and 2614 mg/g for Pb	Xiong et al., [42]
Agave Bagasse	Removal efficiencies of 93.14 mg/g for Pb and 28.50 mg/g for Cd	Cholico-González et al., [43]
Competitive biosorption using *Eichhornia crassipes*	Pb removal remains effective even in the presence of other metals	Mahamadi, Nharingo [44]
microalgae Chlamydomonas reinhardtii	Maximum biosorption capacities were 72.2 mg/g for Hg(II), 42.6 mg/g for Cd (II), and 96.3 mg/g for Pb(II)	Tüzün et al., [45]
green alga (Ulva lactuca)	Maximum biosorption capacities were 34.7 mg/g for Pb(II) and 29.2 mg/g for Cd(II)	Sarı, Tuzen [46]
spergillus flavus	Maximum biosorption capacities were 1550 μmol/g for Cd(II), 950 μmol/g for Hg (II), and 1000 μmol/g for Pb (II)	Mahmoud et al., [47]
alginate and immobilized live and heat inactivated *Phanerochaete chrysosporium*	Alginate beads exhibited a biosorption capacity of 28.9 mg/g for Hg(II) and 34.8 mg/g for Cd(II). Live fungus immobilized beads increased this capacity to 66.1 mg/g for Hg(II) and 50.0 mg/g for Cd(II). Heat-inactivated fungus immobilized beads further increased the capacity to 112.6 mg/g for Hg(II) and 85.4 mg/g for Cd(II). The maximum biosorption capacities were observed between pH 5.0 and 6.0. The biosorbents could be regenerated with up to 97 % recovery using 10 mM HCl and reused across three cycles with minimal loss in capacity.	Kaçar et al., [48]
biosurfactant-producing Pseudomonas sp.	Maximum biosorption capacities were 27.5 mg/g for Cd(II) and 77.8 mg/g for Pb(II)	Huang, Liu [49]
Simultaneous biosorption using brown macroalgae Fucus vesiculosus.	Maximum capacities were 143.2 mg/g for Cd(II), 70.1 mg/g for Ni(II), and 516.3 mg/g for Pb(II)	V.R. et al., [50]
algae Gelidium and granulated agar extraction algal waste	The maximum uptake capacities observed were Algae Gelidium: 0.067 mmol Pb/g and 0.171 mmol Cu/g and Composite Material: 0.014 mmol Pb/g and 0.043 mmol Cu/g. Desorption test successfully 100 % effective using 0.1 M HNO_3 as eluant.	Vilar et al., [51]
Phycoremediation using Nostoc muscorum.	Maximum sorption was 85.2 % for Cd and 93.3 % for Pb	Dixit, Singh [52]
Biosorption using *Paliurus spina-christi*.	Maximum capacities were 231.7 mg/g for Pb(II) and 36.84 mg/g for Cd(II)	Özmal et al., [53]
Hybrid biosorbent matrix of *Phanerochaete chrysosporium* and ibrous network of papaya wood	Maximum biosorption capacity was 141.63 mg/g	Iqbal et al., [54]

conditions, with a focus on the contributions of cell wall structures and bacterial metabolic activity.

Bacterial cell walls play a primary role in metal binding due to their unique structural components, which offer multiple functional groups that can interact with metal ions. The peptidoglycan layer, a major component of the bacterial cell wall, contains carboxyl, hydroxyl, and amino groups that serve as potential binding sites for heavy metals. These sites facilitate adsorption through various mechanisms, including ion exchange, complexation, and precipitation, which can vary depending on the specific metal ion and bacterial strain involved [78]. For instance, carboxyl groups within the peptidoglycan structure have been observed to interact effectively with metal ions such as chromium, enhancing the adsorption capacity of bacteria like *Bacillus cereus* for this and other heavy metals [76]. Moreover, wall teichoic acids (WTA) in the bacterial cell walls enhance the metal-binding affinity, particularly for divalent ions like magnesium and calcium, which further supports the structural integrity and metal-adsorptive properties of the cell wall under different conditions [77,79]. Consequently, the structural characteristics of the bacterial cell wall significantly influence adsorption-biosorption efficiency, with dead bacteria relying heavily on these passive binding sites for metal uptake.

In contrast, live bacteria offer an additional advantage in metal biosorption due to their active metabolic processes. Live bacterial cells not only rely on the structural components of the cell wall for adsorption but also engage in active transport mechanisms that enable metal ions to be transported across the cell membrane. This active transport is often coupled with metabolic activity that may alter the surrounding microenvironment, potentially increasing metal availability and enabling a higher overall biosorption rate. The metabolism-driven uptake can enhance the removal of metal ions beyond passive adsorption, as metabolic activity modifies the chemical environment around the cell wall, allowing for the creation of more binding sites and a greater attraction to metal ions [80,81]. This advantage makes live bacteria particularly effective in environments where metal ion concentrations fluctuate, as they adapt to these conditions and maintain relatively high uptake rates. However, while live bacteria offer enhanced metal uptake, dead bacterial cells, with their stable structural features, are often preferred in scenarios where simplicity and long-term stability are essential.

Environmental factors such as pH and temperature are also crucial in determining the adsorption-biosorption performance of bacteria. The pH of the surrounding solution, in particular, affects the ionization state of functional groups on the bacterial cell wall, thereby influencing metal binding capacity. Lower pH levels increase proton concentration, which can compete with metal ions for binding sites on the cell wall, reducing overall metal adsorption capacity. Conversely, at higher pH values, carboxyl and other groups on the bacterial cell wall become deprotonated, enhancing their ability to attract and bind metal ions through electrostatic interactions [82]. This pH sensitivity plays a significant role

Table 2
Comparison performance of biochar with bacteria vs other conventional materials.

Adsorbent	Toxic Metals Removed	Adsorption Mechanism	Maximum Adsorption Capacity (mg/g)	Reusability	References
Functionalized Magnetite-Biochar with Live and Dead Bacteria	Cd, Pb	Biosorption via surface functional groups, bioaccumulation	93.02 (Cd)	High (73–88 %)	Deng et al., [30]
KMnO4-Treated Magnetic Biochar	Pb, Cd	Surface oxidation, ion exchange	148 (Pb), 79 (Cd)	High (87–95 %)	Sun et al., [62]
Pseudomonas sp. Bacterial Biomass	Cd, Pb	Amine, carboxyl, phosphate group interactions	77.8 (Pb), 27.5 (Cd)	High (>90 % recovery)	Huang, Liu [63]
Magnetic Biochars (Non-MRSB types)	Cd, Pb	Electrostatic interaction, ion exchange	58.65 (Cd), 42.48 (Pb)	High	Huang et al., [64]
Chitosan-Magnetite-Biochar (E-CMBC)	Pb	Functionalized complexation	156.68 (Pb)	Moderate	Zheng et al., [65]
Iminodiacetic Acid Magnetic Biochar	Cd	Complexation, ion exchange	197.96 (Cd)	Moderate	Zhou et al., [66]
Magnetic Clay-Biochar Composite	Cd	Multilayer adsorption, functional group interaction	26.22 (Cd)	High (recovered magnetically)	Zhao et al., [67]
Magnetite-Douglas Fir Biochar (MBC)	Pb, Cd	Ion exchange, surface interaction	27 (Pb), 11 (Cd)	Moderate	Karunanayake et al., [68]
MoS2-Modified Magnetic Biochar	Cd	Complexation with sulfur groups	139 (Cd)	Moderate	Khan et al., [69]
Magnetic Aerobic Granular Sludge Biochar	Pb	Surface complexation, electrostatic attraction	127 (Pb)	High (88 %)	Huang et al., [70]
Thermophilic Bacteria-Immobilized Biochar	Pb, Cd	Preconcentration, biosorption	34.3 (Pb), 37.1 (Cd)	Moderate	Özdemir et al., [71]
tilolite (Zeolite)	Pb, Cd	Ion exchange, surface interaction	~20–30	Moderate	Wang, Ariyanto [72]
Activated Carbon from Waste Biomass	Pb, Cd	Surface adsorption, functional group interactions	Comparable to zeolite (~30–40)	Moderate to High	Minamisawa et al., [73]
Bentonite for Cd and Pb Removal	Cd, Pb	Ion exchange, surface adsorption	10–20	Moderate	Hamidpour et al., [74]
Zeolite-Supported Nanoscale Zero-Valent Iron (Z-NZVI)	Cd, Pb	Electrostatic adsorption, ionic exchange, reduction	48.63 (Cd), 85.37 (Pb)	High (below magnetite-biochar)	Li et al., [75]

in determining biosorption performance, especially in applications involving dead bacterial biomass, where adsorption relies entirely on passive mechanisms. In the case of live bacteria, pH influences not only adsorption but also the metabolic activity that underpins active biosorption. Temperature similarly impacts metal binding as it affects both the diffusion rates of metal ions and the kinetic energy of the adsorbing bacteria. Higher temperatures generally increase diffusion rates, potentially enhancing adsorption rates and capacities [83]. However, for live bacterial cells, elevated temperatures may disrupt metabolic processes if they exceed the bacterial tolerance range, highlighting the need for precise temperature control to optimize biosorption in live bacterial systems.

The concentration of metal ions in solution is another influential factor affecting biosorption performance. Higher metal concentrations can lead to increased competition for available binding sites on the bacterial cell wall, eventually reaching a saturation point where no additional ions can be adsorbed. This saturation effect can limit the efficacy of both live and dead bacteria in high-concentration environments, particularly if there are insufficient functional groups available for binding [80,84]. However, at lower concentrations, the relative uptake rates can be higher, as there is less competition among ions for binding sites. Additionally, live bacteria may adaptively respond to varying metal concentrations through metabolic adjustments, potentially enhancing their metal uptake under suboptimal concentrations compared to dead bacteria, which lack this adaptability [80,81]. The interplay between metal concentration and bacterial physiological state introduces complex dynamics in biosorption, as live bacterial cells tend to perform better in fluctuating or low-metal environments due to their adaptive capacities.

Bacterial cell wall composition also exhibits adaptability to environmental changes, particularly in response to the presence of various metal ions. When exposed to different metals, bacterial cell walls can undergo compositional alterations, which may influence their metal-binding capacity. This dynamic nature allows bacteria to adapt to changing conditions, such as shifts in metal concentration or pH, by restructuring cell wall components to optimize metal uptake [82]. The adaptation capacity is beneficial in environments with fluctuating metal concentrations or chemical compositions, where biosorption performance may otherwise be compromised. This feature is advantageous for both life and dead bacterial cells, although live cells exhibit a more pronounced adaptive response due to their metabolic processes, which support structural modifications and regeneration of cell wall binding sites [82]. Such adaptability underscores the potential of bacteria as efficient biosorbents across various environmental conditions, whether deployed in passive or active forms.

The examination of adsorption and biosorption rates using live and dead bacteria under different environmental conditions reveals a nuanced interplay between bacterial structure, metabolic state, and environmental factors such as pH, temperature, and metal concentration. The bacterial cell wall's structural components, specifically peptidoglycan and teichoic acids, are pivotal in facilitating metal ion binding, with dead bacteria relying heavily on these passive adsorption mechanisms. Conversely, live bacteria benefit from metabolic activity that enhances biosorption rates and offers adaptability to environmental fluctuations. This comparison highlights the importance of understanding the specific interactions between bacteria and metal ions for optimizing biosorption processes in wastewater treatment applications. Recognizing the influence of external conditions on biosorption performance is essential for developing effective bioremediation strategies that exploit the advantages of both life and dead bacterial cells in removing heavy metals from contaminated environments. The illustration of this materials showed in Fig. 6.

3.4. Isotherm and kinetic models

In understanding the adsorption characteristics of magnetite-biochar-bacteria composites, isotherm and kinetic models provide valuable insights into adsorption capacities and rate mechanisms essential for environmental remediation applications. This section discusses the application of prominent isotherm models, such as the Langmuir and Freundlich models, to characterize the adsorption process, as well as kinetic models like the pseudo-first-order (PFO) and pseudo-second-order (PSO) models to analyze adsorption-biosorption dynamics. These models aid in optimizing the use of magnetite-

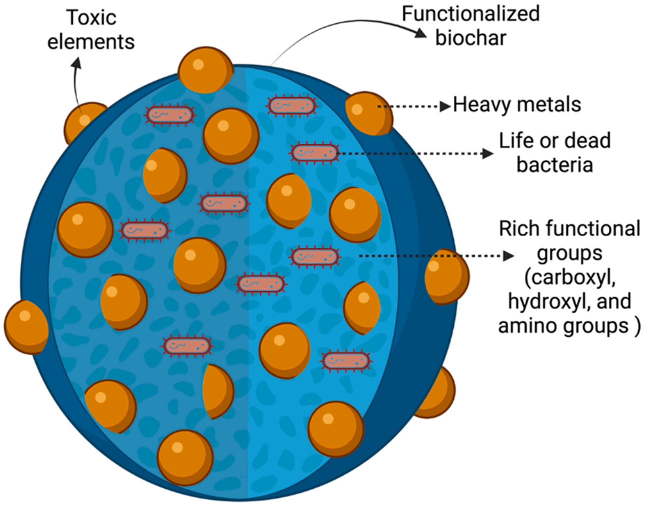

Fig. 6. Illustration of functionalized biochar with live and dead bacteria for the removal of toxic elements and heavy metals.

biochar-bacteria composites in capturing heavy metals and organic pollutants from contaminated environments.

The Langmuir and Freundlich isotherm models represent two primary approaches to understanding adsorption equilibrium, with each model focusing on distinct surface characteristics. The Langmuir model is based on the assumption that adsorption occurs as a monolayer on a homogenous surface with uniform energy distribution across all sites. The Langmuir equation is given by:

$$q_e = \frac{q_{\max} K_L C_e}{1 + \quad K_L C_e}$$

where q_e is the adsorption amount at equilibrium, C_e is the equilibrium concentration of adsorbate, $q_{\max}$ represents the maximum adsorption capacity, and K_L is the Langmuir constant, indicating binding site affinity [85]. This model is particularly relevant to magnetite-biochar-bacteria composites because it provides a way to assess the maximum adsorption capacity for heavy metals, such as Pb and Cd, by indicating a finite number of available sites on the composite's surface. Studies have demonstrated the efficacy of magnetite-biochar composites in following Langmuir-type adsorption, with high correlation coefficients (R^2) suggesting a robust fit to this model for certain heavy metal pollutants, indicating an even distribution of active sites [86,87].

In contrast, the Freundlich isotherm model accounts for adsorption on heterogeneous surfaces, where energy distribution and site affinity are not uniform. The Freundlich equation is:

$$q_e = K_F C_e^{\frac{1}{n}}$$

where K_F is a constant indicative of adsorption capacity, and nnn represents the adsorption intensity. The Freundlich model is useful for describing multilayer adsorption that can occur on the diverse and irregular surfaces of biochar and bacterial cells in composite form [85,88]. This model is effective in describing adsorption behavior for organic pollutants and metals on biochar-based adsorbents, where surface heterogeneity plays a significant role in adsorption efficiency. For instance, magnetite-biochar-bacteria composites often exhibit adsorption behaviors that conform to the Freundlich model when removing complex pollutants such as dyes, reflecting the heterogeneous nature of binding sites and the possibility of multilayer formation [89,90].

When combined, the Langmuir and Freundlich models offer a more comprehensive understanding of adsorption dynamics in magnetite-biochar composites. In particular, the Sips model, which integrates aspects of both isotherms, can be applied to systems where adsorption may exhibit both monolayer and multilayer characteristics depending on pollutant concentration [85]. This model can capture the complexity of adsorption in magnetite-biochar composites, making it a valuable approach for evaluating performance in variable environmental conditions where contaminants exhibit mixed affinities for adsorption sites. Such insights from isotherm models inform the design and selection of magnetite-biochar-bacteria composites for optimized adsorption capacity in real-world applications [91].

Kinetic models are equally important as they provide insights into the rate and mechanism of adsorption-biosorption processes, which are influenced by the interaction dynamics between adsorbate and adsorbent. Two primary kinetic models—the pseudo-first-order (PFO) and pseudo-second-order (PSO) models—are widely applied to evaluate magnetite-biochar composites' adsorption rates. The PFO model, introduced by Lagergren, is expressed as:

$$\frac{dq_t}{dt} = k_1 (q_e - q_t)$$

where q_t is the adsorption amount at time t, q_e is the equilibrium adsorption amount, and k_1 is the rate constant. The integrated form of this model is:

$$\ln(q_e - q_t) = \ln(q_e) - k_1 t$$

This model suggests that the adsorption rate is proportional to the difference between the equilibrium concentration and the concentration at a specific time. Although it effectively describes systems with fast adsorption processes, it often lacks accuracy for heterogeneous systems where chemical interactions play a significant role [92,93]. In magnetite-biochar-bacteria composites, PFO may provide limited insight as it assumes physisorption, which may not account for the chemisorption-driven interactions prevalent in these composites.

The PSO model, in contrast, assumes that the rate of adsorption is more dependent on the square of the difference between q_e and q_t, suggesting that the process is governed by chemisorption mechanisms. This model is represented as:

$$\frac{dq_t}{dt} = k_2 (q_e - q_t)^2$$

where k_2 is the pseudo-second-order rate constant, with the integrated form given by:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e}$$

The PSO model is often a better fit for adsorption on magnetite-biochar composites because it considers chemisorption, which involves electron sharing or exchange between adsorbate and adsorbent. Many studies find that PSO aligns well with experimental data for heavy metal adsorption, particularly for composites containing biochar and bacteria where chemical interactions with adsorbates are predominant [92,94]. This alignment suggests that chemisorption is the primary mechanism, likely due to interactions between functional groups on the composite surface and metal ions.

The structural and chemical properties of magnetite-biochar-bacteria composites enhance their effectiveness in adsorption, with the magnetite component contributing to surface area and magnetic properties that facilitate pollutant removal. Incorporating magnetite increases the adsorption capacity by introducing additional binding sites and enhancing separation capabilities post-adsorption due to magnetic properties. Furthermore, the presence of bacteria on the composite surface contributes additional binding sites, particularly through functional groups on cell walls that interact chemically with heavy metals and organic pollutants [86,87]. This unique combination of magnetite, biochar, and bacterial components allows the composite to exhibit adsorption behaviors that fit both isotherm and kinetic models, supporting its application in water treatment and environmental remediation.

Thus, the application of isotherm models, such as Langmuir and Freundlich, along with kinetic models like PFO and PSO, provides comprehensive insights into the adsorption dynamics of magnetite-biochar-bacteria composites. The Langmuir model's monolayer adsorption assumptions align well with homogenous adsorption processes, whereas the Freundlich model addresses the heterogeneous nature of biochar-based materials. Kinetic studies using PSO models suggest that chemisorption mechanisms govern the adsorption process, which aligns with the composite's reliance on chemical interactions between pollutants and functional groups. This understanding aids in optimizing composite applications for efficient contaminant removal, providing a foundation for future studies on improving adsorption efficiency and expanding practical environmental remediation applications. In addition, Table 3 showed the several studies related the isotherm and kinetic models in heavy metals removal.

3.5. *Mechanism of metals removal*

Magnetite-biochar composites combined with bacterial biomass are emerging as effective materials for removing highly toxic heavy metals like Cd, Hg, and Pb from contaminated environments. This approach combines the adsorptive capabilities of biochar, the magnetic properties of magnetite, and the metabolic detoxification processes of bacteria, leading to a robust and multifaceted metal remediation system. This section explores the chemical and biological mechanisms that drive the removal of these metals, emphasizing the role of functional groups on the biochar surface and the unique contributions of bacterial metabolism.

Table 3
Isotherm and kinetic models in heavy metals removal.

Materials	Isotherm Model	Kinetic Model	References
Magnetic aerobic sludge-biochar	Langmuir	Pseudo-second-order	Huang et al., [70]
Surface-modified magnetic biochar	Langmuir	Not specified	Zahedifar et al., [95]
Chitosan–EDTA magnetic biochar	Langmuir	Avrami fractional-order	Zheng et al., [65]
MnFe2O4 magnetic biochar	Sips (Freundlich–Langmuir)	Pseudo-second-order	Zhang et al., [96]
MoS2-modified magnetic biochar	Langmuir	Pseudo-second-order	Khan et al., [69]
Bacillus cereus on magnetic biochar	Langmuir	Not specified	Deng et al., [30]
Iminodiacetic acid magnetic biochar	Langmuir	Pseudo-second-order	Zhou et al., [66]
Saccharomyces carlsbergensis biomass	Langmuir	Not specified	Sayyadi et al., [97]
Fe3O4-biochar with Bacillus sp.	Langmuir	Not specified	Wang et al., [31]
Magnetic biochar (KMnO4 modified)	Langmuir	Pseudo-second-order	Sun et al., [62]
APTES-functionalized magnetic biochar	Langmuir	Pseudo-first/second-order	Nnadozie, Ajibade [98]
Geobacillus galactosidasius on γ-Fe2O3	Langmuir	Pseudo-second-order	Özdemir et al., [71]
Magnetic clay-biochar composite	Langmuir	Intra-particle diffusion	Zhao et al., [67]
Functionalized magnetic biochar composite	Langmuir	Pseudo-second-order	Singh et al., [86]
Biochar washed with HCl	Langmuir	Not specified	Wu et al., [39]
FMWCNTs vs. magnetic biochar	Langmuir/Freundlich	Pseudo-second-order	Ruthiraan et al., [99]
Ca-based magnetic biochar	Langmuir	Not specified	Wu et al., [100]
Serratia marcescens magnetic biocomposite	Langmuir	Pseudo-second-order	Wu et al., [101]
Bacillus licheniformis on magnetic biochar	Langmuir	Pseudo-second-order	Wen et al., [102]

The magnetite-biochar component of the composite plays a central role in metal adsorption through its diverse functional groups and structural properties. Biochar itself has a high surface area and a porous structure rich in functional groups such as carboxyl, hydroxyl, and phenolic groups, which are known for their strong binding affinity to metal ions. These groups interact with heavy metals primarily through mechanisms like ion exchange, electrostatic interactions, and complexation (Fig. 7.), all of which facilitate the adsorption of Cd, Hg, and Pb [103,104]. For example, Pb ions bind effectively with the carboxyl and hydroxyl groups on biochar, creating stable inner-sphere complexes that prevent the re-release of lead into the environment [105]. The presence of magnetite, an iron oxide, on biochar enhances its adsorption properties by introducing additional binding sites, which contribute to a greater overall metal removal capacity. This also enables magnetic separation, making it easier to retrieve the composite from the solution after metal adsorption, a distinct advantage for practical applications [86].

Ion exchange is another critical process by which magnetite-biochar composites capture metal ions. In this process, metal ions in solution, like Cd and Pb, replace cations such as calcium (Ca^{2+}) on the biochar surface, a mechanism that immobilizes the metal ions and prevents them from leaching back into the water. Research indicates that iron oxide-modified biochar exhibits superior ion exchange capabilities, contributing to the immobilization of toxic metals for prolonged periods [39]. Through ion exchange, these composites not only capture metal ions but also transform them into less mobile forms, making the metals more stable within the environmental matrix. This combination of complexation and ion exchange provides a dual mechanism that is highly effective for long-term containment of heavy metals.

In addition to chemical mechanisms, the integration of bacterial biomass introduces essential biological processes for enhanced metal detoxification. Bacteria such as *Pseudomonas* and *Bacillus* species are particularly useful for biosorption due to the functional groups on their cell walls, which include amine, phosphate, and carboxyl groups. These groups interact with metal ions through covalent and electrostatic bonding, providing additional sites for metal binding beyond those offered by the biochar matrix [49]. Furthermore, some bacterial strains can sequester metal ions within their cellular structures, thus reducing the availability of these metals in the surrounding environment. Studies on magnetite-biochar composites immobilized with *Bacillus cereus* have shown that these systems outperform free bacteria due to the additional surface area and protective environment provided by the composite, which enhances biosorption efficiency and allows the bacteria to function more effectively in toxic conditions [106].

Beyond biosorption, bacteria within the magnetite-biochar matrix play a significant role in metal transformation through enzymatic reduction processes. This transformation capability is particularly important for mercury detoxification, where bacterial metabolic activity can reduce highly toxic Hg^{2+} ions to elemental mercury (Hg^0), a less bioavailable form. The enzymatic reduction of Hg within bacterial cells lowers the toxicity and environmental impact of mercury in contaminated systems. This bacterial reduction adds another layer of detoxification to the magnetite-biochar-bacteria composite, going beyond simple adsorption to actively reduce the toxicity of metal contaminants ([107], p. 20). Similarly, certain bacterial species can reduce other heavy metals, such as Cr(VI) to Cr(III), through metabolic pathways, further contributing to the detoxification capabilities of these composites [108].

The environmental conditions surrounding the magnetite-biochar-

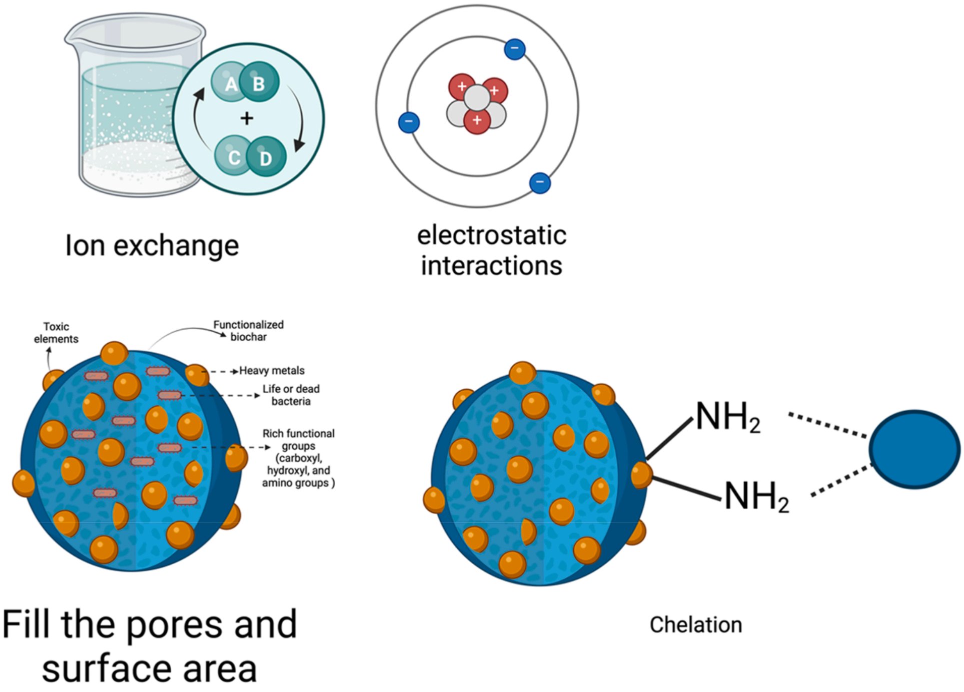

Fig. 7. Mechanisms of heavy metal removal using functionalized-biochar with life and dead bacteria.

bacteria composites, such as pH and temperature, significantly influence their adsorption and detoxification performance. The pH of the solution, for instance, affects the ionization state of functional groups on both the biochar and bacterial surfaces. At lower pH levels, competition between protons and metal ions for binding sites can decrease adsorption efficiency, as the presence of excess H$^+$ ions limits the availability of sites for metal ions [104]. However, at higher pH levels, deprotonation of functional groups like carboxyl and hydroxyl groups on the biochar surface increases, enhancing the electrostatic attraction to positively charged metal ions and improving metal binding [109]. This dependency on pH makes it essential to optimize environmental conditions to maximize metal removal.

Previous studies involving batch adsorption tests across a wide pH spectrum (e.g., pH 2–10) have been instrumental in identifying the optimal pH conditions for adsorption and biosorption. These studies reveal that heavy metal removal is most effective within a pH range of 5–6. At lower pH levels, the high concentration of H$^+$ ions competes with metal cations for adsorption sites, significantly reducing efficiency [110]. Conversely, at higher pH levels, metal ions tend to precipitate as hydroxides, complicating the adsorption process and potentially reducing its efficacy.

Temperature also plays a pivotal role, influencing both the physical adsorption rate and the metabolic activity of bacteria involved in biosorption. Elevated temperatures enhance the kinetic energy of metal ions, leading to increased diffusion rates and potentially higher adsorption capacities. However, excessive temperatures may impair bacterial metabolic functions, undermining the biological mechanisms essential for detoxification [83,93]. Hence, maintaining an optimal temperature range is crucial to balancing the physical adsorption capacity of the composite with the biological activities of bacteria, ensuring maximum efficiency in metal removal.

Several studies have highlighted the importance of pyrolysis temperature in the synthesis of nano-magnetite biochar composites, which serve as effective adsorbents. For instance, biochar pyrolyzed at temperatures between 300°C and 600°C demonstrated significant performance variations based on the pyrolysis conditions. At 500°C, biochar achieved a 96.9 % degradation efficiency for ethylbenzene and a 36.2 % total organic carbon (TOC) removal rate, driven by the generation of hydroxyl and oxygen radicals critical for pollutant degradation [111]. Similarly, biochar prepared at 400°C preserved redox-active moieties, enhancing electron transfer and improving methane production in anaerobic digestion [111]. Magnetite biochar prepared at 400°C preserved redox moieties, enhancing electron transfer and methane production in anaerobic digestion [112]. agnetic biochars synthesized at varying temperatures, such as 300°C, 500°C, and 700°C, exhibited distinct adsorption properties. Pyrolysis at 700°C yielded the highest capacity for removing organic pollutants, with adsorption capacities of 153.2 mg/g for 17β-estradiol and 85.93 mg/g for Cu(II) under optimal conditions [113].

The integration of magnetite-biochar composites and bacterial biomass provides a synergistic approach to metal detoxification. The biochar component offers ample binding sites and enhances surface interactions through chemical processes like ion exchange and complexation. Simultaneously, bacteria contribute to the system through biosorption and metabolic processes, which either sequester or transform metal ions. This dual chemical and biological approach offers superior performance over traditional adsorbents, as it combines passive adsorption with active detoxification, thus enhancing the system's capacity to handle high concentrations of Cd, Hg, and Pb. Studies show that magnetite-biochar-bacteria composites have significantly higher removal efficiencies for these metals, making them highly effective for applications in wastewater treatment and contaminated site remediation [114].

The adsorption process in aqueous systems is significantly influenced by the presence of coexisting ions, which compete for active sites on the adsorbent. For instance, cations such as Ca^{2+} and Mg^{2+} can occupy functional groups, thereby reducing the number of sites available for target metals. This competitive effect has been documented, showing reductions in adsorption capacity by up to 21.79 % in the presence of Pb^{2+} and 12.43 % with PO$_4^{3-}$ for Cr(VI) adsorption onto modified Auricularia auricula substrate [115]. Similarly, anions such as SO$_4^{2-}$ and HCO$_3^-$ can form complexes with heavy metals, altering their mobility and adsorption behavior. For instance, SO$_4^{2-}$ has been found to reduce the adsorption of Cr(VI) ions at the oil-water interface due to competitive adsorption [116]. Additionally, in the presence of PO$_4^{3-}$, the adsorption efficiency of As(V) by Mg-Fe-(CO$_3$) LDH was significantly reduced due to competitive adsorption, illustrating the high impact of specific anions [117].

These effects are especially pronounced in high ionic strength environments, where electrostatic repulsion between adsorbent and adsorbate can further hinder adsorption efficiency. For example, in competitive multi-ion systems, adsorption kinetics and equilibrium align well with models such as Langmuir and Freundlich isotherms, showcasing the role of electrostatic forces [115]. Future studies could include controlled experiments with varied ion concentrations to quantitatively assess these impacts. As observed, modifications to adsorbent surface chemistry, such as embedding functional nanoparticles or altering pH conditions, could mitigate competitive effects and improve selectivity and efficiency [118].

Magnetite-biochar-bacteria composites capitalize on both chemical and biological mechanisms to achieve comprehensive heavy metal detoxification. Functional groups on the biochar and bacterial cell surfaces interact with metal ions through complexation, ion exchange, and biosorption, providing robust adsorption capabilities. The bacterial component further contributes through metabolic detoxification, transforming certain metals into less toxic forms. By optimizing environmental conditions such as pH and temperature, these composites can be tailored to achieve high removal efficiencies for Cd, Hg, and Pb, presenting a sustainable and highly adaptable solution for environmental remediation applications. This combination of physical and biological metal removal mechanisms offers a promising path forward in developing effective strategies to mitigate heavy metal contamination in diverse ecological contexts.

Functionalized magnetite-biochar composites are increasingly utilized for wastewater treatment due to their excellent adsorption capacities and unique secondary reaction mechanisms. These processes, however, often involve the occurrence of secondary reactions and byproducts, influenced by the chemical properties of the adsorbent and surrounding environmental conditions. For instance, magnetite in the composite frequently participates in redox reactions, such as the reduction of Cr(VI) to Cr(III) or the oxidation of elemental mercury (Hg^0) to ionic mercury (Hg^{2+}). Modified magnetite-biochar composites have demonstrated remarkable Cr(VI) removal efficiencies, achieving up to 97 % removal with adsorption capacities reaching 142.86 mg/g in acidic environments [119].

Additionally, reactive oxygen species (ROS) such as hydroxyl radicals (-OH) can form in systems containing embedded nanoparticles. These ROS not only enhance the oxidative degradation of organic contaminants but also play a role in the metal adsorption process, contributing to the system's multifunctionality [120]. Another prevalent mechanism is the formation of stable precipitates, facilitated by functional groups like -COOH and -OH on biochar surfaces. For example, Pb (II) can form precipitates such as $PbCO_3$ through these interactions, with adsorption capacities for Pb(II) reported as high as 64.92 mg/g [98].

In systems using live bacterial biomass, biological activity can produce organic-metal complexes, and under anaerobic conditions, methylation of Hg^{2+} into toxic methylmercury (MeHg) can occur. Such reactions highlight the importance of maintaining carefully controlled conditions, such as adequate oxygen levels, to mitigate unwanted byproducts [121]. Optimization strategies are therefore essential to balance adsorption efficiency with the minimization of undesired secondary reactions. One key optimization strategy involves maintaining a near-neutral pH (6–7), which prevents excessive precipitate formation while maintaining high adsorption performance. For example, thiol (-SH) functionalized biochar has shown over 90 % Hg^{2+} removal while preventing methylation under these conditions [122,123]. The use of mild acids or chelating agents such as EDTA during desorption can further prevent precipitate accumulation and ensure the adsorbent retains high functionality across multiple cycles, with studies reporting over 90 % efficiency retention after several uses [124].

To safely handle spent adsorbents containing heavy metals or byproducts, immobilization in cementitious matrices is often employed. Advanced characterization techniques, such as Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Photoelectron Spectroscopy (XPS), are invaluable for monitoring changes in the adsorbent's surface functional groups and detecting secondary byproducts. These tools help refine operational parameters, improving both efficiency and reliability of the treatment process [125]. While secondary reactions and byproducts are inherent to functionalized magnetite-biochar systems, these challenges can be effectively managed through strategic operational adjustments and advanced adsorbent designs. Such measures enable these composites to deliver high performance and maintain long-term viability in wastewater treatment applications.

3.6. Assessment of stability and long-term viability in real-world wastewater environments

Under representative conditions mimicking real-world wastewater environments, various experimental approaches have been employed to assess the stability and operational long-term viability of functionalized magnetite-biochar composites. These experiments focus on simulating actual environmental and operational challenges, including varying pH levels, temperature fluctuations, competing ions, and continuous flow scenarios.

One effective approach involves batch equilibrium studies and continuous column experiments. Batch experiments are often used to evaluate adsorption capacity under controlled conditions, including varying concentrations of heavy metals like Cd, Pb, and Hg, while adjusting pH, ionic strength, and temperature to mimic real wastewater [30,126]. For example, studies on magnetic biochars derived from rice straw have demonstrated high Cd adsorption capacities of 93 mg/g in column studies under varying pH and contaminant loads, suggesting robust performance even with fluctuating wastewater chemistry [30].

Continuous column experiments provide more realistic assessments of the composite's performance over time. These studies measure parameters such as breakthrough curves, retention time, and reusability across multiple cycles of adsorption and desorption. For instance, Pb removal efficiencies of 95 % have been sustained over five cycles, with high adsorption stability, using magnetite-biochar composites in column setups designed to replicate industrial effluents [65,127].

Regeneration and reuse studies are critical for evaluating the composite's long-term viability. In these experiments, adsorbents undergo multiple cycles of adsorption and regeneration using acid or base eluents. For instance, a study found that magnetite-biochar composites retained 97 % of their initial adsorption capacity for Pb after three cycles, with negligible structural degradation, highlighting their durability under repeated use [65].

To simulate interference from competing ions commonly found in wastewater (e.g., Na^+, Ca^{2+}, and Cl^-), advanced studies introduce these ions during experiments. These studies have shown that ion competition may reduce adsorption efficiency by up to 10–15 %, but functional modifications, such as chelation with EDTA or functionalization with Mg, can mitigate this effect, maintaining high adsorption efficiencies even in complex wastewater matrices [69,126].

Additionally, pilot-scale studies have been conducted to bridge laboratory findings with field applications. These involve larger systems with continuous wastewater inflow and real-time monitoring of adsorption kinetics and desorption efficiencies. For example, a pilot-scale evaluation of magnetic biochar-bacterial composites revealed stable heavy metal removal (e.g., Pb at 90 % efficiency) over continuous operation for several weeks [127].

Finally, mechanistic studies utilizing advanced techniques such as Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), and X-ray Photoelectron Spectroscopy (XPS) are conducted post-adsorption. These analyses assess changes in functional groups, surface morphology, and chemical composition, ensuring that the composite maintains its structural integrity and functional activity across operational cycles [67]. Thus, experimental approaches under representative wastewater conditions have demonstrated that functionalized magnetite-biochar composites are not only highly effective in adsorbing heavy metals but are also resilient and operationally viable for long-term use. Their stability across regeneration cycles, adaptability to complex wastewater matrices, and ease of separation through magnetism make them a promising technology for real-world applications [30,126,65].

In addition, a review also summary the numerous adsorbents with their cost production in USD/g [128]. Various adsorbents, each with unique properties and adsorption capacities, have been investigated for their potential in pollutant removal, demonstrating cost-effectiveness compared to more advanced methods. For instance, tomato seeds, classified as an "A" adsorbent, effectively removed Acid Blue 92 and Acid Red 14, with adsorption capacities of 36.23 mg/g and 125.00 mg/g at costs of $0.1180/g and $0.0340/g, respectively [129]. Similarly, durian shells and tiger nut residues offered highly economical solutions for removing Basic Brown 16 and methylene blue, with tiger nut residue achieving a remarkable capacity of 146 mg/g at an exceptionally low cost of $0.00001/g [130,131].

Modified agricultural by-products, such as Bambusa tulda and kola nut husk, also demonstrated efficiencies in removing pollutants like crystal violet and ibuprofen, with capacities of 20.84 mg/g and 39.22 mg/g, respectively, at costs as low as $0.0009/g and $0.0425/g [132,133]. In comparison, activated carbons derived from waste materials, including sugarcane bagasse and gasification by-products, were particularly effective for dyes like malachite green (226.06 mg/g at $0.0002/g) [134].

Biochars such as distillers grain hydrochar and cherry kernel biochar excelled in heavy metal removal. For example, distillers grain hydrochar removed methylene blue at 340.3 mg/g and phosphate at 96.9 mg/g, with a cost of $0.0009/g [135]. Cherry kernel biochar was effective against Pb(II), Cd(II), and Ni(II), with capacities ranging from 66.22 to 94.48 mg/g, though at a slightly higher cost of $0.0419/g [136].

While this study does not focus on magnetite-biochar functionalized with live and dead bacteria, the adsorbents reviewed are cost-effective alternatives to more advanced methods. For example, nano-zeolites used for nitrophenol removal achieved an adsorption capacity of 156.6 mg/g at $0.0300/g [137]. In contrast, traditional adsorbents like nanoscale zero-valent iron (nZVI) removed phosphate at 0.312 mg/g but with a significantly higher cost of $3.15/m^3 . Advanced composites such as titania/graphene oxide removed Pb(II) at 228.31 mg/g, though the cost was moderate at $0.0460/g [138].

Comparing these adsorbents to other treatment methods highlights their affordability. For instance, chemical precipitation for heavy metal removal costs approximately $0.50–$1.00/m^3 of treated water but generates sludge requiring disposal, adding to operational expenses. Membrane filtration systems like reverse osmosis offer high efficiency but can cost $0.30–$0.80/m^3 for installation and operation, with additional costs for membrane replacement. Ion exchange resins, while highly effective, cost $0.10–$0.20/m^3 but involve periodic regeneration expenses [139].

Thus, these low-cost adsorbents, while not as advanced as magnetite-biochar functionalized with live and dead bacteria, serve as viable alternatives for wastewater treatment. Their affordability and accessibility make them competitive, especially in regions with limited resources for advanced water purification technologies.

3.7. LCA analysis

The environmental sustainability of the magnetite-biochar-bacteria approach for heavy metal remediation is best evaluated through Life Cycle Assessment (LCA) metrics, which provide a comprehensive overview of its ecological footprint compared to conventional treatment methods. This section synthesizes existing research on the environmental impacts, resource efficiency, and effectiveness of this hybrid system, emphasizing its advantages in terms of greenhouse gas emissions, energy consumption, and waste reduction.

The magnetite-biochar-bacteria composite capitalizes on the unique properties of each component to optimize the removal of heavy metals, such as Cd, Hg, and Pb. Magnetite nanoparticles (MNPs) confer magnetic properties that allow for easy recovery of the adsorbent after treatment, thus reducing waste. Biochar, a carbon-rich material produced by pyrolysis of organic waste, enhances adsorption capacity due to its porous structure and functional groups, which can attract and immobilize heavy metals through mechanisms such as ion exchange and complexation [140,141]. Meanwhile, the incorporation of bacterial biomass introduces a biological dimension that can further detoxify metals through biosorption and biotransformation, thus providing an additional layer of treatment [142,143]. This combination not only boosts metal removal efficiency but also reduces reliance on synthetic chemicals, a common practice in conventional methods that often leads to secondary environmental issues.

Life Cycle Assessment (LCA) is a standardized methodology for evaluating the environmental impacts associated with each stage of a product's lifecycle, from raw material extraction to end-of-life disposal. Applying LCA to the magnetite-biochar-bacteria composite reveals its advantages in terms of reduced greenhouse gas emissions, lower energy consumption, and efficient resource utilization [144,145]. The production of biochar from waste materials like agricultural residues or sewage sludge can significantly reduce the carbon footprint by capturing carbon and preventing waste from entering landfills. Furthermore, because the biochar production process sequesters carbon in a stable form, it contributes to long-term carbon storage, which helps mitigate climate change [144,145]. This aspect positions biochar as an environmentally beneficial material within the composite, where it serves as both a highly effective adsorbent and a carbon-storing component.

In contrast, conventional heavy metal treatment methods such as chemical precipitation and ion exchange are typically resource-intensive and environmentally impactful. Chemical precipitation, for instance, often requires large amounts of chemical reagents and generates secondary waste products, which then require additional treatment. Similarly, ion exchange processes, while effective, demand significant energy inputs and often involve synthetic resin materials that contribute to the environmental burden [145]. The magnetite-biochar-bacteria system provides an alternative with potentially lower environmental impacts, as it utilizes natural materials and capitalizes on biological processes that are inherently sustainable and less resource-intensive [146,147]. For example, biochar derived from agricultural waste not only functions as an adsorbent but can also be repurposed as a soil amendment after use, contributing to soil health and fertility by improving soil structure, water retention, and nutrient availability [146].

The addition of magnetite nanoparticles enhances the adsorption capacity of biochar for heavy metals, offering a critical advantage in terms of treatment efficiency. This physical adsorption combined with the bacterial detoxification mechanisms leads to high removal rates for metals such as Cd, Hg, and Pb, making this hybrid system a promising substitute for conventional treatments [142,148]. Furthermore, the magnetic properties of the composite allow for straightforward separation and recovery of the adsorbent from treated water, which reduces the need for extensive and energy-intensive post-treatment steps. The ease of separation also facilitates the reuse of the composite, aligning with principles of resource efficiency and sustainability [140].

Evaluating the LCA metrics of this composite system also highlights its alignment with resource recovery and circular economy principles. The production of biochar from agricultural and industrial waste materials transforms these byproducts into valuable resources for environmental remediation, exemplifying waste valorization [146]. This approach is consistent with a circular economy model, which emphasizes minimizing waste and maximizing resource reuse. By reusing organic waste to produce biochar, this system reduces landfill burden and repurposes waste in a way that benefits the environment and addresses metal contamination simultaneously [145]. Moreover, if the biochar-magnetite-bacteria composite is eventually applied to land as a soil amendment, it could further contribute to resource recovery by enriching soil with organic material, improving agricultural productivity, and promoting sustainable land management practices.

Biochar's role in carbon sequestration also plays a central part in the environmental sustainability of this system. Biochar is known for its ability to sequester carbon, as it is highly resistant to decomposition, allowing it to store carbon in soil over extended periods [144]. Integrating biochar into the magnetite-biochar-bacteria composite thus provides the dual benefit of metal adsorption and carbon sequestration, aligning the treatment process with climate change mitigation efforts. By reducing greenhouse gas emissions associated with waste treatment, this hybrid system contributes to broader climate goals while addressing the immediate need for contaminant removal [144].

Furthermore, the LCA approach sheds light on potential economic advantages. Conventional treatment methods are often costly, not only due to energy and material inputs but also because of the costs associated with secondary waste disposal. The magnetite-biochar-bacteria composite system, in contrast, presents a more cost-effective option. With the use of low-cost, locally available materials such as agricultural waste for biochar production, the overall operational costs are substantially reduced. The ease of separation enabled by magnetite also contributes to cost savings by minimizing the labor and resources required for adsorbent recovery. Additionally, since bacterial biomass can be cultivated from inexpensive organic substrates, this component of the composite is both affordable and scalable, making the system economically viable for large-scale wastewater treatment applications [147].

Thus, evaluating the magnetite-biochar-bacteria composite through LCA metrics highlights its substantial advantages over conventional heavy metal treatment methods in terms of environmental sustainability, resource efficiency, and overall ecological footprint. The hybrid system combines the adsorptive properties of biochar, the magnetic capabilities of magnetite, and the metabolic functions of bacteria to achieve efficient metal removal while minimizing secondary pollution and waste. By transforming agricultural and industrial byproducts into a functional treatment material, the magnetite-biochar-bacteria composite embodies the principles of a circular economy, promoting waste reduction and resource recovery. Its potential for carbon sequestration further supports its role as a climate-friendly solution in environmental remediation. As demand grows for sustainable wastewater treatment solutions, this hybrid system stands out as a promising direction for future research and application, offering a sustainable, adaptable, and cost-effective approach to mitigating heavy metal contamination in diverse environmental contexts.

4. Future prospect

Functionalized magnetite-biochar composites integrated with bacterial biomass offer a versatile and effective solution for large-scale wastewater treatment, particularly in the removal of highly toxic metals like Cd, Pb, and Hg. This technology capitalizes on the high adsorption capacities of biochar, the magnetic properties of magnetite, and the biosorptive abilities of bacterial biomass, positioning it as a promising alternative to conventional treatment methods. As demand for sustainable and efficient wastewater treatment solutions increases, the potential applications and enhancements of this hybrid system make it well-suited for future environmental remediation initiatives.

The potential for large-scale applications of magnetite-biochar composites in wastewater treatment lies in their unique ability to combine both adsorption and magnetic separation. The presence of magnetite imparts magnetic properties to the biochar, which allows for easy retrieval and reuse of the adsorbent material. In industrial or municipal wastewater treatment facilities, this feature reduces operational complexity and costs by simplifying separation processes [149]. Moreover, the porous structure of biochar, coupled with its surface functional groups like carboxyl and hydroxyl, facilitates strong interactions with metal ions, enhancing the adsorption of toxic metals even in low concentrations [150]. The magnetic properties also allow for rapid and thorough separation from the treated water, which is critical in high-throughput, large-scale operations where efficiency and recovery are paramount [149].

The addition of live bacterial biomass to the system brings a biological dimension that further enhances metal removal efficiency. Live bacteria not only serve as biosorbents but also participate actively in metal detoxification through bioaccumulation, extracellular polymeric substances (EPS) production, and biotransformation processes. For instance, bacterial strains like *Lysinibacillus sphaericus* are effective in binding and detoxifying heavy metals through biosorption processes, which reduce the toxicity of metal ions and facilitate their immobilization [151]. When live bacteria are not feasible due to extreme environmental conditions, dead bacterial biomass can still provide significant adsorption capacity through its functionalized cell wall structures. This versatility makes the magnetite-biochar-bacteria composite suitable for diverse and challenging wastewater conditions, further enhancing its applicability on a large scale [152].

For scaling up these composites, there are challenges related to efficiency under high metal concentrations and stability in variable pH environments. Advanced modifications to biochar, including amino-functionalized magnetic biochars and structural alterations, have shown improved stability and performance under various conditions [153]. However, these advancements must be refined to ensure consistent efficacy in real wastewater systems, which contain a mix of metal ions and organic compounds that can interfere with adsorption. Biochar stability is especially important in acidic wastewater, where the durability of magnetite particles and biochar's functional groups can affect adsorption rates and metal binding efficiency [121]. Developing more resilient biochar materials that withstand these environmental variables would be essential for the widespread adoption of this technology in real-world settings.

Another promising area for enhancing the efficiency of this hybrid system is through genetic engineering and chemical modification techniques that improve the specificity of both biochar and bacterial biomass for targeted metal removal. Modifying biochar with chelating agents like ethylenediaminetetraacetic acid (EDTA) or amino groups, for instance, has been shown to increase affinity for particular metals such as Pb and Cd, as it adds functional groups capable of forming stronger metal complexes [154]. Additionally, genetic engineering could be applied to bacterial strains to develop biofilms rich in metal-binding peptides or proteins, enhancing the composite's biosorption selectivity. Engineering bacteria to produce specific cell wall proteins that have a high affinity for target metals could enhance the metal removal efficiency of the composite and make it even more effective in addressing specific contaminants in complex wastewater systems [155].

As the field progresses, biochar and bacterial modifications present a pathway for creating highly selective adsorbents that not only remove metals efficiently but can also be tailored to target specific metals based on the composition of wastewater. Incorporating genetically engineered bacteria, particularly strains that naturally exhibit magnetotactic properties, could further improve the system's effectiveness by creating a targeted approach to metal adsorption. For example, engineered bacterial strains have been investigated for their ability to adsorb precious metals such as gold, suggesting that similar approaches could be used for the selective capture of heavy metals like Hg, Cd, and Pb in wastewater treatment facilities [155].

The future application of these functionalized magnetite-biochar-bacteria composites aligns with principles of the circular economy by promoting waste valorization and resource recovery. Producing biochar from agricultural or industrial waste not only provides a sustainable source of adsorbent material but also reduces waste that would otherwise contribute to landfill mass. After metal adsorption, biochar-based materials could potentially be repurposed as soil amendments, improving soil health and fertility while continuing to immobilize any residual contaminants in a stable, non-bioavailable form. By closing this loop, the functionalized biochar system contributes to resource recovery and reduces environmental impact, supporting broader sustainability goals [149,156].

Regenerating and reusing these composites is another crucial aspect for their application on a large scale. Research shows that magnetite-biochar composites can be regenerated through simple washing or chemical treatments, enabling multiple cycles of heavy metal removal without significant loss in adsorption capacity [156]. This regenerative capability reduces the long-term costs of operating such systems by extending the life of the adsorbent materials, making it an economically viable option for industries looking to adopt sustainable treatment practices. Furthermore, the reduced need for constant replacement of adsorbents also lowers the environmental impact associated with disposal, further supporting the composite's life cycle sustainability [149].

Thus, the future of functionalized magnetite-biochar composites combined with bacterial biomass is highly promising for the field of environmental remediation. The unique properties of this hybrid system—magnetic separation, high adsorption capacity, and biological detoxification—make it well-suited for large-scale wastewater treatment applications. Addressing current limitations, such as stability in variable wastewater conditions and optimizing for high metal concentrations, will be crucial for advancing this technology. Innovations in biochar functionalization and bacterial genetic engineering could further refine the selectivity and efficiency of these composites, transforming them into specialized tools for the targeted removal of specific contaminants. By aligning with the principles of sustainability and resource recovery, this hybrid system not only provides an effective treatment solution but also promotes environmental stewardship by valorizing waste materials and supporting circular economy practices. With continued research and technological refinement, functionalized magnetite-biochar-bacteria composites hold substantial potential for making large-scale wastewater treatment more efficient, adaptable, and environmentally sustainable.

In the last, The approach outlined in this paper supports several Sustainable Development Goals (SDGs) by addressing critical challenges in environmental sustainability and resource efficiency. The development and application of waste-derived nanocomposites directly contribute to SDG 6 (Clean Water and Sanitation) by enabling cost-effective and efficient treatment of AMD, thereby improving water quality and availability. Additionally, by repurposing waste materials, this approach aligns with SDG 12 (Responsible Consumption and Production), promoting waste minimization, recycling, and sustainable resource utilization.

Moreover, the potential to restore AMD-affected ecosystems supports SDG 15 (Life on Land), contributing to the protection, restoration, and sustainable use of terrestrial ecosystems. The economic viability of using waste materials and the accessibility of this technology in resource-constrained regions further align with SDG 9 (Industry, Innovation, and Infrastructure) by fostering innovation and supporting resilient infrastructure development. By reducing environmental hazards associated with AMD and waste accumulation, this approach also indirectly supports SDG 13 (Climate Action)by mitigating ecosystem degradation and reducing pollution impacts.

Future research should focus on integrating advanced characterization techniques, exploring novel waste materials, and conducting comprehensive life cycle assessments to optimize the application of waste-based nanocomposites. Furthermore, partnerships between governments, industries, and academic institutions will be critical to scale up implementation efforts, ensuring that this innovative solution achieves widespread impact in addressing environmental challenges and advancing global sustainability goals.

5. Conclusion

This study explored the potential of functionalized magnetite-biochar combined with live and dead bacteria for the adsorption and biosorption of toxic heavy metals such as Cd, Hg, and Pb from wastewater. The hybrid system demonstrated a high level of efficiency in heavy metal removal, owing to the synergistic action of magnetite-biochar's adsorption capabilities and the biosorptive properties of bacterial biomass. The system offers several advantages, including operational flexibility, environmental sustainability, and potential for regeneration and reuse, making it a viable alternative to conventional heavy metal treatment methods.

However, several limitations must be addressed to enhance the applicability of this technology. These include the stability of the composite under variable environmental conditions such as extreme pH and high metal concentrations, the potential for biochar degradation over prolonged use, and the influence of co-contaminants in real wastewater systems. Additionally, the long-term economic feasibility and scalability of this system require further investigation, particularly regarding the cost of material synthesis and system maintenance.

Future research should focus on improving the structural and chemical stability of magnetite-biochar composites, particularly under acidic and high-temperature conditions. Efforts to functionalize biochar with chelating agents or engineer bacterial strains with specific metal-binding capabilities could improve the selectivity and efficiency of metal removal. Conducting field-scale trials with real wastewater, which typically contains a mixture of contaminants, will provide valuable insights into the composite's performance in practical settings. Comprehensive LCA studies should also be undertaken to evaluate the environmental impact and economic viability of scaling up this technology for industrial applications.

Moreover, advanced and cost-effective methods for regenerating magnetite-biochar composites should be explored to enhance their reuse potential and sustainability. Research into repurposing spent biochar as a soil amendment or in other value-added applications would align with circular economy principles and promote resource recovery. By addressing these limitations and pursuing these recommended research directions, this hybrid system could significantly advance the field of wastewater treatment, providing a sustainable and efficient solution for mitigating heavy metal contamination.

Declaration of Generative AI and AI-assisted Technologies in the Writing Process

During the preparation of this work the author(s) used ChatGPT in order to improve the readability and fix the grammatical errors. After using this tools/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the published article.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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