Science of the Total Environment 886 (2023) 163968

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

The potential of biochar as a microbial carrier for agricultural and environmental applications

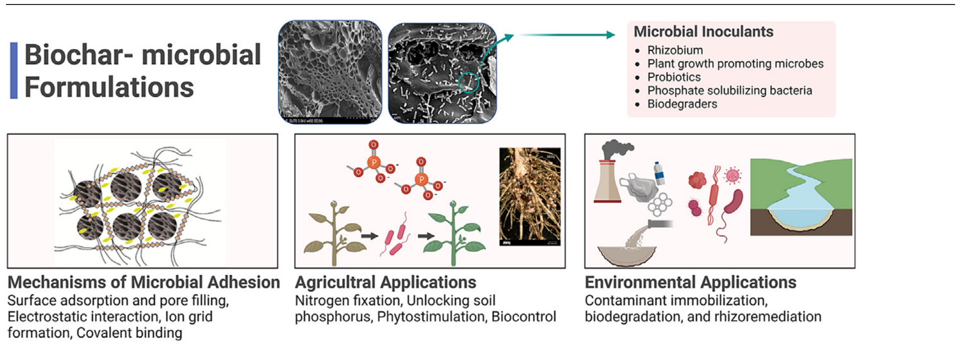
Shiv Bolan [a,b,c], Deyi Hou [d], Liuwei Wang [d], Lauren Hale [e], Dilfuza Egamberdieva [f,g], Priit Tammeorg [h], Rui Li [i], Bing Wang [i,j], Jiaping Xu [k], Ting Wang [k], Hongwen Sun [k], Lokesh P. Padhye [l], Hailong Wang [m], Kadambot H.M. Siddique [b,a], Jörg Rinklebe [n], M.B. Kirkham [o], Nanthi Bolan [a,b,c,*]

[a] *UWA School of Agriculture and Environment, The University of Western Australia, Perth, WA 6009, Australia*
[b] *The UWA Institute of Agriculture, The University of Western Australia, Perth, WA 6009, Australia*
[c] *Healthy Environments and Lives (HEAL) National Research Network, Australia*
[d] *School of Environment, Tsinghua University, Beijing 100084, People's Republic of China*
[e] *USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Avenue, Parlier, CA 93648-9757, United States*
[f] *Institute of Fundamental and Applied Research, National Research University (TIIAME), Tashkent 100000, Uzbekistan*
[g] *Leibniz Centre for Agricultural Landscape Research, Müncheberg, Germany*
[h] *Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland*
[i] *College of Resources and Environmental Engineering, Guizhou University, Guiyang, Guizhou 550025, People's Republic of China*
[j] *Key Laboratory of Karst Georesources and Environment, Ministry of Education, Guiyang, Guizhou 550025, People's Republic of China*
[k] *MOE Key Laboratory of Pollution Processes and Environmental Criteria, College of Environmental Science and Engineering, Nankai University, Tianjin, People's Republic of China*
[l] *Department of Civil and Environmental Engineering, Faculty of Engineering, The University of Auckland, Auckland, 1010, New Zealand*
[m] *Biochar Engineering Technology Research Center of Guangdong Province, School of Environmental and Chemical Engineering, Foshan University, Foshan, Guangdong 528000, People's Republic of China*
[n] *University of Wuppertal, School of Architecture and Civil Engineering, Institute of Foundation Engineering, Water- and Waste-Management, Laboratory of Soil- and Groundwater-Management, Pauluskirchstraße 7, 42285 Wuppertal, Germany*
[o] *Department of Agronomy, Throckmorton Plant Sciences Center, Kansas State University, Manhattan, KS, United States*

HIGHLIGHTS

- Biochar is an ideal microbial carrier due to its properties favoring microbial life.
- Co-location of carbon and nutrients in biochar promotes microbial colonization.
- Biochar-based inoculants enhance plant growth even in hostile environments.
- Biochar-immobilized microbes help in the remediation of contaminated soils.
- Biochar replaces commercially used non-renewable microbial carrier substrates.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Daniel CW Tsang

Keywords:
biochar
Microbial carrier
Agricultural application
Environmental implications

ABSTRACT

Biochar can be an effective carrier for microbial inoculants because of its favourable properties promoting microbial life. In this review, we assess the effectiveness of biochar as a microbial carrier for agricultural and environmental applications. Biochar is enriched with organic carbon, contains nitrogen, phosphorus, and potassium as nutrients, and has a high porosity and moisture-holding capacity. The large number of active hydroxyl, carboxyl, sulfonic acid group, amino, imino, and acylamino hydroxyl and carboxyl functional groups are effective for microbial cell adhesion and proliferation. The use of biochar as a carrier of microbial inoculum has been shown to enhance the persistence,

* Corresponding author at: Healthy Environments and Lives (HEAL) National Research Network, Australia.
E-mail address: nanthi.bolan@uwa.edu.au (N. Bolan).

http://dx.doi.org/10.1016/j.scitotenv.2023.163968
Received 25 February 2023; Received in revised form 6 April 2023; Accepted 2 May 2023
Available online 8 May 2023

Microbial immobilization
Nutrient carrier

survival and colonization of inoculated microbes in soil and plant roots, which play a crucial role in soil biochemical processes, nutrient and carbon cycling, and soil contamination remediation. Moreover, biochar-based microbial inoculants including probiotics effectively promote plant growth and remediate soil contaminated with organic pollutants. These findings suggest that biochar can serve as a promising substitute for non-renewable substrates, such as peat, to formulate and deliver microbial inoculants. The future research directions in relation to improving the carrier material performance and expanding the potential applications of this emerging biochar-based microbial immobilization technology have been proposed.

Contents

1. Introduction

Biochar-based soil amendments are produced through the pyrolysis of various carbon-based biowastes, such as woody biomass, crop residues, animal carcasses, and biosolids (Wu et al., 2021a). During the pyrolysis process, organic matter is broken down into syngas, bio-oil, and biochar, which serves as a stable carbon repository for soil amendment (Woolf et al., 2010; Wang et al., 2021; Liu et al., 2022a, 2022b). However, biochar has evolved beyond its initial purpose and offers multifaceted applications in agriculture and environmental remediation (Bolan et al., 2022a, 2022b). As a soil conditioner, it can improve soil properties, processes, and health, thereby enhancing productivity (Palansooriya et al., 2019; Wang et al., 2020a).

Additionally, as organic feedstocks are required for biochar synthesis, it provides a nutrient source for agricultural and horticultural production (Khadem et al., 2021; Hossain et al., 2021). However, the availability of nutrients in biochar is highly dependent on the feedstock and production process, with negligible available nutrients for most woody feedstocks used for carbon sequestration (Shaheen et al., 2019; Li et al., 2019a; Hossain et al., 2021). Enriched biochar, however, can serve as a nutrient source and microbial carrier for soil application due to its highly porous nature, which increases the carrying capacity of these nutrients and microbes (Ajeng et al., 2020; Khadem et al., 2021). Farmers have also utilized biochar feed supplements to enhance livestock nutrient intake, health, and economic value (Man et al., 2021).

The increase in porosity and thus surface area during pyrolysis, coupled with its affinity for organic and inorganic solutes, also allows for the use of biochar as a potential amendment for the remediation of soil and water contaminated with organic and inorganic contaminants (Lu et al., 2020; Chen et al., 2022). The alternative use of biochar as a highly porous and carbon-rich material provides an option in place of traditional carbon-based catalysts (Yang et al., 2022). This porosity and surface chemistry enables the function of biochar as a carbon sink to help capture and store greenhouse gas (GHG) emissions. Additionally, by modifying the surface properties of biochar, its ability to act as a greenhouse gas sink and catalyst can be enhanced (Lyu et al., 2022).

Several studies have shown that biochar is an effective carrier for microbial inoculants due to its favourable properties that promote microbial life and enable them to resist external environmental changes, which is beneficial for improving their activity in degrading organic contaminants (Zhang et al., 2013; Tu et al., 2020; Zhang et al., 2022). The excellent physicochemical properties of biochar enable the immobilization of bacteria on its surface, leading to the removal of organic contaminants (Zhang et al., 2013; Tu et al., 2020). With its high porosity and specific surface area, biochar can provide living space for microorganisms (Zhang et al., 2022).

Features of biochar that favor microbial habitation include vast amounts of organic carbon, nutrients including N, P, and K, increased porosity, and high water-holding capacity (Li et al., 2019b; Wu et al., 2021b). Microorganisms introduced with biochar allow for increased rates of survival and, thus, improve microbial integration and proliferation in the soil and plant rhizosphere (Azeem et al., 2021). These soil microbes are necessary to improve soil health and mediate carbon and nutrient cycles, soil biochemical processes, and remediation of contaminated soils (Palansooriya et al., 2019). Biochar-based inoculants have been found to be effective in enhancing plant growth and remediation of soil contaminated with organic contaminants (Tu et al., 2020). These studies illustrate the potential of biochar to formulate microbial inoculants. It could contribute successfully as a replacement for other commercially used non-renewable substrates, such as peat, regardless of the inhospitality of the environmental matrix.

Several reviews have focused on applying biochar on a case-by-case basis, including carbon sequestration, soil conditioning for remediation of contaminants, greenhouse gas emissions reduction, and catalytic performance (Bolan et al., 2022a, 2022b; Wu et al., 2021b). However, the majority of these reviews concentrate on the production, characterization, and engineering of biochars, as well as their utilization for specific purposes (Albert et al., 2021; Basak et al., 2022; Gao et al., 2022). The reviewed topics primarily focus on renewable energy and environmental remediation, with applications such as contaminant adsorption, precursors of catalytic functions, and electrochemical energy storage substrates (Cheng et al., 2017; Li et al., 2022a; Qiu et al., 2022). For instance, Wang et al. (2022c) conducted a systematic review of engineered biochars' synthesis, characterization, engineering, and potential value for different environmental applications, including soil remediation, carbon storage, organic waste composting, contaminant removal from water and wastewater sources, as well as their applications as catalysts, activators, and electrode composites.

Despite the increasing use of biochar as a nutrient, moisture, and microbial carrier (Ajeng et al., 2020; Dong et al., 2020; Wong et al., 2022), there is little published information about the application of biochar-based microbial inoculants in agricultural and environmental applications. Although several reviews have covered the environmental application of biochar concerning soil and water remediation and carbon storage (Kookana et al., 2011; He et al., 2017; Novak et al., 2016; Vithanage et al., 2017; Rajapaksha et al., 2016; Bolan et al., 2022a, 2022b; Lu et al., 2020; Qiu et al., 2022), there have been limited reviews on the value of biochar as a microbial carrier (Egamberdieva et al., 2018; Wu et al., 2022; Ajeng et al., 2020; Li et al., 2022a, 2022b). These reviews mainly focused on either the agricultural application (Ajeng et al., 2020; Egamberdieva et al., 2018) or environmental remediation (Wu et al., 2022; Li et al., 2022a, 2022b) of biochar-based microbial inoculants. The present review proposes to fill knowledge gaps about the increasing application of biochar-immobilized microbial inoculants for enhancing nutrient cycling, soil health and remediation of soil contamination. This information is essential for developing guidelines for optimizing feedstock and pyrolyzing conditions for producing biochar suitable to immobilize microbes and the beneficial application of biochar-immobilized microbes for agricultural production and soil remediation. Moreover, improved knowledge about biochar synthesis and modification for utilizing biochar as a microbial carrier will benefit the circular economy of waste management industries in promoting biowastes for biochar synthesis. Future research priorities for sustainable biochar management as a microbial carrier are also proposed.

This review comprehensively examines the effectiveness of biochar as a carrier for microbial inoculum in agricultural and environmental applications. The primary objective is to provide a comprehensive overview of the current understanding of the immobilization processes of microbes on biochars, including the mechanisms and factors involved in the process. Additionally, this review covers the various environmental and agricultural applications of biochar-immobilized microbial inoculants and the potential unintended consequences of such applications. Data from the literature were obtained from databases of Web of Science, Scopus, and Pubmed and screened following the PRISMA guideline. Search terms and a flow diagram of literature screening are provided in SI (Text S1, Fig. S1). Literature were then visualized via VOSviewer (version 1.6.19) (Fig. 1). It was found that the number of publications in this field of biochar as a carrier has grown rapidly in the last 10 years (Fig. 1). Major topics include utilization of microbial-immobilized biochar for nutrient delivery and crop yield improvement, the role of colonized Arbuscular mycorrhizal fungi in agricultural applications, and use of these novel amendments for bioremediation (Fig. 1). A detailed discussion on these topics is provided in the following sections.

2. Synthesis and characteristics of biochar

Biochar is a carbonaceous material derived from thermal conversion under oxygen-limited conditions (Hou, 2021a, 2021b; IBI, 2015). Via heating the biomass feedstock under an optimum temperature (pyrolysis temperature) for a certain time (residence time) with an optimum temperature elevation rate (heating rate), biochar as the solid product can be obtained along with its byproducts, bio-oil, and syngas. Typically, the biochar yield falls within 30 % ~ 60 % (Al-Rumaihi et al., 2022; Elkhalifa et al., 2022). Owing to its sustainable production from a broad array of waste materials, biochar is globally-available and does not need to be mined, two critical drawbacks of the two commercially used carriers, peat and vermiculite (Herrmann and Lesueur, 2013; Schoebitz et al., 2013).

A variety of biomass feedstocks have been reported to fabricate biochar, including crop residues (such as wheat straw and rice husk), wood chips, grass, bone, manure, digestate, and sewage sludge (Alkurdi et al., 2019; Bruun et al., 2022; Li et al., 2022a, 2022b; Wang et al., 2022c). Thermal conversion can also be achieved via different methods, including slow pyrolysis, fast pyrolysis, or hydrothermal carbonization (Wang et al., 2020c). Slow pyrolysis is typically achieved via heating the biomass at 300– 700 °C at a heating rate of 0.1– 10 °C/min; it is the most widely used approach to fabricate biochar (Li et al., 2022a; Manyà, 2012; Wang et al., 2020a). Fast pyrolysis refers to the process where biomass feedstock is heated at a much higher heating rate (over 10 °C/min), thus favoring the generation of bio-oil and syngas instead of the solid product biochar (Butler et al., 2011; Kostetskyy and Broadbelt, 2020). Hydrothermal carbonization refers to the process of thermally converting biomass at 180– 240 °C under subcritical water pressures. Its product is hydrochar, exhibiting distinct physicochemical properties compared to pyrolyzed biomass (Liu et al., 2021; Zhang et al., 2019a; Padhye et al., 2022). For comparison among these feedstocks and thermal conversion methods, readers are referred to Al-Rumaihi et al. (2022) and Kambo and Dutta (2015).

The physicochemical properties of the resulting biochar vary greatly. Firstly, the porous structure of biochar is a key factor determining its performance in environmental applications. A high specific surface area favors physical adsorption of microbes including bacteria via van der Waals interactions (Tran et al., 2017). This adsorption mechanism is rather weak compared to chemisorption; however, it is a nonspecific adsorption mechanism responsible for the adsorption of bacteria and various contaminants on biochar surfaces (Sizmur et al., 2017; Zhang et al., 2020a).

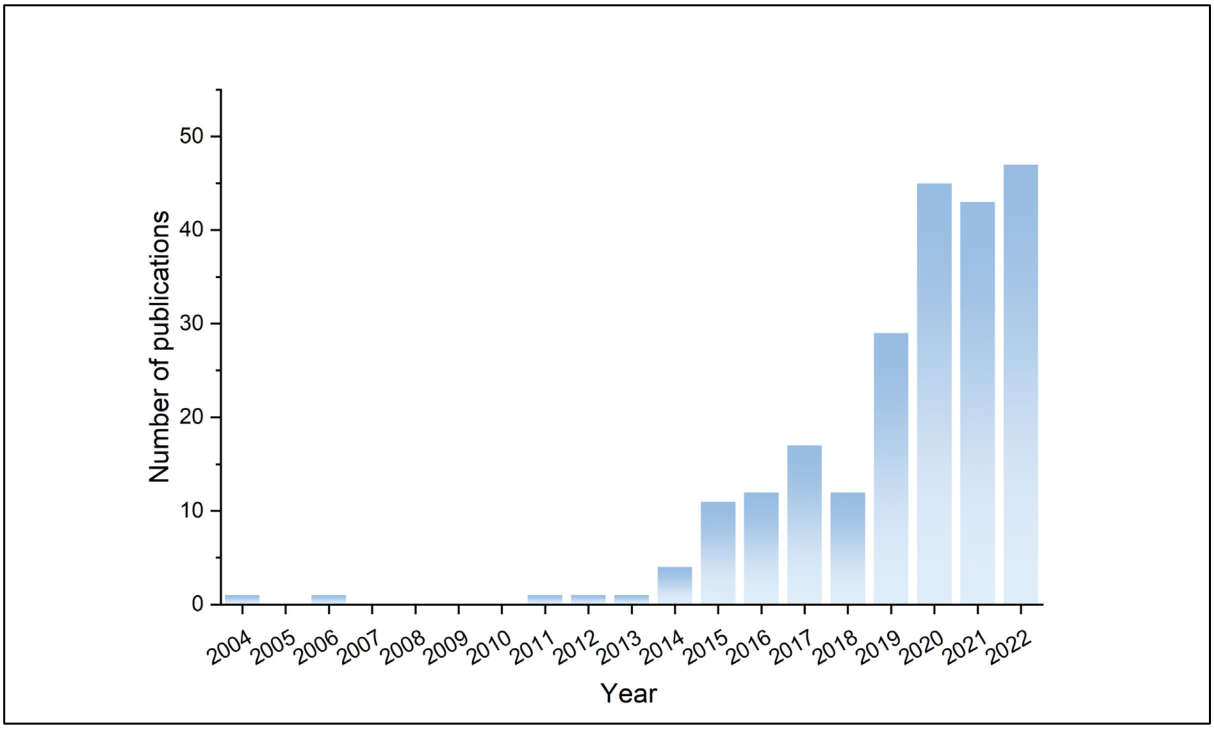
A number of properties including surface area, surface charge, porosity, functional groups and pH of biochar can influence of adsorption of microbes including bacteria by biochar during the microbial immobilization process to produce microbe-enriched biochar products for agricultural and environmental applications. One can take standard biochar produced from a UK biochar research center as an example. Their specific surface areas range from 7.3 to 162.3 mg/g, which are much lower than those of activated carbon (Yang et al., 2022). However, extensive research has demonstrated that biochar materials possessing surface areas within the above range are suitable for a plethora of applications (Atinafu et al., 2020; Dissanayake et al., 2022; Shen et al., 2017b; Vikrant et al., 2020).

Quantitative analysis of surface area values of different biochars has suggested that a plant feedstock, along with a high pyrolysis temperature, produces biochar with the highest surface area (Wang et al., 2020c). It is also noteworthy that biochar is often a micro- or mesoporous material, with the majority of its pores below 50 nm. A quantitative analysis of a biochar's porous structure suggested that the micropore volume of biochar typically ranges from 0.012 to 0.060 $cm^3\ g^{-1}$ with a mean value of 0.024 $cm^3\ g^{-1}$, whereas the mesopore volume ranges from 0.007 to 0.020 $cm^3\ g^{-1}$ with a mean value of 0.009 $cm^3\ g^{-1}$. The percentage of micropores falls within 12.1– 58.0 %, whereas the mesopore figure is 18.9– 31.7 % (Leng et al., 2021). Several attempts have been made to produce hierarchical biochars to improve their porous structures further. A 3D interconnected porous structure with micropores, mesopores, and macropores is the distinct characteristic of hierarchical biochar (Cuong et al., 2021).

Surface charge is another crucial parameter affecting the environmental performance of biochar. In particular, its affinity towards charged surfaces, including living microorganisms, is important. When the pH of soil pore water is below the point of zero charge (pH_{PZC}), biochar carries a positive charge, and vice versa (Mia et al., 2017; Silber et al., 2010). An elevation of pyrolysis temperature leads to higher pH_{PZC}, and the resulting biochar is less negatively charged under the same soil pore water condition (Banik et al., 2018). This is also attributed to the disappearance of negatively-charged functional groups with higher pyrolysis temperatures. Harvey et al. (2012) provided a molecular understanding of biochar surface charge formation using two-dimensional perturbation-based correlation infrared spectroscopy (2D-PCIS). They concluded that the disappearance of O-H···O type hydrogen bonds, hydroxyl oxidation to carboxyl below 500 °C, and the dehydrogenation/dehydroxylation of carboxyl over 500 °C lowered the surface charge negativity of biochars at high temperatures (Harvey et al., 2012). It is noteworthy that under typical soil pH values, biochar surfaces are typically negatively charged (as revealed by negative zeta potential values) (Fang et al., 2014; Yuan and Xu, 2011).

The aromaticity and carbon stability of biochar are crucial factors in determining whether soil microorganisms can readily use biochar (Hou, 2021a, 2021b, 2022; Steinbeiss et al., 2009). Biochar carbon is believed

(a)



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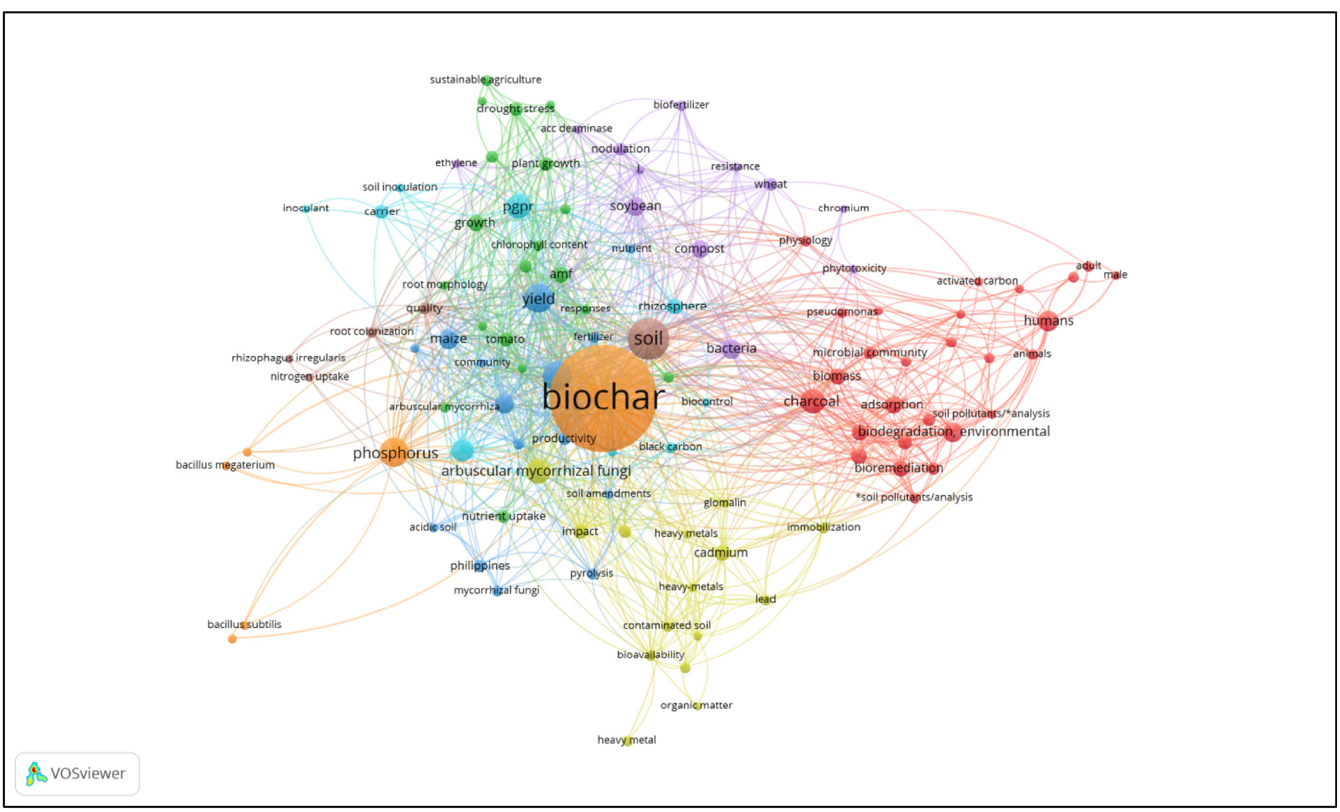

Fig. 1. Systematic literature search results on biochar as a microbial carrier topic. (a) The number of publications related to biochar as a microbial carrier topic in each year following literature screening. (b) Keyword co-occurrence map in this field showing the most frequently investigated topics. The procedure of literature search is provided in Text S1 and Fig. S1.

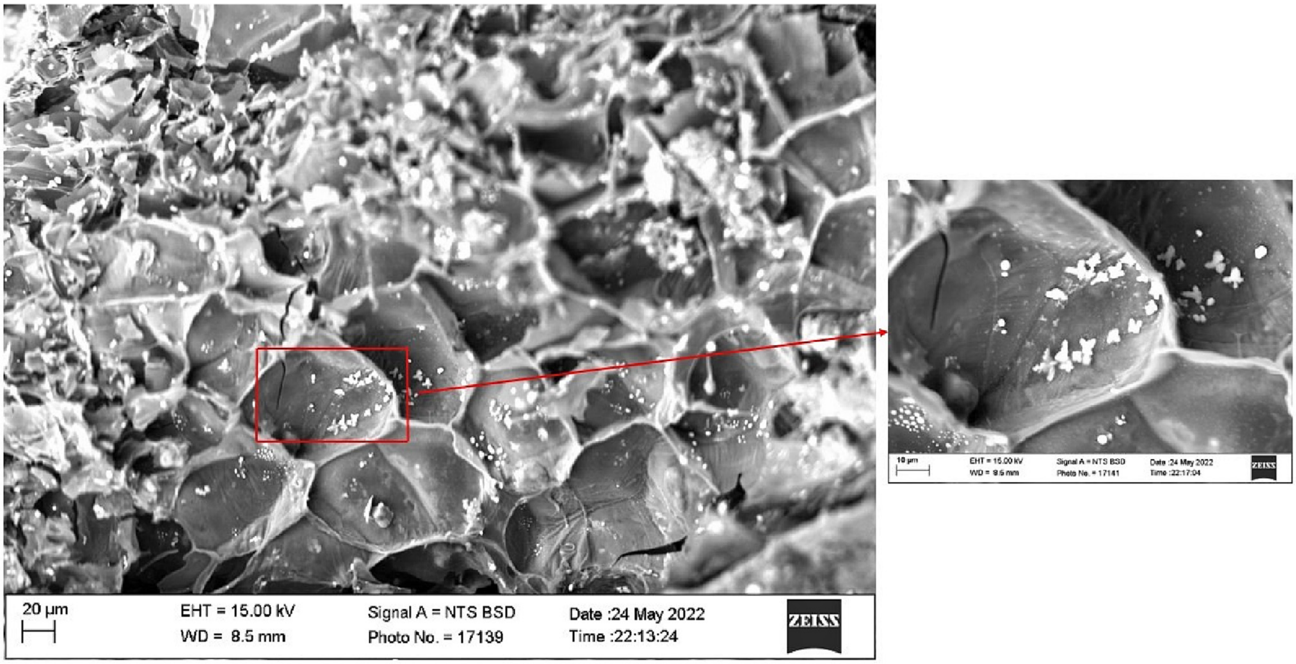

Fig. 2. SEM photomicrograph of bacterial colonization on the surface of biochar.

to be much more recalcitrant than native organic matter in the soil, taking hundreds to thousands of years to reach full mineralization. The recalcitrance of biochar to mineralization is a function of its ultimate properties as well as its pyrolysis temperature. Lehmann et al. (2021) recently suggested that the majority of biochars with $H/C_{org} < 0.5$ exhibited a carbon persistence of over 50 % after 100 years, with the average value being 82 %. Higher pyrolysis temperature generated more aromatic moieties that are much harder for soil microorganisms to metabolize (Keiluweit et al., 2010; Lehmann et al., 2021). Long-term field trials have also shown that biochar may not be a suitable habitat for the colonization of native soil microorganisms (Quilliam et al., 2013a). The higher lignin content of the biomass feedstock results in higher aromaticity and carbon stability (Chen et al., 2022; Jing et al., 2022). Therefore, the half-life of wood biochars is much longer than other biochars (Spokas, 2010).

The abundance of surface functional groups is another important characteristic of biochar, useful for environmental remediation. Various oxygen-containing functional groups, such as hydroxyl, carbonyl, and carboxyl, and nitrogen-containing functional groups, including pyridinic,

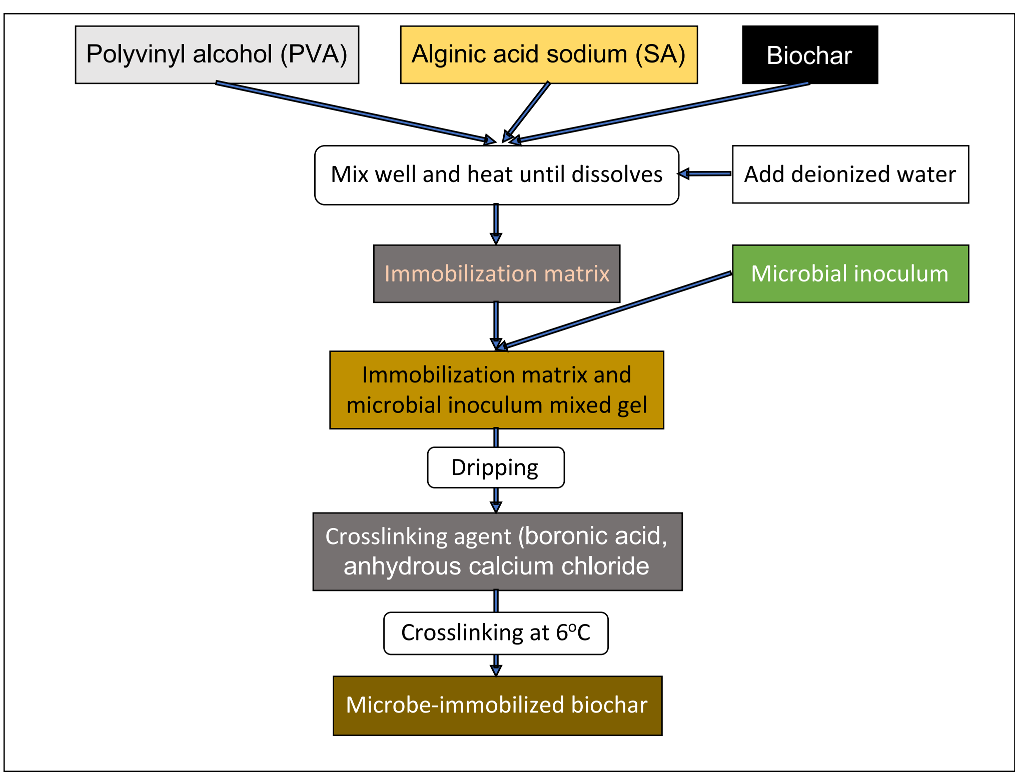

Fig. 3. Protocols for the immobilization of microbes onto biochar (Yang et al., 2020a, 2020b).

pyrrolic, and quaternary moieties play vital roles in the adsorption of microbes during the immobilization process (Başer et al., 2021; Leng et al., 2020; Wu et al., 2022; Wang et al., 2022). Biochar is typically alkaline, which makes it a suitable amendment to restore acid soils and immobilize microbial cells and cationic potentially toxic elements (PTEs) (Hou et al., 2022; O'Connor et al., 2018; Bolan et al., 2023). Ash content is a key factor affecting biochar pH (Wang et al., 2020b). Increasing pyrolysis temperature leads to higher biochar pH because a greater proportion of volatile carbon is burned off, leaving more inorganic components in the solid phase. The surface chemistry of biochar can be altered through physicochemical modification processes to target the adsorption of specific microorganisms during the immobilization processes and contaminants during the remediation process using microbial-immobilized biochar products (Sumaraj et al., 2020).

3. Immobilization of microbes in biochar

3.1. Microbial immobilization processes

Microbial immobilization is a biotechnology in which microorganisms are immobilized in a specific space by different means of immobilization to preserve their inherent microbial activity (Karel et al., 1985) (Fig. 2). Li et al. (2022a, 2022b) summarized microbial immobilization mechanisms and biochar-immobilized microorganisms' environmental application in a recent review. The process of immobilizing microorganisms usually includes attachment on the surface and pores through adsorption, electrostatic interaction, ion grid formation, and covalent binding (Liu et al., 2020; Vasilieva et al., 2021; Zhang et al., 2014). The immobilization of microorganisms by biochar embedding method is achieved by using stabilizers such as calcium alginate and ferric alginate to produce microbe-immobilized biochar beads for agricultural and environmental applications (Zhao et al., 2021; Zheng et al., 2021). Yang et al. (2020a, 2020b) provided a detailed protocols for the synthesis of microbe-immobilized biochar beads using calcium alginate and polyvinyl alcohol in the presence of crosslinking agents (Fig. 3). The immobilization processes of biochar immobilized microorganisms and their applications in agriculture and environmental remediation are presented in Fig. 4.

Surface adsorption and pore filling are based on the interaction of nonspecific forces (such as adhesion) between biochar and microbial surface functional groups (such as -OH and -COOH) (Wu et al., 2022). Microorganisms tend to adhere to carrier materials by producing adhesion substances (known as extracellular polymers), which allow them to grow and spread throughout the surface and inner pores of the carrier material. (Li et al., 2022a, 2022b). Chen et al. (2021) used biochar as a carrier for the immobilization of *Bacillus cereus* WHX-1 to remediate Cr(VI) contaminated soil. Analysis by SEM and FTIR indicated that the rough and porous surface of biochar provided active sites for microorganism inhabitation. Direct contact of a contaminant with a microbe can cause a loss of microbial activity. Maintaining microbial activity is an important issue that needs to be addressed.

Electrostatic interaction is one of the common processes involved in the immobilization of microbes by biochar. In general, microbial surfaces are negatively charged, and using surface positively charged carrier materials helps accelerate the immobilization process. Shen et al. (2017a) investigated the remediation of Cd by biochar-immobilized microalgae and found that microorganisms were immobilized on the biochar surface by electrostatic action. For example, biochar produced at low temperature tends to contain more abundant O- and N-containing functional groups, can be more effective in immobilizing microorganisms and metal(loid)s (Başer et al., 2021). Nevertheless, there are obstacles related to unstable immobilization and low tolerance of microorganisms. Subsequent research should prioritize enhancing microbial resilience to the harmful effects of contaminants at high concentrations. (Li et al., 2022a, 2022b).

The embedding of microorganisms in fine grids of a polymeric carrier is termed as ion grid formation. The grid structure can inhibit microorganisms from being exuded from the carriers, while the external environment's small molecule substrates and components can readily enter and exit the carriers (Lu et al., 2020; Wu et al., 2022). This process promotes the growth and proliferation of microorganisms, increasing their tolerance to contaminants and enhancing their ability to degrade them (Wu et al., 2022). However, only small molecule substrates have free access to the particle's interior, which is unsuitable for the degradation of macromolecular contaminants. Ion grid formation also causes high mass transfer resistance and hinders the electron transfer of substrates. Thus, it is ideal for reaction systems where both substrate and product are composed of small molecules (Jiang et al., 2022).

Covalent bonding is the use of chemical covalent bonds formed between functional groups on microbial cells and chemical groups (sulfhydryl, amino, hydroxyl, etc.) on the surface of a carrier to immobilize cells (Ahmad and Khare, 2018). The process is characterized by strong binding and adequate stability. Thus, the binding of microbial cells with the carrier substrates such as biochar through functional groups becomes a bottleneck problem in the process of immobilization of microbial cells by biochar, and the persistence of immobilized microbial cells, and the stability of the biochar-based microbial immobilization products for agricultural and environmental applications (Wu et al., 2022). There are relatively few relevant

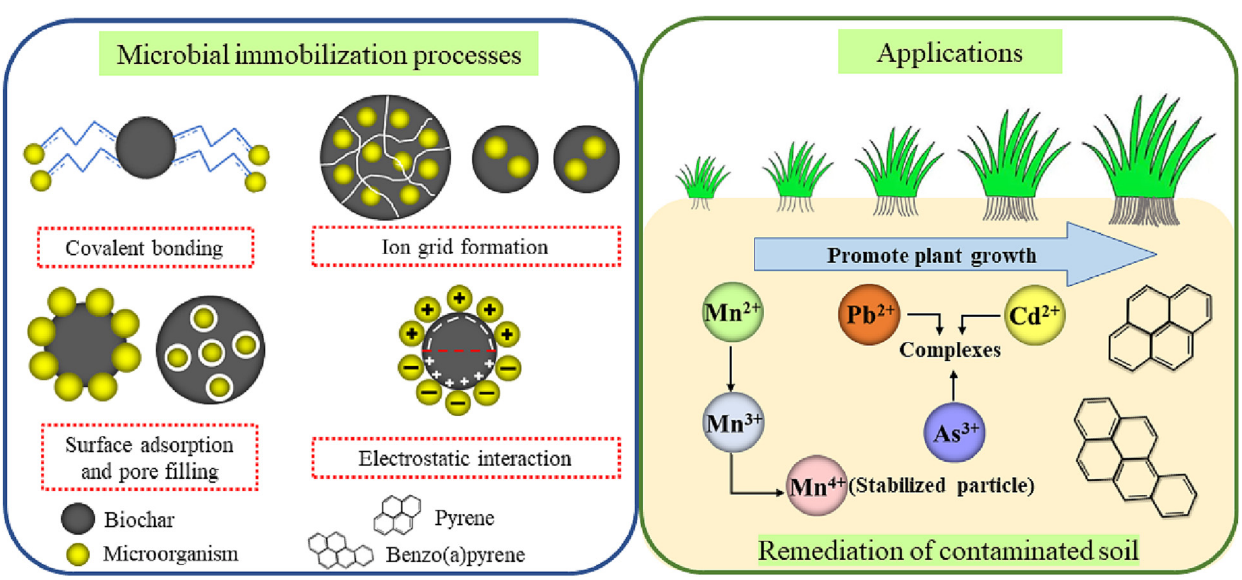

Fig. 4. Immobilization processes of biochar immobilized microorganisms and their applications in agriculture and environmental remediation.

studies on this aspect. For example, Bianco et al. (2022) immobilized phenanthrene (PHE) degrading microbes using biochar involving covalent bonding and achieved 96 % degradation of PHE through coupling of desorption of PHE from marine sediments and biodegradation of the sediment washing solution in a novel biochar immobilized–cell reactor, In the future, more research can be conducted in this area.

Some studies have used composites (biochar-microorganism), with immobilization carried out by gel embedding or surface adsorption, to remove acenaphthene from wastewater (Lu et al., 2021; Lu et al., 2018). Both immobilization methods showed different removal effects (embedding: 50.60 %; adsorption: 38 %). The study results indicated that compared to adsorption (pH = 4.5–10.5), the embedding method maintains higher effectiveness over a wide pH range (pH = 2.5–10.5). In contrast to the above results, Yang et al. (2020a, 2020b) found that surface adsorption was superior to gel embedding in the bioremediation of contaminated soils. Ha et al. (2022) compared the effects of covalent bonding and surface adsorption on the degradation of paraquat. The results indicated that the removal percentage of the former (90.61 %) was greater than that of the latter (82.05 %). Based on the above results, it can be said that different microbial immobilization methods significantly impact the removal of contaminants. Therefore, immobilization methods should be carefully chosen based on the target contaminants after evaluating the advantages and disadvantages of different methods.

3.2. Factors affecting immobilization

Many internal and external factors affect the process of immobilizing microorganisms with biochar. The internal factors include nutrients and the properties of the biochar, while the external factors include the environmental pH and the initial concentration of contaminants (Wu et al., 2022). These influencing factors are essential to determine biochar-immobilized microorganisms' contaminant removal and degradation efficiency.

The properties of biochar play a critical role in the interaction between carbon and bacteria during immobilization. The pore structure and specific surface area of biochar create a suitable habitat for microorganisms (Zhao et al., 2020). The properties of biochar, including specific surface area and pore size and distribution, usually depend on the pyrolysis temperature and biomass type. Das et al. (2021) studied various properties of biochar from different raw materials at three different carbonization temperatures (400, 500, and 600 °C). Their results showed that all studied biochars' pore diameter and specific surface area were optimum at 600 °C. Biochar produced by high-temperature pyrolysis had more aromatic carbon ring layers and higher stability (Das et al., 2021), which was more conducive to microbial enrichment. Nutrient conditions of biochar also largely depend on its pyrolysis temperature and biomass type (Hossain et al., 2021). However, the nutrient contents (e.g., nitrogen and sulphur) and functional groups can be lost at high temperature (>600 °C), and hence low-temperature biochar is recommended to preserve nutrients (Tomczyk et al., 2020). The ash of biochar can provide inorganic nutrients to microorganisms and is a major factor driving microbial metabolic stability. Biochars produced from grass materials, and manure usually have a higher ash content and provide more nutrients than wood biochars (Adnan et al., 2015). Xu and Chen (2013) found a positive correlation between the ash content and pyrolysis temperature of crop straw biochar and manure biochar. In addition, another benefit of biochar providing nutrients to microorganisms may be that it can promote microbial functions critical for nutrient cycling. For example, biochar can increase the abundance of rhizosphere bacteria capable of converting organic S and P into bioavailable forms, thereby promoting ryegrass growth (Fox et al., 2014).

Environmental pH plays an important role in immobilization by influencing cell viability and charges on the surface of biochar (Wu et al., 2022). Microorganisms are susceptible to environmental pH variations, and under field conditions, the optimal pH for their reproduction and viability ranges from 6 to 8 (Zhang et al., 2022). The pH value can also affect the charge on the biochar surface and thus affect the immobilization process. It was found that increasing pH (when initial pH < 7) enhanced the adsorption of cationic PTEs and basic dyes by biochar-microbial composites and decreased the removal of anionic PTEs and acid dyes (Hu et al., 2019; Huang et al., 2020a, 2020b). Similar results were also found in the study of Wang et al. (2021b), which utilized magnetic biochar-microbial composites for the remediation of Cd(II) and As(III). The results showed that the composites' Cd(II) removal percentage was low at pH = 3, while it increased when the pH increased from 4 to 7. Also, As(III) removal percentage increased from 18 % to 61 % by increasing pH from 2 to 5. The environmental pH mainly affects the adsorption efficiency and contaminant removal capacity by affecting the activity of microorganisms and the point charge on the surface of microorganisms. Therefore, it is necessary to consider the effects of environmental pH on the immobilization process.

The initial concentration of the contaminant is an important influencing factor. High contaminant concentration affects microbial activity and causes microbial death (Wahla et al., 2020). Zhao et al. (2020) immobilized bacterial strains on biochar by gel embedding to remove phenol. According to the findings, biodegradation was considerably hindered by high initial phenol concentrations (>400 mg/L), and the bacteria failed to survive when the phenol concentration surpassed 1000 mg/L. However, including biochar increased phenol degradation from 46 % to 99 % at a concentration of 600 mg/L. The results indicated that adding biochar in the immobilization process could enhance the toxicity resistance of microorganisms and improve the contaminant removal efficiency of biochar-immobilized microorganisms.

The effectiveness of biochar-immobilized microbial technology in practical applications is limited due to complex and variable environmental conditions. More consideration should be given to external factors affecting the immobilization process, such as competition with indigenous microorganisms, which are critical in advancing the efficient application of biochar-immobilized microorganisms. Efficient contaminant removal is of great significance when using biochar-immobilized microorganisms.

Multiple properties of biochar materials are important for their success as inoculum carriers. For example, biochars typically have large quantities of pores, suitable for microbial colonization and their protection from predators such as protozoan, nematodes, and mites. Those can also retain or adsorb nutrients essential for microbial growth (Wildman and Derbyshire, 1991; Brewer et al., 2014; Das and Ghosh, 2021; Labanya et al., 2022). Such pores also provide active sites on biochar surfaces which can sorb contaminants allowing simultaneous adsorption and biodegradation (Ha et al., 2022; Wu et al., 2022).

The pyrolysis of organic residues to produce biochar results in a sterile product, providing a solid support on which inoculated organisms do not compete with established microbial populations. Also, biochar pH is often neutral to slightly alkaline and can confer some pH buffering capacity (Shi et al., 2018; Shi et al., 2019), providing a habitat suitable for many soil microorganisms, which tend to be most active at neutral pH (Rousk et al., 2010). In a comparison of multiple biochar materials, According to Hale et al. (2015), the survival rate (i.e., shelf life) of plant-growth-promoting bacteria (PGPR) after inoculation was correlated with the chemical properties of biochar, such as its nitrogen content and pH, while biochar's physical properties (e.g., porosity and pore opening diameters) were better predictors of inoculum survival after soil incorporation (Hale et al., 2015). This was further supported by the work of Vanek et al. (2016), who evaluated *Rhizobium* inoculated onto biochar. Pore size distribution, plant-derived ash nutrients, and volatile organic matter influenced inoculum survival in storage and under drying conditions.

Microorganisms have been shown to colonize tubular biochar pores and surfaces (Tao et al., 2018). Pore structure and distribution have been consistently documented as important carrier properties. Cell immobilization onto biochar may be achieved by adsorption (e.g., by mixing liquid cultures promoting surface tension and adhesion interactions between functional groups of microbial and biochar surfaces (Wu et al., 2022)), covalent bonding, leveraging the functional groups onto microbial surfaces to form covalent bonds with groups on carrier surfaces (Ha et al., 2022); or embedding/encapsulation via sodium alginate or agar (Jiang et al., 2022; Wang et al., 2022). Carrier colonization may be further facilitated via the formation of extracellular polysaccharide (EPS) matrices (e.g., biofilm), by inoculating organisms on biochar surfaces (Hale et al., 2014; Bertola et al., 2019; Tu et al., 2020). Mechanistically, EPS not only protects microorganisms from shear stress, desiccation, toxic compounds, and protozoan grazing, but it also enhances extracellular enzyme retention/ efficacy and facilitates nutrient transport (Sauer et al., 2022a, 2022b; Sooriyakumar et al., 2022). On biochar surfaces, microbial EPS has been associated with increased extracellular enzymatic activity and facilitation of enhanced electron transport (Sathishkumar et al., 2020).

3.3. Modification of biochar for enhanced microbial immobilization

Modified biochar improves the adsorption capacity of contaminants by improving electronic shuttle ability and specific surface area and increasing oxygen-containing functional groups, which are conducive to the immobilization of microorganisms (Jiang et al., 2022). Currently, biochar modification methods applied to immobilization technology mainly include layered double hydroxides (Zheng et al., 2021), H_2O_2 (Youngwilai et al., 2020), and Fe_3O_4 modification (Wang et al., 2021b). Table 1 summarizes the removal effects of immobilized microorganisms from modified biochars under different influencing factors. Zheng et al. (2021) utilized layered double hydroxide-modified biochar to immobilize *Acinetobacter* sp. FYF8, which exhibits excellent denitrification and phosphorus removal capabilities in neutral conditions. It should be noted that strong acids and bases can impact the functional activity of microorganisms. The study results demonstrated an 86.11 % efficiency in phosphorus removal and a maximum denitrification efficiency of 95.32 %. Similarly, Teng et al. (2020) immobilized *Leclecia adecarboxylata*, a phosphorus lytic bacterium, to prepare composites with nano zero-valent iron biochar. This composite facilitated the passivation effect of Pb(II). Nano zero-valent iron biochar can rapidly reduce the toxic effects of Pb(II) on microorganisms and reduce the redox potential of soils, thus enhancing phosphate release. Insoluble lead phosphate compounds are formed by phosphorus-dissolving bacteria, thereby enhancing the stability of Pb(II). Studies have proposed using immobilized microorganisms in magnetic biochar gel beads to remove PAHs with high molecular weight (Qiao et al., 2020). Magnetic biochar gel beads solve most of the problems of poor buoyancy and difficult collection of carriers. Compared with single strains, immobilizing microorganisms by magnetic biochar gel beads, which have a high removal capacity and good buoyancy and magnetic properties, and which can also be collected by an external magnetic field, is a promising method for removing PAHs after an oil spill.

Youngwilai et al. (2020) immobilized the manganese-oxidizing bacterium *Streptomyces violarus* SBP1 using H_2O_2-modified biochar for the adsorption and biotransformation of Mn(II). The modified biochar increased Mn(II) adsorption and cell attachment sites. Microbial cells converted Mn (II) to Mn(III) through a biological oxidation process, and Mn(III) was further converted to Mn(IV) (stable particles). The Mn(II) removal rate reached 74.80 %. This study demonstrated the feasibility of using biochar-immobilized cells to remediate Mn(II). Wang et al. (2021b) achieved efficient removal of cadmium (Cd) and arsenic (As) using a novel composite synthesized using Fe_3O_4-modified biochar with *Bacillus* sp. K1. The addition of *Bacillus* sp. K1 increased Cd(II) and As(III) adsorption sites (amines, carboxylates, etc.). Fe_3O_4-modified biochar can protect *Bacillus* sp. K1 from PTE stress. The composite of Fe_3O_4-modified biochar with microorganisms can also be easily separated by a magnetic field to mitigate secondary pollution, making it a highly promising adsorbent for clean and sustainable environmental remediation.

When different modified biochars are compared, the microbial immobilization technology based on magnetic nanomaterials is regarded as a promising approach. It can enhance the adsorption sites, physicochemical characteristics, and mechanical stability of the material by adjusting the morphology and composition of the material, thus facilitating effective microbial immobilization (Wang et al., 2021a). Li et al. (2022a, 2022b) showed that biochar mixed with metal or non-metal materials as carrier substrates could enhance electron mobility and increase the adsorption of contaminants. Therefore, modified biochar can provide more adsorption sites for contaminants and microorganisms and improve the toxicity tolerance of microorganisms. In summary, modified biochar improves the contaminant removal capacity of composites and maintains the activity of microorganisms. Although modified biochar is effective, it poses problems of high cost and complicated preparation.

4. Agricultural application of microbial-immobilized biochar

Although carrier materials are important for the protection of introduced strains or consortia in the agricultural context, it is also critical that, once introduced into an environment, an inoculum can colonize host tissue (e.g., roots or root surfaces). Several studies have demonstrated that once immobilized in biochar pores and on surfaces, inoculated microorganisms did not show reduced capacity to colonize plant host roots (Douds Jr et al., 2014; Hale et al., 2014), nor did biochar impede root colonization by native soil microorganisms (Solaiman et al., 2010) or when co-applied with inoculum (Liu et al., 2018; Hashem et al., 2019). Once introduced into soils, plant-beneficial traits of plant growth-promoting bacteria, mycorrhizal fungi, endophytes, and biocontrol agents are numerous and varied. Many microbial inoculants possess nutrient-acquisition strategies, competitive mechanisms, and phytohormone interactions that concomitantly promote plant health while benefiting their own growth and reproduction. There are many reviews on plant-growth-promoting microbial traits and their functionalities in agriculture (Bashan, 1998; Compant et al., 2010; Ramakrishna et al., 2019). This area has received attention

Table 1
Removal effects of differently modified biochars used as microbial carriers on pollutants in soil and the environment.

Immobilization processes	Biochar raw materials	Pyrolysis temperature (°C)	Modifiers	Microorganisms	Pollutants	Optimal pH	Initial concentrations of pollutants	Removal rates (%)	References
Gel embedding	Orange peel	/	Layered double hydroxide	Acinetobacter sp. FYF8	Nitrate Phosphate	7.0	5 mg/L 3 mg/L	95.32 86.11	(Zheng et al., 2021)
Gel embedding	Rice straw	500	Fe_3O_4	Bacillus sp. K1	Cd(II) As(III)	7.0	50 mg/L 20 mg/L	87.00 61.00	(Wang et al., 2021b)
Gel embedding	Rice hull	/	$FeSO_4 \cdot 7H_2O$	*Leclercia adecarboxylata*	Pb(II)	5.0	1 mM	93.00	(Teng et al., 2020)
Gel embedding	*E. prolifera*	500	Fe_3O_4	Pseudomonas sp., Thalassospira sp., Shewanella sp. and Alcanivorax sp.	Pyrene	8.0	20 mg/L	91.90	(Qiao et al., 2020)
				Halomonas sp. and Shewanella sp.	Benzo(*a*) pyrene		10 mg/L	69.20	
				Thalassospira sp., Joostella sp., and Pseudomonas sp.	Indeno (1,2,3 *cd*) pyrene		10 mg/L	77.30	
Surface adsorption and pore filling	Eucalyptus leaves	800	H_2O_2	*Streptomyces violarus*	Mn(II)	/	3 mg/L	74.80	(Youngwilai et al., 2020)

from researchers for decades. However, modern advances in "omics" tools, as well as needs for sustainable alternatives to heavy agrochemical inputs, have prompted additional research on the subject and development of biologicals by agrochemical manufacturers (Xu and Geelen, 2018). This has led to a body of literature wherein researchers have evaluated biochar-microbial formulations for agricultural purposes.

Many soil organisms contribute to plant nutrient acquisition, such as nitrogen (N) via symbiotic and free-living N-fixation, phosphorus (P) through its solubilization by the production of small organic acids or phytase enzymes, iron via the production of siderophores, and/or by the direct colonization of roots and uptake of nutrients as is the case with arbuscular mycorrhizal (AM) fungi (Egamberdieva et al., 2019a, 2019b). A nitrogen-fixing bacterial inoculum is one of the most common, commercially-available biological amendments, often applied as seed coats, and it has been inoculated onto biochar to serve as a soil amendment or seed coating. In comparison to perlite, pine bark biochar enhanced the shelf life of an N-fixing *Rhizobium* strain, whereas sludge biochar did not, but nodulation of pigeon pea was not enhanced using the *Rhizobium*-pine bark biochar formulation (Araujo et al., 2020). A biochar produced from rice prolonged *Rhizobium* survival in storage, and similar formulations enhanced nodule weight, plant weight, and plant height of kidney beans (Ghazi, 2017). Seven of 13 biochar materials evaluated promoted *Rhizobium* sp. survival at least as well as peat under moist storage conditions. Survival was further improved when biochar was supplemented with montmorillonite prior to pyrolysis to increase macropores of size <0.3 μm (Vanek et al., 2016).

Bradyrhizobium and maize silage hydrochar formulations had high inoculum survival compared to pyrolyzed wood and enhanced lupin growth and N and P uptake (Egamberdieva et al., 2017). Two of five biochars evaluated enhanced *Bradyrhizobium* shelf life, and the best performer, softwood biochar, was shown to increase soybean nodulation in the absence of chemical fertilizer (Głodowska et al., 2017). It is known that abiotic stress factors inhibit nodule formation in legumes, disturbing symbiotic association of legumes with rhizobia. In such conditions, biochar as a source of nutrients supports the abundance of bacteria, protects bacteria against desiccation, and maintains their survival in the rhizosphere which is a critical issue for positive effects on plant growth under extreme conditions (Pietikainen et al., 2000). A free-living, N-fixing *Azotobacter* strain applied as a single species inoculum or in consortia with a P-solubilizing *Bacillus* sp. and *Tricoderma* fungi showed prolonged shelf life on a rice husk biochar compared to biochar prepared from coconut shell or palm fruit residues (Maftuah et al., 2020). Acacia wood biochar sourced from the UK, but not India, promoted survival in storage for up to six months with high population densities of another free-living N-fixer, a strain of *Azosprillum* (Kuppusamy et al., 2011).

Multiple studies have also evaluated biochar-microbe formulations to deliver P solubilizing bacteria (PSB) into soils. A PSB *Pseudomonas* sp. inoculated onto hardwood biochar as a seed coat treatment enhanced corn biomass, whereas softwood biochar stimulated earlier germination (Głodowska et al., 2016). Of the four biochar materials evaluated in the *Pseudomonas* bioformulation, three improved inoculum shelf life beyond that determined for peatmoss (Głodowska et al., 2016). A P solubilizing consortium of three bacteria and one fungus maintained high population densities in the storage on oil palm, coconut shell, corncob, and rice husk biochars, with the coconut biochar retaining the highest viable populations after six months of storage (Husna et al., 2019). Tea leaf biochar prepared at 600 °C, but not 350 °C, enhanced the shelf life of a PSB *Bacillus* sp. and improved mung bean nodule number and yields over inoculated peat (Azeem et al., 2021). Zheng et al. (2019) developed biochar-formulated phosphate solubilizing bacteria (PSB) and studied its effect on plant growth and P uptake in rape. They observed a positive correlation between the abundance of the phosphate solubilizing bacteria community, plant biomass, P concentration in rhizosphere soil and P content in plant tissue. The available-P content of the biochar maintained the proliferation and abundance of PSB and supported their survival.

Arbuscular mycorrhizal fungi grown within pelletized switchgrass biochar effectively colonized bahia grass (*Paspalum notatum*) roots (Douds Jr et al., 2014). While not all biochar materials performed similarly, biochar formulations with N-fixing bacteria, PSB, or AM fungal strains or consortia have been widely recognized as promising for use in agriculture.

Beneficial microbes for soil application can also be classified as phytostimulants or rhizosphere probiotics or PGPR, and various mechanisms have been proposed for the effects on improving soil health and plant productivity (Vassileva et al., 2020; de Souza Vandenberghe et al., 2017). Some direct probiotics' action mechanisms include biological nitrogen fixation, release of nutrients such as nitrogen, phosphorous, sulphur and iron, and plant hormone production. Indirectly, these microorganisms can release biomolecules such as antibiotics, enzymes, and antimicrobial and pathogen-inhibiting volatile compounds, which can help to mitigate abiotic and biotic stresses impacting plants growth. For example, bacteria can produce plant growth hormones (e.g., auxins, gibberellins, and cytokinins) or impede the accumulation of a stunting hormone, ethylene, via 1-aminocyclopropane-1 carboxylic acid (ACC) deaminase activity, which degrades its precursor. A few phyto-stimulant strains have also been evaluated in biochar formulations. Their involvement in root growth and development can benefit agricultural productivity when challenged by abiotic stressors, such as drought and PTEs, or salinity (Vessey, 2003; Yang et al., 2009). Pinewood biochar promoted inoculum survival of an auxin-producing strain of Enterobacter in soil and enhanced cucumber growth, irrespective of inoculum addition (Hale et al., 2014). Many studies show the positive effects of biochar application on *microbial diversity and activity* in soil (Hardy and Knight, 2021; Zhao et al., 2022). Root-associated bacterial diversity exhibiting plant growth-promoting activities was higher in biochar- amended soil than untreated soil (Egamberdieva et al., 2017). There results indicate that improved plant growth, and development is an indirect effect that relates to microbial activities in soil and in the rhizosphere.

Biochar from agricultural wastes pyrolyzed at 600 °C prolonged the survival of auxin-producing strains of *Burkholderia* and *Bacillus* in storage, and the bioformulations promoted tomato biomass and yield (Kumar et al., 2017). A pinewood biochar formulation with a strain of *Pseudomonas,* with ACC deaminase activity, maintained high inoculum populations. It enhanced maize growth in salinized soil, effects which were independent of biochar nutrient supplementation (Sun et al., 2016). Importantly, the pinewood biochar did not impede the ACC deaminase activity of the *Pseudomonas* sp. (Hale et al., 2015). Plant-induced stress from PTEs can also be combated via microbial immobilization or uptake. When combined with sludge or rice husk biochar, a PSB *Enterobacter* strain removed lead (Pb) from a medium. It contributed to its stabilization on biochar surfaces, a process that can, in turn, support crop development in Pb-contaminated soils (Chen et al., 2019). Provided that biochar can sorb contaminants that may be detrimental to plant development or enhance soil water status and bulk density, formulations of phyto-stimulants and biochar materials may be especially promising for agricultural regions challenged by abiotic stressors.

Additionally, introduced organisms can promote plant disease resilience by producing antibiotic or antifungal compounds, competitively displacing pathogens via resource acquisition, or by stimulating plant immune responses. Biochar from sawdust and peat moss improved the survival of a consortium of three *Fusarium* wilt biocontrol bacterial strains, also reducing disease incidence and enhancing tomato yield (Elhadidy, 2019). Cotton straw biochar prepared at 400 °C or 600 °C showed greater benefits to pepper growth when inoculated with a *Bacillus* strain with biocontrol potential than when either amendment was incorporated independently (Tao et al., 2019). Overall, in most reports, biochar has been demonstrated to either promote the survival or shelf life of the inoculum or to enhance the benefits of biochar on plant growth properties (e.g., root length, shoot height, yield). Table 2 shows that biochar-microbial bioformulations have been assayed for bacterial and fungal species and consortia that encompass a range of plant beneficial characteristics.

5. The utilization of microbe-immobilized biochar for environmental purposes

Biochar is considered an effective microbial carrier through adsorption, embedding, and electrochemical immobilization of microbes (Cheng et al.,

Table 2
Examples of biochar-microbial bioformulations evaluated in agricultural contexts.

Crop-beneficial function	Microbial inoculants	Synergistic impact	Reference
Plant growth regulation			
ACC deaminase	Pseudomonas sp.	Pinewood biochar- *Pseudomonas* sp. formulation did not impede or enhance ACC deaminase activity (Hale et al., 2015). Pinewood biochar-*Pseudomonas* sp. formulations maintained high population densities and enhanced maize growth in salinized soil, effects which were independent of biochar nutrient supplementation (Sun et al., 2016)	(Hale et al., 2015; Sun et al., 2016)
Auxin production	Enterobacter sp.; Burkholderia sp.; Bacillus sp	Pinewood biochar promoted inoculum survival of *Enterobacter* sp. in soil and enhanced cucumber growth, irrespective of inoculum addition (Hale et al., 2014). Biochar from agricultural wastes pyrolyzed at 600 °C prolonged the survival of a *Burkholderia* sp. and a *Bacillus* sp. in storage, and the bioformulations promoted tomato biomass and yield (Kumar et al., 2017)	(Hale et al., 2014; Kumar et al., 2017)
Nutrient acquisition			
Nitrogen fixation	Rhizobium spp.; Bradyrhizobium spp.; Azotobacter sp.; Azospirillum sp.	In comparison to perlite, pine bark biochar enhanced the shelf life of *Rhizobium* sp., and sewage sludge biochar did not. *Rhizobium* nodulation of pigeon peas was not enhanced when pine park biochar was used as a carrier (Araujo et al., 2020) Rice biochar enhanced *Rhizobium* survival in storage, and these formulations enhanced nodule weight, plant weight and plant height of kidney beans (Ghazi, 2017). Seven of 13 biochar materials evaluated promoted *Rhizobium* sp. survival as well as peat under moist storage conditions. When biochar was supplemented with montmorillonite prior to pyrolysis with the goal of increasing macropores of size <0.3 μm, the benefits to inoculum survival were much greater (Vanek et al., 2016). *Bradyrhizobium* and maize silage hydrochar (BR-HTC) formulations had high inoculum survival compared to pyrolyzed wood and maize chars. The HTC-BR formulation enhanced lupin growth, N, and P uptake (Egamberdieva et al., 2017). Two of five biochars evaluated enhanced *Bradyrhizobium* shelf life. The best performer, softwood biochar, was shown to increase soybean nodulation in the absence of chemical fertilizer (Głodowska et al., 2017). An N-fixing *Azotobacter* sp. applied as a single species inocula or in consortia with a P-solubilizing *Bacillus* sp. and *Trichoderma* fungi showed prolonged shelf life on a rice husk biochar compared to biochar prepared from coconut shell or palm fruit residues (Maftuah et al., 2020) Acacia wood biochar sourced from the UK, but not India, promoted *Azospirillum* survival in storage for up to 6 months and high population densities (Kuppusamy et al., 2011)	(Kuppusamy et al., 2011; Vanek et al., 2016; Egamberdieva et al., 2017; Ghazi, 2017; Głodowska et al., 2017; Araujo et al., 2020; Maftuah et al., 2020) (Azeem et al., 2021)
Inorganic phosphorus solubilization	Pseudomonas spp.; Paenibacillus sp.; Burkholderia sp.; Acinetobacter sp.; Penicillium sp.; Bacillus sp.	A *Pseudomonas* sp. inoculated onto hardwood biochar as a seed coat treatment enhanced corn biomass, whereas softwood biochar stimulated earlier germination. Of the four biochar materials evaluated, three improved inoculum shelf life beyond that determined for peat moss (Głodowska et al., 2016). A P solubilizing consortia of three bacteria and one fungus maintained high population densities in the storage on oil palm, coconut shell, corncob, and rice husk biochars. Coconut biochar had the highest viable populations after six months of storage (Husna et al., 2019) Tea leaf biochar prepared at 600 °C but not 350 °C enhanced *Bacillus* shelf life and improved mung bean nodule number and yields over inoculated peat (Azeem et al., 2021)	(Głodowska et al., 2016, Husna et al., 2019, Maftuah et al., 2020, Azeem et al., 2021)
Arbuscular mycorrhizal fungi	Funneliformis sp.,; Glomus sp.; Rhizophagus sp.	AM fungi grown within pelletized switchgrass biochar colonized bahiagrass roots (Douds Jr et al., 2014)	(Douds Jr et al., 2014)
Biocontrol	Bacillus spp.; Brevibacillus sp.,; Stenotrophomonas sp.	Biochar from sawdust and peat moss improved the survival of a consortium of three *Fusarium* wilt biocontrol bacterial strains, also reducing disease incidence and enhancing tomato yield (Elhadidy, 2019) Cotton straw biochar prepared at 400 or 600 °C showed greater benefits to pepper growth when inoculated with a *Bacillus* strain with biocontrol potential than when either amendment was incorporated independently (Tao et al., 2019)	(Elhadidy, 2019; Tao et al., 2019)
Heavy metal tolerance	Enterobacter sp.	When combined with sludge biochar and rice husk biochar, P solubilizing *Enterobacter* strain removed lead from the medium (Liu et al., 2018; Chen et al., 2019)	(Liu et al., 2018; Chen et al., 2019)

2020; Li et al., 2022a, 2022b; Teng et al., 2020; Wang et al., 2020c). Biochar can provide habitat and nutrients for microorganisms, favoring the growth and colonization of microorganisms. The growth and reproduction of microorganisms can further promote the removal efficiency of contaminants (Chen et al., 2019; Li et al., 2022a, 2022b; Wahla et al., 2020; Zheng et al., 2022). Thus, microbial-immobilized biochar is increasingly being used for the removal of various contaminants in industrial and domestic wastewater, soil, and air through biosorption or/and biodegradation process (Li et al., 2022a, 2022b; Wu et al., 2021a, 2021b; Zheng et al., 2022) (Table 3).

Up to now, wastewater and soil are the two primary environmental media that have been frequently investigated (Bolan et al., 2022a, 2022b; Deng et al., 2022; Gong et al., 2022; Li et al., 2022a, 2022b; Wu et al., 2021a, 2021b; Zheng et al., 2022). The investigated contaminants include organic contaminants and their intermediate metabolites, PTEs (metals, metalloids, and radionuclides), and inorganic salts and nutrients (Deng et al., 2022; Li et al., 2022a, 2022b; Wu et al., 2021a, 2021b; Zhang et al., 2021). Commonly investigated organic contaminants include phenols (Lou et al., 2019; Wang et al., 2021; Wang et al., 2020b), pesticides/fungicides (Sun et al., 2020; Wahla et al., 2020), dyes (Abu Talha et al., 2018; Bharti et al., 2019), estrogens (Dai et al., 2019), pharmaceuticals (Sun et al., 2020), antibiotics (Zhang and Wang, 2021; Zhang et al., 2022), antibiotic resistance genes (Cheng et al., 2020; Zhang et al., 2022), polycyclic aromatic hydrocarbons and their derivatives (Kong et al., 2018; Liang et al., 2021; Piscitelli et al., 2019), polybrominated biphenyl ethers (Guo et al., 2022), and petroleum hydrocarbons (Zhang et al., 2019a, 2019b). The PTEs include Cu(II) (Huang et al., 2020a, 2020b), Cd(II) (Shen et al., 2019), Pb(II) (Chen et al., 2019; Teng et al., 2020), As(V) (Simiele et al., 2021), Mn(II) (Deng et al., 2022), and others (Zhang et al., 2022).

Table 3
Examples of biochar-immobilized microorganisms in environmental applications.

Environmental applications	Microbial inoculants	Synergistic impact	References
Heavy-metal removal	Enterobacter sp.; *Leclercia adecarboxylata*; *Bacillus subtilis* (B38); Chlorella sp.; Acinetobacter sp. AL-6; *Stenotrophomonas maltophilia*	When coupled with alkaline and slightly acidic biochar derived from rice husk and sludge, respectively, the microbe can remove Pb^{2+} by 24.11 % and 60.85 % from water (Chen et al., 2019).	Chen et al., 2019
		The immobilized *Leclercia adecarboxylata* by rice hull biochar/nZVI shows excellent remediation efficiency, and the highest removal rate of Pb^{2+} in beef peptone liquid medium could reach 93 % (Teng et al., 2020).	Teng et al., 2020
		Two kinds of biochar pyrolyzed at 350 °C, one derived from corn straw and one from pig manure, were used as the carriers of bacterium (B38, a mutant genotype from *Bacillus subtilis* using UV irradiation) to immobilize heavy metals (Cd, Cr, Hg, and Pb) in solution and contaminated soil (Wang et al., 2018, 2017).	Wang et al., 2018; 2017
		The feasibility of the bioremediation of Cd using microalgal-water hyacinth biochar immobilized complex was achieved (Shen et al., 2017a, 2017b).	Shen et al., 2017a
		The capacity for Mn^{2+} remediation by grapefruit peel biochar and strain AL-6 was checked in the sequencing batch bioreactor and soil column (Deng et al., 2022).	Deng et al., 2022
		Biochar derived from different parts of dried corn (root, stalk, leaf, and cob) was used to immobilize microorganism cells to remove Cu^{2+} in a water solution, and the corn stalk biochar (pyrolyzed at 250 °C) had the strongest Cu^{2+} removal ability (Huang et al., 2020a, 2020b).	Huang et al., 2020a, 2020b
	Herbaspirillum huttiense	The chaff-based biochar exhibited high immobilization of Cu (85.5 %) and Zn (64.4 %) and great removals of oxytetracycline (41.9 %) and enrofloxacin (40.7 %) in contaminated soil (Zhang et al., 2020a, 2020b).	Zhang et al., 2022
Organic pollutant removal	*Brevibacillus parabrevis*; *Alcaligenes faecalis*	Compared with those without immobilization, better bioremediation of Congo red dye was achieved by the immobilized batch and continuous packed bed bioreactor of *Brevibacillus parabrevis* using coconut shell biochar (Abu Talha et al., 2018). The *Casuarina* seed-biochar with *Alcaligenes faecalis* immobilization has excellent removal capability of methylene blue dye (Bharti et al., 2019).	Abu Talha et al., 2018; Bharti et al., 2019
	Shewanella oneidensis MR-1; Nonylphenol degrading bacteria; Microorganism mixture	The reed straw biochar enhanced the removal of 17 β estradiol by *S. oneidensis* MR-1 (Dai et al., 2019). The cells immobilized on bamboo charcoal and wood charcoal biochar achieved effective removal of nonylphenol, and bamboo charcoal biochar showed better performance (Lou et al., 2019). The peanut shell biochar, coupled with the microorganisms, exhibited the best sorption and degradation ability of 2,4-dichlorophenol in the soil bio-reactive layer (Wang et al., 2020a, 2020b, 2020c).	Dai et al., 2019; Lou et al., 2019
	Phenanthrene-degrading bacteria; Petroleum-degrading bacteria	Fe-rich sludge biochar with bacteria immobilization (removal efficiency: 58.15 ± 4.90 %) facilitated phenanthrene biodegradation in soil compared with that of free bacteria treatment (removal efficiency: 38.73 ± 3.98 %) (Liang et al., 2021). The immobilized microorganisms on mushroom biochar (pyrolyzed at 550 °C) accelerate the biodegradation of petroleum in petroleum hydrocarbon-contaminated soil (Zhang et al., 2019a).	Liang et al., 2021; Zhang et al., 2019a
	Bacillus cereus LZ01;	Chlortetracycline in water can be removed by co-effects of microbial degradation and surface adsorption biochar acquired from forsythia, erding, and honeysuckle (Zhang et al., 2021).	Zhang et al., 2021
	Alcaligenes faecalis WZ-2; bacterial consortium MB3R	The straw biochar-immobilized WZ-2 accelerated the degradation of tebuconazole in soil with a reduced half-life of tebuconazole (Sun et al., 2020). Augmentation with MB3R immobilized-rice husk biochar in potato vegetated soil achieved a degradation rate of 96 % for metribuzin herbicide as compared to only 29.3 % in un-augmented soil (Wahla et al., 2020).	Sun et al., 2020; Wahla et al., 2020
Inorganic salts removal	*Pseudomonas mendocina* GL6; Acinetobacter sp. AL-6 Acinetobacter sp. FYF8	Immobilized *P. mendocina* GL6 on bamboo biochar showed higher nitrate removal than bacteria-free biochar (Zhang et al., 2021). Grapefruit peel biochar and strain AL-6 remediate NH^{4+}-N in sequencing batch bioreactor and soil column (Deng et al., 2022). A novel double-layered hydroxide-orange peel biochar/sodium alginate composite act as a carrier for *Acinetobacter* sp. FYF8 and improve the removal of nitrogen and phosphorus in the bioreactor (Zheng et al., 2021).	Deng et al., 2022; Zhang et al., 2021; Zheng et al., 2021

Water bodies are common media for the application of microbial-immobilized biochar. Wu et al. (2022) have provided a detailed overview on the application of microbe-immobilized biochar in removing contaminants from water and soil environments. Microbial biomass can be used as an effective biosorption strategy for contaminant removal from an aqueous environment. However, one of the limitations restricting the direct application of microbial biomass for contaminant removal comes from the post-adsorption process, where there can be difficulties in separating the microbial biomass from the contaminant media for the safe disposal of contaminant-enriched spent microbial biomass. Hence, it is important to create optimal carrier matrices for the application of biosorption so that the microbe-immobilized carrier matrices such as biochar can be readily separated from the contaminant media for safe disposal and reuse of spent carrier (Baskar et al., 2022).

Although polymeric materials have been used as carrier matrices, there are specific issues regarding the practical application of polymeric materials. Those include their potential toxicity to microorganisms, short service life, high cost, the requirement for recycling, and environmental risks (Alhakawati et al., 2003; Kim et al., 2014; Yakup Arıca et al., 2003). Biochar is a suitable alternative because of its cost-effectiveness and environmentally friendly nature. Plentiful pores with suitable size distribution create ideal conditions for microorganisms. They provide structural protection from hostile organisms and desiccation and allow access to carbon (C), energy, and mineral nutrients (Saito and Marumoto, 2002; Warnock et al., 2007). Varied and functionally differentiated microbial communities have been detected and isolated in biochar pores (Jaafar et al., 2015; Theis and Rillig, 2009). Thus, biochar can act as a functional carrier for microorganisms.

Liu et al. (2021) observed that using different biochar substrates could result in varying structures and properties of the final product. Biochar produced from plant residues typically contains lower amounts of inorganic ash (Brewer et al., 2014; Spokas et al., 2011) and reduced levels of macro- and micro-nutrients (Glaser and Birk, 2012). Conversely, biochar derived from livestock manures tends to have higher ash content (Cao et al., 2011a; Zhang et al., 2013), leading to enrichment with primary nutrients compared to plant-derived biochar (Sarkhot et al., 2012).

In a study by Wang et al. (2018), two types of biochar were used as carriers for a mutant genotype bacterium, B38 (source: *Bacillus subtilis*), to adsorb PTEs in an aqueous medium. The biochars, derived from corn straw (CBC) and pig manure (PBC), exhibited significant differences in surface structural characteristics, as shown in SEM photographs (Fig. 5). Both biochars were effective carriers for microorganisms to form co-sorbents, contributing to the removal of PTEs. The study also found that the co-sorbents had an advantage in removing mixtures of PTEs, and the contributions of biochars and microorganisms varied for different PTEs.

In soil, microbial bioaugmentation is a minimally invasive technique. It is, therefore, a feasible option for mitigating the hazardous effects of contaminants on microorganisms without impacting soil properties and interrupting agricultural activities during the soil remediation period (Ledin et al., 1999; Udeigwe et al., 2011). However, one of the limitations of the bioaugmentation process is that the contaminated soils are often deficient in biologically essential nutrients and, hence, may not be able to promote the rapid growth of the bioaugmented microbes (Reddy et al., 2003). Biochar can release nutrients to provide carbon and energy sources for microbial growth (Jaiswal et al., 2017; Lehmann et al., 2011). Furthermore, as a porous material, biochar can provide a good habitat for microorganisms (Wang et al., 2018). Hence, biochar is a potential micro-environment for soil microorganisms (Jaafar et al., 2014).

The relative ratio of carbonized and non-carbonized fractions of biochar depends on the pyrolysis conditions, including temperature, which determines the characteristics and behavior of biochar (Cao et al., 2011b). Generally, a lower pyrolysis temperature stabilizes the PTEs and releases P, K, Ca, and other plant nutrients into the soil (Han et al., 2022). Two types of biochars pyrolyzed at 350 °C (CBC and PBC) were tested as carriers of B38 to promote bioaugmentation efficiency by Wang et al. (2017). The surface area of PBC was almost 2-fold higher than that of CBC (Wang et al., 2017). Thus, the surface of pig-manure-derived biochar contained more adsorption sites, thereby providing greater adhesion space for B38 growth and reproduction. Wang et al. (2017) reported that PBC had an ash content almost three times higher than that of CBC and had greater concentrations of nutrient elements compared to CBC. Therefore, PBC has great potential to provide abundant nutrients for B38 growth to utilize its functional activity. The polymerase-chain-reaction denaturing-gradient-gel-electrophoresis (PCR-DGGE) profiles provided evidence that PBC could promote the growth and proliferation of both the exotic B38 bacteria and native microbes (Wang et al., 2017). Moreover, after treatment, Cd and Pb concentrations in lettuce plants were measured and found to be less than the standard threshold levels of China (Wang et al., 2017). In addition, the concurrent application of B38 and PBC promoted plant growth (37.9 % increment of plant biomass) (Wang et al., 2017). Thus, the low-pyrolysis-temperature pig-manure-derived biochar was a good carrier for B38, and co-amendment with B38 displayed a synergistic effect on the immobilization of PTEs in the soil.

6. Economic feasibility and unintended consequences of the application of microbial-immobilized biochar

Evaluation of the economic feasibility of biochar-microorganism composites is a prerequisite and key for their use in commercial applications (Singh and Srivastava, 2022). At present, many studies are focused on the cost-effectiveness analysis of biochar. For example, Sakhiya et al. (2022) evaluated the preparation cost of rice straw biochar from the perspectives of raw material collection, adsorbent production, and indirect cost. It is reported that rice straw biochar has certain application potential in commercial areas. In addition, Alhashimi and Aktas (2017) compared the economic performance of biochar and activated carbon, and the results show that biochar has the same effect as activated carbon, but the cost of biochar is lower.

Currently, there are relatively few studies on cost analysis of biochar-microorganism composites at present, and only some studies were evaluated from the perspective of the reaction process and economic benefits. Through literature research, it was found that Bianco et al. (2022) conducted an economic evaluation of the process of removing phenanthrene from sediment by biochar-immobilized cells. The study showed that parts of the pollutants are constantly degraded by microorganisms, thus reducing the regeneration cost and improving the economic feasibility of composites, the cost of the whole process is 346.2 €/ton (Bianco et al., 2022). On the other hand, Ijaz et al. (2019) studied the effect on wheat growth when biochar and plant growth-promoting rhizosphere bacteria (PGPR) were applied simultaneously. The potential economic benefits (such as production costs and revenues) of growing wheat were also explored. This study showed high environmental benefits for the combined application of biochar and PGPR. Reducing the fertilizer dose by 50 % under conditions that did not affect yield and net benefit, and the net benefit (per ha) was approximately $400. However, Hussain et al. (2016) found that the net benefit of wheat (per ha) could only reach about $260 and the fertilizer dose was only decreased by 38 % when PGPR is applied alone. Although the simultaneous application of biochar and microorganisms increases input costs, their gains in economic and environmental benefits are concomitantly high.

In summary, there are currently limited studies on the systematic evaluation of the economics of biochar-microorganism composites. Although the presence of microorganisms improves the reusability of biochar-microorganism composites, it is still necessary to analyze their input costs to make them more widely used in the commercial field in future studies.

Although microbial-immobilized biochar has shown great potential in the various aforementioned environmental applications, several unintended consequences should be considered.

Firstly, certain types of biochar may possess toxic substances, leading to toxicity to soil microorganisms, and they may cause secondary pollution following their use as soil amendments. In this case, inoculated biochars also face the same problem. For instance, considering that PTEs (except for Hg) cannot be burnt off during pyrolysis, biochars made from

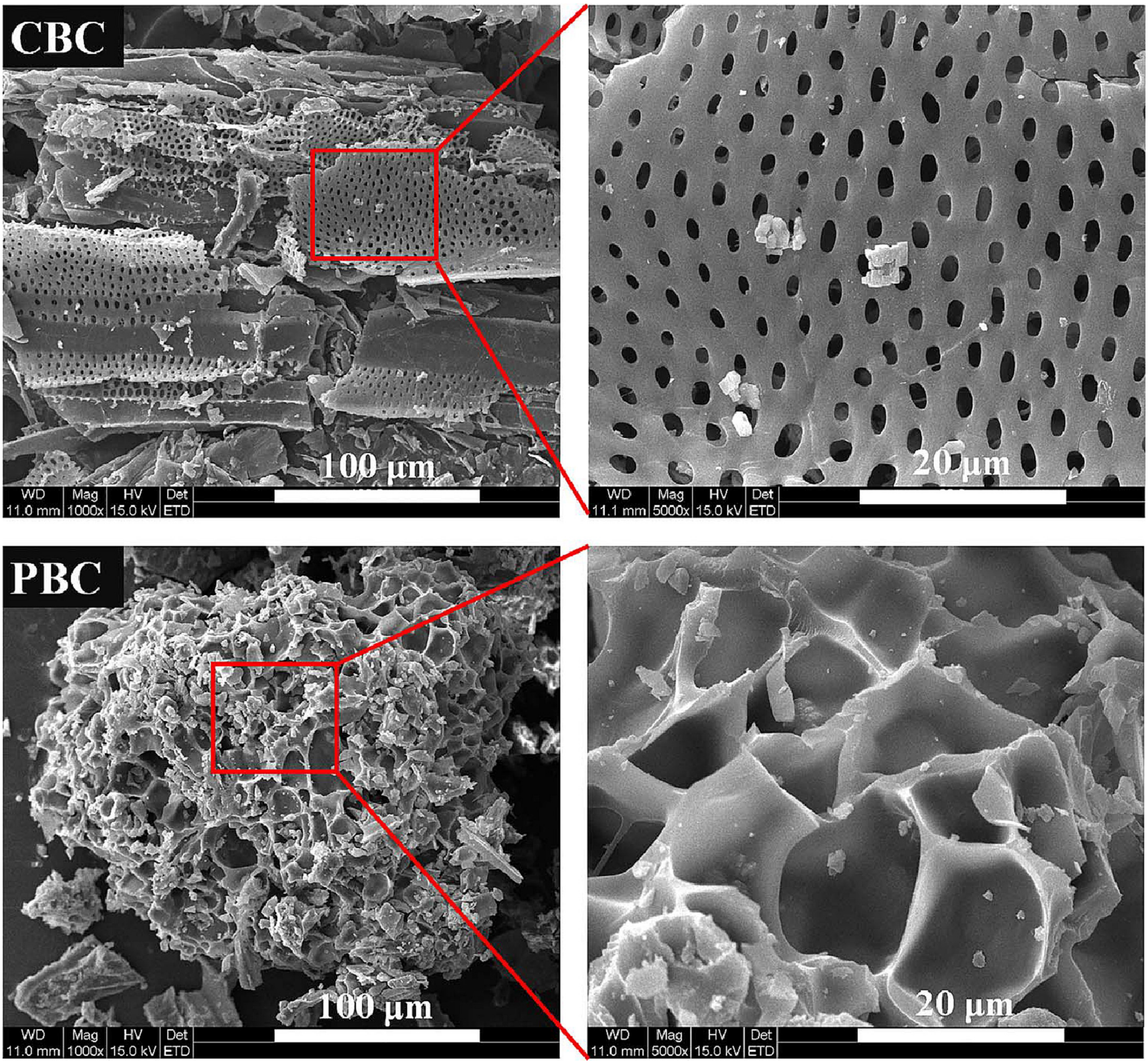

Fig. 5. SEM photography of corn straw biochar (CBC) and pig manure biochar (PBC) (Wang et al., 2018).

contaminated biomass may contain considerable amounts of PTEs (Agrafioti et al., 2013; Van Wesenbeeck et al., 2014). In this context, special care must be taken when using sewage sludge as biomass feedstock. For instance, Lu et al. (2016) found that sewage sludge biochar was enriched with PTEs and metalloids, including Pb, Zn, Cu, Cr, Ni, and As, whose concentration even exceeded that of the sludge feedstock. However, one should note that the labile capacity of these elements decreases substantially following pyrolysis. Jin et al. (2016) suggested that the geochemical fractions of PTEs in sludge biochars were mainly less labile forms, including the oxidizable and residual forms, as compared with the labile ones. Other examples of metal-enriched biomass feedstock include PTE-contaminated crop residues (Bian et al., 2018; Shen et al., 2019) or hyperaccumulators harvested from phytoextraction practices (Cui et al., 2018; Wang et al., 2022d).

Biochar toxicity can also come from biomass pyrolysis, which may generate considerable amounts of polycyclic aromatic hydrocarbons (PAHs). PAH concentration in biochars can vary greatly from 0.07 mg/kg to 100 mg/kg (Han et al., 2022). Slow pyrolysis is much safer as compared with fast pyrolysis in terms of PAH generation (Fig. 6) (Hale et al., 2012). Typically biochars fabricated at a relatively low pyrolysis temperature (below 500 °C) are enriched with low molecular weight PAHs with 2– 3 rings, whereas high molecular weight PAHs with 5– 6 rings are generated in high temperature (above 500 °C) biochars (Han et al., 2022). Whether biochar serves as a sink or a source of soil PAHs is a question with no definite answer yet. A 3-year field study suggested that soil enriched with unmodified biochar had significantly higher concentrations of PAHs (1.95 mg/kg) as compared with the unamended soil (1.13 mg/kg) because biochar itself acted as a sorbent for PAH retention in the soil and that biochar amendment decreased PAH catabolism (Quilliam et al., 2013b). By contrast, other studies using microbial-immobilized biochar have shown that inoculated microorganisms accounted for the successful degradation of soil PAHs. For instance, inoculation of the PAH-degrading bacteria *Pseudomonas putida* (Chen et al., 2012), *Pseudomonas aeruginosa* (Lu et al.,

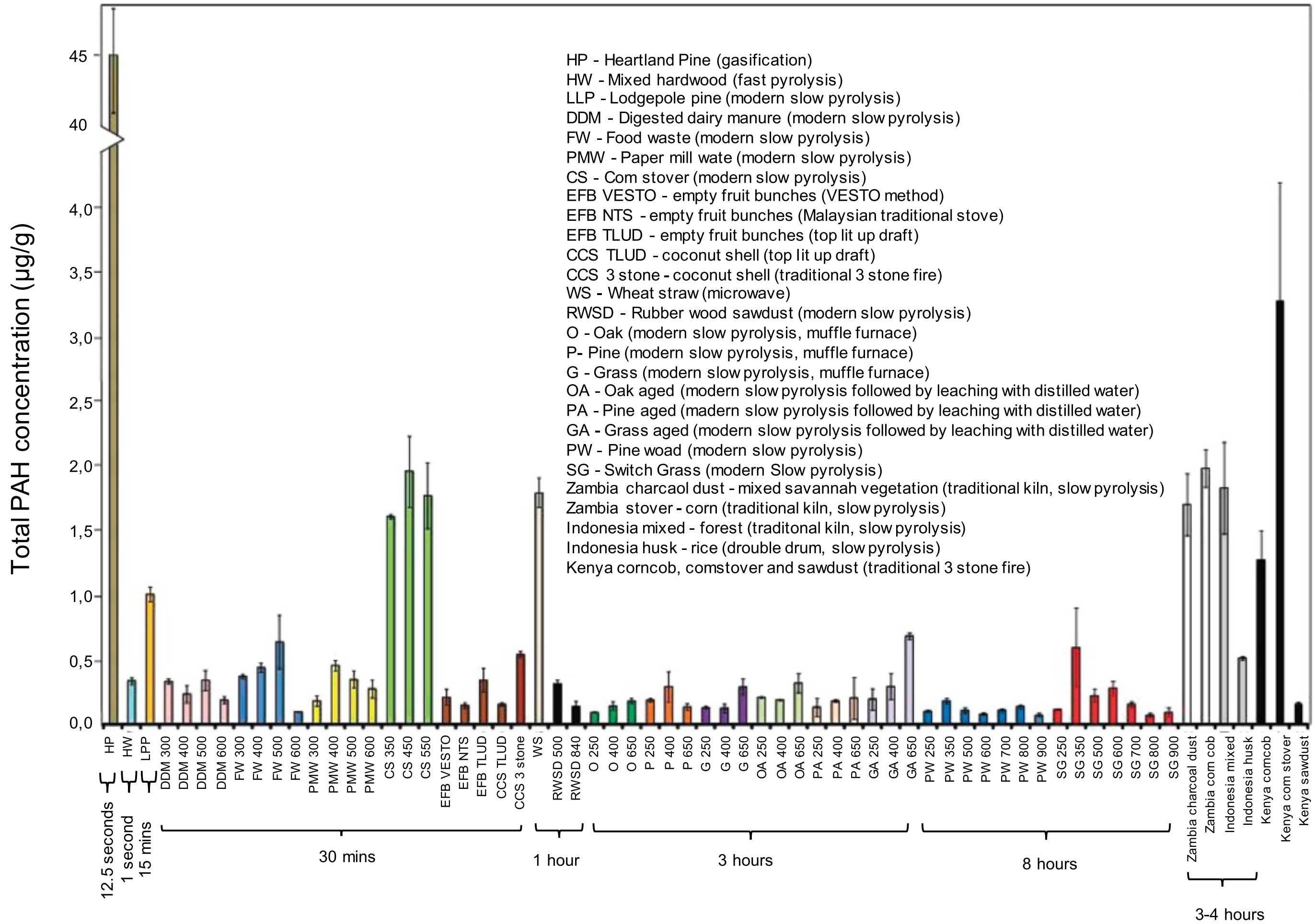

Fig. 6. Total PAH concentration (µg/g) in different biochars. Bars are grouped by color according to the biochar source material and country of production. Reproduced with permission from Hale et al. (2012). Copyright 2012 ACS Publications.

2021), *Bacillus* sp. (Wang et al., 2019a), and *Sphingomonas* sp. (Song et al., 2021) onto a biochar carrier results in successful degradation of PAHs.

Secondly, the introduction of external microbes may cause disturbance to the native microbial community, which has been largely neglected in previous attempts where microbial-immobilized biochar has been applied to the soil. The disturbance may lie either in the biochar host or the immobilized strains. In the former case, a decrease in the fungi-to-bacteria ratio has been reported to occur in certain biochar-amended soils (Brtnicky et al., 2021). For instance, in a field trial conducted in an acid paddy soil in Southwest China, a normal wheat straw biochar application rate at 40 t/ha led to a significant increase in bacteria 16S rRNA gene copy numbers (by 64 %) along with a significant decrease in fungi 18S rRNA gene copy numbers (by 46 %) (Chen et al., 2013). Considering that a fungi-dominated microbial community is more favourable for carbon stabilization as compared with a bacteria-dominated community, it is likely that soil carbon loss would be stimulated in this case (Chen et al., 2013; Six et al., 2006).

Han et al. (2016) reported that switchgrass biochar negatively affected arbuscular mycorrhizal fungi colonization, which was possible because the salty conditions this high ash biochar created were not favourable for fungal growth. Exogenous bacteria directly affect the community structure of native microorganisms. Hg-volatilization bacteria *Pseudomonas* sp. DC-B1 and *Bacillus* sp. DC-B2 immobilized onto pine sawdust biochar were reported to alter the soil bacterial community slightly, but the ecological consequences remained to be explored (Chang et al., 2019). Another study found that the application of a *Bacillus* sp. K1 inoculated magnetic biochar to a Cd-contaminated rice paddy resulted in a slight decrease in indigenous microbial alpha diversity, providing direct evidence of competition between exogenous bacteria with soil native microbes (Wang et al., 2021b).

Finally, the long-term behavior of microbial-immobilized biochar remains uncertain. Biochar undergoes an aging process following soil amendment, which may lead to unpredictable consequences, such as physical fragmentation, ash dissolution, and (photo)oxidation, all of which will affect microbe-biochar interactions in the long term (Jin et al., 2021; Mia et al., 2017; Wang et al., 2022a; Wang et al., 2020b). As previously mentioned, weak interactions, such as electrostatic interactions, may play a vital role in microbial immobilization within the biochar matrix. With progressive aging, this weak interaction will likely diminish because the surface of biochar would become more negative due to the formation of more oxygen-containing functional groups (Mia et al., 2017; Wang et al., 2019b). Whether immobilized strains would remain active following detachment from the porous biochar matrix should be investigated. Besides, labile carbon may be preferentially metabolized within a few months of biochar amendment, leaving recalcitrant aromatic moieties that microorganisms can hardly use (Rasul et al., 2022; Wang et al., 2022b; Zhong et al., 2020). Whether biochar would remain effective at supporting the growth and the performance of immobilized microbes also remains unclear. Therefore, more field trials should be conducted to test the long-term performance of microbial-immobilized biochars.

7. Summary and conclusions

The present review provides an overview concerning the interactions between microorganisms and biochar as a carrier and the agricultural and environmental effects after applying biochar-immobilized microorganisms. Ion grid formation and electrostatic interaction have been shown to be the primary mechanisms for immobilizing microorganisms by biochars due to the high surface area, porosity, surface charge, and stability of biochars.

There have been increasing research interests in using biochar as an alternative microbial carrier to replace commercially available non-renewable microbial carrier substrates such as peat. As noted, biochar is an effective carrier due to its surface properties that favor microbial life. The colocation of carbon and nutrients in biochar promotes microbial colonization. Biochar-based inoculants enhance plant growth even in hostile environments. Biochar-immobilized microbes help in the remediation of contaminated soils. Adsorption of contaminants by biochar avoids microbial inactivation by contaminants. Colocation also provides contact between microorganisms and contaminants, which is conducive to their subsequent biodegradation. When biochar-immobilized microbes for nutrient management and remediation of contaminated soils are used, secondary toxicity caused by the contaminants remaining on the biochar and the carrier's direct toxicity should be considered. Although biochar can be effectively used as a carrier for microbial cells, thereby the immobilized microbes can be used to improve soil health and environmental remediation, some of the issues with the overall application of biochar technology in relation to energy cost for the synthesis of biochar and air quality in terms of greenhouse emission need to be taken into consideration for large scale applications of this emerging biochar-based microbial immobilization technology.

Given the limitations on the use of non-renewable substrates, such as peat, for microbial inoculum and the growing use of biochar-immobilized-microorganism technology, we propose exploring the following future research avenues to improve carrier material performance and expanding the potential applications of this emerging technology:

- Determine if the immobilization of microorganisms by biochar can be enhanced by improving the surface characteristics of biochar by optimizing the feedstock and the pyrolyzing conditions
- Develop cost-effective techniques for large-scale microbial immobilization of microbes including rhizosphere probiotics onto biochar
- Monitor the survival and activity of biochar-immobilized microbes during the storage, transport and field application of these microbial-enriched biochar products.
- Optimise the storage and transport conditions to maintain the long-term survival and activity of immobilized microbes in microbial-enriched biochar products used for field application
- Determine if the long-term persistence of immobilized microorganisms can be improved by examining the stability of biochar in soils
- Determine if the recovery and reuse of ‘spent’ microorganism-immobilized biochar can be facilitated by the application of magnetic biochar
- Determine if the selection of microorganisms is critical for the successful utilization of biochar-based inoculants for agricultural and environmental applications
- Demonstrate the agronomic (e.g., plant-growth-promoting probiotic microbes) and environmental remediation (e.g., contaminant degrading microbes) benefits of biochar-based microbial immobilization technology using large-scale field experiments.
- Explore the opportunities for the immobilization of microbial extracellular enzymes onto biochar so that enzyme-immobilized biochar products can be utilized directly for improving soil health and environmental remediation

CRediT authorship contribution statement

Shiv Bolan, Deyi Hou and Nanthi Bolan - Conceptualization, Writing – original skeleton of the entire draft, contributed Sections 1, 2 and 6, data extraction for Tables and prepared Figures.

Lauren Hale, Dilfuza Egamberdieva, Priit Tammeorg, Rui Li– Contributed Sections 4 and 5, data extraction for Tables and prepared Figures.

Rui Li, Bing Wang, Jiaping Xu, Ting Wang, Hongwen Sun Contributed Sections 3, 5 data extraction for Tables and prepared Figures.

Hailong Wang, Lokesh Padhye, Jörg Rinklebe, M.B. Kirkham and Kadambot H.M. Siddique – covered Section 7 and contributed to the interpretation of the discussion of various sections and provided critical revision and editing of the article.

Data availability

No data was used for the research described in the 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.

Acknowledgement

We acknowledge the partial support by Healthy Environments And Lives (HEAL) National Research Network, which receives funding from the National Health and Medical Research Council (NHMRC) Special Initiative in Human Health and Environmental Change (Grant No. 2008937).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2023.163968.

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