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Review

The co-application of biochar with bioremediation for the removal of petroleum hydrocarbons from contaminated soil

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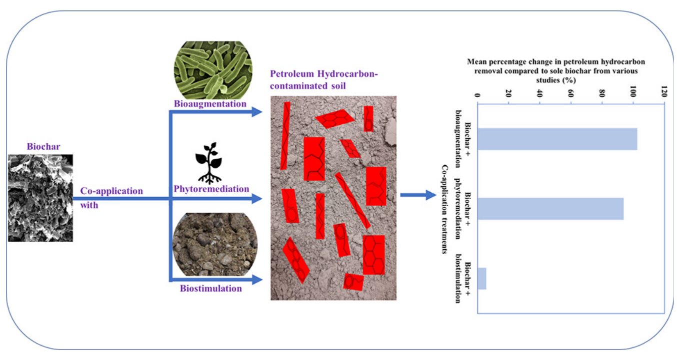
HIGHLIGHTS

- Biochar co-application can achieve up to 4-fold higher hydrocarbon removal than biochar treatment.
- Co-application with bioaugmentation was most effective followed by phytoremediation.
- No synergistic effect was observed in most of the co-application studies.
- Modification of biochar before application can enhance hydrocarbon removal.

GRAPHICAL ABSTRACT



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ABSTRACT

Soil pollution from petroleum hydrocarbon is a global environmental problem that could contribute to the non-actualisation of the United Nations Sustainable Development Goals. Several techniques have been used to remediate petroleum hydrocarbon-contaminated soils; however, there are technical and economical limitations to existing methods. As such, the development of new approaches and the improvement of existing techniques are imperative. Biochar, a low-cost carbonaceous product of the thermal decomposition of waste biomass has gained relevance in soil remediation. Biochar has been applied to remediate hydrocarbon-contaminated soils, with positive and negative results reported. Consequently, attempts have been made to improve the performance of biochar in the hydrocarbon-based remediation process through the co-application of biochar with other bioremediation techniques as well as modifying biochar properties before use. Despite the progress made in this domain, there is a lack of a detailed single review consolidating the critical findings, new developments, and challenges in biochar-based remediation of petroleum hydrocarbon-contaminated soil. This review assessed the potential of biochar co-application with other well-known bioremediation techniques such as bioaugmentation, phytoremediation, and biostimulation. Additionally, the benefits of modification in enhancing biochar suitability for bioremediation were examined. It was concluded that biochar co-application generally resulted in higher hydrocarbon removal than sole biochar treatment, with up to a 4-fold higher removal observed in some cases. However, most of the biochar co-applied treatments did not result in hydrocarbon removal that was greater than the additive effects of individual treatment. Overall, compared to their complementary treatments, biochar co-application with bioaugmentation was more beneficial in hydrocarbon removal than biochar co-application with either phytoremediation or biostimulation. Future studies should integrate

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the ecotoxicological and cost implications of biochar co-application for a viable remediation process. Lastly, improving the synergistic interactions of co-treatment on hydrocarbon removal is critical to capturing the full potential of biochar-based remediation.

Contents

1. Introduction

In 2015, the United Nations mapped out seventeen sustainable development goals (SDGs) aimed at protecting the planet, ending poverty, and promoting peace and prosperity (UNDP, 2021). Although soil pollution was only considered in targets 3.9 and 12.4 of the SDGs, soil pollution can hinder the actualisation of many SDGs, such as “clean water and sanitation for all”, and “zero hunger” (FAO, 2021a,b). This is unsurprising considering the environmental, socio-economic, and human health impacts of soil pollution (FAO, 2021b). Petroleum and its derived product are pollutants present in the soil in many countries. In Canada's federal contaminated site inventory, at least 50 % of the active and suspected soil and surface soil media are contaminated by petroleum hydrocarbons (PHCs), polycyclic aromatic hydrocarbon (PAH), and benzene, toluene, ethylbenzene, and xylene (BTEX) (Treasury Board of Canada Secretariat, 2022). In the Russian Federation, a 2018 report stated that the area polluted by

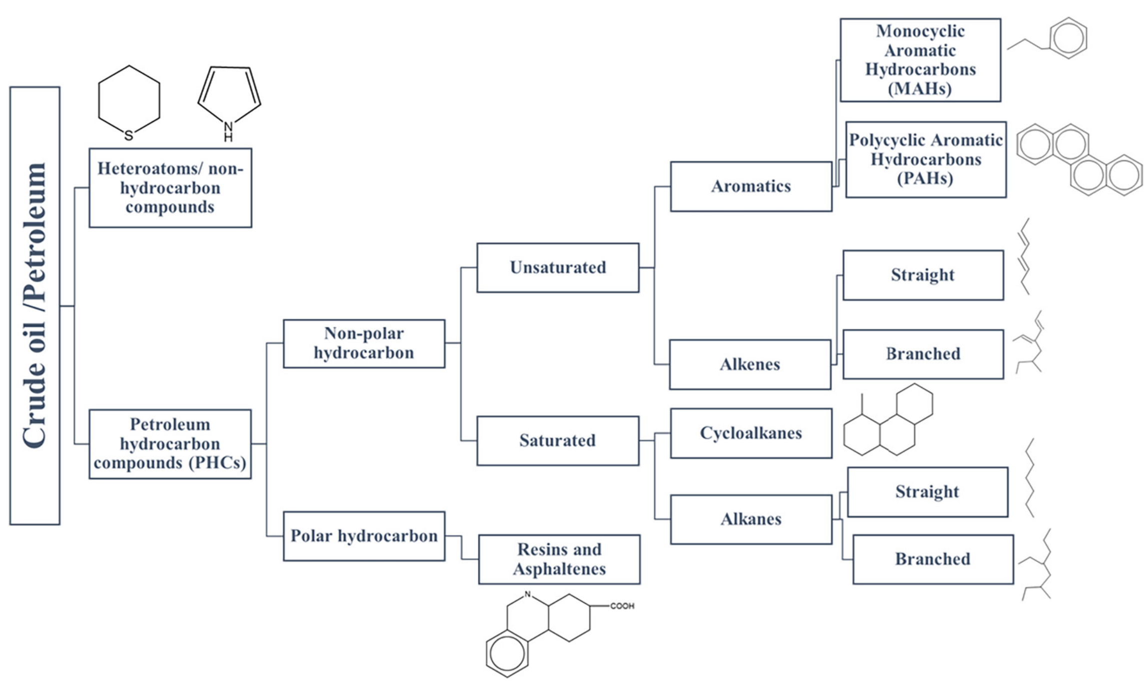


Fig. 1. Classification of petroleum hydrocarbons (Coulon and Wu, 2014; Logeshwaran et al., 2018; Speight, 2014).

petroleum and its by-products was >100,000 ha, while in Azerbaijan, about 11,143 ha was stated to be polluted by petrochemical products (FAO, 2018). Similarly, a total of 4,102 crude oil and refined product spills on land have been reported in the Nigerian oil spill monitor between 2006 - June 2022, representing 253,143 barrels released to the land within the period (NOSDRA, 2022).

Petroleum is composed mainly of hydrocarbons and heteroatomic compounds (containing non-hydrocarbons, such as sulphur, nitrogen, and metals) in minor amounts (Fig. 1) (Logeshwaran et al., 2018). Petroleum and its derived products are of great relevance as energy and fuels, as well as in petrochemicals production. Increasing oil production and consumption have further increased the risk of oil pollution (Fingas, 2012). The presence of petroleum contaminants in soils can affect the activity and diversity of soil microbes; plant growth, root development, stem diameter, and grain yields are reduced on exposure to petroleum hydrocarbons (Ahmed and Fakhruddin, 2018). Exposure to this class of contaminants can result in carcinogenic, mutagenic, immunotoxic, haemotoxic, cardiotoxic, neurotoxic, nephrotoxic, genotoxic, teratogenic, and hepatotoxic effects on humans and animals (Ossai et al., 2020). Furthermore, the listing of the members of the hydrocarbon family (PAH and BTEX) in the 2019 substance priority list suggests that they are contaminants of concern since this ranking was based on a combination of the toxicity, frequency, and potential for human exposure at the National Priorities List site (Agency for Toxic Substances and Disease Registry, 2020).

The remediation of petroleum hydrocarbon-contaminated soils has been carried out using several approaches, such as biological, physicochemical, chemical, thermal, acoustic, and electrical/electromagnetic methodss (Ossai et al., 2020). Table 1 shows the advantages and limitations of some examples of these remediation methods. Biochar, a product derived from the thermal decomposition of carbon-based biomass, has gained relevance in remediation studies due to its cost-effectiveness, and environmental sustainability (Singh et al., 2021; Zama et al., 2018). Biochar is produced when biomass, such as agricultural residues, forest wastes, manure, and biosolids, are heated at elevated temperatures (typically 300–700 °C) in an oxygen-limited environment. As a remediation technique, biochar can stimulate microbial communities by modifying soil physicochemical properties, as well as providing shelter, nutrients, and protection to soil microbes (Dike et al., 2021). In addition, the role of biochar in remediation is linked to its sorption ability (Yang et al., 2017). Aside from remediation, biochar also plays a vital role in other environmental areas such as waste management, climate change, carbon sequestration, and soil improvement (Yuan et al., 2019).

The sole application of biochar to hydrocarbon-contaminated soils has been assessed in several works; the outcomes from these studies suggest

Table 1
Comparison of methods for remediation of petroleum hydrocarbon-contaminated soil.

Methods	Example	Advantages	Limitations	References
Biological	Bioaugmentation	- Low cost - Environmentally friendly	- The introduced organism may be affected by competition, predators, and harsh environmental conditions. - Time-consuming - Alteration of natural microbial community structure - Inconsistent results	(Gentry et al., 2004; Lim et al., 2016; Shahsavari et al., 2015)
Bio-physicochemical	Biochar	- Low cost - Environmentally friendly - Improves soil physicochemical properties - Reduces bioavailability - Addresses other environmental issues	- Sorption of contaminant - Time-consuming - Affected by environmental factors - Concerns about biochar toxicity - Long-term fate uncertain - Inconsistent results - Nutrient immobilisation	(Dike et al., 2021)
Physicochemical	Soil vapour extraction	- Complete destruction of contaminant - Appropriate for heavily polluted sites - Quick (several days to months) - Promotes the growth of microbes	- Liable to secondary pollutants - Expensive - Ineffective for low volatility compounds - Transport issue if treated ex-situ - Destructive - Ineffective for soils with low air permeability	(Koshlaf and Ball, 2017; Lim et al., 2016; Ma et al., 2016)
Chemical	Chemical oxidation	- Fast (up to 72 h) - By-products of remediation are environmentally friendly - Ease of operation - High efficiency	- pH has a significant effect on efficiency - Soil microbes are destroyed - Expensive compared to biological - Limited by low soil permeability	(Lim et al., 2016)
Thermal	Incineration	- Complete destruction of contaminants - Very fast (several seconds – 2 h) - Effective - Established technology - Can be used for large amounts of contaminated soil	- Expensive - Energy-intensive - Secondary waste generation (fly ash) - Loss of soil minerals and organic matter - Transport and chances of spillage if treated ex-situ - Lack of societal acceptability	(Lim et al., 2016; Ossai et al., 2020; Vidonish et al., 2016)
Electrical/electro-magnetic	Electrokinetic	- Remediation efficiency is best in clay soil - Less energy-demanding compared to other ex-situ methods - Highly specific - Competitive in efficiency and cost compared to other methods - Not too time consuming (14–45 days)	- Affected by soil conditions - Not suitable for non-polar chemicals - Secondary pollutant - Dependent on desorption of contaminant - Not environmentally friendly - Microbial activity may be affected	(Kuppusamy et al., 2016a; Lim et al., 2016)
Acoustic	Ultrasonic	- No use of chemicals - Environmentally friendly - Very fast (several seconds – 45 min)	- Expensive - High energy requirement - Difficulty in site implementation	(Dos Santos and Maranho, 2018; Lim et al., 2016)

that in terms of hydrocarbon removal, biochar application resulted in a positive and negative outcome compared to the control (Agarry et al., 2015; Aziz et al., 2020; García-Delgado et al., 2015; Gielnik et al., 2019; Kong et al., 2018; Li et al., 2019c; Zhang et al., 2018b). In these studies, biochar was applied in its pristine form, without any prior modification. For example, Aziz et al. (2020) and Wang et al. (2017b) observed higher total petroleum hydrocarbon (TPH) removal in the biochar treatment (47–76 %) compared with the control (28–36 %). In contrast, no significant difference was found between biochar amended treatment and the control in other studies (Galitskaya et al., 2016; Gielnik et al., 2019; Uyizeye et al., 2019).

Aside from the use of sole or unmodified biochar for remediation, attempts have been made to assess the effect of co-applying biochar with other bioremediation techniques or modified biochar on hydrocarbon remediation. Some of these initiatives could potentially address the problems of sole or unmodified biochar use, such as sorption, toxicity concerns, and nitrogen immobilisation as well as improve other remediation techniques (Dike et al., 2021; Hoang et al., 2021; Zhang et al., 2019). These approaches involve the co-application of biochar with bioaugmentation, phytoremediation, and biostimulation, as well as the modification of biochar, which demands a critical review. Previous reviews discussing various aspects of biochar co-application in petroleum hydrocarbon-contaminated soil exist (Hoang et al., 2021; Hussain et al., 2018b; Meki et al., 2022; Sui et al., 2021; Zahed et al., 2021). However, no review provides a comprehensive insight into the co-application of biochar with bioaugmentation, phytoremediation, and biostimulation, as well as modification of biochar in the remediation of petroleum hydrocarbon contaminated soils. For example, Sui et al. (2021) provided a brief discussion on biochar co-application with microbes (bioaugmentation) while examining different microbial combined remediation methods. Hoang et al. (2021) focused on rhizoremediation with an overview of the biochar-assisted rhizoremediation (phytoremediation) technique. Therefore, this review provides detailed insight into the effect of biochar co-application with bioremediation techniques (bioaugmentation, phytoremediation, and biostimulation) and the use of modified biochar in the remediation of petroleum hydrocarbon contaminated soils. Examination of different co-application strategies in a single review provides an opportunity to critically compare their potential. Specifically, this review examines the various strategies when biochar is co-applied with bioaugmentation (microbial cells and enzymes), phytoremediation (plants and root exudate), and biostimulation (surfactant and nutrient) and hybrids of these techniques in the remediation of petroleum hydrocarbon-contaminated soil. Additionally, an overview of the potential of biochar modification for the remediation of petroleum hydrocarbon-contaminated soil is examined.

2. Co-application of biochar with non-hybrid bioremediation techniques

Co-application of biochar refers to the use of biochar in conjunction with other remediation techniques such as bioaugmentation, phytoremediation, biostimulation, and any of their combination (hybrid remediation techniques). In this section, the focus will be on the co-application of biochar with one bioremediation technique, such as bioaugmentation, phytoremediation, and biostimulation.

2.1. Co-application of biochar with bioaugmentation

Biochar, given its porous nature, can act as a matrix for organic contaminants, and thus its sole application to the soil could result in the reduction of the organic contaminant's bioavailability and biodegradation (Kuppusamy et al., 2016b). In addition, the contaminated soil where biochar is intended to be added may lack appropriate hydrocarbon-degrading microbial communities or the hydrocarbon-degrading population is low even after biostimulation (Couto et al., 2010; Da Silva and Alvarez, 2010). In such circumstances adding biochar alone may not result in the anticipated enhanced hydrocarbon removal. Also, in some soils, the build-up of toxic by-products and the presence of harsh biological environmental conditions may make the indigenous microbes unable to efficiently degrade the contaminant (Da Silva and Alvarez, 2010). To address these challenges, biochar can be co-applied with hydrocarbon-degrading microorganisms (bacteria, fungi, yeast, and algae) or enzymes.

One way of studying the effect of the co-application of biochar with bioaugmentation is by introducing both amendments separately to the soil without any prior immobilisation (Guo et al., 2021). Another way involves introducing biochar and the bioaugmentation agent in the immobilised form (Song et al., 2021). Further details of both approaches are discussed in the subsequent subsections.

2.1.1. Bioaugmentation and biochar without prior immobilisation

In this approach, the biochar and bioaugmentation agents are introduced separately to the contaminated soil without any interaction or immobilisation before introducing them to the soil. In this case, the biochar acts as a biostimulator to the introduced or autochthonous organism. A recent study examined the effect of co-applying 2–10 % (w/w) wheat straw-derived biochar with free-living microorganisms on the bioremediation of benzo(*a*)pyrene-contaminated soil (Guo et al., 2021). The co-applied treatment achieved higher hydrocarbon removal after 30 days (35–89 %) compared to the sole biochar (8–41 %) and bioaugmented treatments (24 %) (Table 2). Hydrocarbon removal in the co-applied treatment increased as the biochar application dose increased. Biochar amendment promoted the activity of microbes in the biochar-assisted bioaugmentation treatment, which was confirmed by increased CO_2 evolution in the biochar-assisted bioaugmentation treatment, relative to the sole treatment (Guo et al., 2021). This was reconfirmed by the results from assessing microbial biomass, enzyme activities, and soil organic matter, which were higher in the combined treatment compared to the sole bioaugmentation treatment. In other studies, the co-application of sugar cane bagasse biochar with either *Bacillus* sp. MN54 or *Enterobacter* sp. MN17 resulted in statistically significantly higher diesel removal ($p < 0.05$) compared to their biochar or bioaugmentation treatment (Ali et al., 2021; Ali et al., 2020) (Table 2).

Piscitelli et al. (2019) observed that the co-application of biochar with fungi (*Trichoderma harzianum*) did not result in a significant difference in pyrene removal compared to the sole biochar or fungal bioaugmentation treatment at day 28. Results from this study showed that pyrene removal in the co-applied and sole fungal treatment decreased gradually during incubation, while in the control or sole biochar treatment, an abrupt removal occurred on day 7 (Piscitelli et al., 2019). Biochar enhanced the growth of *Trichoderma harzianum* in the co-applied treatment compared to other treatments from day 14 until the end of the incubation (day 28). The lack of efficiency in pyrene removal in the co-applied treatment compared to the sole biochar treatment or the control could be due to the choice of fungi used. This is because the autochthonous soil microflora was partially inhibited by the fungi and the fungi used did not show any distinct ability to degrade the contaminant, compared to the indigenous microorganism (Piscitelli et al., 2019). The authors suggested that when a microbial inoculum is used in pyrene degradation, the test organism should have no inhibitory effect on the indigenous pyrene degrading organism (Piscitelli et al., 2019). In another study involving fungal cells (*Pleurotus ostreatus*) and wood chip biochar, hydrocarbon removal was higher in the fungal-biochar co-applied treatment (58 %) than in the sole biochar treatment (14 %) but lower than the fungal treatment (73 %) (García-Delgado et al., 2015). The lower removal observed in the combined treatment in comparison to the fungal sole treatment may be linked to the biochar used in this study (wood chip) since the removal efficiency in the sole biochar treatment (14 %) was lower than the control (17 %). Moreover, biochar application resulted in the inhibition of the PAH-degrading population on days 21 and 42 in the sole biochar treatment. The wood chip biochar was reported to have a high C/N ratio, which could cause nitrogen immobilisation. Further, in this study, biochar and *Pleurotus ostreatus* (inoculated on wheat straw) were introduced into a PAH-contaminated soil on days 0 and 21, respectively (García-Delgado et al., 2015). The goal of this application method is to immobilise the contaminant to the biochar and add the fungi after immobilisation to degrade the contaminant. There is no doubt

Table 2
Remediation studies showing the co-application of biochar with bioaugmentation.

Biochar biomass/ pyrolysis temp (°C)	Biochar dose (% w/w)[a]	Microorganism	Immobilisation of microbes on biochar	Immobilisation parameters	Hydrocarbon removal (%)				Contaminant (Amount – mg/kg)	References
					Control	Biochar	Bioaugmentation	Co-application		
Wheat straw/ 500	2	PAH-degrading microbes	×	NA	0	8	24	35	Benzo(*a*)pyrene (43)	(Guo et al., 2021)
	4				0	16	24	50		
	6				0	24	24	64		
	8				0	33	24	77		
	10				0	41	24	89		
Sugarcane bagasse /400	1	*Bacillus* sp. MN54	×	NA	18	32	38	45	Diesel (10000)	(Ali et al., 2021)
Sugarcane bagasse /400	1	*Enterobacter* sp. MNI7	×	NA	42	63	59	69	Diesel (5000)	(Ali et al., 2020)
Pine wood chip/ 450	2.5[b]	*Pleurotus ostreatus*	×	NA	17	14	73	58	PAH (1212)	(García-Delgado et al., 2015)
Olive mill pomace/ 450	–	*Trichoderma harzianum*	×	NA	35	37	33	30	Pyrene (43)	(Piscitelli et al., 2019)
Pine needle /400	NA	*Sphingomonas* sp. PJ2	✓	10 g biochar; 50 mL bacteria suspension (optical density: 1.6–1.8); Incubation at 30 °C for 48 h at 100 rpm; Washing and resuspension in deionised water	15	38	21	50	PAH (2)	(Song et al., 2021)
Pine needle/600			✓		15	43	21	59		
Birch waste/ 450	NA	*Pseudomonas aeruginosa*	✓	Biochar plunged into bacterial culture; Stored for 1 day; Final concentration of bacteria on biochar was 5–9 × 10^8 Colony Forming Unit	56	59	–	66	Oil (47000)	(Galitskaya et al., 2016)
	NA	*Acinetobacter radioresistens*	✓		56	59	–	66		
Corn cob/ 300	NA	–	✓	1 g biochar into 50 mL immobilisation medium; 10 % domesticated bacteria; Incubation at 35 °C for 130 rpm at 18 h; Centrifugation.		12[c, d]	42[d]	61[d]	Petroleum (48000)	(Ren et al., 2020)
Corn cob/ 400		–	✓			12[c, d]	42[d]	64[d]	Petroleum (48000)	
Corn cob/ 500		–	✓			12[c, d]	42[d]	71[d]	Petroleum (48000)	
Corn cob/ 600		–	✓			12[c, d]	42[d]	59[d]	Petroleum (48000)	
Straw/ 300	NA	–	✓	1 g biochar into 50 mL immobilisation medium; 10 % domesticated bacteria; Incubation at 35 °C for 130 rpm at 18 h; Centrifugation.		12[c, d]	42[d]	49[d]	Petroleum (48000)	(Ren et al., 2020)
Straw/ 400		–	✓			12[c, d]	42[d]	53[d]	Petroleum (48000)	
Straw/ 500		–	✓			12[c, d]	42[d]	58[d]	Petroleum (48000)	
Straw/ 600		–	✓			12[c, d]	42[d]	58[d]	Petroleum (48000)	
Sawdust/ 300	NA	–	✓	1 g biochar into 50 mL immobilisation medium; 10 % domesticated bacteria; Incubation at 35 °C for 130 rpm at 18 h; Centrifugation.		12[c, d]	42[d]	46[d]	Petroleum (48000)	(Ren et al., 2020)
Sawdust/ 400		–	✓			12[c, d]	42[d]	48[d]	Petroleum (48000)	
Sawdust/ 500		–	✓			12[c, d]	42[d]	55[d]	Petroleum (48000)	
Sawdust/ 600		–	✓			12[c, d]	42[d]	57[d]	Petroleum (48000)	
Wheat bran/ 300	NA	Bacterial consortium[e]	✓	30 mL biochar solution (biochar/distilled water, 1/30 *w/v*); 20 mL bacteria solution; incubation in a shaker for 2 h; Air drying.	23	31	44	47	Oil (6603)	(Guo et al., 2022)
Wheat bran/ 500		Bacterial consortium[e]	✓		23	37	44	58	Oil (6603)	
Wheat bran/ 700		Bacterial consortium[e]	✓		23	31	44	53	Oil (6603)	

✓: Microbial immobilisation on biochar; ×: No microbial immobilisation on biochar; NA: Not applicable.
[a] Only applicable in studies where the microbes were not immobilised on the biochar before introduction.
[b] %.
[c] Representative biochar treatment- Only one biochar treatment was reported out of the 12 biochar type studied here.
[d] Oil removal rate determined by $\left(\frac{Amount\ of\ oil\ in\ control\ -\ Amount\ of\ oil\ in\ the\ sample}{Amount\ of\ oil\ in\ control} \times 100\right)\%$.
[e] *Pseudomonas guguanensis* (NR_135725.1), *Pseudomonas pseudoalcaligenes* (NR_037000.1), *Sphingobacterium pakistanense* (NR_113311.1) and, *Acinetobacter venetianus* (MN542884.1).

that the biochar may immobilise the contaminant, but the introduced fungi may not be in close contact with the biochar (containing the PAH) in the soil. The introduced fungi may find it difficult to penetrate/colonise the biochar at day 21 because clumping and attachment of soil on the biochar particle may occur after the introduction of biochar to the soil, which could make the site (pore the pores and surface area) for microbial colonisation

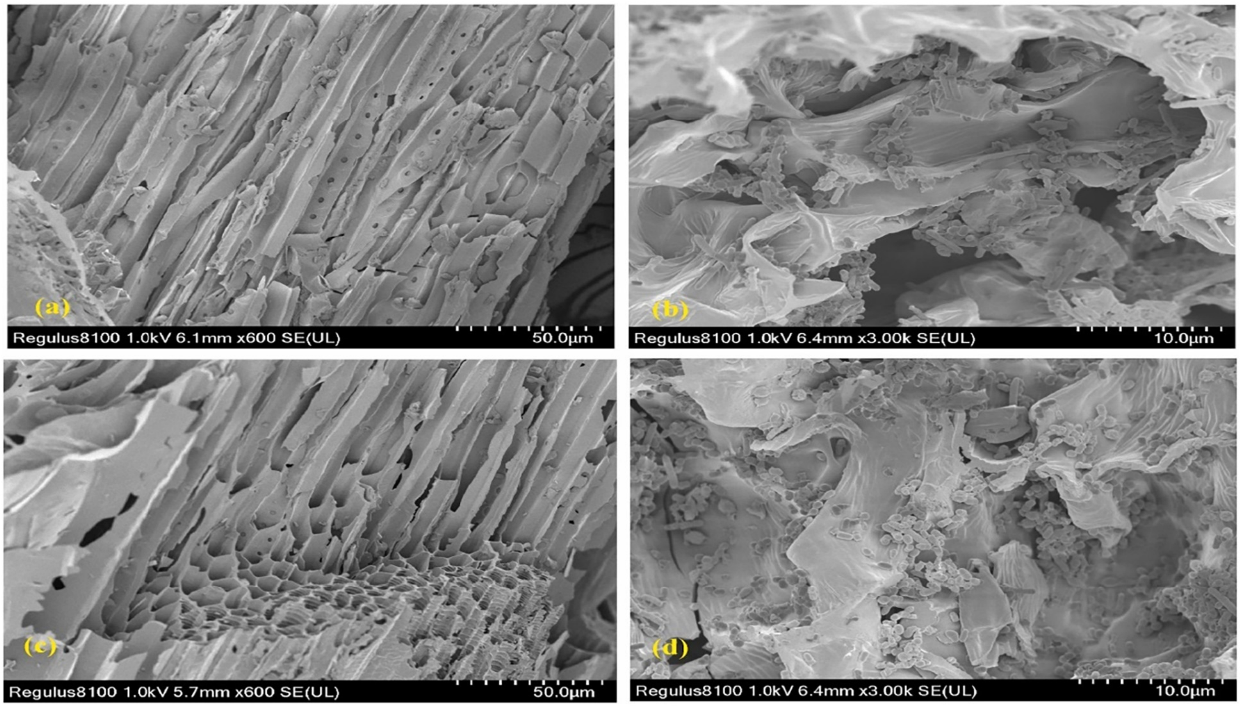


Fig. 2. SEM image of a). Pine needle biochar produced at 400 °C; b). *Sphingomonas* sp. PJ2 immobilised at biochar produced at 400 °C; c.). Pine needle biochar produced at 600 °C; d). *Sphingomonas* sp. PJ2 is immobilised at biochar produced at 600 °C (Copyright permission obtained) (Song et al., 2021).

unavailable (Jaafar et al., 2015). In addition, other organisms already present in the soil may also have attached to the biochar (Jaafar et al., 2015).

2.1.2. Bioaugmentation immobilised biochar

Although introducing microbes in the free-form alongside biochar individually may be beneficial in dealing with the challenge of the lack of efficient or sufficient hydrocarbon-degrading microorganisms, the introduced microbes may be exposed to harsh environmental conditions, competition, or attack from predators (Gentry et al., 2004). Additionally, the problem of reduced contaminant bioavailability associated with sole biochar application may not be fully addressed when the biochar-microbial composite is introduced in the free form (no prior immobilisation). This is because the introduced microbes may not be in proximity to the sorbed contaminant. The above reasons make biochar immobilised microbes more beneficial in biochar-bioaugmentation-based remediation than their individual introduction to the soil without prior immobilisation; specifically, prior immobilisation of bacteria on biochar has been shown to be more beneficial in terms of hydrocarbon removal (18–22 % higher), enzyme activity, microbial respiration, and the microbial population (Wei et al., 2021; Zhang et al., 2019). Microbes have mostly been used for immobilisation-based hydrocarbon studies. The use of microbial enzymes for biochar immobilisation in remediation studies may offer more potential than the use of microbial cells (fungi and bacteria) since enzymes can operate at a faster speed and in the absence of nutrients (Imam et al., 2021; Saravanan et al., 2021). Enzymes can readily be immobilised, are substrate-specific, and degrade the contaminant faster than microbial cells (Gaur et al., 2021). Microbial enzymes such as laccases, lipases, lignin peroxidases, cytochrome-P450, catalases, and manganese peroxidases can be used in place of microbes to degrade hydrocarbons (Imam et al., 2021). The enzymes function similarly to the microbial cell because, during whole cell-mediated degradation, enzymes are secreted first (Imam et al., 2021). Future studies should aim at examining the impact of enzyme-immobilised biochar on the remediation of hydrocarbon-contaminated soil. It is important to ensure that the environmental conditions (temperature, pH) and immobilisation method (agitation, enzyme dosage) are carefully selected (Saravanan et al., 2021).

Microbes can be immobilised on biochar via different fixation methods such as adsorption, entrapment, cross-linking, and covalent bonding or a combination of two methods (Lu et al., 2020; Wu et al., 2022).

2.1.2.1. Biochar immobilisation via adsorption fixation. Immobilisation via adsorption is a weak, reversible, and likely the simplest microbial immobilisation fixation method, which involves the physical interaction between microbes and the carrier (biochar) (Nwankwegu and Onwosi, 2017). Physical interactions may be ionic, van der Waals forces, or hydrogen bonding (Jesionowski et al., 2014). The process of adsorption involves firstly, the microbial cell transfer from the bulk phase to the surface of the biochar, followed by the adhesion to the surface of the biochar and the subsequent colonisation of the microbial cells on the surface of the biochar (Kilonzo et al., 2011). Microbes secrete multiple polymeric substances on the biochar surface, which are used for attachment to the biochar surface, thus forming an extracellular-enclosed microbial biofilm (Frankel et al., 2016; Zhou et al., 2021).

Scanning electron microscopy (SEM) has been used to examine microbial colonisation on biochar (Ren et al., 2020; Song et al., 2021; Xiong et al., 2017; Zhang et al., 2016). Song et al. (2021) reported the presence of many of the *Sphingomonas* sp. PJ2 bacteria in the biochar pores (Fig. 2), while Xiong et al. (2017) observed the adherence of *Mycobacterium gilvum* cells to the biochar, with frequent colonisation on the surface and pores of the biochar. Additionally, there is evidence that biochar immobilisation was beneficial to the introduced microbes (Galitskaya et al., 2016; Song et al., 2021). Song et al. (2021) reported that the relative abundance of the *Sphingomonas* genus was higher in the biochar immobilised treatment compared to other treatments, while Galitskaya et al. (2016) observed the dominance of *Pseudomonas aeruginosa* or *Acinetobacter radioresistens* in their respective immobilised treatment on day 1.

The mechanism of enhanced hydrocarbon removal following the application of biochar immobilised microbes (via adsorption) to the soil is summarised in Fig. 3. The mechanism is centred around:

(i.) combining the benefits of both techniques. Biochar will be useful for hydrocarbon adsorption and stimulating microbes, while

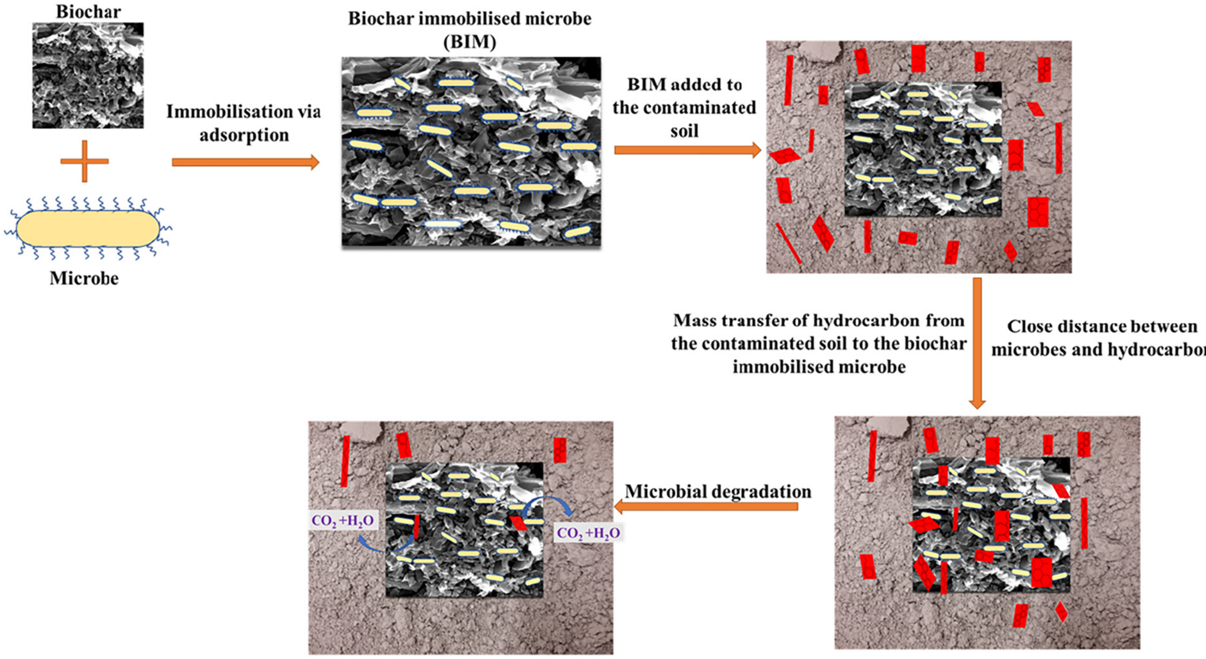


Fig. 3. Mechanism of enhanced hydrocarbon removal in biochar immobilised microbe treatment.

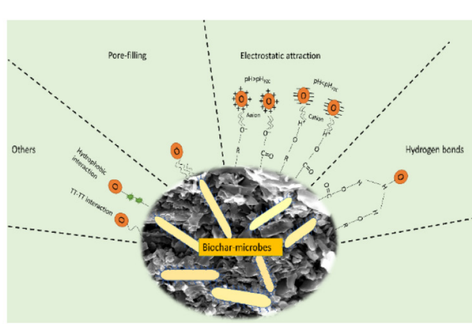
bioaugmentation will be useful in the microbial degradation of the contaminant (Li et al., 2022). The mechanism of adsorption of organic contaminants by the biochar immobilised microbe is shown in Fig. 4A;

(ii.) addressing the limitation of one technique with another (Fig. 4B).

Attempts have been made to assess the effect of biochar immobilised microbe in the remediation of hydrocarbon-contaminated soil (Table 2). Song et al. (2021) observed that PAH removal was higher in *Sphingomonas* sp. PJ2-immobilised biochar treatment (50–59 %) than the sole bioaugmentation (21 %), or their respective biochar treatments (38–43 %). Similarly, Ren et al. (2020) reported a higher hydrocarbon removal rate in their different biochar-microbial immobilised treatments than the sole bioaugmentation, and representative biochar treatments (Table 2). In another study, immobilisation of a bacterial consortium on biochar produced at different temperatures (300, 500, and 700 °C) resulted in significantly higher hydrocarbon removal compared to their respective sole biochar treatments (Guo et al., 2022). However, compared to the bioaugmentation treatment, only 2 out of 3 immobilised biochar pyrolysis temperature treatments resulted in increased hydrocarbon removal (biochar at 500 and 700 °C) (Guo et al., 2022). In contrast, Galitskaya et al. (2016) observed a greater difference in hydrocarbon removal on day 14 between the best immobilised biochar treatment and the biochar treatment, compared to day 84, which may be due to the weak immobilisation (Table 2). The lack of agitation during the incubation may have resulted in weak immobilisation. This observation was corroborated by the community structure and 16S rDNA. The number of 16S rDNA gene copies, as well as the relative abundance of Proteobacteria phylum, Pseudomonas, and Acinetobacter were higher in the immobilised treatment on day 1, followed by a decrease afterward (Galitskaya et al., 2016).

As seen in Table 2, the biochar used for the immobilised microbe has been produced from different feedstock and pyrolysis temperatures. These differences in feedstock and pyrolysis temperature affect the properties of biochar and their potential as a microbial carrier. Influencing biochar properties include surface area, pore-volume, pore size distribution, ash content, zeta-potential, and surface properties (hydrophobicity, metallic oxides, and functional groups) (Guo et al., 2022; Ren et al., 2020; Xiong et al., 2017). Ren et al. (2020) evaluated the microbial-immobilisation efficiency of biochar produced from corn cob, straw, and sawdust at different pyrolysis temperatures. Higher microbial immobilisation rates

A.



B.

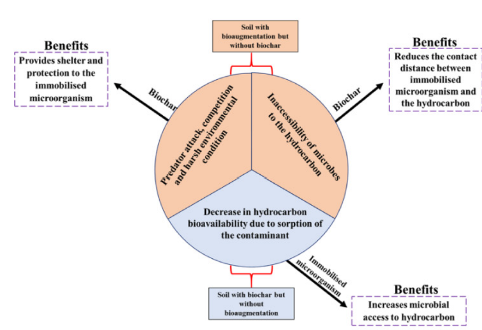


Fig. 4. (A). Proposed mechanism of organic contaminants adsorption by biochar immobilised microbes interaction (Adapted and redrawn from Wu et al. (2022)); (B.) An overview of the potential benefits of co-applying biochar with bioaugmentation (via immobilisation) in the remediation of hydrocarbon-contaminated soil, in relation to the limitation of sole bioaugmentation and biochar treatment. The orange and blue sector in the circle represents the limitations associated with sole bioaugmentation and biochar separate application to hydrocarbon-contaminated soil, respectively. The dashed purple boxes outside the circle show how immobilisation of microbes to biochar can address the limitations in each sector (Dike et al., 2021; Gentry et al., 2004).

were observed in corncob- (62–71 %), followed by straw- (45–58 %) and sawdust- (42–53 %) immobilised biochar; hydrocarbon removal followed a similar trend, i.e., corncob biochar (59–71 %) > straw biochar (49–58 %) > sawdust biochar (46–57 %) (Table 2). This suggests the role of biochar immobilisation efficiency in hydrocarbon removal. The enhanced microbial immobilisation and hydrocarbon removal by corn cob biochar may be due to its higher surface area, pore-volume, and zeta-potential (Ren et al., 2020). Overall, the TPH removal rate was consistent with the surface area and total pore volume (Ren et al., 2020). In contrast, Guo et al. (2022) showed that hydrocarbon removal was not dependent on the biochar surface area but the ash content. The ash content represents the mineral status of the biochar; Zhang et al. (2020) observed that ash content correlated negatively with the residual PAH concentration in the soil ($p < 0.05$ for PAHs with 3–6 rings, except for phenanthrene). In another study, higher PAH removal was observed when *Sphingomonas* sp. PJ2 was immobilised on biochar with higher total organic carbon, C/N, and electrical conductivity (Song et al., 2021). Guo et al. (2022) and Song et al. (2021) observed that immobilizing bacteria on biochar with a basic pH (8.6–9.52) was more beneficial in terms of remediation efficiency than in biochar with neutral biochar pH (6.56–7.31). Further studies should be carried out to understand the main property/properties of biochar affecting immobilisation and remediation efficiency. Once identified, biochar can be modified before immobilisation with microbes. Similarly, the effect of other factors such as microorganism, application dose, and immobilisation method deserve further attention.

2.1.2.2. Biochar immobilisation via adsorption and entrapment fixation. Although biochar has been reported to be a suitable carrier for microbes, Wang et al. (2019a) speculated that since biochar does not immobilise microbes tightly, they are readily displaced from the biochar. This displacement could occur because the bond between the microbes and the carrier in adsorption-based immobilisation is weak (Dzionek et al., 2016). Combining biochar adsorption with entrapment can address this limitation since the grid structure of entrapment can prevent the displacement of the microbes from the biochar (Wu et al., 2022). Few studies have combined microbial immobilisation on biochar (via adsorption) with entrapment in a crosslinking material (alginate) (Chen et al., 2012). Chen et al. (2012) observed that PAH removal was significantly enhanced in the different treatments with the combined application of both immobilised bacteria on biochar and entrapment on alginate compared to PAH removal in treatments with the entrapped bacteria without biochar. When immobilisation with pine needle biochar at 400 °C was further examined, higher hydrocarbon removal was observed in the combined treatment of biochar with microbial entrapment on alginate than treatment with free bacteria, entrapped bacteria on alginate, or entrapped biochar on alginate, irrespective of the bacteria. Wang et al. (2019a) examined the effect of immobilised biochar (via adsorption) and bacteria with or without entrapment to alginate on pyrene degradation in pyrene-Cr(VI) co-contaminated soil. Increased pyrene removal was observed in the treatment where bacteria were immobilised in both biochar and alginate (82 %) compared to when it was immobilised (via adsorption) in biochar only (65 %) or entrapped in alginate only (73 %). Further, soil enzyme activity and microbial diversity were significantly greater when bacteria were immobilised on both biochar and alginate than only on biochar. The higher pyrene removal in the alginate-biochar treatment was likely due to the incorporation of the bacterial consortium in the sodium alginate beads allowing the substrate (contaminant) to move without restriction through the bead micropores (Deng et al., 2016; Wang et al., 2019a). In addition, the alginate gel protected the microbes from predators (Deng et al., 2016; Wang et al., 2019a). Entrapment on the bead also prevented the release of the degradative microorganisms from the bead and colonisation of the beads by native microbes into the bead (Lu et al., 2020). However, it may be difficult to make a firm conclusion in terms of hydrocarbon removal as the case study involved soil co-contaminated with pyrene and Cr (VI), and modified biochar was used. The results may differ if the soil is only contaminated with hydrocarbon and non-modified biochar is used. Entrapment suffers from some limitations, such as costs of immobilisation, deactivation during immobilisation, abrasion of biocarriers upon application, cell leakage, and diffusional and cell loading limitations (Nwankwegu and Onwosi, 2017).

2.2. Co-application of biochar with phytoremediation

In this section, biochar co-application with phytoremediation in form of plants or root exudate is discussed.

2.2.1. Phytoremediation using plants

Although the use of plants is an efficient technique for soil remediation, its efficacy in remediation is affected by the properties of the soil such as the soil pH, nutrient bioavailability, aeration, and water status (Hajabbasi, 2016). Therefore, the co-application of plants with amendments like biochar in the remediation of contaminated soil may be beneficial. Biochar can improve soil properties, such as the pH, nutrient content, oxygen supply, and water holding capacity in the soil (Hussain et al., 2018a), likely resulting in enhanced plant growth and development. This is one of the possible explanations for why higher hydrocarbon removal was reported in the co-applied treatment. The enhanced hydrocarbon removal in the co-applied treatment may also be associated with the activities taking place in the root (rhizosphere) (Li et al., 2020a; Valizadeh et al., 2022). Fig. 5 provides an overview of the mechanism for enhanced hydrocarbon removal in biochar-assisted phytoremediation.

Several authors have investigated the effect of combining biochar with phytoremediation on the remediation of hydrocarbon-contaminated soil (Table 3). Yousaf et al. (2022) evaluated the effect of biochar-assisted phytoremediation involving Fabaceae/leguminous (white clover and alfalfa) and Poaceae plants (ryegrass, maize, and wheat), combined with wood chip-derived biochar separately. Petroleum hydrocarbon removal in the biochar-assisted phytoremediation treatments (34–68 %) was higher than the sole biochar (27 %) and their respective phytoremediation treatments (9–60 %). Hydrocarbon removal was higher when biochar was combined with leguminous plants rather than with the Poaceae plants (Table 3). Furthermore, the TPH tolerant rhizospheric bacterial populations were higher in the biochar-assisted phytoremediation treatment than in the biochar or phytoremediation treatments (Yousaf et al., 2022). Other authors have observed a higher hydrocarbon-degrading rhizospheric bacterial population in biochar-assisted phytoremediation relative to sole phytoremediation treatment (Hussain et al., 2022; Hussain et al., 2018a).

Similarly, Li et al. (2020b) studied the effect of combining biochar and rye grass plant on the remediation of PAH (phenanthrene, pyrene, and benzo(*a*)pyrene) in a root box. After 100 days, increased hydrocarbon removal was found in the biochar-rhizosphere zone (49–51 %) compared to either the rhizosphere zone (41–49 %) or sole biochar treatment (39–44 %), especially with pyrene. Another study showed that phenanthrene, pyrene, and benzo(*a*)pyrene removal was significantly higher in the biochar-rhizosphere zone compared to the sole biochar treatment (Li et al., 2020a). However, only pyrene removal was significantly higher in the biochar-rhizosphere zone than in the sole rhizosphere zone. The combined biochar and rhizosphere treatment resulted in increased relative abundances of PAH degraders, with the cooperation between PAH degraders enhanced in the combined treatment (Li et al. (2020a). Further, the upstream functional gene responsible for PAH degradation was enhanced in the combined treatment (Li et al., 2020a). Li et al. (2020b) reported that in addition to modulating soil microbial community structures, microbial metabolic activity and specific carbon metabolism were enhanced with the combined treatment. In addition, the combination of biochar with the rhizosphere resulted in tighter co-occurrence networks between soil properties, the bacterial community, and metabolites, suggesting the role of energy resources and available nutrients for PAH removal (Li et al., 2020b).

A recent study observed that co-applying biochar at different application doses with rye grass resulted in increased PAH removal compared to phytoremediation or any of their respective biochar treatments (Zhao et al., 2022). In addition, PAH removal increased with biochar application

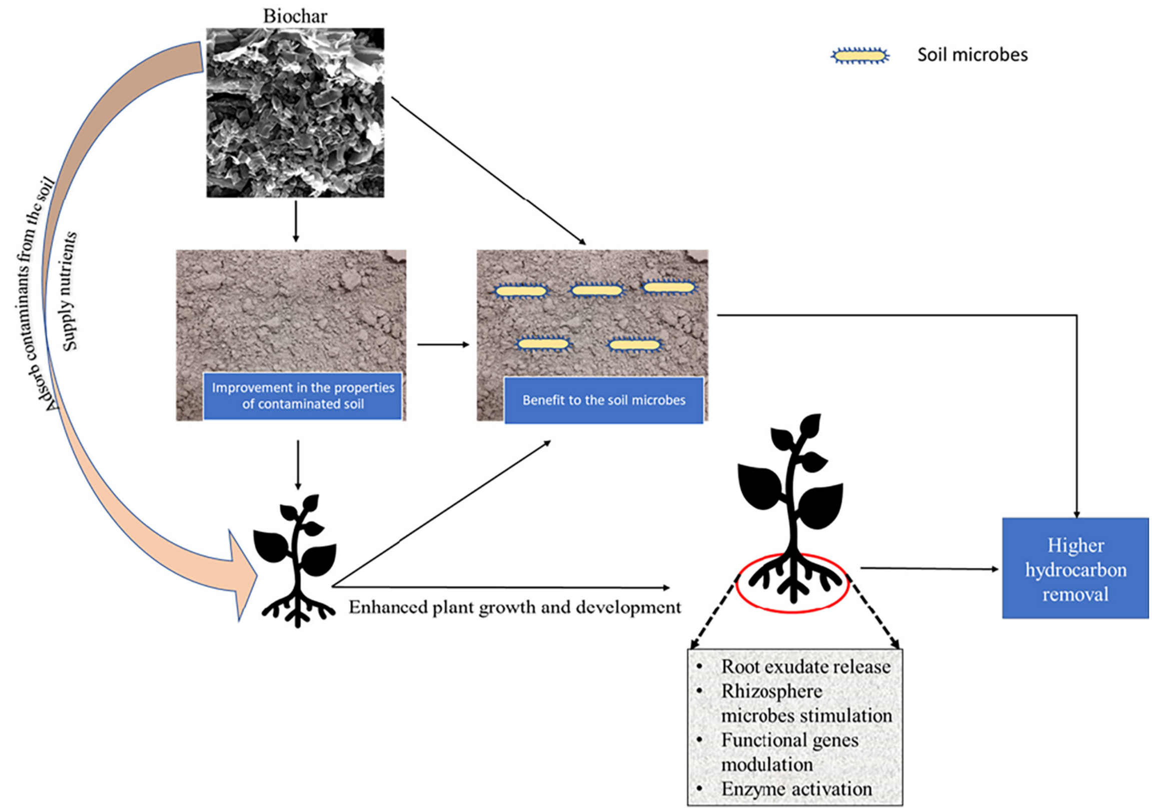


Fig. 5. Mechanism of enhanced hydrocarbon when biochar is co-applied with phytoremediation.

dose from 1 % to 2 % but decreased with a further increase in biochar application dose to 4 %. This decrease in PAH removal was suggested to be due to the reduction in the rhizospheric effect resulting from increases in soil pH, exchange capacity, and free radical injection (Zhao et al., 2022). In another study, the effect of combining carrot plant with either bamboo biochar produced at 700 °C (BB 700) or corncob biochar produced at 300 °C (CB 300), at different application doses (0.5 and 2 %) was assessed (Ni et al., 2017). The results of the study showed that in the non-rhizospheric soils, none of the biochar applications resulted in higher PAH removal compared to the control. However, in the rhizospheric soil, PAH removal was significantly higher in the 2 % CB 300 treatment than in the control and other biochar treatments (Ni et al., 2017). Differences in the biochar feedstock and pyrolysis temperature make it difficult to draw firm conclusions; however, the study suggests the influence of biochar application and production conditions in phytoremediation. Compared to BB 700, CB 300 has a higher nutrient status (ammonium N, nitrate N, P, K, and dissolved organic matter) and a lower sorption capacity (lower surface area, lower aromaticity, and higher polarity). The lower sorption capacity implies that more of the soil contaminant will be available for microbial degradation in soil amended with CB 300 than BB 700. This could have been responsible for the higher hydrocarbon removal observed in CB 300-rhizospheric-assisted treatment, relative to BB700 rhizospheric-assisted treatment. The increase in hydrocarbon removal with application dose in the CB 300 biochar-assisted phytoremediation treatment was due to an increase in the nutrient status of the soil with biochar application dose. In contrast, the decrease in PAH removal with increasing application dose in BB 700 treatment may be due to an increase in the PAH sorption and decrease in PAH bioavailability with application dose since BB 700 has a higher sorption capacity. The microbial diversity (Shannon index) and community composition in the 2 % BB 700-rhizospheric treatment did not differ from the rhizospheric control, while the Shannon index in the 2 % CB 300 rhizospheric treatment was significantly lower, compared to the rhizospheric control (Ni et al., 2017). In addition, the mean proportion of bacterial dioxygenase and dehydrogenase genes associated with PAH degradation (3–hydroxyanthranilate 3, 4–dioxygenase, NADP-dependent aldehyde dehydrogenase, and 4–hydroxyphenylpyruvate dioxygenase) were significantly higher in the 2 % CB300 rhizospheric treatment than the rhizospheric control or 2 % BB700 rhizospheric treatment (Ni et al., 2017).

In other studies, co-applying biochar with phytoremediation also resulted in higher hydrocarbon removal compared to their sole phytoremediation treatment (Table 3) (Deebika et al., 2021; Hussain et al., 2018a; Hussain et al., 2022; Sushkova et al., 2021; Zhen et al., 2019). For example, Hussain et al. (2018a) observed that hydrocarbon removal was higher in the biochar and ryegrass phytoremediation co-applied treatment (65 %) than in the phytoremediation treatment (47 %). Similarly, Deebika et al. (2021) and Zhen et al. (2019) reported a 32–45 % higher hydrocarbon removal in the biochar-assisted phytoremediation treatment than in the phytoremediation treatment. Hussain et al. (2022) and Saeed et al. (2021) observed that hydrocarbon removal was significantly higher in the biochar and phytoremediation co-applied treatment than in the phytoremediation treatment. In the study of Saeed et al. (2021), plant seeds were introduced after 20 days of biochar amendment. Some authors who found significantly higher hydrocarbon removal in the biochar-phytoremediation co-applied treatment reported that the studied plant parameters were significantly increased in the co-applied treatment in most cases (Hussain et al., 2018a; Yousaf et al., 2022; Zhen et al., 2019). For example, Hussain et al. (2018a) found that the germination rate, chlorophyll content, performance index (chlorophyll-based) and physiological parameters (fresh shoot biomass, dry shoot biomass and height of aerial parts) were significantly higher in the biochar-co-applied Italian ryegrass phytoremediation treatment than the sole phytoremediation treatment. However, others reported that sole phytoremediation performed better in enhancing the studied plant parameters than the biochar-assisted phytoremediation treatment (Deebika et al., 2021; Hussain et al., 2022).

In contrast to the above studies, Saum et al. (2018) found that hydrocarbon removal in the corn stalk biochar and mesquite plant phytoremediation

Table 3
Remediation studies showing the co-application of biochar with phytoremediation (plants) and biostimulation (surfactant and nutrients).

Biochar biomass/ pyrolysis temp (°C)	Biochar dose (%)	Plant/ biostimulation agent	Hydrocarbon removal (%)				Contaminant (Amount – mg/kg)	References
			Control	Biochar	Phytoremediation/ biostimulation	Co-application		
Rape straw/ 500	1	Plant – Rye grass	4	14	17	37	PAH (1.2)	(Zhao et al., 2022)
	2	Plant – Rye grass	4	27	17	53		
	4	Plant – Rye grass	4	39	17	41		
Wood chip	5[a]	Plant - Wheat	1	27	9	34	Oil (40000)	(Yousaf et al., 2022)
		Plant - Maize			22	51		
		Plant - Alfalfa			35	66		
		Plant - Ryegrass			60	61		
		Plant - White clover			11	68		
Corn stalks/ 350	1.5	Plant - Mesquite amargo	26	–	38	22	Automobile motor oil (2 % w/w)[b]	(Saum et al., 2018)
Rice husk/ 700	2[c]	Plant - *Spartina anglica*	9	–	19	28	Petroleum (30000)	(Zhen et al., 2019)
Green garden waste/ 500	5[d]	Plant - Italian ryegrass	12	–	47	65	Crude oil (3700)	(Hussain et al., 2018a)
	5		12	–	21	27	Crude petroleum (33800)	(Hussain et al., 2022)
Maize straw/ 500	5[e]	Plant - *Calendula officinalis* L	6	–	50	40	Crude oil (20000)	(Wang et al., 2021)
Bamboo/ 700	0.5	Plant - Carrot roots	21	13	35	31	PAHs (7481)	(Ni et al., 2017)
	2		21	8	35	23		
Corn straw/ 300	0.5	Plant - Carrot roots	21	20	35	38		
	2		21	14	35	52		
–	50[f]	Plant - Nutgrass	41	–	50	66	Crude oil (17)	(Deebika et al., 2021)
Maize Straw /500	1[e]	Plant - Ryegrass	44	39	49	51	Phenanthrene (47–51)	(Li et al., 2020b)[g]
			33	44	41	51	Pyrene (50–54)	
			27	39	42	49	Benzo(*a*)pyrene (10)	
Maize Straw /500	1[e]	Plant - Ryegrass	29	29	32	33	Phenanthrene (9)	(Li et al., 2020a)[g]
			13	27	31	37	Pyrene (9)	
			9	15	25	27	Benzo(*a*)pyrene (4–5)	
Sugarcane residues/ 550	1[h]	Biostimulation - Surfactant (rhamnolipid)	56	75	67	68	Crude oil (495–548)	(Wei et al., 2020a)
Rice husks/ 700	2.5[c]	Biostimulation - Surfactant (rhamnolipid)	6	32	–	30	Crude oil (50048)	(Zhen et al., 2021)
	2.5[i]	Biostimulation - Surfactant (rhamnolipid)	6	32	–	34		
Sugarcane residues/ 550	1[h]	Biostimulation - Inorganic nutrient (urea)	56	75	85	87	Crude oil (511–543)	(Wei et al., 2020a)
Bulrush straw/ 300	5	Biostimulation - Inorganic nutrient (N & P)	28	47	–	51	Petroleum (9620)	(Wang et al., 2017b)
Rice straw	195[j]	Biostimulation - Inorganic nutrient (N)	7	63	<11 > 7	78	Diesel (100)[b, k]	(Lawson et al., 2019)
		Biostimulation - Inorganic nutrient (P)	7	63	<11 > 7	75		
		Biostimulation - Inorganic nutrient (N & P)	7	63	11	80		
Wood brick	5	Biostimulation - Inorganic nutrient (N & P)	0.2	3	0.5	3	Oil	(Yu et al., 2019)
			0.2	3	1	2		
			0.2	3	2	4		
			0.2	3	0.3	2		
–	5	Biostimulation - Organic nutrient (corn straw)	58	61	–	73	PAH (2.2)	(Bao et al., 2020)
		Biostimulation - Organic nutrient (compost)				53		
Hardwood cordwood/ 400–430	2.5	Biostimulation - Organic nutrient (compost)	53	53	60	62	Diesel (95333)	(Uyizeye et al., 2019)
Sewage sludge/ 550	5[h]	Biostimulation - Organic nutrient (Manure)	36	76	–	83	Diesel (0.03)	Aziz et al. (2020)
Fruit/vegetable waste/ 550			36	72	–	83		
Wheat Straw/ 300	10	Biostimulation - Organic nutrient (Lignocellulosic substrate)	100	100	100	100	Phenanthrene (100)	(Cao et al., 2016)
			100	100	100	100	Benzo[*a*]pyrene (50)	
Pig bone/ 500		Biostimulation - Organic nutrient (Pig droppings)	4	67	77	72	Crude oil (2270–2302)	(Ugwoha et al., 2020)
			4	67	77	87		
			4	67	77	76		

[a] w/v.
[b] Soil was contaminated with the amount of the contaminant stated.
[c] wt%.
[d] % v/v.
[e] %.
[f] g.
[g] Carried out in a root box, which was separated into a root growth, rhizosphere, and non-rhizosphere zone with nylon sieve.
[h] % by weight.
[i] The biochar (1 g of biochar per 100 ml of water) was modified with the surfactant (600 mg). The dose here is the amount of the surfactant modified biochar added to the soil.
[j] mg/ha.
[k] mL/kg.

co-applied treatment was significantly lower than the sole phytoremediation treatment. In another study, the co-application of maize straw biochar with *Calendula officinalis* plant (40 %) did not result in higher hydrocarbon removal compared to sole phytoremediation (50 %) (Wang et al., 2021). Saum et al. (2018) and Wang et al. (2021) reported that the studied plant parameters were not enhanced by the application of biochar. Additionally, biochar application did not enhance the hexadecane-degrading and PAH-degrading bacterial population (Saum et al., 2018), nor enzyme activity (Wang et al., 2021). The results of these soil biological and plant parameter results indicate a non-beneficial role of biochar in phytoremediation. According to Wang et al. (2021), alteration of the rhizospheric soil microbial community resulting from increased pH and lower nutrient (phosphorus and nitrogen) content affected the ability of biochar to enhance phytoremediation. Nitrogen deficiencies were also suggested by Saum et al. (2018) to be responsible for reduced tree seedling shoot growth and oil degradation efficiency. During nitrogen deficiency, the root: shoot ratio of the plant is altered to increase the plant's capacity to uptake nutrients (Saum et al., 2018). This suggestion is supported by the observation that hydrocarbon removal in the biochar-assisted phytoremediation treatment increased following co-amendment with organic nutrient source (compost) (Saum et al., 2018; Wang et al., 2021).

Nitrogen deficiency in the biochar-amended soil is due to the increased demand for nitrogen due to the high C/N ratio of biochar (Brewer and Brown, 2012). Adding nutrients to the soil with biochar and phytoremediation may address this issue. Other authors have also reported biochar enhanced phytoremediation in the presence of nutrients (Abbaspour et al., 2020; Barati et al., 2017, 2018); however, others reported contrasting results in the presence of supplementary nutrients (Han et al., 2016; Ni et al., 2018; Zhang et al., 2021a). These contrasting observations on the role of additional fertiliser/nitrogen on biochar-assisted phytoremediation suggest that other factors reasons could have been responsible. These may include differences in feedstock type, application dose, and pyrolysis temperature. For example, Ni et al. (2018) under fertiliser-amended conditions, observed that the co-application of 2 % corn straw biochar (produced at 300 °C) with rice plants enhanced PAH removal, while bamboo biochar produced at 700 °C applied at the same dose inhibited PAH removal. The differences in the biochar properties contributed to this discrepancy.

Generally, biochar and phytoremediation co-application studies have not been carried out uniformly (Deebika et al., 2021; Hussain et al., 2022; Li et al., 2020a; Saum et al., 2018; Wang et al., 2021; Yousaf et al., 2022). For example, some authors cultivated the plant used in their study in another soil before transplantation (Deebika et al., 2021; Wang et al., 2021), while others planted seeds directly in the tested soil (Hussain et al., 2022; Li et al., 2020a; Saum et al., 2018; Yousaf et al., 2022). In some studies, seeds were introduced 20 days after biochar application to the contaminated soil (Saeed et al., 2021), while others co-applied seeds and biochar at the same time (Hussain et al., 2022; Li et al., 2020a; Saum et al., 2018; Yousaf et al., 2022). Assessing the impact of experimental methodology on the effect of biochar co-application is required since this could influence the efficiency of biochar-assisted phytoremediation. In addition, assessing the effect of different plants and biochar conditions (production and application-related) will be important in future research.

2.2.2. Phytoremediation using root exudates

Root exudates are considered the main driver responsible for controlling the composition, activity, and diversity of microbes in the rhizosphere (Correa-García et al., 2018). They release enzymes, such as peroxidases, laccases, cytochrome P450 monooxygenases, and dehalogenases capable of decomposing soil contaminants (Hoang et al., 2021). In addition, the exudates released from plant roots include specialised antimicrobials, signaling molecules, nitrogen, and carbon compounds that are of benefit to soil microbes and plants (Correa-García et al., 2018). However, some compounds in root exudates, such as glucose and glutamate can repress gene expression, while others can stimulate gene expression (Phillips et al., 2012). Antimicrobials can be detrimental to microbes. However, root exudates can be beneficial in biochar-based remediation owing to their properties. For example, co-applying biochar with root exudates can address the problem of reduced bioavailability of hydrocarbon associated with biochar studies because the root exudate acts as a surfactant by releasing sorbed hydrocarbon from the biochar and suppressing sorption to biochar (Ni et al., 2018; Song et al., 2016). The root exudate functions as a surfactant through:

(i) the production of low molecular weight aliphatic carboxylates that promotes desorption from the soil,
(ii) enhancement of hydrocarbon bioavailability because of increased hydrocarbon solubility,
(iii) the presence of biosurfactant-producing microorganisms in the root (Hussain et al., 2018b; Martin et al., 2014).

The mechanism for the first is not fully understood, although some assumptions have been proposed (Martin et al., 2014). Ni et al. (2018) demonstrated that root exudates can function as a surfactant in hydrocarbon-contaminated soil amended with biochar. The desorption of PAHs (release and extractability) from the soil was enhanced by artificial root exudate (ARE), mostly at a concentration of 10–20 mg/L, but not at low ARE concentrations (1 mg/L). Furthermore, Song et al. (2016) reported that sorption of hexachlorobenzene (HCB) to biochar was suppressed by oxalic acid (a low molecular weight organic carbon found in ryegrass root exudate), while the desorption of HCB from biochar and biochar-amended soil was enhanced by the root exudate. In another study, pyrene sorption to biochar was inhibited by different concentrations of oxalic acid, while phenanthrene solution was inhibited only at low oxalic acid concentration (1 mg/L), but not at high concentration (20–100 mg/L) (Li et al., 2019a). Some studies examined the effect of root exudate and biochar on hydrocarbon removal (Li et al., 2019b; Li et al., 2019c). Li et al. (2019c) observed that the co-application of biochar and oxalic acid resulted in significantly higher phenanthrene removal in soils contaminated with different concentrations of phenanthrene (2.5 and 650 mg/kg) in comparison to the sole biochar or oxalic acid treatment. In another study, the co-application of maize straw biochar with any oxalic acid concentration studied (0.5 and 20 mg/kg) resulted in significantly higher PAH removal than the sole biochar application ($p < 0.05$) (Li et al., 2019b). However, the co-applied treatment was only significantly higher than the sole oxalic acid treatment at a high oxalic concentration (20 mg/kg). The removal of PAH was enhanced in biochar amended soil with oxalic acid because root exudate enhanced the bioavailability of PAH sorbed to the biochar and the subsequent PAH degradation. Among the different PAH rings, the results of this study showed that the surfactant property of the root exudate was predominant in 2(+3)-ring PAHs because 2(+3)-ring PAH degradation was inhibited in the biochar treatment, likely due to sorption in comparison to the control. However, the inhibition effect was counteracted, and removal increased in treatments with biochar and oxalic acid, suggesting that the oxalic acid either suppressed its sorption to biochar or promoted its desorption from biochar. The relative abundance of PAH-degrading genera was significantly higher in the co-applied treatment than in the sole oxalic acid (not in all cases) or biochar treatment (Li et al., 2019b). Further analysis showed a positive correlation between these genera and PAH removal, suggesting the role of the promotion of these genera in PAH removal. Future studies should take advantage of the unique properties of root exudates (enzyme source, rich nutrient status, and surfactant nature) in improving the efficiency of biochar-mediated hydrocarbon removal. Since root exudates are nutrient-rich, applying them alongside biochar with low nutrient status may be a valuable intervention to make up for the nutrient deficiency in this type of biochar. Also, higher removal may be achieved by modifying biochar with root exudates using conventional methods, compared to introducing the biochar and root exudate individually without any interaction.

2.3. Co-application of biochar with biostimulation

This subsection deals with biochar co-application studies with either surfactants or nutrients (Table 3).

2.3.1. Biostimulation using surfactant

One of the challenges associated with the use of biochar alone to remediate hydrocarbon-contaminated soil is that it slows down the removal efficiency of the contaminant in the soil as it sorbs the hydrocarbon in the soil, making it unavailable for microbial metabolism (Xiong et al., 2017). Co-applying biochar with a surfactant can be useful in mitigating this challenge because a surfactant enhances the desorption of the hydrocarbon from the biochar (Kang et al., 2019). Based on this, it is anticipated that co-applying biochar with surfactant should result in higher hydrocarbon removal. However, Wei et al. (2020a) observed lower hydrocarbon removal in crude-oil contaminated soil co-applied with biochar and rhamnolipid (68 %) compared to sole biochar treatment (75 %). The results of this suggest that the surfactant slowed down the efficiency of biochar in remediation as the surfactant treatment (67 %) did not differ greatly from the co-applied treatment (68 %). However, the combined treatment was more effective in the removal of the heavy fraction (HFA) and high molecular weight PAHs than the sole biochar treatment, which could be linked to the relative abundance of the Proteobacteria phylum, which was higher in the combined treatment (Wei et al., 2020a). Zhen et al. (2021) found that biochar co-application with rhamnolipid did not result in enhanced hydrocarbon removal (30 %) in comparison to the sole biochar treatment (32 %). The negative result observed with the co-applied treatment in these two studies, despite the supposed benefit of co-applying both techniques could be due to the hindrance of the emulsifying effect of the surfactant by adsorption of clay or its degradation by soil microbes (Zhen et al., 2021). In addition, the ratio used may not be appropriate since Wei et al. (2020b) demonstrated the impact of rhamnolipid on the microbial community in oil-contaminated soil amended with biochar varied, based on the amount of rhamnolipid added to the biochar-amended soil. Since microbial communities are vital in soil remediation, there is a possibility that this will also affect hydrocarbon removal. In contrast, several studies carried out in the presence of fertiliser/nutrient source have demonstrated that biochar co-application with surfactant resulted in higher hydrocarbon removal (Brown et al., 2017; Wei et al., 2020a; Xiong et al., 2017).

One research group has conducted research based on modifying biochar with a surfactant (Zhen et al., 2019; Zhen et al., 2021). This approach may potentially address the issue of surfactant adsorption to the clay or microbial degradation of surfactants. Zhen et al. (2021) observed that the hydrocarbon removal rate in the surfactant (rhamnolipid)-modified biochar treatment was higher than the biochar and surfactant co-applied treatment without modification (biochar + rhamnolipid). The surface area and pore volume of the rhamnolipid-modified biochar decreased compared to the original biochar, which could suggest a reduction in the sorption capacity of the biochar and a resultant increase in the bioavailability of hydrocarbon. This is likely responsible for the higher hydrocarbon removal in the rhamnolipid-modified biochar. Also, dehydrogenase activity, an indicator of microbial activity, was slightly higher in the rhamnolipid-modified biochar treatment compared to the treatment with biochar or biochar + rhamnolipid treatment (Zhen et al., 2021), which corroborates the suggestion that the bioavailability of the hydrocarbon increased with the addition of rhamnolipid-modified biochar. In another study from the same group, surfactant-modified biochar performed slightly better in enhancing phytoremediation (35 %) compared to the biochar + rhamnolipid separate treatment (32 %) (Zhen et al., 2019). Although, these present studies suggest only a small difference between the surfactant modified biochar and the biochar + rhamnolipid co-application, there appears to be potential in the application of surfactant-modified biochar for hydrocarbon degradation considering the benefits of the interactive co-application. What is now required is to alter the amendment type (biochar and surfactant) and co-application ratios.

There is a possibility that co-applying biochar with surfactant in a sequential manner may result in higher TPH removal than simultaneous application, since Wei et al. (2020a) reported that the simultaneous application of biochar and rhamnolipid would act opposing each other, with the surfactant increasing the contaminant in the soil available for microbial degradation, while biochar decreases the accessibility of microbes to the contaminants. The sequential co-application entails adding the biochar at the beginning of the incubation and adding the surfactant at the later stage. If the biochar is added at the beginning, the biochar will sorb some of the hydrocarbons in the soil, leaving some for the microorganisms to metabolise. As the remediation progresses, the amount of contaminant in the soil will reduce. Adding the surfactant at the later stage may desorb the sorbed hydrocarbons from the biochar and thus make them available for microbial metabolism. In doing this, the hydrocarbon will be slowly released to the soil microbes.

2.3.2. Biostimulation using nutrients

Although biochar has been associated with the provision of nutrients to soil microbes (Zhu et al., 2017), it may not be a substitute for nutrients in soils, especially in biochar with a high C/N ratio. Soils contaminated by oil are characterised by nitrogen deficiency since oil is composed mainly of C and H (Chorom et al., 2010), and adding biochar (usually high C/N biomass) to the soil may further increase the soil C/N ratio and cause nitrogen immobilisation. A previous study reported that the high biochar C/N ratio and aromaticity of most of the biochar C resulted in low microbial (bacterial and fungal) development and the subsequent lower PAH removal in biochar-amended treatment (García-Delgado et al., 2015). Co-applying contaminated soils amended with biochar with nutrients or fertiliser could address this problem.

2.3.2.1. Inorganic nutrient/fertiliser. Biochar-based remediation of hydrocarbon-contaminated soils has been carried out in the presence or absence of supplementary inorganic nutrient/fertiliser (Agarry et al., 2015; García-Delgado et al., 2015; Guirado et al., 2021; Kong et al., 2018; Qin et al., 2013; Zhang et al., 2018b; Zhang et al., 2020). The results have been both positive and negative compared to the non-biochar treatment, irrespective of nutrient presence or absence. Some authors observed that amending biochar with fertiliser/inorganic nutrient resulted in enhanced hydrocarbon removal compared to the fertiliser control (Qin et al., 2013; Zhang et al., 2019), whereas others did not (Guirado et al., 2021). Furthermore, some studies have assessed the effect of biochar co-application with inorganic nutrients on hydrocarbon removal (Lawson et al., 2019; Wang et al., 2017b; Wei et al., 2020a; Yu et al., 2019). Higher hydrocarbon removal was observed in soils amended with both biochar and nutrient compared to sole biochar or nutrient amendment (Lawson et al., 2019; Wang et al., 2017b; Wei et al., 2020a). Wang et al. (2017b) found that the hydrocarbon removal was 9.4 % higher in soils amended with both biochar and inorganic nutrient (K_2HPO_4 and $(NH_4)_2SO_4$), compared to sole biochar treatment. A slightly higher microbial population was observed in the co-applied treatment compared to their sole application, confirming that supplementing biochar amended soils with inorganic nutrients was beneficial to the microbial population. Another study observed that the hydrocarbonoclastic population was significantly higher in treatments with biochar co-application with different nutrients ($p < 0.05$) at day 40 (Lawson et al., 2019). Biochar was able to retain more nutrients on its surface and pore volume when it was co-applied with nutrients, compared to sole biochar application (Wang et al., 2017b). This resulted in increased nutrients (P_2O_5 and total N) in the co-applied treatment, which could have contributed to enhanced degradation and microbial population (Wang et al., 2017b). Wei et al. (2020a) observed that the community structure of the dominant bacterial phyla in the sole biochar treatment showed a close resemblance to the oil-contaminated control at week 7. However, when the contaminated soil was co-applied with both biochar and nitrogen, the dominant bacterial phyla differed from the biochar-amended soil (Wei et al., 2020a). For example, *Epsilonbacteraeota* and *Chloroflexi* were no longer the dominant phyla in the biochar and nitrogen co-applied treatment. The disappearance of *Chloroflexi* as an abundant phylum in the biochar and nitrogen co-applied treatment could have contributed to higher hydrocarbon removal in this treatment since another study has observed lower hydrocarbon removal in treatment where *Chloroflexi* replaced *Proteobacteria* as the most abundant phyla (Bao et al., 2020). Further, various bacterial diversity measures (Shannon, ACE, and Chao1) were lower in biochar and nitrogen co-applied treatment at week 7, while Simpson diversity slightly increased in the co-applied treatment (Wei et al., 2020a).

Yu et al. (2019) examined the effect of wood brick biochar (5 % wt) and nutrients at different ratios (C/N/P of 100:5:1, 100:10:1, 100:15:1, and 100:25:1) on petroleum degradation. They observed that the removal of TPH was higher with biochar and nutrient co-application at C/N/P of 100:15:1 (4 %), compared to a sole biochar treatment (3 %). However, the sole biochar application was higher in terms of TPH removal, relative to when biochar was co-applied with nutrients at other ratios (2–3 %) (Yu et al., 2019). The findings of this study suggest that nutrient application was not beneficial when it was applied at low or very high concentrations. In another study, biochar was co-applied with nitrogen and/or phosphorus (Lawson et al., 2019). Hydrocarbon removal was found to be higher in all biochar and nutrient co-applied treatments (75–80 %) compared with the sole biochar (63 %) (Lawson et al., 2019); however co-applying biochar with both nitrogen and phosphorus (80 %) did not result in a significant difference in hydrocarbon removal compared with biochar co-application with only nitrogen (78 %) or phosphorus (75 %). Although this result suggests no difference in terms of remediation efficiency among the various biochar and nutrient co-applied treatments, nitrogen was more beneficial in promoting biochar-based remediation.

So far, in the discussion, nutrients and biochar have been added separately to the soil without any interaction. However, biochar-based fertiliser has gained relevance as a beneficial soil amendment (Sim et al., 2021). In biochar-based fertiliser, biochar is impregnated with fertiliser/nutrient, or co-pyrolysed with nutrient/fertiliser, or mixed and ground with solid nutrients/fertiliser (Fig. S1) (Sim et al., 2021). Biochar serves as a carrier of nutrients and a slow releaser of nutrients to soils (Ghezzehei et al., 2014; Gwenzi et al., 2018). Biochar-based fertiliser has found application in agriculture (El Sharkawi et al., 2018; Qian et al., 2014; Yang et al., 2021). However, no reports have assessed the effect of biochar-based fertiliser on the remediation of hydrocarbon-contaminated soil. Hydrocarbon removal may be greater in soils amended with the biochar-based fertiliser than where biochar and fertiliser are added individually, due to the prevention of nutrient loss through biochar retention of nutrients. In addition, the slow release of nutrients to the soil may prevent the presence of an excessive amount of nutrients in the soil. Future studies are required to examine the use of biochar-based fertiliser in remediation. Understanding the cost as well as the ecotoxicological impact of the tandem combination of biochar and nutrient associated with changes in biogeochemical cycling, and ecosystem functioning are imperative in biochar research.

2.3.2.2. Organic nutrients. The use of organic nutrients may be more economical than inorganic nutrients as they can be sourced from a wide range of waste materials and at a significantly lower cost. Biochar has been co-applied with several organic substrates like crop residues, manure, and compost (Table 3) (Aziz et al., 2020; Bao et al., 2020; Cao et al., 2016; Uyizeye et al., 2019). Crop residues can be a source of nutrients and microbes to the soil because crop residues have their microflora and possess degrading intermediates (Shahsavari et al., 2015). Because of these benefits, co-applying biochar with crop residues in remediation may be useful in promoting biochar-based remediation. Bao et al. (2020) found that co-applying biochar with corn straw was more effective in PAH remediation (73 %) than sole biochar application (61 %). Soil respiration, dissolved organic carbon (DOC), and microbial biomass carbon were increased, confirming that the co-application of biochar with the crop residue was beneficial in promoting microbial activity and population. The bacterial community structure and the relative abundance of bacteria at the phylum level differed when biochar was co-applied with corn straw, compared to sole biochar treatment (which was similar to the control). At the genus level, the abundance of *Bacillus*, *Porphyrobacter*, *Sphingomonas*, *Lysobacter*, *Ohaekwangia*, and *Rhizobium* significantly increased when biochar was co-applied with corn straw in comparison to the control and biochar treatments. Most of these genera of bacteria have been reported as PAH degraders (Li et al., 2019b; Wang et al., 2016). Additionally, mean proportions of the functional gene associated with PAH degradation (4-hydroxyphenylpyruvate dioxygenase, salicylate hydroxylase, 3-hydroxyanthranilate 3,4-dioxygenase, 1,3,7-trimethyluric acid 5-monooxygenase, and NADP-dependent aldehyde dehydrogenase) were significantly higher in the co-applied treatment (Bao et al., 2020). The enhanced PAH degradation observed in the combined treatment may be due to the significant increase ($p < 0.05$) in DOC, the abundance of PAH degrading bacteria, and functional genes (Bao et al., 2020). In contrast, Cao et al. (2016) reported that the application of 10 % (*w*/w) biochar and lignocellulosic substrate was not beneficial for benzo[*a*] pyrene and phenanthrene removal (100 %), relative to the sole biochar treatment (100 %) at day 56.

Co-applying biochar with manure has also found relevance in the remediation of hydrocarbon-contaminated soil. Aziz et al. (2020) examined the effect of cow dung manure and biochar derived from either vegetable/ fruit waste or sewage sludge on diesel degradation. Co-applying any biochar with manure resulted in at least 8 % higher diesel degradation than their respective sole biochar treatment. Total nitrogen, potassium, phosphorus, organic matter, and total organic carbon were found to be higher in any of the co-applied treatments on day 180, in comparison to their respective sole biochar treatment. This suggests that supplementing the biochar treatment with additional nutrient sources resulted in the availability of more nutrients in the soil. The availability of more nutrients may have resulted in improved microbial activities in the co-applied treatment since enzymatic activities were generally higher in the co-applied treatment. The application dose of manure/biochar plays a vital role in the efficiency of biochar-manure-based degradation. Ugwoha et al. (2020) examined the effect of different ratios of biochar and pig droppings (biochar:pig droppings ratio of 20:80, 50:50, and 80:20 %). Hydrocarbon removal in the different biochar:pig droppings ratio was found to be higher than the sole biochar treatment; however, only a 50:50 % ratio was higher than the sole pig-dropping treatment. Among the co-applied treatment, the 50:50 % biochar:pig droppings ratio performed best, followed by the 20:80 and 80:20 % ratios. The maximum removal observed when biochar and pig droppings were applied in equal proportion was because of the complementary effect of both substrates in providing different soil nutrients (Ugwoha et al., 2020). Biochar and pig droppings are richer in phosphate and nitrate, respectively.

Compost, a low-cost organic material has also been co-applied with biochar. Uyizeye et al. (2019) reported a higher TPH removal in biochar and compost co-applied treatment (62 %) than the sole biochar (53 %) or compost treatment (60 %) (Uyizeye et al., 2019). The compost was derived from yard waste, garden waste, and kitchen scraps. Results of their study showed that the organic matter, respiration, extractable phosphorus, aggregate stability, extractable potassium, magnesium, and zinc were higher in the co-applied treatment than the sole biochar treatment, while iron and manganese were lower in the co-applied treatment than the sole biochar treatment. In another study, the co-application of biochar with compost (53 %) resulted in lower PAH removal than the sole biochar (61 %) or control treatment (58 %) (Bao et al., 2020). The inhibition of PAH degrading bacteria and functional genes in the co-applied treatment was suggested as the reason for the lower hydrocarbon degradation in the biochar and compost co-applied treatment (Bao et al., 2020). Compared to the control and biochar treatment, the combined treatment significantly decreased ($p < 0.05$) the mean proportion of 18 PAH functional genes and the relative abundances of PAH degrading genera like *Porphyrobacter*, *Rhizobium*, and *Lysobacter*. Microbial biomass carbon, DOC, and microbial respiration were higher in soil amended with both biochar and compost relative to the control or biochar treatment, which suggests that co-applying biochar with compost could have increased soil nutrient concentrations and the microbial population and related activities (Bao et al., 2020). Despite higher microbial respiration in the combined treatment, PAH degradation was not enhanced in comparison to the biochar or control treatment. Another study reported no correlation between TPH removal and microbial respiration (Uyizeye et al., 2019). The introduction of other microbial species that inhibited the growth of PAH degraders could be responsible (Bao et al., 2020). For example, *Proteobacteria* was displaced by *Chloroflexi* as the most dominant bacterial phylum in the co-applied treatment on day 28 (Bao et al., 2020). *Proteobacteria* have been reported as the dominant bacteria in a previous bioremediation study (Qin et al., 2013). The high abundance of *Chloroflexi* was suggested to originate from the compost (Bao et al., 2020) because studies have shown that *Chloroflexi* is one of the most dominant phyla in compost (Liu et al., 2018; Yang et al., 2019).

To date, all reported studies on biochar and compost co-application in hydrocarbon remediation have involved adding biochar (B) and compost (C) as a mixture to hydrocarbon-contaminated soil without any interaction (B + C). Aside from this, biochar and compost can be introduced into the soil in an interactive format as either a composted biochar (BCed, without compost) or by biochar-compost (BCing, biochar, and biomass mixed then composting) (Wu et al., 2017). Karami et al. (2011) recommended that future research should be directed towards the interaction between biochar and other amendments if the full potential of using both amendments is to be realised. These two interactive methods of biochar introduction (BCed or BCing) may be more effective for the remediation of hydrocarbon-contaminated soil than the conventional way (B + C) because when applied as a mixture (B + C), the benefits of the strong interaction of the two organic substrates may be lacking (Zeng et al., 2015). Co-applying biochar with compost in an interactive form can alter the properties of biochar such as the water extractible organic carbon, O/C ratio, moisture content, cation exchange capacity, organic matter, surface area, and the functional group in biochar (Wu et al., 2017). There is need for future studies to assess the effect of BCed and BCing on the remediation of hydrocarbon-contaminated soil.

Overall, the introduction of biochar and organic nutrient to the soil in the form of biochar-based fertiliser appears promising in hydrocarbon removal (Section 2.3.2.1, Fig. S1). Also, further research utilising advanced analytical techniques such as Fourier transformed ion cyclotron resonance mass spectrometry (FTICR-MS) are likely to provide valuable insights into the molecular structure of the soil organic matter (Zhang et al., 2021b).

2.4. Summary of biochar co-application with non-hybrid bioremediation technique

Data obtained from published studies were used to assess the general effectiveness of the three co-application techniques compared to their sole treatment (biochar, bioaugmentation, phytoremediation, and biostimulation). The result is presented in Fig. 6. Studies, where both sole biochar and complementary treatment (bioaugmentation, phytoremediation, and biostimulation) were not provided were omitted. In comparison to the sole treatment (biochar, bioaugmentation, phytoremediation, and biostimulation), co-applying biochar with bioaugmentation was more beneficial in terms of hydrocarbon removal (mean = 54–72 % higher than their respective sole biochar or bioaugmentation treatment, n = 16), followed by biochar co-application with phytoremediation (mean = 43–68 % higher than their respective sole biochar or phytoremediation treatment, n = 17) and biostimulation (mean = 11–15 % higher than their respective sole biochar or biostimulation treatment, n = 12). The Tukey Post HUC test showed that biochar co-application with bioaugmentation or phytoremediation differed significantly from their sole biochar or complementary (bioaugmentation or phytoremediation) treatment ($p < 0.05$). However, co-application with biostimulation did not differ significantly from either sole biochar or biostimulation treatment ($p < 0.05$). In cases where biostimulation involves a surfactant, the counteracting effect of biochar and surfactant may be responsible for their reduced efficiency (Wei et al., 2020a). Biochar and surfactant would act in opposing directions when co-amended in the soil since the biochar and the surfactant will work to sorb and desorb the contaminant from the soil, respectively (Wei et al., 2020a).

Although there was evidence to show that biochar co-application resulted in higher hydrocarbon removal, most results did not suggest any synergistic or additive interactions occurring between biochar and its co-applied treatment (Fig. 6, Tables 2 and 3). The study of Ren et al. (2020) was excluded due to their experimental setup (only one sole biochar treatment result was provided out of the 12 different biochar types used). Considering that bioaugmentation, phytoremediation, or biostimulation could on their own enhance hydrocarbon removal, a synergistic or additive effect on hydrocarbon removal would have been generally expected in most of the comparative studies. However, the results of Guo et al. (2021) involving biochar co-application with bioaugmentation (free-living organism) revealed that the co-application technique resulted in a synergistic effect on hydrocarbon removal. Similarly, Yousaf et al. (2022) observed that co-applying biochar with white clover resulted in a synergistic effect; however, the co-application of the same biochar with other plant types (alfalfa, ryegrass, maize, and wheat) did not result in a synergistic effect. Lawson

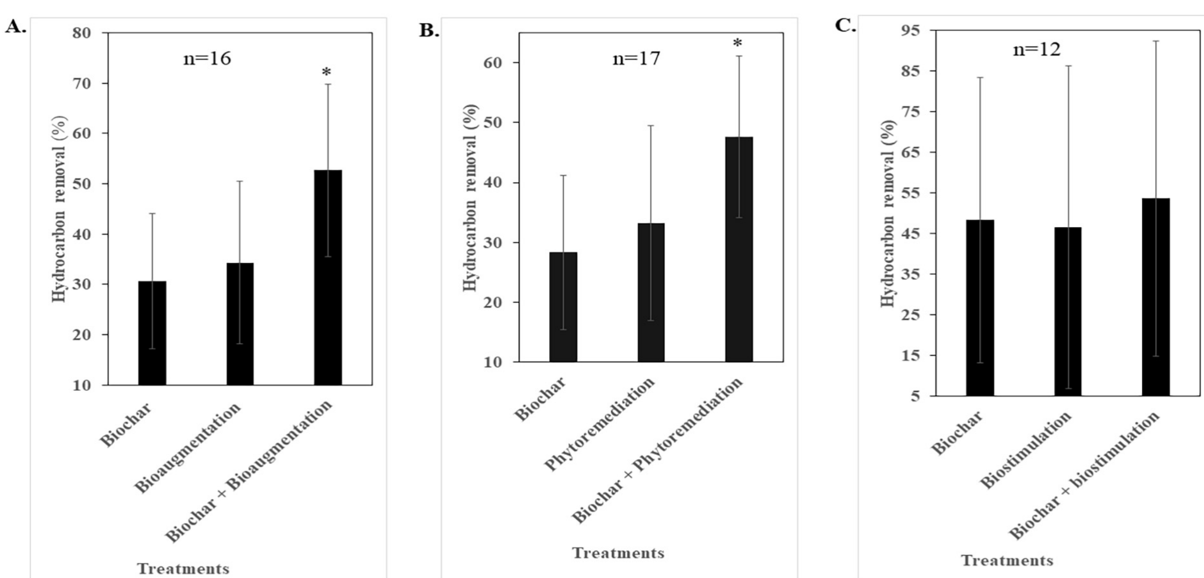


Fig. 6. Comparison of the mean of hydrocarbon removal in studies on biochar co-application with A. bioaugmentation; B. Phytoremediation; and C. Biostimulation. Values are the mean of the data from different studies, while the error bar is the standard deviation of the mean. * signifies that the mean of the co-application differs significantly ($p < 0.05$) from their biochar treatment and respective complementary treatment, based on Tukey Post HUC test. The number of biochar results used for the biochar co-application with bioaugmentation, phytoremediation, and biostimulation is 16, 17, and 12, respectively. Only co-application studies with the comparison with both biochar and any of the bioremediation techniques (bioaugmentation, phytoremediation, and biostimulation) were included. (Cao et al., 2016; Chen et al., 2012; García-Delgado et al., 2015; Guo et al., 2021; Guo et al., 2022; Lawson et al., 2019; Li et al., 2020a; Li et al., 2019b; Li et al., 2019c; Li et al., 2020b; Ni et al., 2017; Piscitelli et al., 2019; Song et al., 2021; Ugwoha et al., 2020; Uyizeye et al., 2019; Wei et al., 2020a; Yousaf et al., 2022; Yu et al., 2019; Zhao et al., 2022).

et al. (2019) observed that co-application resulted in higher hydrocarbon removal than the sum of the hydrocarbon removal efficiency of the sole biochar and biostimulation. What we can infer from the results of Yousaf et al. (2022), Guo et al. (2021) and Lawson et al. (2019) is that co-application would result in an additive or synergistic effect if the complementary treatment (bioaugmentation, phytoremediation, and biostimulation) and controls are less effective in hydrocarbon removal, while the sole biochar treatment have a high removal efficiency (Tables 2 and 3). For example, Yousaf et al. (2022) examined the effect of co-applying biochar with different plants (Table 3) and found that co-application resulted in an additive or synergistic effect on hydrocarbon removal only when co-applied with plants with lower hydrocarbon removal than those with higher hydrocarbon removal (Table 3). This observation may not apply in all cases considering the heterogeneity of co-application parameters and treatment condition as seen in Table 3. For example, a recent study showed that synergistic effect was found in co-applied treatments when biochar showed lower removal; however, antagonistic effects were reported in the co-applied treatment when biochar showed higher removal (Zhao et al., 2022).

While we anticipate more biochar co-application studies, it is important for researchers to critically consider biochar co-application, since the advantage of co-application may be reduced if it is not resulting in at least an additive effect. One challenge so far in biochar co-application-based studies is the lack of complementary sole treatment with any biochar, phytoremediation, bioaugmentation, and biostimulation sole treatment in several studies (Aziz et al., 2020; Bao et al., 2020; Deebika et al., 2021; Galitskaya et al., 2016; Hussain et al., 2018a; Hussain et al., 2022; Saum et al., 2018; Hussain et al., 2022; Wang et al., 2021; Wang et al., 2017b; Zhen et al., 2019; Zhen et al., 2021). The consequence of this is the inability to understand if the co-application resulted in an antagonistic, additive, or synergistic effect on hydrocarbon removal. The use of appropriate sole treatments should be considered in future studies.

3. Co-application of biochar with hybrid bioremediation techniques

Several studies have examined the effect of co-applying biochar with more than one bioremediation technique in any combination (Table 4). Some observed that biochar co-application with two techniques resulted in higher hydrocarbon removal than co-applying biochar with one technique (Abbaspour et al., 2020; Brown et al., 2017; Deebika et al., 2021; Wei et al., 2021; Wei et al., 2020a; Zhang et al., 2019). Zhang et al. (2019) observed that biochar co-application with both nutrient (fertiliser) and bioaugmentation resulted in 52–95 % higher hydrocarbon removal than the soil amended with both biochar and fertiliser. Similarly, Brown et al. (2017) reported a higher hydrocarbon removal of 53 % in treatments where biochar was co-applied with fertiliser and surfactant (rhamnolipid) compared with treatment with biochar and fertiliser (44 %). Abbaspour et al. (2020) observed that hydrocarbon removal was higher in soils when biochar was co-applied with fertiliser and phytoremediation (53–55 %) than in soils amended with both biochar and fertiliser (27 %). In another case, co-applying biochar with surfactant (68 %) in an oil-contaminated soil did not result in higher removal compared to sole biochar treatment (75 %); however, when biochar was co-applied with both surfactant and nitrogen, hydrocarbon removal was higher (87 %) (Wei et al., 2020a). Nitrogen played a dominant role in hydrocarbon removal in this study because hydrocarbon removal in biochar and nitrogen co-applied treatment (87 %) did not differ to a great extent from the sole nitrogen treatment (85 %) (Table 4). In other studies, co-applying biochar with fertiliser and phytoremediation resulted in higher hydrocarbon removal than fertiliser-assisted phytoremediation treatment (Barati et al., 2017, 2018).

Other studies have examined the effect of co-applying biochar with more than two techniques (Table 4) (Abbaspour et al., 2020; Ali et al., 2021; Ali et al., 2020; Hussain et al., 2018a). The results from these studies showed that the hydrocarbon removal generally increased as the number of remediation techniques biochar is co-applied with increased. Hussain et al. (2022) observed that co-application of biochar with three techniques (bioaugmentation, compost, and phytoremediation) resulted in significantly higher hydrocarbon removal compared to co-applying biochar with one or two techniques. Similarly, Ali et al. (2021) reported that the diesel removal efficiency increased as the number of remediation techniques biochar is co-applied with increased. In another study, Hussain et al. (2018a) combined biochar with one, two, or three techniques (Table 4). They observed that biochar co-application with only phytoremediation resulted in 65 % hydrocarbon removal, while a further combination with either microbial consortia or compost increased the removal efficiency to 75–82 %. When all the techniques were combined (biochar, phytoremediation, microbial consortia, and compost), hydrocarbon removal increased to 85 % (Hussain et al., 2018a). In another study, combining biochar and nutrient (minimal medium) with either bioaugmentation or surfactant resulted in higher PAH removal (Xiong et al., 2017). A further combination involving biochar, nutrient (minimal medium), bioaugmentation, and surfactant resulted in both increased or reduced PAH removal compared to combined treatment without bioaugmentation or surfactant, respectively (Table 4).

In contrast, other studies have reported that biochar co-application with more than one technique was not beneficial in terms of hydrocarbon removal (Han et al., 2016; Hussain et al., 2022; Ni et al., 2018; Wang et al., 2021; Zhang et al., 2021a). Hussain et al. (2022) reported that although hydrocarbon removal in the biochar-phytoremediation treatment was significantly higher than in the phytoremediation treatment, co-applying both techniques (biochar and phytoremediation) with either bioaugmentation or compost did not enhance hydrocarbon removal. Wang et al. (2021) and Saum et al. (2018) similarly reported that compared to the sole phytoremediation treatment, biochar co-application with phytoremediation was not beneficial in terms of hydrocarbon removal. When phytoremediation and compost were further co-applied with biochar, hydrocarbon removal was significantly higher than biochar-assisted phytoremediation treatment but did not differ significantly from the phytoremediation treatment (Saum et al., 2018; Wang et al., 2021). Abbaspour et al. (2020) also found no significant difference in hydrocarbon removal when the number of techniques biochar was co-applied with increased from two (phytoremediation and fertiliser) to three (phytoremediation, fertiliser, and fungal cells). Other authors observed that even combining biochar with two techniques (nutrient and phytoremediation) did not enhance hydrocarbon removal compared to the nutrient-assisted phytoremediation treatment (Han et al., 2016; Ni et al., 2018; Zhang et al., 2021a). Cao et al. (2016) showed that combining biochar with the organic substrate was not beneficial in phenanthrene and benzo[*a*]pyrene removal, compared to the biochar or control treatment. When biochar was further combined with both surfactant and organic substrates, no beneficial effect on hydrocarbon removal was observed in comparison to the biochar (Table 4) (Cao et al., 2016).

While it appears that in some cases biochar co-application with more than one technique could result in enhanced hydrocarbon removal relative to the sole biochar or biochar tandem application with one technique, the remediation cost will increase as the number of techniques used increases. Brown et al. (2017) reported that the estimated cost to achieve 50 % hydrocarbon removal using a combined treatment of biochar and fertiliser was US$12/m^3 soil. However, when surfactant was further combined with both amendments (biochar and fertiliser), the estimated cost increased to US$165/m^3 soil. Biological techniques must be able to compete favourably with other remediation techniques in terms of cost to get wider acceptance. Caution should be applied in combining biochar with hybrid techniques, since the goal of bioremediation may be defeated if cost-effectiveness is not achieved. For example, Hussain et al. (2018a) observed that increasing the number of techniques biochar combined from two to three did not cause a significant difference in hydrocarbon removal (Table 4).

4. Modification of biochar

The properties of biochar can influence their efficacy in hydrocarbon bioremediation. Some of the properties of biochar that could impact the

Table 4
Some summarised studies showing biochar co-application with hybrid bioremediation techniques.

Techniques	Treatments	Hydrocarbon removal (%)	Contaminant (Amount-mg/kg)	References
Biochar, biostimulation (inorganic nutrient - fertiliser) & bioaugmentation	Biochar + fertiliser + bioaugmentation	45–58	Petroleum (47,700)	(Zhang et al., 2019)
	Biochar + fertiliser	30		
	Nutrient + bioaugmentation	39		
	Fertiliser	9		
Biochar, biostimulation (inorganic nutrient - fertiliser), phytoremediation & bioaugmentation	Biochar + Fertiliser + Phytoremediation +bioaugmentation	57–58	Oil (16,790)	(Abbaspour et al., 2020)
	Biochar + fertiliser + bioaugmentation	20		
	Biochar + fertiliser + phytoremediation	53–55		
	Biochar + fertiliser	26		
	Phytoremediation + fertiliser	13–30		
	Fertiliser + bioaugmentation	15		
	Fertiliser	9		
Biochar & biostimulation (surfactant and inorganic nutrient - fertiliser)	Biochar + fertiliser + surfactant	53	Crude (9957–11,736)	(Brown et al., 2017)
	Biochar + fertiliser	44		
	Surfactant + fertiliser	33–37		
	Fertiliser	43		
Biochar, phytoremediation, bioaugmentation, biostimulation (organic nutrient – compost)	Biochar + phytoremediation + bioaugmentation + compost	85	Crude oil (3,700)	(Hussain et al., 2018a)
	Biochar + phytoremediation + compost	75		
	Biochar + phytoremediation + bioaugmentation	82		
	Biochar + phytoremediation	65		
	Phytoremediation	47		
Biochar, bioaugmentation & biostimulation (organic nutrient - digestate)	Biochar + bioaugmentation + digestate	24	Oil (6,200)	(Gielnik et al., 2019)
	Biochar	10		
	Digestate	9		
Biochar, bioaugmentation & biostimulation (organic nutrient - digestate)	Biochar + bioaugmentation + digestate	37	Motor oil (32,600)	(Gielnik et al., 2019)
	Biochar	24		
	Digestate	24		
Biochar, phytoremediation & biostimulation (organic nutrient -compost)	Biochar + phytoremediation + compost	42	Motor oil (20,000)	(Saum et al., 2018)
	Biochar + phytoremediation	23		
	Phytoremediation + compost	44		
	Phytoremediation	39		
Biochar, phytoremediation & biostimulation (surfactant)	Biochar + phytoremediation + surfactant	32–35	Petroleum (30,000)	(Zhen et al., 2019)
	Biochar + phytoremediation	28		
	Phytoremediation	20		
Biochar, phytoremediation & biostimulation (organic nutrient -compost)	Biochar + phytoremediation + compost	60	Crude oil (200,000)	(Wang et al., 2021)
	Biochar + phytoremediation	40		
	Compost + phytoremediation	73		
	Phytoremediation	50		
Biochar, phytoremediation & biostimulation (inorganic nutrient - fertiliser)	Biochar + phytoremediation + fertiliser	74–77	PAH (19)	(Zhang et al., 2021a)
	Phytoremediation + fertiliser	77		
Biochar & biostimulation (surfactant & organic nutrient – lignocellulosic substrates)	Biochar + lignocellulosic substrates + surfactant	100	Phenanthrene (100)	(Cao et al., 2016)
	Biochar + lignocellulosic substrates	100		
	Biochar	100		
	Lignocellulosic substrates	100		
Biochar & biostimulation (surfactant & organic nutrient – lignocellulosic substrates)	Biochar + lignocellulosic substrates + surfactant	100	Benzo[*a*]pyrene (50)	(Cao et al., 2016)
	Biochar + lignocellulosic substrates	100		
	Biochar	100		
	Lignocellulosic substrates	100		
Biochar & biostimulation (surfactant & nutrient)	Biochar + surfactant + nutrient	91	Crude oil (495–548)	(Wei et al., 2020a)
	Biochar + surfactant	68		
	Biochar + nutrient	87		
	Biochar	75		
	Surfactant	67		
	Nutrient	85		
Biochar, bioaugmentation & biostimulation (surfactant & nutrient (minimal medium))	Biochar + nutrient (minimal medium)	17	PAH (668)	(Xiong et al., 2017)
	Biochar + surfactant + nutrient (minimal medium)	23		
	Biochar + bioaugmentation + nutrient (minimal medium)	46		
	Biochar + bioaugmentation + nutrient (minimal medium) + surfactant	27		
	Surfactant + nutrient (minimal medium)	23		
	Nutrient (minimal medium)	3		

bioremediation of hydrocarbon-contaminated soil are highlighted in Table 5. Biochar modification, which could be pre or post-modification, may improve the properties of biochar for enhanced hydrocarbon removal (Zhang et al., 2018a). Several modification methods exist and have been reviewed extensively (Rajapaksha et al., 2016; Wang et al., 2017a; Wang and Wang, 2019; Yaashikaa et al., 2019). Examples of modification that can be used to functionalise some properties of biochar relevant in the bioremediation of hydrocarbon-contaminated soil are presented in Table 5.

Table 5
Some biochar properties, modification methods and their potential to improve the efficacy of biochar on the bioremediation of hydrocarbon-contaminated soil.

Biochar properties	Direct/indirect relevance of biochar property on hydrocarbon removal	Example of biochar modification	References
Surface area and pore volume	May enhance biochar-microbe interactions and colonisation May improve sorption of the contaminant Can change the soil water holding capacity and structure Can enhance soil nutrient retention	Steam activation, CO_2 activation, acid treatment, alkali treatment	(Al-Wabel et al., 2018; Gul et al., 2015; Kołtowski et al., 2016)
Ash content	Can affect the concentration of micro and macro nutrients	Co-pyrolysis; acid pre-treatment	(Hakeem et al., 2022; Lehmann et al., 2011; Rathnayake et al., 2022)
C/N ratio	Can affect the ability of biochar to release inorganic N	Co-pyrolysis, alkali modification, amino modification	(Ahmed et al., 2016; Jin et al., 2016; Yang and Jiang, 2014)
CEC	May enhance the retention of nutrients on the biochar	Oxidizing agent activation (H_2O_2)	(Huff and Lee, 2016; Nachenius et al., 2013)
pH	Can affect the pH of the soil environment	Washing with acidic or basic solvent	(Yakout, 2015)
Labile fraction	Important source of carbon to microbes May improve the microbial co-metabolism of hydrocarbon Possible delay in hydrocarbon degradation due to competition for consumption with contaminant in the soil	Acid, base and hot water modification	(Bakshi et al., 2018; Dai et al., 2021; Kong et al., 2018)
O/C and H/C	Key ratios that affect biochar stability	Acid and alkali modification, co-pyrolysis	(Ahmed et al., 2016; Leng et al., 2019; Wang et al., 2019b)
PAH	Presence of PAH on biochar may present ecotoxicity issues Possible delay in hydrocarbon degradation due to competition for consumption with contaminant in the soil	Co-pyrolysis, catalytic pyrolysis	(Carlson et al., 2011; Dike et al., 2021; Han et al., 2021; Huang et al., 2017)
Heavy metals	Heavy metal could be toxic to soil microbes	Co-pyrolysis, catalytic pyrolysis	(Carlson et al., 2011; Han et al., 2021; Huang et al., 2017; Xiang et al., 2021)

Despite the importance of biochar modification, its effect on hydrocarbon removal has rarely been reported apart from the application of microorganism-immobilised biochar (Section 2.1.2). Ding et al. (2021) observed that the application of modified biochar resulted in a 28 % higher phenanthrene dissipation ratio relative to the unmodified biochar. The biochar was modified with NaOH to remove leachable pyrogenic organic carbon (LPyOC). Modification of biochar resulted in a decrease in pH, nitrogen, and ash content, as well as an increase in the surface area, C, O, O/C, and (N + O)/C ratio (Ding et al., 2021; Ding et al., 2019). Changes in the microbial community structure between the modified biochar and unmodified biochar along the second redundancy analysis (RDA2) was observed, which was attributed to higher bioavailable nitrogen in the modified biochar treatment (Ding et al., 2021). At the genus level, the relative abundance of *Azospirillum*, *Magnetospirillum*, *Thermincola*, and *Desulfotomaculum* was higher in the modified treatment, while *Pseudomonas*, *Geobacter,* and *Cristensenellaceae_R-7_*group were lower in the modified treatment. Degraders of PAHs were found to be higher in modified biochar, perhaps due to the removal of LPyOC from the biochar, making phenanthrene the major substrate (Kong et al., 2018). In other studies, the effect of modified biochar was assessed on the total concentration of bioavailable and bio-accessible PAHs (Kołtowski et al., 2017; Kołtowski et al., 2016).

5. Conclusion and future research perspectives

This review has critically examined the impact of biochar in the co-application with other remediation techniques on the removal of petroleum hydrocarbons from contaminated soil. The principle for most of these advances is built on addressing some of the problems associated with the sole use of biochar (reduced hydrocarbon bioavailability, nutrient immobilisation, biochar toxicity), improving other remediation techniques, and addressing the problem of inefficient soil microbial communities. Biochar co-application with other remediation techniques was found to be more effective in hydrocarbon removal, especially when co-applied with bioaugmentation. However, in some cases, the combined treatment was less effective. Biochar co-application can enhance hydrocarbon removal; however, in most cases, no synergistic or additive effect was observed in the co-applied treatment. Overall, biochar co-application with bioaugmentation was the most effective co-application strategy; this was followed by biochar co-application with phytoremediation and then with biostimulation. Although there is a growing interest in co-applying biochar with more than one remediation technique (hybrid), the cost implications must be fully assessed. There exists significant potential for enhanced hydrocarbon removal with modified biochar. To enhance biochar-based co-application or modified biochar research in hydrocarbon remediation, the following research gaps have been identified:

- Microbial immobilisation on biochar via adsorption is facilitated by a weak bond and as such, microbes can readily be dislodged from the biochar. Combining immobilisation via adsorption with entrapment appears a promising approach.
- The use of enzyme immobilised biochar also offers promise in the biochar-based remediation of hydrocarbon-contaminated soil, compared to the use of immobilised microorganism biochar.
- Assessing the effect of co-application ratios of biochar with bioaugmentation (enzymes and microorganisms), phytoremediation (root exudate), and biostimulation (surfactant, compost, and inorganic nutrients) is required in enhancing bioremediation in biochar-co-application based remediation.
- Modifying biochar with root exudate or surfactant appears to be promising, because of a stronger interaction between biochar and either root exudate or surfactant.
- Sequential application of biochar with either surfactants or root exudates may be more effective in remediation than their simultaneous application. Future studies are required to assess this hypothesis.
- Biochar-based fertiliser promises to be an effective technique in petroleum hydrocarbon remediation involving biochar and nutrients (inorganic and inorganic) co-application.
- Due to the positive interaction between biochar and compost, it is expected that higher remediation will be achieved when they are combined with interaction. The BCed or Bcing appears to be more effective than biochar and compost mixture without interaction (B + C).
- Assessing the ecotoxicological and cost implications of biochar co-application is necessary because sustainable remediation must also encompass low cost and environmental impact.
- Different co-implementation strategies should also be considered. For example, the remediation effect of co-application of biochar with multiple techniques. However, cost implications need to be considered.
- Modification of biochar offers significant potential although its effect on hydrocarbon soil remediation has not been well studied. Also, the cost and ecotoxicological impact of modification should be considered.

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Definition of terms used in this paper

Co-application: Using biochar in conjunction with other remediation techniques such as bioaugmentation, phytoremediation, biostimulation, and any of their combination (hybrid remediation techniques).

Bioaugmentation: Introduction of microbial cells or enzymes to remediate contaminated soil.

Phytoremediation: Use of plants or their parts to remediate contaminated soil.

Biostimulation: The use of nutrients and surfactants to remediate contaminated soil.

Hybrid remediation techniques: Use of biochar in conjunction with more than one remediation technique in any combination.

CRediT authorship contribution statement

Charles Chinyere Dike: Conceptualization, Writing – original draft, Investigation, Writing – review & editing. **Ibrahim Gbolahan Hakeem:** Writing – review & editing, Writing – original draft. **Alka Rani:** Visualization, Writing – review & editing. **Aravind Surapaneni:** Supervision, Writing – review & editing. **Leadin Khudur:** Writing – review & editing. **Kalpit Shah:** Supervision, Writing – review & editing. **Andrew S. Ball:** Supervision, Writing – review & editing.

Data availability

This is a review article. Data was obtained from published literatures. The literatures were cited.

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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