

Running Title: ASSISTED BIOREMEDIATION TECHNOLOGY FOR PHC

Plants-Microbes Assisted and Biochar Amendment Technology for Petroleum Hydrocarbon Remediation in Saline-Sodic Soil

Kudakwashe MEKI*, Qiang LIU, Shuai WU and Yanfei YUAN¹

Institute of Coastal Environmental Pollution Control, Key Laboratory of Marine Environment and Ecology, Ministry of Education, Institute for Advanced Ocean Study Ocean University of China, Qingdao 266100, (China).

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ABSTRACT

Soil degradation through salinization and pollution (by toxic compounds such as petroleum hydrocarbon (PHC)) in the coastal wetlands became a significant threat to ecosystem health, biodiversity, and food security. However, the traditionally used remediation technologies' insignificant effects include the generation of secondary pollutants, high operating costs, and high-energy consumption. Bioremediation (plants, biochar, and microbes) is currently an innovative and cost-effective option to remediate contaminated soil. Biochar as a plant/microbe growth enhancer is a promising green approach for sustainable phytoremediation of PHC in salinized soil. As such, the review seeks to summarize the effects of plants-microbes assisted and biochar amendment technology for PHC and salinization remediation. As a result of the plant-microbes mediated rhizodegradation despite increasing hydrocarbon sorption. The overall microbial respiration was more active in biochar amendments due to more rapid biodegradation of the PHC and improved soil properties. The use of biochar, plant, and microbes was recommended as this offers an emerging trend for remediation of hydrocarbons and salinity. The findings of plants, biochar, and microbes interactions are significant concerning sustainable management of the PHC polluted environment in ecology and economy. It offers the possibility of further development of new green technologies.

Key Words: microbial community, phytoremediation, polluted, remediation, salinity.

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Highlights

- Plants, microbes, and biochar are novel technologies for remediating petroleum hydrocarbons contaminated soils.

Corresponding author. E-mail: m17664050571@163.com

- High adsorption capacity, porous structure, large surface area, high minerals, and functional groups of biochar significantly affect petroleum hydrocarbons degradation.
- The major obstacle in bioremediation is the choice of plant species, microbes and biochar production.
- Applications of plants, microbes, and biochar in degraded soil and removing pollutants have been reported.

INTRODUCTION

Food security continues to be on the hot spot due to a growing global population and shifting of diets (David Eckstein *et al.*, 2020). Soil degradation such as erosion, desertification, acidification, salinization, and pollution are significant challenges to the sustainability of agricultural production and food security (Elleby *et al.*, 2020). The persistent imbalance between the world population and the need for a more sustainable food supply has increased pressure on food security (Krishna Koyande *et al.*, 2020, Gonzalez-Balderas *et al.*, 2020). The current world population is around 7.8 billion, and it is projected to reach 9.9 billion by 2050, an intensification of more than 25% of the current population (David Eckstein *et al.*, 2020). However, soil degradation such as erosion, desertification, acidification, salinization, and pollution forms the major challenges of our time and, in turn, threatens the long-term sustainability of agricultural production to ensure food security (Deepranjan Sarkara *et al.*, 2020, Kramer and Mau, 2020). The existence of petroleum hydrocarbons in soils, especially wetland ecosystems, is an adverse factor for human health and a negative impetus for plant growth and development. Petroleum hydrocarbons (PHC) in natural gas, crude oil, tars, and asphalts are composed of various alkanes, aromatics, and polycyclic aromatic hydrocarbon (Zhang *et al.*, 2010, Moubasher *et al.*, 2015). The hydrocarbons enter the environment from leaking storage tanks, pipelines, and land disposal of waste petroleum and oil (Zhu *et al.*, 2018, Sam and Zabbey, 2018).

The deterioration of soil quality and fertility resulting from salinity and PHC pollution has become a critical limitation in restoring these degraded soils (Zhang *et al.*, 2010, Moubasher *et al.*, 2015). However, the influence of salts and PHC leads to a decrease in soil productivity, restricts plant growth, and adversely affects the soil microbial community structure (Peng *et al.*, 2009). The harmful effects of PHC include inhibition of seed germination, reduction of photosynthetic pigments, the slowdown of nutrient assimilation, and shortening of roots and aerial organs. It is also expected that some petroleum fractions can dissolve biological membranes and, consequently, disrupt the plant root architecture. (Shi *et al.*, 2019, Huang *et al.*, 2019, Zhu *et al.*, 2020). In previous years various approaches have been developed for remediation of the contaminated areas. The removal of pollutants has been done through different techniques such as chemical oxidation, photocatalytic oxidation, volatilization, sedimentation, and fertilization (compost manure, ammonium sulfate, and microbial fertilizer). Fertilization has been used on unsuitable or degraded soil for agricultural production. However, many problems have been encountered, such as high cost and secondary pollution (Yang *et al.*, 2018, El-Naggar *et al.*, 2018). In addition, manures and composts contain pathogens, heavy metals, and pharmaceuticals, which may cause long-term contamination of farmland (Zhu *et al.*, 2020). Therefore, developing an environment-friendly technology, sustainable strategies to remediate the salinity and PHC contaminated soils is an

essential task for soils to restore soil ecosystem function and service; alleviate adverse effects on global climate change, and protect biodiversity (Varjani, 2017).

Trends of developing innovative technologies for removing PHC and salinity have been the debate of the century (Dzionic *et al.*, 2016). Among these technologies, phytoremediation is the most eco-friendly, economically sustainable, cost-effective technology and offers ecological and natural aesthetic benefits. The use of plants and associated with rhizospheric microbial populations to degrade organic pollutants is becoming a promising method for remediation of polluted soil (Zhang *et al.*, 2010, Moubasher *et al.*, 2015). Plants used in phytoremediation could promote the dissipation of PHC in soils through phytostabilisation, phytoextraction, phytovolatilization, and phytodegradation. The enhancement of microbial activities and diversity through the improvement of physical and chemical conditions in rhizosphere soils and the phytoremediation process significantly reduced the toxicity of the high molecular weight PHCs (Wenzel, 2008, Moubasher *et al.*, 2015). Several plant species have been positively used to soil phytoremediation contaminated with organic and inorganic pollutants (Khan *et al.*, 2013). The question of how to improve efficiency and optimize phytoremediation conditions is one of the significant trepidations. Studies revealed that soil physicochemical properties and microbial activities substantially impacted phytoremediation effectiveness (Zhen *et al.*, 2019). In addition, the use of microbial biomass and activity might be stimulated in the rhizosphere because roots excrete significant amounts of sugars, amino acids, organic acids, hormones, vitamins, mucilage, and other substances together with a sloughed-off root cap.

On the same note, recently, scientists have proposed using biochar as a similar material with the capacity to act as an adsorbent of the pollutant (Anyika *et al.*, 2015). In recent years, biochar application as a soil amendment has increased attention (Xiao *et al.*, 2018, Leng *et al.*, 2019, Xiao *et al.*, 2020). Biochar is solid and highly aromatized C-rich material formed by low-temperature pyrolysis (< 700 °C) of biomass under oxygen-limited conditions, with stability, large specific surface area, developed pore structure, rich surface functional groups, and other excellent characteristics (Xiao *et al.*, 2018), widely used in multiple fields including carbon sequestration. The addition of biochar to contaminated soils results in improved soil fertility, nutrient retention, water holding capacity, and oxygen supply and remediate contamination by surface adsorption, precipitation, partitioning, and sequestration. Microbial growth and biomass can be promoted by the addition of biochar as it also provides shelter for microbes in the soil (Mahmoud *et al.*, 2019, Chaganti *et al.*, 2015, Lashari *et al.*, 2015). For instance, biochar is applied as a novel carbonaceous material to adsorb metals in soil and water (Kwoczynski and Čmelík, 2021). It was reported that biochar could decrease many toxic metals toxicity and mobility (Yaashikaa *et al.*, 2020). Its characteristics, such as high surface area and microporosity, have also proven efficient in adsorbing organic contaminants in water (Suman and Gautam, 2017). Current studies evaluated plant-microbes assisted and biochar amendment technology's effectiveness, but the research has been reported insufficiently. Several studies have focused on using biochar to bind or inspire the microbial degradation of organic pollutants (Qin *et al.*, 2013; Xin *et al.*, 2014). Relevant critical reviews have been published encompassing the working mechanisms and the factors influencing the degradation of PHC. To our knowledge, there are limited studies that evaluate plant-microbes assisted and biochar amendment technology on promoting the rate and efficiency of PHC degradation under salinity conditions. Thus, the review provides a comprehensive summary of the plants-microbes assisted and biochar amendment technology for PHC and salinization remediation. The specific objectives of this study include; (1) evaluate the potential of effectiveness of plants-

microbes assisted, and biochar amendment technology in remediation of petroleum hydrocarbons and salinity; (2) Identify gaps and future consideration for PHC and salinity remediation.

PROBLEMS OF SALINE-SODIC SOIL

The relative significant soil problem includes key soil structural devastations from physical, chemical, and biological properties (Verheijen *et al.*, 2019, Abd El-Mageed *et al.*, 2020). The physical properties become worse with increasing exchangeable sodium levels and high swell-shrink potential, poor hydraulic properties due to aggregate breakdown, and poor drainage (Wong *et al.*, 2010, Wu *et al.*, 2014a, Sun *et al.*, 2018). The effects result in low plant growth (Abou-Shady, 2016), reduced plant water availability, increased runoff, and soil erosion. Studies further describe that the saline soils became dense, cloudy, and structureless due to natural aggregation loss (Day *et al.*, 2018). Furthermore, it is associated with high bulk density, and poor porous structure as the permeability of soils to water and air is restricted due to its porous structure (Wang *et al.*, 2019, Mehdizadeh *et al.*, 2020) and very low hydraulic conductivity. It then reforms and solidifies into almost cement-like soil with little or no structure (Amini *et al.*, 2015). The problems caused by sodium result in reduced infiltration, hydraulic conductivity, and surface crusting (Lashari *et al.*, 2015) and increased runoff and soil erosion. On the other side, the soil is compromised with high electrical conductivity (EC) values due to salt concentration and increases the osmotic pressure in soil solutions (El-Naggar *et al.*, 2019). The soil has a lower cation exchange capacity (CEC) due to the immobilization of cations such as K, Ca, and Mg. It has been noted that anions are bound very poorly by soils, especially the salt-affected. By this, the plant growth in sodic soil is adversely restricted (Abou-Shady, 2016). These result in nutrition disorders and limit the uptake of essential plant nutrients (K, Ca, Mg, P) and eventually in poor crop yields (Amini *et al.*, 2015, Akhtar *et al.*, 2015). The soil organic carbon is also an essential resource owing to a negative effect on the nutrient supply, detoxification of harmful soil constituents, moisture and nutrient retention, and soil formation (Chávez-García and Siebe, 2019).

Furthermore, the increase in salinity results in a decrease in total N and organic C mineralization (Dugdug *et al.*, 2018, Sadegh-Zadeh *et al.*, 2018). High salt concentrations in soils cause osmotic stress and dehydration of microbial cells. Though having discovered the problems mentioned earlier on, more new emerging challenges are found in salt-affected soils that require urgent attention. For instance, the wetlands also face issues of PHC, which critically requires attention for sustainable agriculture (Wenzel, 2008, Moubasher *et al.*, 2015). In general, deterioration of soil's physical and chemical properties causes limited oxygen supply. It results in poor growth of seedlings and roots and even seedling death. All these limiting factors work simultaneously, harming the rhizosphere environment, including microbial communities, thereby reducing crop yields. Therefore, how to repair and maintain the sustainable use of these soils has become a top priority.

Pollutants a treat to terrestrial biodiversity

The existence of pollutants is the primary source of degenerative diseases that affect human life, such as cancer, Alzheimer's disease, atherosclerosis, Parkinson's disease, etc. (Al-Baldawi *et al.*, 2015). The degree of toxicity of each metal depends on exposure and the dose absorbed by the organism. The organisms most affected by the toxicity of pollutants are plants because their normal

physiological activities are severely hindered (Day *et al.*, 2018). It can be exemplified, the practices of respiration, photosynthesis, electron transport chain and cell division are adversely affected by increased heavy metal content, as demonstrated by laboratory experiments. In addition, high metal toxicity can inhibit cytoplasmic enzymes in plant cells and damage the cell structure due to oxidative stress (Karppinen *et al.*, 2017a), thereby affecting plant growth and metabolism. Human exposure to pollutants can cause serious health risks such as lack of coordination and paralysis.

In contrast, severe exposure to cadmium can damage the human body's internal organs such as kidney, liver, and heart tissue. Hydrocarbons are the most common cause of acute heavy metal poisoning in adults and children (Ren *et al.*, 2014, Zhen *et al.*, 2019). The central nervous system is affected by mercury, a neurotoxin that can damage speech and hearing and then cause muscle weakness (Sarma *et al.*, 2019). It accumulates in the microbial cells of aquatic organisms and is converted into methylmercury in the microbes, which is harmful to marine organisms. Human consumption of fish and other aquatic animals can lead to the transfer of toxic methylmercury to humans. There is currently limited data in accordance with the degree to which PHC negatively affects the environment and sustainable measures to reduce its existence. However, due to these pollutants' harmful effects, joint efforts must eliminate them from the environment and ecosystem stabilization.

VARIOUS WAYS FOR SOIL REMEDIATION

Continuous efforts have been directed towards reclaiming degraded soils with a long history (M and J, 2002, Arora and Singh, 2017, Seenivasan *et al.*, 2015, Sastre-Conde *et al.*, 2015). The traditional approaches have been employed to remediate soil pollution by PHCs and salinity, including physical engineering (e.g., underground drainage, physical barrier), the application of chemical agents (e.g., gypsum) (Hafez *et al.*, 2015, Zhu *et al.*, 2020) and bioremediation methods (e.g., phytoremediation, microbial) (Yu *et al.*, 2019). Nevertheless, the ways require significant investments due to high costs. In addition, manures and composts contain pathogens, heavy metals, and pharmaceuticals which may cause long-term contamination of farmland (Yang *et al.*, 2018, El-Naggar *et al.*, 2018). However, there is an imperative need to establish low-cost, environmentally friendly, and sustainable technologies for salt-affect soil remediation. The most appropriate choice is decisive for minimizing environmental impact, thus reducing salinity and the concentration of PHC or toxicity of pollutants (Al-Baldawi *et al.*, 2015). Some contaminants (such as agricultural chemicals) are applied to the soil surface. Due to leakage from buried storage tanks, sewage pipes, or landfills, other pollutants are released below the surface (Qin *et al.*, 2013). Atmospheric pollutants containing harmful substances can also cause problems. Besides, It can also be noted that pollution is not always limited to specific locations but can penetrate groundwater through the soil or be brought to nearby land and waterways in rainwater or dust.

Chemical decontamination methods usually focus on chemical oxidation which injects reactive chemical oxidants into the soil and groundwater quickly and then destroys pollutants. In-situ chemical oxidation (ISCO) is a universal solution, especially when remediating contaminants in areas that are difficult to access, such as deep soil or soil under buildings (Hussain *et al.*, 2018b). Chemical oxidation has a wide range of applications and can be used to treat various organic pollutants. By effectively locking PHC pollutants in the soil, stabilization reduces the risk of pollution. It can be achieved in two ways: firstly, by modifying the contaminants in the ground to a less dangerous form; secondly, by curing, reducing the migration rate of the pollutants and combining them in an appropriate location, making it impossible to reach any receptor (Sanusi *et*

al., 2016, Varjani, 2017). Although chemicals effectively reduce pollutants in contaminated areas, they are not highly recommended as they come as second pollutants and simultaneously cause underground water pollution. In addition, manures and composts contain pathogens, heavy metals, and pharmaceuticals, which may cause long-term contamination of farmland. Moreover, manures and composts have the potential to lead to ammonia and methane releases, which can aggravate global warming, severe groundwater and stream nutrient pollution (Hussain *et al.*, 2018b).

Soil stability depends on the addition of fixatives which reduces the leaching capacity and bioavailability of pollutants. Due to the higher resistance and lower permeability, the technology can also improve the ground's geotechnical engineering capacity, making it more suitable for construction projects. Soil washing eliminates harmful pollutants by washing the soil with a liquid washing solution. In this process, fine-grained soil (such as silt and clay) will be washed away with contaminants, and the pollutants are more likely to combine with fine soil. Therefore, the contaminated fine powder will be separated from clean coarse-grained soil (such as sand and gravel) and safely reused. Due to soil pollution's health risks to humans, animals, and plants, soil remediation is essential in many cases (Alessandrello *et al.*, 2017).

Bioremediation uses biological processes to degrade, transform or substantially remove soil pollutants (Chen *et al.*, 2015a). The process relies on microorganisms, including bacteria and/or fungi, which use contaminants as a food source (Chen *et al.*, 2015a, Hilber *et al.*, 2017). Composting is a technology that utilizes microbes to clean up or stabilize the pollutants (Zhang *et al.*, 2010). A large number of studies showed that many kinds of microbes had a strong ability to degrade various organic pollutants and imposed excellent passivation effect on heavy metals (Yu *et al.*, 2011, Chen *et al.*, 2015a, Pi *et al.*, 2017). Bacteria and fungi, the main pollutant-degrading microbes in composts, have been widely considered the most crucial factors governing the remediation of contaminated soils. Remediation of contaminated soils by composting or compost addition mainly relies on two mechanisms (Hussain *et al.*, 2018a) (i) adsorption by organic matter and (ii) degradation by microorganisms. The decomposition of organic pollutants in soil/compost mixture relies mostly on microbial activity (Chen *et al.*, 2015a). Hence, more research needs to be implemented to discover mechanisms that come with plant species. Care must be taken to introduce genetically engineered microbes into the environment for bioremediation. Horizontal gene transfer can occur between the engineered microbes and natural microbes in the environment. Environmentalists are thoughtful of horizontal gene transfer of the engineered microorganisms with the indigenous microbes. The reason is that the microbes are capable of spreading rapidly in the environment and transfer-resistant genes from one microbe to another via plasmids, which enables them to adapt to new ecological environments.

The mechanisms that come with the traditional methods for rehabilitating the degraded soil require an alternative technological supplement for better environmental purification. Moreover, alternative technologies are considered less harmful to the environment, more comfortable to apply in any contaminated environment, and cost-effective than traditional technologies. In general, using plant-microbes assisted and biochar amendment technology stimulates contaminants degradation as they complement each other's weaknesses for sustainable recovery.

RECOVERY OF PETROLEUM HYDROCARBONS AND SALINIZATION

Environmental problems such as the accumulation of PHC, salinity, deterioration of groundwater quality, soil degradation, and various threats to human, animal, and ecosystem health

are closely related to the presence of high concentrations of pollutants in the environment. Employing appropriate technologies to remediate contaminated soils is crucial due to most remediation methods' site-specificity (Yavari *et al.*, 2015, Harindintwali *et al.*, 2020). According to the nature of the pollutants and the diversity of the biosphere. Soil degradation has led to shortages of food security due to salinity and contamination (Harindintwali *et al.*, 2020). However, limitations of conventional remediation technologies include low environmental compatibility, high cost of implementation, and inadequate public acceptability. This raises the call to employ biological remediation methods. Plants-microbes assisted and biochar amendment offers many ecological and cost-associated benefits (Gouda *et al.*, 2018). For this reason, plants, biochar, and microorganisms that are tolerant and capable of growth in the presence of contaminants are commonly used.

Effects of biochar on petroleum hydrocarbons degradation.

In recent years, biochar was used as a soil improver. It has become a subject with increased scientific attention due to its capability for soil remediation (Wu *et al.*, 2014b, Sun *et al.*, 2016b, Sun *et al.*, 2018). Many studies reported that biochar could change soil attributes' physical and chemical properties (Mukherjee and Lal, 2013, Tang *et al.*, 2010). For instance, it can increase the pH of the soil (Abit *et al.*, 2012), strengthen water retention capacity (Sun *et al.*, 2016a), improve soil fertility (Liu *et al.*, 2019), reduce the leaching of soluble micronutrients (Amini *et al.*, 2015), and enhance carbon sequestration (Barati *et al.*, 2017). Moreover, it is potentially beneficial to crop productivity and growth, mitigating climate change by reducing greenhouse gas emissions (Ji *et al.*, 2020). At the same time, biochar is a promising material in environmental restoration. The application of biochar was reported to immobilize heavy metals in the environment and reduce heavy metals bio toxicity to the organisms (Gong *et al.*, 2019, Zhang *et al.*, 2019). For instance, Xu *et al.* (2013) demonstrated that bamboo biochar could immobilize Cd and Pb in the soil and reduce bioavailability. They ascribed these results to the adsorption of metals to the surface of biochar by complexation and ion exchange.

Nevertheless, biochar benefits in soil remediation generally depend on its properties, including physical and chemical properties. It exhibits high biodegradability, high contents of total and organic carbon, as well as optimal concentrations of micro and microelements (potassium, sodium, magnesium, calcium, copper, zinc, *etc.*) (Han *et al.*, 2016, Gong *et al.*, 2019). The application of biochar increases cation charges that contribute to increasing of CEC in soil. For instance, Mehdizadeh *et al.* (2020) reported an increase in CEC by 8.2% after 2% (w/w) woody branches biochar produced at 530°C. Similarly, Mahmoud *et al.* (2019) used maize stalk, wood sawdust derived biochar made at 500°C variation in application rates 5, 10, 19 t ha⁻¹ respectively, and the soil CEC increases 44.1-142.4%. The mechanisms for the improvement of soil CEC following biochar application: (1) biochar has a high specific surface area, negative surface charge, and oxygen-containing functional groups, which can directly increase replacements of Na from exchange sites through the provision of Ca and Mg in the soil colloids (Lashari *et al.*, 2015). (2) the high adsorption capacity of biochar and the CEC can reduce the sodium ion content in the soil solution, thereby increasing the exchange site of soil colloids by holding cations that eventually precipitate with those of negatively charged elements such as Ca²⁺, Mg²⁺, and Fe³⁺ (Luo *et al.*, 2016, Zheng *et al.*, 2018). (3) Biochar promotes the increase of soil organic matter content and the increase of CEC. Biochar is crucial because nutrients are low in PHC contaminated soils; thus,

plants and microbes compete for the same resource. Therefore, using biochar and phytoremediation methods is essential due to the nutrient content in biochar.

Moreover, biochar as the soil conditioner, when applied in the soil, creates more avenues for creating favorable soil conditions to enhance plant and microbe growth. In addition, when bacteria are applied to the soil for PHC degradation, biochar might act as a habitation for the microbes and provide nutrients. To date, insufficient data is available on the implications of using the trio in bioremediation technologies hence the need for further studies on the subject.

Mechanisms of biochar application on PHC removal

Studies have demonstrated that biochar could reduce contaminant bioavailability and biodegradation in soil (Anyika *et al.*, 2015, Han *et al.*, 2016, Zhen *et al.*, 2019). In these studies, pollutant concentrations in soil were generally lower ($<500 \text{ mg kg}^{-1}$), and apparent sequestering effects may occur, leading to a reduction in pollutant mobility and bioavailability. The incorporation into contaminated soil with thorough mixing, biochar promptly interacts with organic contaminants and soil microorganisms. Organic pollutants are stabilized on the biochar surface and in pores and may be further decomposed by microbes as stimulated by biochar amendment. The porous, functional-group-abundant aromatic-C-condensed biochar surface can adsorb various organic compounds through different mechanisms. As organic pollutants are adsorbed by biochar, their soil water concentrations decrease, and bioaccessibility to soil organisms, including plant roots, is reduced.

Meanwhile, biochar amendment enhances overall soil health by improving soil physical, chemical, and biological properties. Furthermore, microbial growth and biomass can be promoted by the addition of biochar as it also provides shelter for microbes in the soil (Tang *et al.*, 2010). However, Denyes *et al.* (2013) reported that biochar and soils improve plant growth and physiological developments (Chlorophyll content, shoot, and root biomass). Similarly, augmentation of microbial consortia (selected microbial strains) is also well documented to promote the vigor and growth of plants and hydrocarbon degradation, thereby improving phytoremediation (Zhen *et al.*, 2019).

Biochar has been used in remediating a variety of inorganic and organic contaminants in soils. Besides providing mineral nutrients such as N, P, K, Ca, Mg, and S, the biochar modifier also introduces a large amount of biodegradable OC as a soil microbial substrate (Zhang *et al.*, 2017). After the initial disturbance period, the soil modified by biochar usually shows an improved microbial community structure. It promotes microbial activity (using soil respiration rate, soil enzyme activity, and soil microbial biomass as indicators). The mechanism for organic pollutants (included PHCs) remediation is increased sorption by the processes of adsorption, partition, and sequestration (Beesley *et al.*, 2011). Remediation of PHCs and PAHs by biochar was reported widely where sorption of recalcitrant molecules is considered the primary mechanism (Bushnaf *et al.*, 2011, Zhen *et al.*, 2019). In some studies, biochar was used as a stimulating agent (Sarma *et al.*, 2019, Qin *et al.*, 2013) and a carrier for selected microbial strains during remediation trials (Zhang *et al.*, 2017). In addition, biochar contains more or less multivalent metal elements such as Fe, Al, Ca, and Mg on the surface polar and ionized organic compounds may form complexes with the metal ions and be deposited on the biochar surface or precipitated in soil (Song *et al.*, 2017).

Therefore, besides abiotic factors, biochar addition may change the microbial community's structure, enzyme activity, and decomposition of carbon substrates and cycling of other soil

elements (Kuzyakov and Razavi, 2019, Lehmann *et al.*, 2011). It has further alluded that biochar absorbs organic and inorganic compounds on its surface. According to studies, this is an advantage as sorption decreases the liability and availability of toxicants in soils leading to decreased phytotoxicity (Beesley *et al.*, 2011, Lu *et al.*, 2015). Thus, biochar absorption ability is used for bioremediation of soils polluted by petroleum hydrocarbons such as alkanes, polycyclic aromatic hydrocarbons, and asphaltenes (Beesley *et al.*, 2011). The influence of biochar on oil-polluted soils is different and dependent, e.g., on doses and time of use, type of biochar (initial substrates and method of preparation), and soil quality (Lehmann *et al.*, 2011). A more recent study, a novel biochar-plant tandem approach was used to understand the rhizoremediation stimulation mechanism by biochar addition (Harindintwali *et al.*, 2020). They concluded that the first recalcitrant organic molecules are adsorbed to biochar. Root exudates may help in desorption that will subsequently be available to the degenerative microbial community in the rhizosphere.

Moreover, it has also been used as an adsorbent due to its surface area, aromatic and aliphatic structures (Zhang *et al.*, 2016). Moreover, due to its inflexibility, pore structure and nutrient characteristics can affect the microbial degradation of PHCs in the soil. For instance, Barati *et al.* (2017) compared granular biochar (GBC) and powdered biochar (PBC) that was produced at low temperatures. They found a positive effect of pore size on microbial habitat. However, it is not clear whether this response is also the same result of soil acid conditions (pH >5). The potential of biochar serves as a shelter for microbes and decrease the rate of de-functional of microbial the soil; hence this results in greater bacterial biomass (Galitskaya *et al.*, 2016), while the fungal biomass remains unchanged as a result of decreased fungal mobility due to their hyphal arrangement (Gong *et al.*, 2019). This also favors microbes that rely on their extracellular enzymes to degrade PHCs in soils into compounds that can be absorbed by their cells and consumed during metabolic activity. However, it has been reported that the microbes prefer to remain closer to surfaces where they release extracellular enzymes into their surrounding (Rillig, 2009). The microbial respiration rate increased in the presence of plant species in biochar amendments because there will be good conditions in the rhizosphere following the PHC degradation rate (Ren *et al.*, 2014, Galitskaya *et al.*, 2016). The prevention of leaching of nutrients enables the biochar amendments to have enough nutrients (Hussain *et al.*, 2018a). However, insufficient data concerning the use of biochar for immobilization of hydrocarbon-degrading microbes.

The surface properties of biochar, particularly surface area, pore size, pore volume, polarity, aromaticity, and hydrophobicity, have a predominant effect on biochar–organic compound interactions. In general, biochar produced at higher pyrolysis temperatures is more significant in surface area, aromaticity, and hydrophobicity and lower in surface polarity due to the loss of O- and H-containing functional group (Barati *et al.*, 2017, Karppinen *et al.*, 2017b). Additionally, it is reported that the surface of biochar is a hydrophobic or electrostatic attraction (Lehmann *et al.*, 2011), thus enables it to enhance its adsorption capacity. However, biochar has a low isoelectric point (i.e., they are electrical Neutral) (pH <4) (Cheng *et al.*, 2019). The presence of minerals can promote electrostatic attraction or bio-oil on the surface of biochar. For example, Galitskaya *et al.* (2016) also claimed that the biochar surface could absorb nutrients and cations in the soil solution, resulting in an increased concentration of sufficient nutrients for microbial metabolism.

Similarly, Zhang *et al.* (2016) found that adding biochar helped overcome polychlorinated biphenyls toxicity to microorganisms. Currently, studies showed that a reduction in the mobility and bioaccessibility of soil contaminants caused by biochar addition might be lower since the PHC concentration might be relatively high. Karppinen *et al.* (2017b) reported that biochar produced under lower temperatures might absorb contaminants via a partition mechanism that is relatively

more accessible to microbes than the dominant adsorption process, which appears in biochar produced at higher temperatures (600-800°C). The low-temperature biochar contains abundant nutrients with good adsorption performance, making the biochar an excellent candidate in practice for remediation of PHC in soil. Admittedly, due to the complexity and dynamic of PHC in field conditions, the effectiveness of biochar may be widely different. The pollutant stabilization effect of biochar in field soils, however, may diminish over time. Field studies at larger scales are needed to examine the long-term impact of biochar amendment on mitigating soil contamination under practical circumstances (Peng *et al.*, 2009, Behera *et al.*, 2019). Based on these potential changes in soil properties, biochar could stimulate PHC degradation while utilizing waste products from local industries. However, its success and practicality under field conditions have not been well studied, primarily in wetland ecosystems.

Remediation of PHC and salinity by phytoremediation and microbes

Phytoremediation includes a set of technologies that use plants and their associated microorganisms to remove pollutants from the environment or make them harmless. In addition, plant microbiology can also promote the removal of organic pollutants (Maqbool *et al.*, 2012). In particular, some studies have conducted research on the purpose of combining plants and biodegradable bacteria to remove petroleum products (Peng *et al.*, 2009, Behera *et al.*, 2019), which seems to be a promising remedy. Phytoremediation is one of them since it is the most beneficial repair technology because it is both economical and environmentally friendly (Fig 1). The planted soils harbored more oil-utilizing bacteria than the non-planted soils (Sorkhoh *et al.*, 2010). Several plants, e.g., ryegrass (*Lolium Linn.*) and alfalfa (*Medicago sativa Linn.*), have been used in phytoremediation and including the additional use of bacteria such as *Proteobacteria*, *Firmicutes*, *Chloroflexi*, *Acidobacteria* in reconstructing the bacteria community (Kirk *et al.*, 2005, Gansberger *et al.*, 2015), which enhanced petroleum hydrocarbon degradation in the contaminated soils (Chen *et al.*, 2015b, Varjani, 2017). Therefore, re-construction of contaminated soil texture before phytoremediation was an excellent solution to the nutrient's deficient soils (Maqbool *et al.*, 2012).

Fig. 1 The phytoremediation of pollutants by plant species including different processes such as phytodegradation and phytoextraction.

Although many plants can absorb, degrade, and accumulate large amounts of pollutants. The pollutants inhibit the number of toxic contaminants in the soil (Chen *et al.*, 2017). The reduction in plant growth due to toxic pollutants ultimately reduces the plant's potential for contaminated soil phytoremediation. Additionally, there is inadequate research on the application of plants for remediation of PHC in soil. However, some studies indicate that applying bacteria that promotes plant growth and degradation of pollutants feasibly is the first step in improving plant growth and plant remediation activity (Behera *et al.*, 2019). Moreover, the inoculated bacteria must show survival and colonization in the rhizosphere and plant tissues such as roots (Bleicher, 2016).

Microbial assisted-phytoremediation is an ecologically sound technology that uses microorganisms (bacteria, yeast, algae, protozoa, fungi, etc.) with distinctive features of metabolic potential and their products enzymes and bio-surfactant to assist plants for the decontamination of

pollutants. Different technologies of phytoremediation benefit from the use of intrinsic or extrinsic microorganisms to promote pollutant remediation.

However, bacteria known as plant growth-promoting bacteria (PGPB) has been described as the top performers in the phytoremediation of agricultural soils due to their fast growth, diversity, ubiquity, adaptability, versatility, and abilities to promote plant growth and health by fixing nitrogen, solubilizing phosphate, potassium and producing phytohormones, antibiotics, and siderophores (Sarma *et al.*, 2019). Within bacterial groups, members of the phylum Actinobacteria, notably the *Streptomyces* genus, have the most useful physiological and metabolic properties for PHC bioremediation (Zhen *et al.*, 2019).

Furthermore, studies found that expected changes in pH in the rhizosphere soil are often caused by acid and alkaline cations present in the soil. The number of hydrocarbon-degrading bacteria in the rhizosphere varied greatly in response to the plant species and PHCs concentration. High plant-to-plant variations in the number of PHCs degraders and the microbial community structure and diversity among treatments with different PHCs concentrations were detected (Hussain *et al.*, 2018a, Cheng *et al.*, 2017). The variation was caused by the accumulation of acidic metabolites (e.g., aliphatic acids) produced by microbes during hydrocarbon degradation.

It is well known that bioavailability is one of the most important limiting factors in the bioremediation of persistent organic pollutants in soils (Gao *et al.*, 2010, Hilber *et al.*, 2017). Nevertheless, there is a need to combine the technologies in plants-microbes assisted and biochar amendment technology on contaminated soil; thus, biochar assist will enable smooth plant growth and microbe's functionality.

COMPARISON OF BIOCHAR APPLICATION, MICROBES, AND PLANTS OF DEGRADED SOIL

It can be contended that there are limited studies that focused on assisted bioremediation (biochar, phytoremediation, and microbes) on petroleum hydrocarbon's fate in saline-sodic soil (Han *et al.*, 2016). However, several studies have shown that biochar can reduce the bioavailability of organic pollutants (Hussain *et al.*, 2018a, Barati *et al.*, 2017) by improving soil's chemical properties (Fig 2). In general, plant-microbes assisted, and biochar amendment technology improves soil nutrient and potentially toxic environment for plants and microorganisms to colonize. Nonetheless, it has been noted that the use of different plant species results in biodegradation of hydrocarbons (Al-Baldawi *et al.*, 2015, Cheng *et al.*, 2017); thus, some plants-associated bacteria can produce biosurfactants that can boost the bioavailability of hydrocarbons and may be useful for bioremediation (Dos Santos and Maranhão, 2018). The use of assisted bioremediation (plants, microbes, and biochar technology results in a significant effect in reducing salinity and PHC degradation. Recent studies indicated that biochar could stimulate plant growth by facilitating beneficial microorganisms, making the combination methods environmentally friendly and sustainable. The microorganisms can secrete plant hormones and promote absorption of nutrients in the soil, which does not directly enhance plant growth but also improve adaptation to drought; salinity and toxicity; and organic pollutant. The comparative analysis studies between biochar and phytoremediation are yet to be stretched, and more research still needs to be done. The exploration of the mechanism that comes with biochar in the degradation of petroleum hydrocarbon and the effective species that enhance microorganisms' survival requires more depth studies.

Fig. 2 The application of biochar improves chemical properties through several mechanisms for the reduction of PHC degradation.

FURTHER RESEARCH AND DEVELOPMENT NEEDS

The following were generally proposed in line with bioremediation strategies' potential attributes: (1) phytoremediation of PHC through phytodegradation, specifically using microbial-enhanced systems. Further efforts should focus mainly on publishing field trials and associated costs to make bioremediation (plants, microbes, and biochar) a common choice for sites impacted with PHC. There have been cost implications noticed in bioremediation, particularly for inorganic contaminants.

However, studies have reviewed that biochar reduces vitality by ensuring rapid sorption, reducing the risk of split PHC into crops, and leaching into groundwater or surface runoff. Biochar rehabilitates the soil's physicochemical properties for greater microbes' stimulation to enhance biodegradation of PHC. Biochar heterogeneous nature due to production temperature and feedstock, there is a need to examine the proper type before making biochar recommendations that can absorb pollutants.

The proper examination of the experimental design is an essential aspect in evaluating biochar amendment's efficiency compared to non-amended treatments. Studies recommended that biochar on sorption and biodegradation of PHC should be designed so that the concentration of biodegraded could be determined through comparison analysis on each amendment, hence the need to use sterile soil to validate results. Pieces of evidence for the concept have been elucidated in this review, and the bases rely on future work.

CONCLUSIONS

The plants-microbes assisted and biochar amendment technology interactions in correspondence with the soil amendments offer an emerging trend of remediation technologies for several persistent organic pollutants, especially PHCs. The usefulness of bioremediation as a better substitute for removing heavy metals from contaminated sites compared to the physiochemical techniques, which are less efficient and expensive due to the amount of energy, expended. Microorganisms and plants possess inherent biological mechanisms that enable them to survive under heavy metal stress and remove the metals from the environment. The microbes use several processes such as precipitation, biosorption, the enzymatic transformation of metals, complexation, and phytoremediation techniques, of which phytoextraction and phytostabilization have been very useful. The findings indicated that that bioremediation approaches for petroleum-contaminated soil with plants are more efficient than non-amended soil. The investigation concluded that the combined application of plants, microbes, and biochar affected the rhizosphere effect. However, individually each of these amendments significantly improved the plant's tolerance and increased dissipation rate; the combined use of organic modification and synergistic augmentation of microbial consortia is more advantageous for accelerating widespread use of on the fate of petroleum hydrocarbon in saline-sodic soil. The study recommends focusing on long-term field studies, discovering suitable removal methods, and treating potentially toxic biomass. The research further suggests other researches to fill the knowledge gaps and determine the best biomass valorization options.

Corresponding author. E-mail: m17664050571@163.com

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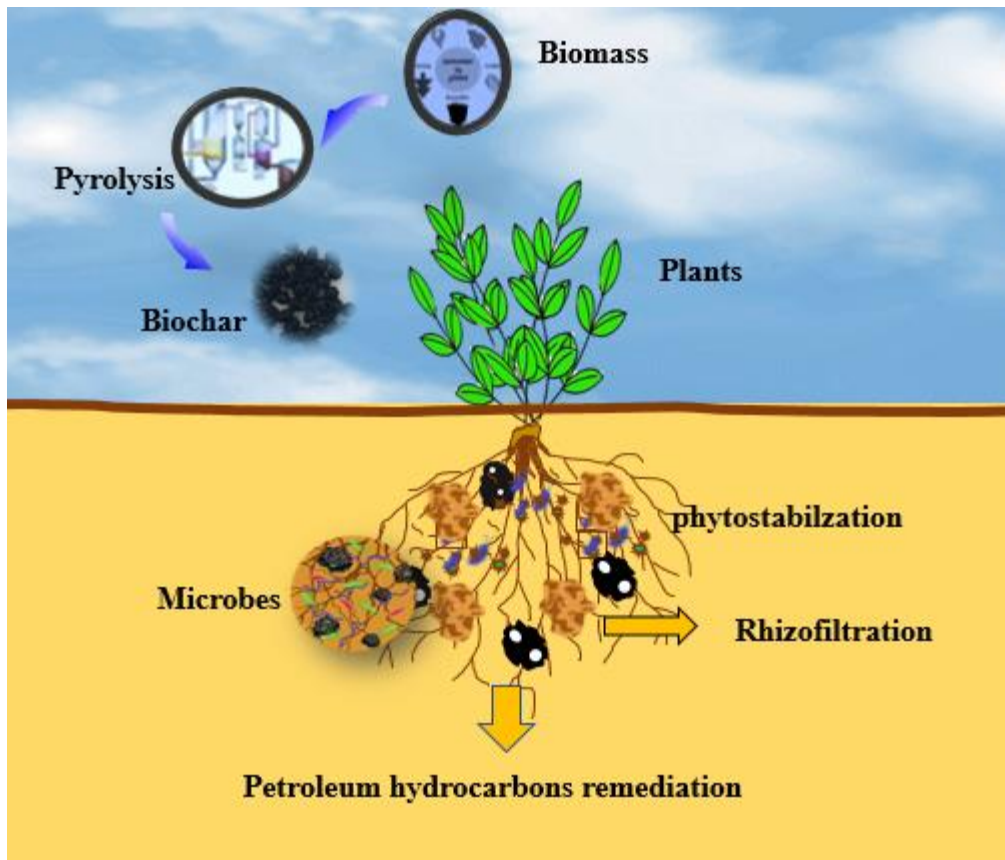
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Graphical Abstract

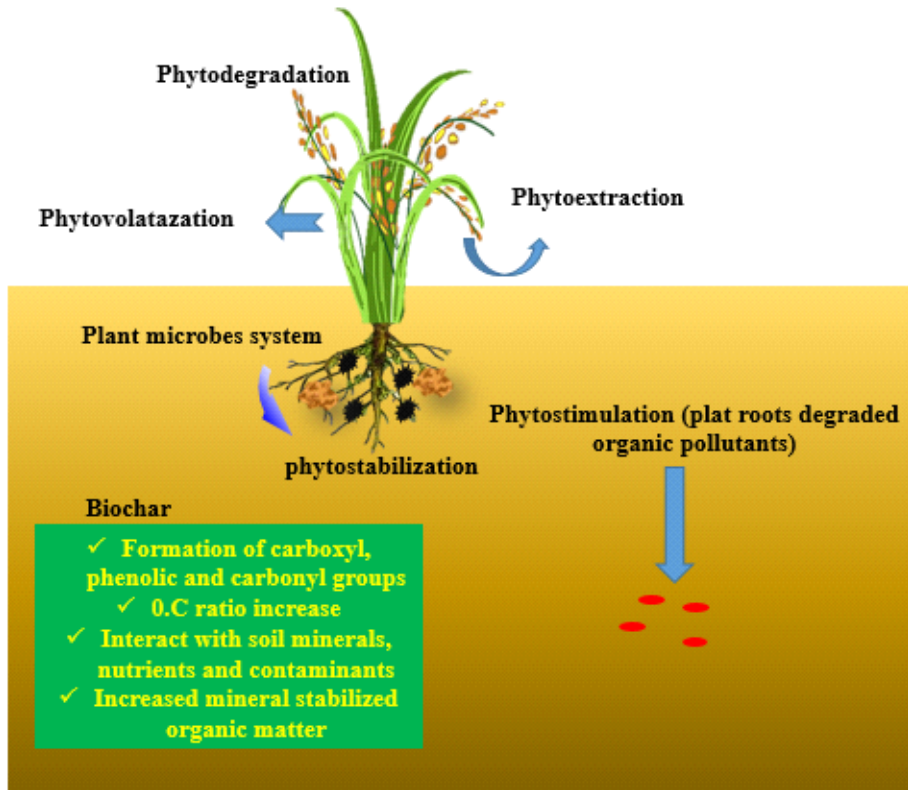


Fig 1. The phytoremediation of pollutants by plant species including different processes such as phytodegradation and phytoextraction.

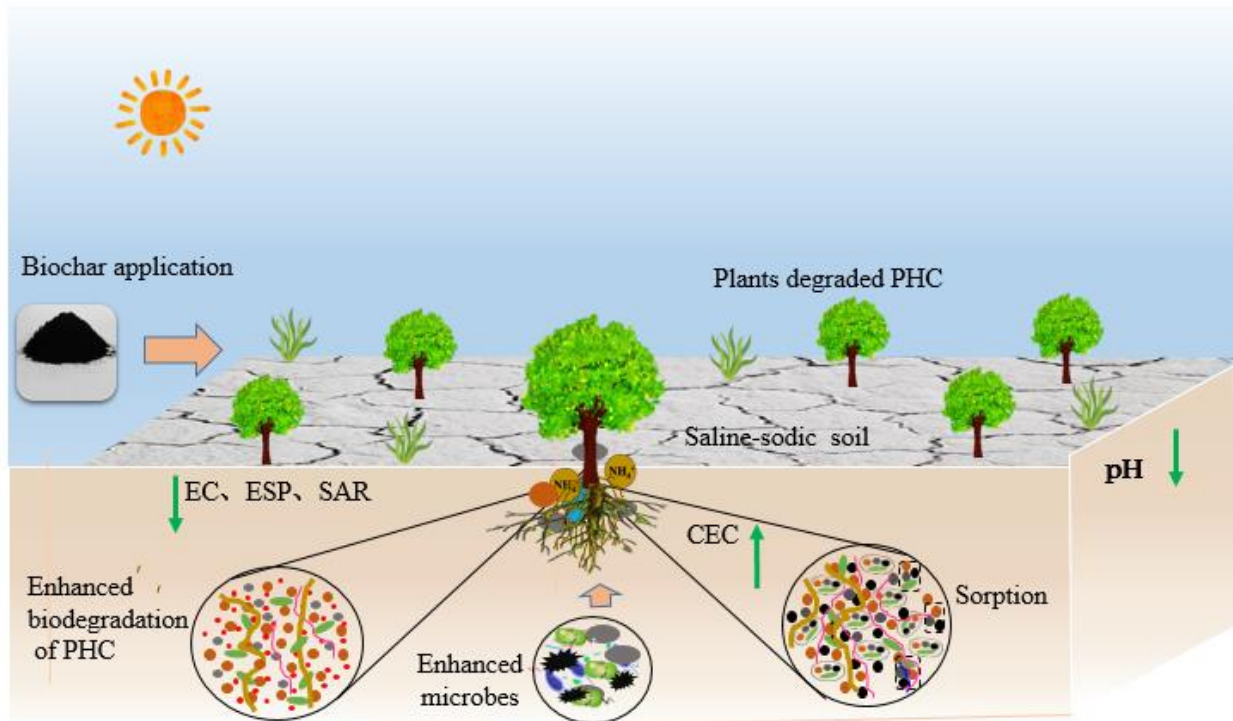


Fig 2. The application of biochar improves chemical properties through several mechanisms for the reduction of PHC degradation.