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Stimulation of phenanthrene and biphenyl degradation by biochar-conducted long distance electron transfer in soil bioelectrochemical systems

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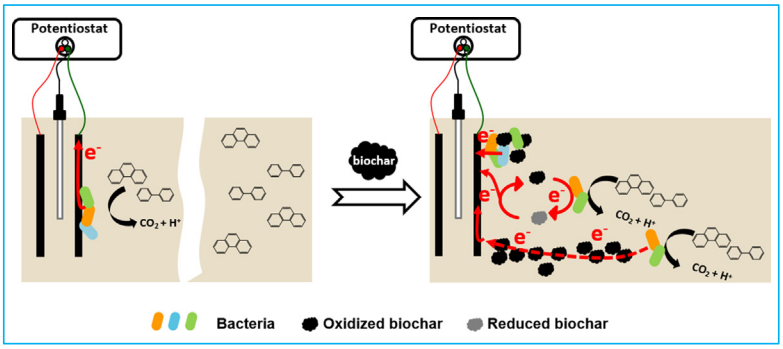
HIGHLIGHTS

- Facilitated BP and PHE degradation by biochar in SBESs was explored.
- Redox ability and conductivity of biochar promoted distance of influence in SBESs.
- Biochar accelerated the enhancement of *Proteobacteria* and *Azotobacter* in SBES.
- Biochar was beneficial to the enrichment of active BP- and PHE-degrading bacteria.

GRAPHICAL ABSTRACT



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ABSTRACT

The bioelectrochemical degradation of organic pollutants has attracted considerable attention owing to its remarkable sustainability and low cost. However, the application of bioelectrochemical system (BES) for the degradation of pollutants in soils is hindered by limitations in the effective distance in the soil matrix. In this study, a biochar-amended BES was constructed to evaluate the degradation of organic pollutants. This system was expected to extend the electron transfer distance via conductive biochar in soils. The results showed that biochar pyrolyzed at 900 °C facilitated the degradation of phenanthrene (PHE) and biphenyl (BP) in the soil BES (SBES), reaching 86.4%–95.1% and 88.8%–95.3% in 27 days, respectively. The effective distance of SBESs was estimated to be 154–271 cm away from the electrode, which increased 1.9–3 fold after the addition of biochar. Microbial community and functional gene analysis confirmed that biochar enriched functional degrading bacteria. These findings demonstrate that the promotion of long-distance electron transfer and the formation of soil conductive networks can be achieved by biochar amendment. Thus, this study provides a basis for the effective degradation of for persistent organic pollutants in petroleum-contaminated soils using bioelectrochemical strategy.

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1. Introduction

In industrialized countries, soils are frequently exposed to persistent organic pollutants (POPs) from various discharges (Uhlik et al., 2012). POPs are generally hydrophobic, with soils serving as the ultimate depository. Polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs) coexist in petroleum-contaminated soil and are becoming a growing concern (Gao et al., 2015). PAHs are ubiquitous products of the incomplete combustion of fossil fuels. They are complex organic chemicals with fused rings and at least two benzene rings (Ene et al., 2012; Gao et al., 2015). PCBs are commercially produced by the catalytic chlorination of biphenyls (BPs), which are widely used in a variety of industrial applications (Goel et al., 2016). These pollutants often pose a serious threat to both human health and ecosystem, even at low concentrations (Samanta et al., 2002). Bioremediation is considered as a low-cost and eco-friendly remediation method, and is commonly used to treat PAH- and PCB-contaminated soils (Li et al., 2019a). However, bioremediation has a number of limitations, including low abundance and diversity, as well as slow growth rates of indigenous degrading bacteria, which result in a limited soil bioavailability of PAHs and PCBs (Kronenberg et al., 2017). Therefore, effective technologies are required to improve the efficiency of soil bioremediation.

Bioelectrochemical systems (BESs) are emerging technologies that use microorganisms to catalyze oxidation/reduction reactions through donor/accept electrons from an electrode, and have been found to improve PAHs and PCBs removal (Hennebel et al., 2011; Kronenberg et al., 2017). However, it is noticed that the enhanced pollutant removal is often limited to a few centimeters near the surface of the electrode in soil-BESs (SBESs) due to the large internal resistance of soils (Wang et al., 2012). By using a multi-anode configuration, the bioelectrochemical remediation of petroleum hydrocarbons in SBESs can be improved to go beyond a certain distance (Lu et al., 2014b). However, multi-electrode systems significantly increase the cost of SBESs. Therefore, there is an urgent need to identify cost-effective alternatives that support long-distance PAHs and PCBs degradation.

Beyond a certain distance from the anode, biological stimulation is limited by the fact that electrochemical active bacteria need to connect to the electrode, either by direct contact or by a conductive network, which is of a limited length (Cai et al., 2020). The performance of SBESs can be enhanced by establishing conductive networks of exoelectrogenic bacteria and conductive materials. Li et al. (2016) reported that conductive carbon fiber could significantly enhance the bio-power generation and remediation efficiency of petroleum hydrocarbon-contaminated SBESs by improving soil conductivity. In a previous study, we applied biochar as a model conductor to increase soil conductivity and build conductive networks with microbes in the soil matrix of SBESs (Cai et al., 2020). As a result, in SBES amended with biochar pyrolyzed at 900 °C (BC900), the effective distance of pentachlorophenol (PCP) conversion was found to extend ~16 cm toward the electrode (Cai et al., 2020). This bioelectrochemical success resulted from the promotion of electron transfer and the collection of current by biochars in the reductive dichlorination process of PCP. However, little is known about how conductive networks affect the effective radius of the biological oxidation process in SBESs. Furthermore, most POPs remain adhered to soil particles because of their low solubility; therefore, the process of bio-oxidation remains unclear because the mechanisms influencing the survival and activity of microbial communities in PAH- and PCB-contaminated SBESs have not been investigated.

To address these issues, research should focus on the relationships between conductive material properties and dosage, microbial communities, and the biostimulation success of PAHs and PCBs by SBESs. In this study, phenanthrene (PHE) and BP were used as model petroleum pollutants to study PAH and PCB degradation (Li et al., 2019a). BC900-amended SBESs were used to degrade PHE and BP in petroleum-contaminated soils. The effective radius stimulated by BC900 in SBESs was determined by analyzing the spatial oxidation efficiencies of PHE and BP. In addition, the microbial community and some functional genes (PAH-ring hydroxylating dioxygenase, PAH-RHD; BP dioxygenase, *bph*A) were analyzed. These were linked to the spatial distribution to determine the conductive networks established by microbes and biochar, which can better explain the biodegradation mechanisms in SBESs. Therefore, this study provides insights into the establishment of conductive networks between functional bacteria and biochar, providing an effective strategy for the management of petroleum-contaminated soil.

2. Materials and methods

2.1. Soil sampling and biochar preparation

POP-contaminated soil was sampled from the surface soil (0–20 cm) near a petroleum production platform in Dongying, Shandong Province, China. The soil sample was air-dried at room temperature, separated with a 2 mm sieve, and stored at 4 °C until use. The soil was well-aged, as per the previous analysis (Li et al., 2019a). The main characteristics of the soil are provided in Table S1. PHE and BP were chosen as representative POP contaminants. The soil PHE and BP concentrations were adjusted to a final concentration of ~5 $mg \cdot L^{-1}$ to enrich indigenous PHE- and BP-degrading bacteria. Before their addition to the reactor, PHE and BP were spiked again in the soil and stirred thoroughly to obtain a PHE and BP concentration of 10 $mg \cdot L^{-1}$.

Biochar was produced using wheat straw at 900 °C in a cylindrical quartz tube and pyrolyzed under N_2 flow, as previously described (Cai et al., 2020). The characteristics of the biochar are summarized in Table S2. The surface morphology and pore size were visually observed using scanning electron microscopy (SEM), as shown in Fig. S1 (a) (Hitachi S-4800, Japan). Fourier transform infrared spectroscopy (FTIR) (Bruker VERTEX 70, Germany) was conducted to identify the functional groups of BC900.

2.2. Batch experiments of PHE and BP degradation in SBESs

A plexiglass reactor (30 × 5 × 5 cm) was used for the analysis (Fig. S2). Carbon cloths (7.1 cm diameter) were used as the counter and working electrodes, respectively, and the reference electrode was a saturated calomel electrode (SCE). The reactors were connected to a multichannel potentiostat (CHI 1000C, Chenhua Instrument, China), and the working electrode was applied at a constant potential of +200 mV vs. SCE. Seven sites located at x = 4, 8, 12, 16, 20, 24, and 28 cm (distance from the working electrode) were used as soil sampling sites for PHE, BP, and biological analyses. The SBESs were filled with biochar at four different mass ratios of 1%, 3%, 6%, and 10% and marked as 1% BC900-SBES, 3% BC900-SBES, 6% BC900-SBES, and 10% BC900-SBES, respectively. A closed-circuit control without biochar and open-circuit controls with biochar were marked as SBES, 1% BC900, 3% BC900, 6% BC900, and 10% BC900. In addition, an open circuit control without biochar was used to reflect the baseline natural attenuation as representative "Soil" treatment. All treatments were performed in triplicates at room temperature (25–28 °C) without light.

2.3. Pollutants analyses and electrochemical characterization of the bioelectrode

The extraction of PHE and BP was carried out in centrifuge tubes using an equal volume of ethanol and shaking on a horizontal shaker at 180 rpm for 2 h at 30 °C. The samples were subjected to high-performance liquid chromatography (HPLC) using a UV-VIS detector at 254 nm with a C18 column (Waters, USA) and methanol: water (80: 20) mobile phases (sample injection, 10 μL; flow rate, 0.8 mL/min) (Chakraborty and Das, 2016; Yang et al., 2008). The distance of effective influence (DOI) of SBES was defined as the distance between the working electrode of the SBES and the location where the PHE and BP concentrations were lower than the baseline control.

The particle size distribution of the biochar was determined using a Mastersizer 2000 particle size analyzer (Malvern Instruments Ltd., USA). Sixteen priority PAHs from the original soil were measured according to previous methods (Wang et al., 2012).

SBES performances were assessed based on the bioelectrochemical behavior, in terms of current and charge output. The charge output (Q, C) was obtained from $Q = \int_0^t I\,dt$, where I (A) is the current and t (d) is the cycle time. The PHE or BP biodegradation rate (K, d^{-1}) was obtained from the slope of the linear relationship of $-\ln(C/C_0) \sim t$, where C (mg/L) is the determinate concentration and C_0 (mg/L) is the initial concentration.

The electrical conductivity (EC) of soil and biochar-amended soil was studied using a typical double-probe bed method (Yu et al., 2015). Soils amended with different doses (0%, 1%, 3%, 6%, and 10%, w/w) of BC900 were visually characterized by scanning electrochemical microscanning (SECM) spectroscopy using a VersaSCAN SECM instrument (AMETEK, Berwyn, PA) (Qin et al., 2019). The detailed procedures for the SECM tests were obtained from previous studies (Cai et al., 2020; Qin et al., 2019). The electron exchange capacity (EEC) of biochar represents the entire electron-accepting capacity (EAC) and electron-donating capacity (EDC), and was obtained from their sum (Klüpfel et al., 2014). To quantify the EAC and EDC of BC900, the mediated electrochemical oxidation (MEO) and reduction (MER) were performed at an applied potential of +0.61 V and −0.49 V (vs. SCE), respectively. 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and zwitterionic viologen 4,4′-bipyridinium-1,1′-bis(2-ethylsulfonate) were used as electron shuttles for MEO and MER, respectively (Gorski et al., 2013; Klüpfel et al., 2014). Electrochemical characterization was carried out at an electrochemical workstation (CHI660D, Chenhua Co., Ltd., Shanghai, China) with a three-electrode cell at room temperature. A graphite plate (7 cm^2), platinum sheet, and SCE were used as the working electrode, counter electrode, and reference electrode, respectively. Chronoamperometry (CA) methods were performed in a phosphate buffer solution with 0.1 M KCl electrolyte, under constant stirring and N_2 flow.

2.4. DNA extraction and biological analysis

Genomic DNA was extracted from soil samples using a Power Soil DNA Isolation Kit (Mobio, USA) according to the manufacturer's instructions. The bacterial V4–V5 16S rRNA gene was pyrosequenced using high-throughput 454 GS-FLX pyrosequencing to analyze the microbial communities. The primers 515f/806r and 57f/1492r, with a barcode on the forward primer were used for the PCR reactions, performed in triplicate with a 25 μL mixture containing 12.5 μL of rTaq premix buffer (TaKaRa Bio Inc., Shiga, Japan), 0.5 μL of each primer (100 nM), and 1 μL of template DNA. Pyrosequencing and bioinformatics analyze were carried out based on previously described methods (Li et al., 2018). Soil samples were collected from the closed and open circuits of the SBES or 3% BC900-SBES.

The quantitative determination of PAH-RHD, *bph*A, and total bacterial populations (16S rRNA gene) was performed using an ABI 7500 real-time PCR system (Applied Biosciences, Waltham, MA, USA) with the primers RHD_f/RHD_r, bp_f/bp_r, and Bac519F/Bac907R (Table S3). The PCR mixture (20 μL) contained 10 μL of SYBR green PCR Premix Ex Taq II (TaKaRa), 0.2 μL of each primer (10 μM), and 1 μL of template DNA. The PCR conditions included an initial denaturation step at 94 °C for 10 min, 40 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, and extension at 72 °C for 15 s.

3. Results and discussion

3.1. Biochar stimulates anodic current generation in SBESs

The electric current increased gradually in all SBESs on the day 9 (Fig. 1a), indicating the adaptability of the indigenous microbial community to the electrochemical facilities. The peak current densities appeared around day 13, which may be due to the microbial utilization of electron donors around the working electrode. The subsequent decrease in current densities may be related to the discrepancy between high microbial PHE and BP requirements and relatively slower mass transfer to the working electrode. Lu et al. (2014a) argued that if the diffusion could not provide sufficiently fast hydrocarbon transfer in petroleum hydrocarbon-contaminated SBES, a decrease in the pollutant concentration on the anode would lead to a reduced current production. The maximum current densities of all treatments were enhanced compared to SBES: 1% BC900-SBES (24.2 μA/cm^2), 3% BC900-SBES (30.3 μA/cm^2), 6% BC900-SBES (33.95 μA/cm^2), and 10% BC900-SBES (51.3 μA/cm^2) were 22.1%, 53.0%, 71.5%, and 159.1% higher than SBES (19.8 μA/cm^2), respectively. In contrast, the accumulated charge output of 30.9 ± 1.4C for SBES was reached at day 27, and was 9.6%, 28.5%, 32.4%, and 50.3% lower than those of 1% BC900-SBES (33.9 ± 1.3C), 3% BC900-SBES (39.7 ± 1.6C), 6 %BC900-SBES (40.9 ± 0.8C), and 10% BC900-SBES (46.4 ± 1.2C), respectively (Fig. 1b). SBESs amended with biochar had a relatively higher current peak and charge output, owing to the faster electron transfer resulting from an improved soil conductivity or electroactive components in the structure of biochar (Li et al., 2019b).

Increasing the biochar content could improve soil EC, which was also predicted in our previous study (Cai et al., 2020). In the present study, the ECs of soil with different doses of BC900 (0%, 1%, 3%, 6%, and 10%) were 0.0005, 0.0024, 0.0042, 0.0222, and 0.0436 μS·cm^{-1}, respectively (Fig. S3). The further detection of soil EC by SECM could determine the distribution of electrochemical active sites in the soil matrix. As shown in Fig. S4(a–e), significant topographic changes were observed in the SECM images of the soil and BC900-amended soil samples. More high current peaks appeared in BC900-amended soil samples, indicating that the addition of BC900 increased the density of conductive species in the soil matrix at the micron scale. The maximum current densities of the 1%, 3%, 6%, and 10% BC900-amended soil were 0.16, 0.59, 6.07, and 19.3 mA, which were 7, 28, 302, and 964 times higher than the control soil (0.02 mA), respectively. These results indicate that conductive materials could provide additional electroactive sites for electron transfer in the soil matrix. Although conductivity is necessary, the EEC of biochar is mainly attributed to the electroactive groups on its surface, which can reversibly transfer electrons through geobattery mechanisms (Prado et al., 2019). The chemical moieties of BC900 were determined using FTIR spectroscopy. As shown in Fig. S1(b), the FTIR spectra indicated the presence of –OH (3462 cm^{-1}), C═O stretching of quinones (1656 cm^{-1}), and –COH (1445 cm^{-1}). Quinone groups were currently recognized as electroactive groups, suggesting that biochar acts as an electron shuttle (Yuan et al., 2018). The EEC of BC900 was studied further using the MER and MEO methods. As shown in Fig. S4(f), the response current after adding each dose of BC900 was compared. As a result, EAC and EDC were found to increase with an increasing biochar addition. The EAC and EDC obtained by integral calculations were 272.55 ± 26.45 $\mu mol_{e^-}/g_{BC}$ and 118.88 ± 8.21 $\mu mol_{e^-}/g_{BC}$, respectively. The EDC was less than that of EAC, indicating that most of the electroactive groups in BC900 were found in an oxidation state.

3.2. Biochar stimulated BP and PHE degradation in petroleum contaminated soil

During bioremediation, PHE and BP can be degraded by soil microorganisms. In this study, this process was found to be significantly enhanced by increasing the biochar content. After 27 days, the degradation efficiencies of PHE were 31.5%, 44.9%, 53.4%, 61.6%, and 73.9% in the 0%, 1%, 3%, 6%, and 10% BC900-amended soil, respectively. Similarly, the degradation efficiency of BP increased with the BC900 content, yielding BP degradation efficiencies of 27.6% (soil), 42.7% (1% BC900), 54.1% (3% BC900), 71.7% (6% BC900), and 75.7% (10% BC900). The K of PHE was 0.02, 0.03, 0.04, and 0.05 d^{-1} for soils amended with 1%, 3%, 6%, and 10% BC900, respectively

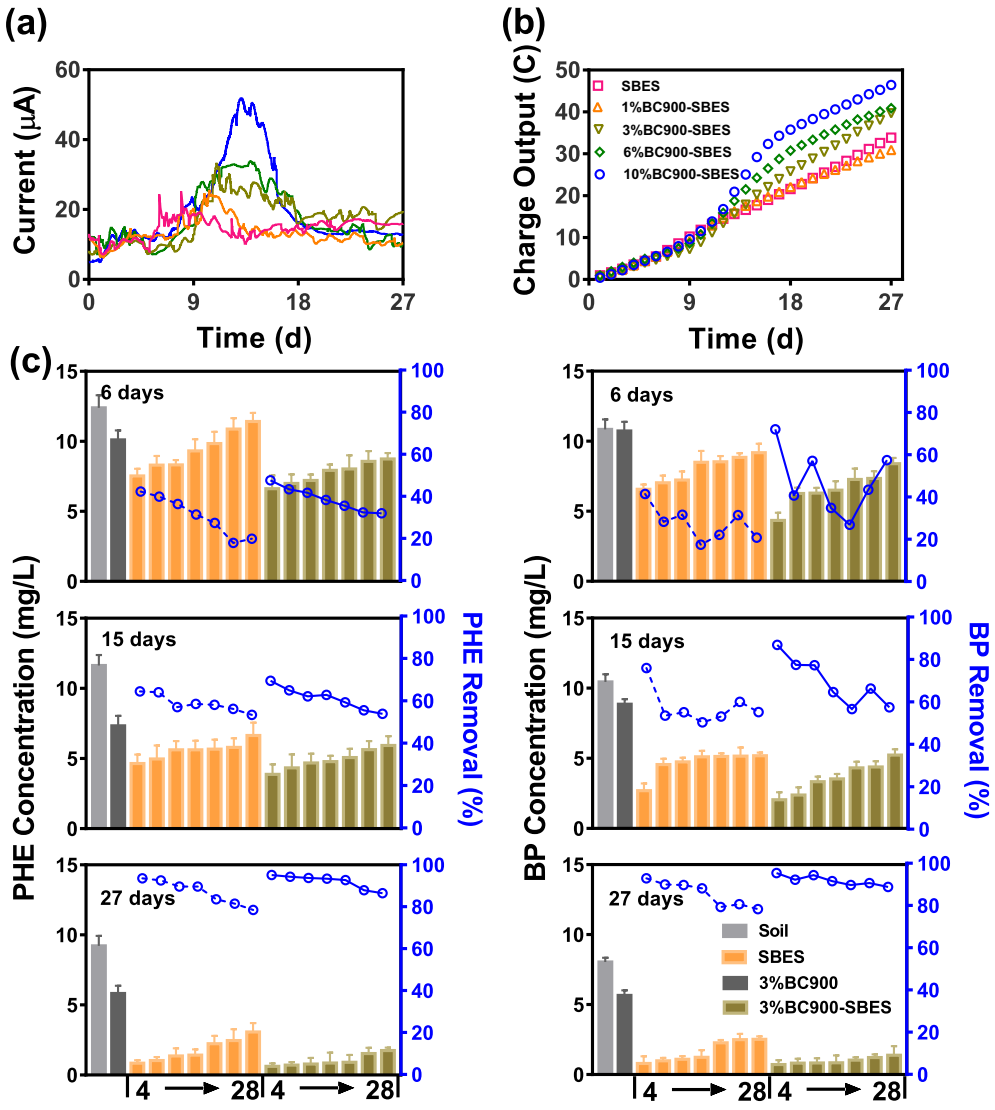

Fig. 1. (a) Biocurrent and (b) charge output of soil bioelectrochemical system (SBES) and SBESs amended with different contents of biochar pyrolyzed at 900 °C (BC900: 1%, 3%, 6%, and 10%). (c) Phenanthrene (PHE) and biphenyl (BP) removal from soil, 3% BC900, SBES, and 3% BC900-SBES treatments at different distance of influence (DOI) and sampling times.

(Fig. 2a, b). Enhanced PHE biodegradation by biochar has been previously demonstrated in an aqueous solution system that simultaneously decreased PHE concentrations of up to 90% (Leglize et al., 2008). These authors hypothesized that bacteria could form biofilms on biochar particles, allowing them to directly utilize PHE adsorbed on the surface of particles (Leglize et al., 2008). Sterilized controls were also performed simultaneously. As shown in Figs. S5 and S6, the degradation efficiency of PHE and BP was markedly inhibited after the sterilization of the soil, demonstrating the important role of the indigenous microorganisms in the removal of PHE and BP in natural environments.

3.3. Biochar stimulated long-distance electron transfer for BP and PHE degradation in SBESs

The PHE and BP degradation at increasing distances (4, 8, 12, 16, 20, 24, and 28 cm) from each SBES were monitored every three days. The spatial and temporal distributions of PHE and BP degradation and removal rates are shown in Figs. 1c and S6, respectively. The concentration of PHE on day 6, 4 cm away from the anode, was decreased to 42.3% and 47.7% for SBES and 3% BC900-SBES, respectively (Fig. 1c). This represents a 509.2% and 153.1% increase in the degradation efficiency compared to the soil and 3% BC900 treatments, respectively, in which 7.8% and 18.8% of PHE was removed. The degradation of PHE on day 15 at 4 cm away from the anode, increased significantly to 64.5% for SBES and 69.5% for 3% BC900-SBES, indicating an increase of 21.2% to 22.2% from day 6. In addition, an increase of 21.9%–33.5% at 28 cm was observed from day 6 to day 15. Similar to the observations on day 6, the control soil and soil amended with 3% BC900 were found to have significantly lower PHE degradation rates (13.5% and 41.2%, respectively). On day 27, the PHE removal at different distances from the anode ranged from 78.5% to 93.4%, and 86.4% to 95.1% in the SBES and 3% BC900-SBES treatments, compared to 31.5% and 53.4% in the control soil and 3% BC900 treatments, respectively. As shown in Fig. 1c, the degradation rates of BP in 3% BC900-SBES were also significantly higher than those in the soil, 3% BC900 and SBES treatments, respectively. Although a similar trend was observed during the degradation of BP in SBESs, the removal rate was found to fluctuate on day 6, most likely due to the strong adsorption of BP by biochar. As a result, BP dispersion in the soil was uneven. However, on day 15, the BP degradation rates at 4 cm were 76.0% and 86.9% for SBES and 3% BC900-SBES, respectively. The degradation rate was 1.7–13.5 times higher than that of the control soil and 3% BC900 treatments. On day 27, BP removal in SBESs at different distances from the anode ranged from 78.2% to

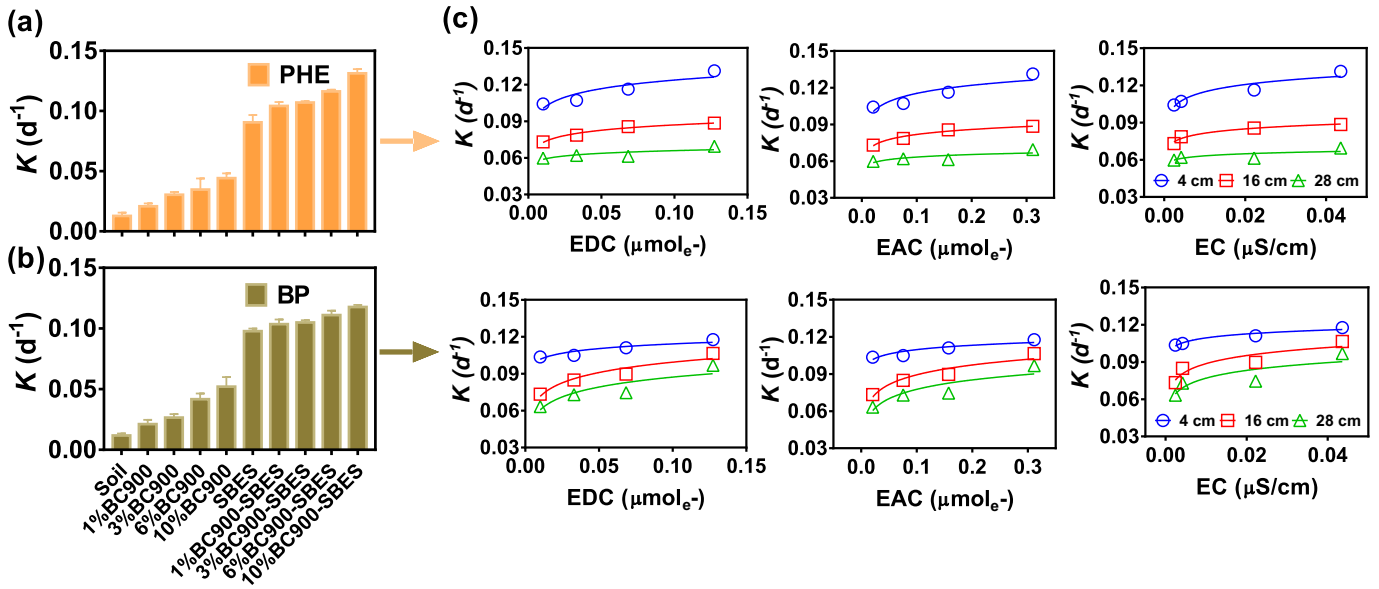

Fig. 2. (a, b) The phenanthrene (PHE) and biphenyl (BP) degradation rate (*K*) in the soil bioelectrochemical systems (SBESs) and biochar pyrolyzed at 900 °C (BC900)-amended SBESs at a distance of 4 cm toward the electrode. (c) The relationships between of the electron-donating capacity (EDC), electron-accepting capacity (EAC), and electrical conductivity (EC) of different contents of BC900-amended soils and the PHE or BP transformation rate.

92.9%, and 88.8% to 95.3% in the SBES and 3% BC900-SBES treatments, respectively, compared to 27.6% and 54.1% in the control soil and 3% BC900 treatments.

BESs is an emerging technology for the remediation of aromatic pollutant-contaminated soils (Li et al., 2015). However, the biodegradation efficiency and charge output of these systems may be limited by the difficulty of mass transfer in soil or sediment matrices (Li et al., 2019b). A similar effect was associated with SBES treatment in the present study. The prediction of the maximum DOI is an effective way to measure the performance of SBESs (Lu et al., 2014b). As shown in Fig. 3 (a)–(d), the slope between DOI and enhanced BP removal was steeper than that between the DOI and enhanced PHE removal, indicating a

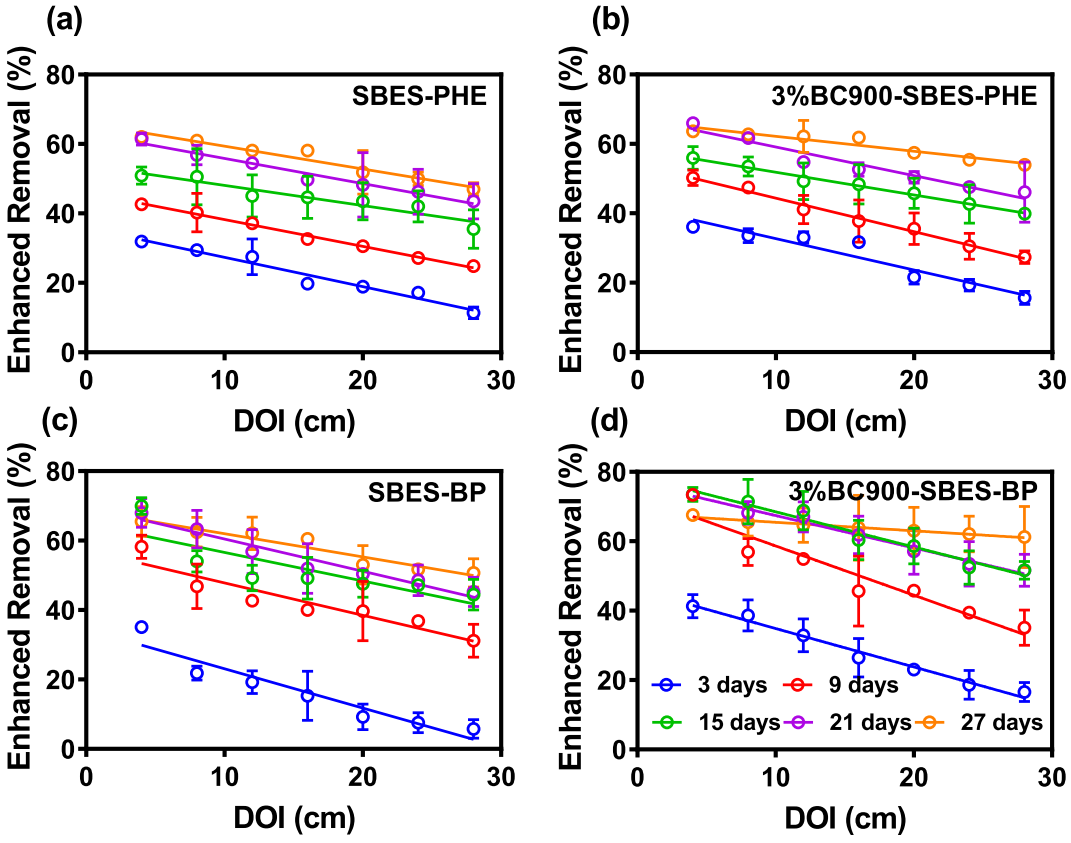

Fig. 3. Relationship between phenanthrene (PHE) and biphenyl (BP) enhanced removal and the distance of influence from the soil bioelectrochemical systems (SBESs) anode at different sampling times. Graphs were plotted based on the SBESs enhanced PHE or BP removal by deducting the value from the natural attenuation based on the absolute PHE or BP removal in the SBESs.

greater effect of electrochemical treatment on PHE degradation. Interestingly, Fig. 2c shows that the *K* of PHE increased with an increasing EC and EEC in the amended biochar, 4 cm away from the anode, whereas *K* was not significantly affected by the addition of biochar at >4 cm from the anode. However, the addition of biochar can promote the *K* of BP in SBESs within 28 cm. After microbial colonization, the biodegradation of pollutants may depend on the adsorption capacity of the biochar to pollutants. The high adsorption capacity of biochar may also facilitate the mass transfer of aromatic pollutants from the surrounding soil matrix toward the biochar (Lu et al., 2014a). BP was found to have a higher affinity to biochar than PHE, and colonized microbes tended to degrade BP more than PHE at a distance from the anode (> 4 cm). Therefore, biochar is expected to readily sequester organic contaminants and thus affect their fate and transport in natural environments (Zhang et al., 2010). In Fig. S7, a negative linear correlation appeared between the amount of PHE or BP removal and the DOI, and the slope became less negative over time. As shown in Fig. 3, the slope of the DOI gradually flattened during the experiment, suggesting that the DOI continued to extend. The interception with the *X*-axis could be considered as the predicted maximum DOI of the SBESs, as described by Lu et al. (2014b). The maximum DOI inferred from SBES increased from 30–42 cm to 99–102 cm from day 3 to day 27. As reported by Lu et al. (2014b), the maximum DOI increased from 37–47 cm to 81–90 cm after 45 days of SBES startup. However, the maximum DOI was enhanced from 99–102 cm to 154–271 cm in 3% BC900-SBES from day 3 to day 27, indicating a 1–2 fold increase in DOI due to the addition of biochar to the SBESs within 24 days. These results demonstrate that SBESs coupled with biochar could effectively degrade POPs, revealing the promotion mechanism of biochar on long-distance electron transfer. Future studies will need to analyze the intermediate products and degradation pathways in the process of mineralization and transformation of POPs (Qin et al., 2020; Zhang et al., 2021), as well as evaluating the potential risks to the environment before their application to petroleum-contaminated soils.

3.4. Spatial distribution characteristics of bacterial communities in SBESs

PHE and BP can be oxidized to less-toxic products via redox reactions mediated by microbial metabolism. In this study, the shifts and spatial distributions of bacterial communities after the addition of biochar were analyzed by 16S rRNA high-throughput sequencing. A comparison of the overall phylum-level phylogenetic characteristics is shown in Fig. 4a. *Firmicutes* and *Proteobacteria* were the most dominant microbial components in the control soil, soil with 3% BC900, SBESs without biochar, and SBESs with 3% BC900 treatments. Variations were observed in *Firmicutes* between the closed and open circuits of SBESs. Although the relative abundance of *Firmicutes* was 26.5%, it was enhanced by 24.4% for amendment with 3% BC900 and 11.9%–72.5% for SBESs. *Firmicutes* are reported to be electrochemical active bacteria and can improve electron transfer via biofilm matrices, such as nanowire-like appendages (Goud and

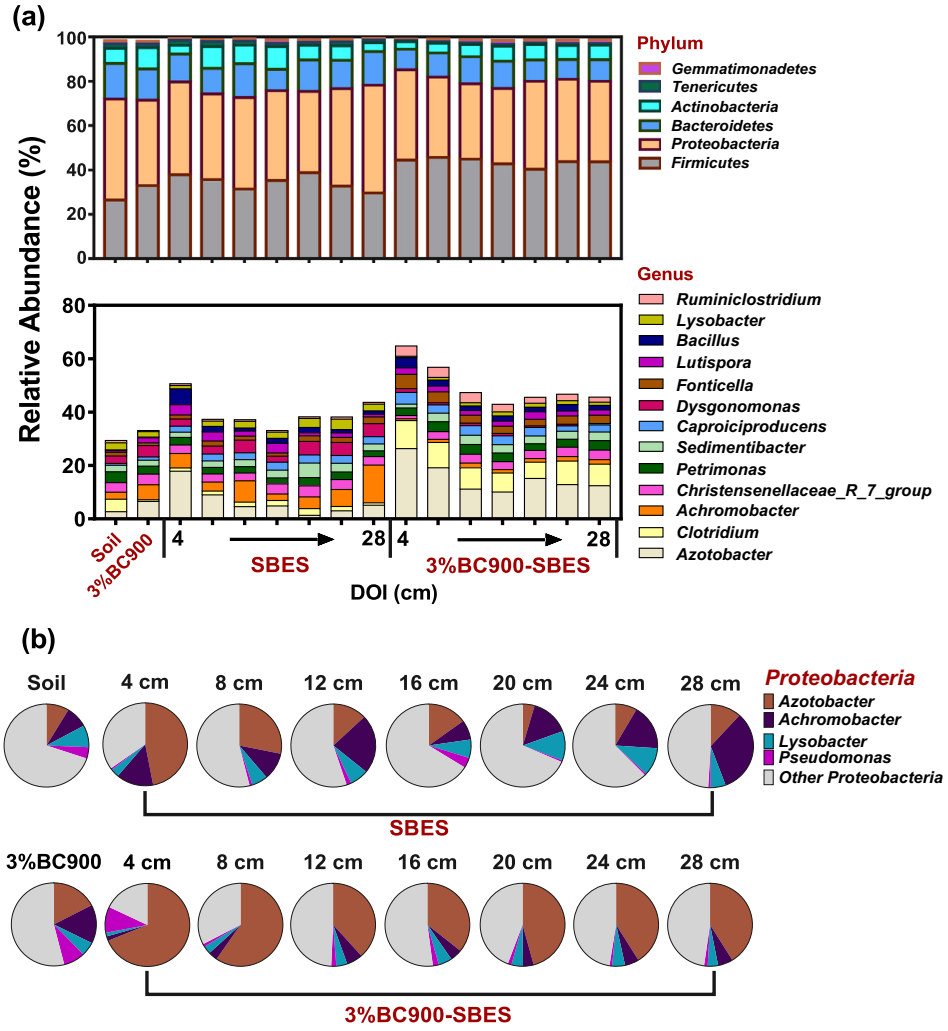

Fig. 4. Taxonomic classification of bacteria DNA sequences from communities at the (a) phylum or (b) genus level, and (c) the phylogenetic distribution of Proteobacteria with or without 3% biochar pyrolyzed at 900 °C (BC900)-amended soil bioelectrochemical systems (SBESs).

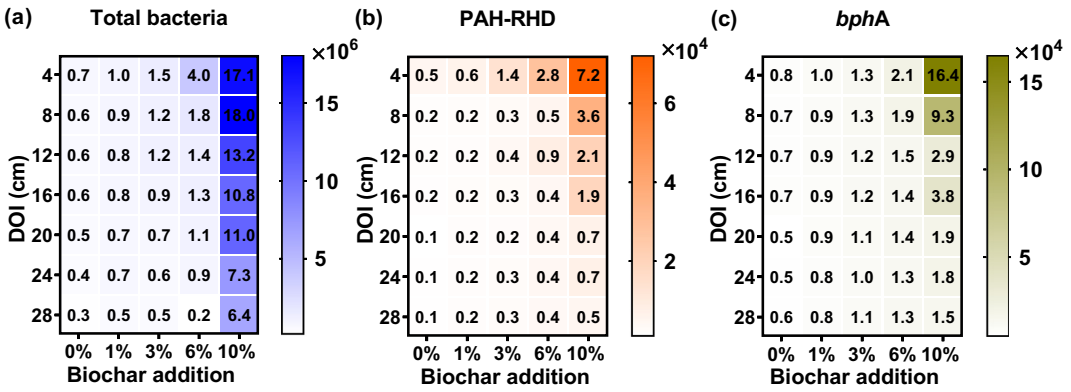

Fig. 5. DNA copy numbers of 16S rRNA genes copies of (a) total bacteria, (b) PAH-ring hydroxylating dioxygenase (PAH-RHD), and (c) *bphA* genes with an applied bias-potential of +200 mV vs. SCE at various distances (4, 8, 12, 16, 20, 24, and 28 cm) toward the electrodes.

Mohan, 2013; Reguera et al., 2005). This suggests that biochar or SBESs also alter the microbial communities to enhance electron transfer. As gram-negative bacteria, *Proteobacteria* possess a thin cell wall that allows the redox mediators or metabolites to diffuse inside and outside the cell, facilitating efficient electron transfer and enhancing the formation of biocurrents (Goud and Mohan, 2013). At the genus level, *Azotobacter* and *Achromobacter* were found to be dominant in SBES, while *Azotobacter* and *Clostridium* were dominant in SBES+BC900 (Fig. 4b). The relative abundance of the three dominant bacteria was markedly higher than that in the soil. *Azotobacter* is often used as a plant growth-promoting rhizobacteria (PGPR) that promotes plant growth and is easily enriched in PAH- and TPH-contaminated soils (Cheng et al., 2012; Urana et al., 2019). *Azotobacter* is also capable of utilizing benzoate (Langenheder and Prosser, 2008), BP (Uhlik et al., 2012), and pyrene (Singleton et al., 2006). The *Azotobacter* species in the *Proteobacteria* phyla were highly enriched in the 3% BC900-SBES treatment (Fig. 4b), with 26.3%, 19.1%, 11.1%, 10.1%, 15.2%, 12.9%, and 12.4% at 4, 8, 12, 16, 20, 24, and 28 cm away from the anode, respectively. Similarly, the relative abundance of *Azotobacter* around the electrode was also higher than that at farther locations in the SBES, which were 17.8%, 9.0%, 4.5%, 4.8%, 1.3%, 3.0%, and 5.1% at 4, 8, 12, 16, 20, 24, and 28 cm away from the anode, respectively. *Achromobacter* has been previously shown to completely remediate PAH-contaminated soils, including low- and high-molecular-weight PAHs (Cutright and Lee, 1994; Dave et al., 2014). In addition, *Clostridium* capable of degrading PAHs and PCBs in aquatic environments or mangrove sediment has also been previously described (Chang et al., 2008; Hou and Dutta, 2000; Yuan and Chang, 2007). In this study, while biochar amendment or SBES treatment had little effect on soil microbial diversity, they were found to increase the abundance of PAH-degrading bacteria in petroleum-contaminated soils. Biochar coupled with SBESs were found to significantly enhanced the in situ functional microbial abundance, which in turn promoted PHE and BP degradation. Thus, this is an effective method for the site remediation of contaminated soils.

Many labile components that may stimulate microbial activity exist in biochars (Kong et al., 2018). To further confirm the role of biochar in the conversion of PHE and BP in SBESs, the functional genes (PAH-RHD and *bphA*) were quantified for SBES and biochar-amended SBESs at each sampling point. The functional genes encoding the alpha subunit of PAH-RHD and *bphA* are commonly used as biomarkers to quantify bacterial populations that are able to degrade PAHs and PCBs. As shown in Fig. 5, in both SBES and BC900-SBES microcosms after an incubation period of one month, the abundance of the total 16S rRNA and functional genes at each sampling point was significantly higher in the BC900-SBES treatments than in the SBES control. The total 16S rRNA gene abundance of SBES at 4, 8, 12, 16, 20, 24, and 28 cm was 7.27, 6.17, 6.02, 5.87, 5.35, 4.30, and 2.62 ($\times 10^5$) copies/g, accounting for 48%, 50%, 51%, 65%, 73%, 73%, and 54% of that in the 3% BC900-SBES treatment, respectively (Fig. 5a). Accordingly, the relative abundance of PAH-RHD and *bphA* after 3% BC900-amendment increased to 75%–175% and 64–112%, respectively (Fig. 5b, c). Similarly, the total 16S rRNA, PAH-RHD, and *bphA* copy numbers in soils amended with biochar microcosms were significantly higher than in the control soil (Fig. S8). This indicated that the addition of conductive biochars enriched microbial quantities, accompanied by an overall decrease in the copy numbers of 16S rRNA, PAH-RHD, and *bphA* with an increasing distance from the electrode. However, the quantitative results were not consistent with the spatial distribution results of the PHE or BP degradation, which suggests that PHE and BP-degrading microbial communities may possess other functional genes or enzymes not targeted by the primers used in this study, as many microorganisms were able to colonize the pores of biochar.

4. Conclusion

Biochar can increase soil conductivity and redox ability, thereby increasing long-distance electron transfer in soils. In this study, the removal of POPs (PHE and BP) and the biocurrents were found to be improved in biochar-amended SBESs. The DOI reached 154–271 cm in terms of PHE and BP degradation, representing a 1.9–3.0 fold increase compared to SBESs without biochar. Upon biocurrent stimulation, specific species, such as electroactive bacteria (*Proteobacteria*) and POP-degrading bacteria (*Azotobacter*), were found to establish an interacting metabolic network for better adaption to SBESs. The spatial variability of bacterial communities and functional genes reported in this study demonstrates the need to understand biological interactions in this emerging technology.

CRediT authorship contribution statement

Xixi Cai: Conceptualization, Methodology, Writing – original draft. **Xiaoshan Luo:** Formal analysis, Software, Investigation. **Yong Yuan:** Writing – review & editing, Project administration, Supervision. **Jibing Li:** Writing – review & editing. **Zhen Yu:** Writing – review & editing. **Shungui Zhou:** Writing – review & editing.

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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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2021.149124.

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