Research Article

Enhanced biodegradation of phenanthrene using different inoculum types in a microbial fuel cell

Environmental pollution by petroleum hydrocarbons from contaminated groundwater and soils is a serious threat to human health. Microbial fuel cells (MFCs) could be employed in the treatment of these recalcitrant pollutants with concomitant bioelectricity generation. In this study, the use of MFCs in biodegradation of phenanthrene, a model hydrocarbon, was investigated with respect to its biodegradation rate, biodegradation efficiency, and power production using a range of inocula (Shewanella oneidensis MR1 14063, Pseudomonas aeruginosa NCTC 10662, mixed cultures, and combinations thereof). All the inocula showed high potentials for phenanthrene degradation with a minimum degradation efficiency of 97%. The best overall performing inoculum was anaerobically digested sludge supplemented with P. aeruginosa NCTC 10662, having a degradation rate, maximum power density and chemical oxygen demand removal efficiency of 27.30 μM/d, 1.25 mW/m² and 65.6%, respectively. Adsorption of phenanthrene on the carbon anode was also investigated; it conformed to a Type II adsorption isotherm and could be modelled using a modified Brunauer, Emmett and Teller model with a maximum monolayer capacity of 0.088 mg/cm². This work highlights the possibility of using MFCs to achieve high degradation rates of phenanthrene through co-metabolism and could potentially be used as a replacement of permeable reactive barriers for remediation of hydrocarbon-contaminated groundwater.

Keywords: Adsorption / Biodegradation / Co-metabolism / Microbial fuel cell / Phenanthrene

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1 Introduction

Environmental pollution by petroleum hydrocarbons resulting from oil spillages, leaks or indiscriminate disposal is a serious problem. The hydrocarbons, most notable of which are polycyclic aromatic hydrocarbons (PAHs) among others, are toxic, posing serious threats to human health from contaminated groundwater and soils [1]. While a number of approaches such as chemical oxidation, photo-oxidation, use of dispersants etc. have been employed in the cleaning up of hydrocarbon-contaminated sites [2], most of the processes are expensive, ineffective, too slow or environmentally unfriendly.

Biodegradation could be a cost-effective and environmentally friendly way of PAH removal. However, aerobic degradation requires injection of oxygen into the subsurface environment which is capital intensive, difficult to implement and unsustainable [3]. Anaerobic degradation may require the presence of terminal electron acceptors (TEAs) such as nitrate, sulphate or metallic oxides but even then it is a slow process. The deployment of TEAs for in situ treatment is also not without its problems; for example, the high solubility of TEAs in water makes them to easily diffuse away from the point of application due to hydrodynamic forces [3]. Also, as anaerobic degradation proceeds, the amount of TEAs depletes and thus becomes a rate-limiting factor for degradation. Continuous supply of TEAs is not sustainable due to high cost resulting from maintenance and energy costs.

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Abbreviations: COD, chemical oxygen demand; CE, coulombic efficiencies; CO, a co-culture of S. oneidensis and P. aeruginosa; MC, anaerobic-digested sludge; MCO, anaerobic-digested sludge with the co-culture; MCS, anaerobic-digested sludge with S. oneidensis; MCP, anaerobic-digested sludge with P. aeruginosa; MFC, microbial fuel cell; PA, P. aeruginosa; PAH, polycyclic aromatic hydrocarbon; S.O, S. oneidensis; TEAs, terminal electron acceptors
Microbial fuel cells (MFCs) have recently been proposed as having the potential for bioremediation of various contaminants [4–7]. MFCs are unique in the sense that the microorganisms are able to transfer (or receive) electrons extracellularly to a solid material like an anode electrode. The presence of these insoluble and inexhaustible electrodes allows continuous transfer of electrons to the cathode (which could be exposed to air in the atmosphere) where they are consumed by oxygen. This use of oxygen as an indirect TEA would be expected to enhance hydrocarbon degradation compared to degradation via anaerobic respiration.

MFCs have been investigated in the bioremediation of hydrocarbons in model contaminated soil and groundwater systems but only a few studies have been undertaken [6,8–11]. Morris et al. [10] achieved 82% DRO (diesel range organics) removal with an MFC over 21 days compared to an anaerobic incubated control, which achieved 31% removal. Yan et al. [11] studied the degradation of phenanthrene and pyrene in freshwater sediment using sediment microbial fuel cells. It was found that high removal efficiencies of 99.5 and 94.8% were recorded for phenanthrene and pyrene, respectively, over a period of 240 days.

Most studies investigating the feasibility of treating petroleum hydrocarbons in wastewater, sediments and groundwater systems used undefined mixed cultures or indigenous microbes as inocula [6,8,10,12]. Most of the researchers recorded prolonged experimental durations ranging from 20–240 days. Unfortunately, this could limit the potential application of this unique technology in real scenarios.

The use of pure or defined co-cultures in the presence of co-substrates could reduce the period required to degrade hydrocarbons. In the case of cocultures, there could be potential for synergistic utilisation of the metabolic pathways from the microorganisms involved [13]. Such synergy may involve one organism reducing available oxygen in the anode thus enhancing growth of another microaerophilic microorganism or strict anaerobe. Alternatively, by-products of one microorganism may be used by another microorganism as substrate, redox mediator, surfactant etc. The aforementioned two examples may be representative of the coculture of Pseudomonas and Shewanella. Mixed cultures were reported to show good process stability in MFCs [14]; the two strains could have potential for bioaugmentation of mixed cultures used in MFCs.

Carbon materials used in MFCs as anodes can adsorb petroleum hydrocarbons in aqueous or particulate media [15]. Therefore, they could play an essential role in initial removal of hydrocarbons from any medium during MFC operation. However, this adsorption effect has rarely been quantified in the literature.

This study therefore investigated the biodegradation of phenanthrene in a microbial fuel cell using a range of inocula (Shewanella oneidensis, Pseudomonas aeruginosa, mixed cultures and combinations thereof). Of interest were the degradation rates, degradation efficiency, power production and the assessment of phenanthrene’s adsorption on a carbon electrode.

2 Material and methods

2.1 Chemicals

Phenanthrene (97.0% purity) was obtained from Acros (UK). Sodium pyruvate, D-glucose and all other chemicals used were purchased from Sigma Aldrich (UK). Chemical solvents including Ficodox Plus™ mixed chemical oxygen demand (COD) reagent were obtained from Fisher Scientific (Loughborough, UK). All chemicals were of analytical grade and used without further purification.

2.2 Microbial inocula and growth media

Two pure strains along with an undefined anaerobic consortia were used as anodic biocatalysts. Shewanella oneidensis (MR1 14063) and Pseudomonas aeruginosa (NCTC 10662) were obtained from microbial culture collections in our laboratory. Anaerobic-digested sludge samples were obtained from Mogden Sewage Treatment Works London (UK). Anaerobic sludge inoculum was grown anaerobically in serum vials in minimal medium supplemented with D-glucose and subsequently incubated at 30°C for 48 h. S. oneidensis was grown in minimal medium supplemented with sodium pyruvate (500 mg L⁻¹) and 500 mg L⁻¹ Casein hydrolysate (Sigma Aldrich, UK) while P. aeruginosa was sub-cultured in minimal medium supplemented with 300 mg L⁻¹ D-glucose. The defined minimal medium was prepared as described by Fernando et al. [16] but modified by adding 8.24 g L⁻¹ Na₂HPO₄, 5.08 g L⁻¹ NaH₂PO₄, 1.0 g L⁻¹ NH₄Cl, 0.5 g L⁻¹ NaCl and 0.25 g L⁻¹ MgSO₄.

2.3 Experimental design

2.3.1 Experiment 1: Biodegradation of phenanthrene in MFCs

The influence of inoculum type on phenanthrene degradation and MFC performance was investigated using S. oneidensis (S.O.), P. aeruginosa (P.A.), a co-culture of S. oneidensis and P. aeruginosa (CO), anaerobic-digested sludge (MC), anaerobic-digested sludge with the co-culture (MCO), anaerobic-digested sludge with S. oneidensis (MCS) and lastly anaerobic-digested sludge with P. aeruginosa, MCP (Table 1). Each inoculum was 10% v/v of the working volume of the anode chamber (200 mL) while in instances where mixing of different strains occurred, mixing was done in a 1:1 ratio by volume. The anodic chamber had a total capacity of 250 mL and a working volume of 200 mL. The anolyte medium consisted of 100 mg cosubstrate (pyruvate or glucose) per litre of minimal medium, 20 mg L⁻¹ phenanthrene (taken from a 1000-fold concentrate in 100% methanol) and the inoculum (20 mL). In each treatment, three controls were employed: an abiotic MFC, disconnected MFC and non-MFC (anaerobic control using the same strains as in the test). The methanol used in dissolving the phenanthrene (0.1% v/v of the working volume) is considered to be nontoxic since the concentration used is far below the minimum inhibitory concentration.
2.3.2 Experiment 2: Adsorption of phenanthrene onto the anode (carbon electrode)

Experiment 2 determined the adsorption equilibrium of phenanthrene between the bulk solution in the anode and the carbon-felt electrode, (C-TEX 27; surface density 110 g/m²; surface area 1100 m²/g, Mast Carbon Inc., Basingstoke, UK). Various phenanthrene concentrations—20, 40, 80, 140 and 200 ppm were prepared in conical flasks containing 100 mL minimal medium (similar to anolyte medium) and a carbon electrode (total surface area of 40 cm²). The pH of the medium was buffered at pH 7 by PBS (which is part of the medium composition). The flasks were placed in an incubator with agitation speed set to zero. This was intentionally set to zero to mimick conditions used in the running of the MFCs in Experiment 1. Flasks containing no electrode were used as controls. All flasks were kept at a constant temperature of 30°C. The contact time was 180 mins. This contact time was long enough for equilibrium to be attained as determined from a separate study (details not given). Another experiment was conducted using an abiotic MFC with its anode poised at a negative potential (ca. −200 mV Vs Ag/AgCl reference electrode) using a Potentiostat–Galvanostat (PG581, Uniscan Instruments, Buxton, UK) under similar conditions as discussed above (i.e., same temperature, electrode specifica-

2.4 MFC configuration and operation

A dual-chamber MFC was used. The MFC consisted of two identical chambers made of glass bottles with an anode and cathode chamber total volume 250 mL each. The glass bottles were held together by a metallic holder with two rubber gaskets placed in-between to avoid leakage. The diameter of the chambers was 7.0 cm with both chambers separated by a cationic exchange membrane (20 cm², CMI-7000 Membranes International, NJ, USA). The electrodes were made of carbon felt of the same size (4.0 cm × 5.0 cm) and the cathode was coated with a platinum (Pt) catalyst (0.50 mg/cm²) on both sides. The anode chamber was filled with anolyte medium (pH 7.0). The cathode chamber was filled with PBS, pH 7.0 and continuously sparged with air (at a flow rate of 100 mL air/min) using an aquarium pump (KOI Air, UK). Copper wires were used for the connections after sealing with an epoxy sealant.

The experiments were carried out in batch mode with both anodic and cathodic compartments having the same working volume of 200 mL each. The MFCs were sterilised by autoclaving at 121°C for 15 min while anolyte were added to the anode compartment aseptically. The anodic compartments were kept anaerobic by purging them with 100% N₂ for 15 min before starting the experiments. Closed circuit connections were made over an external load of 1000 Ω in all experiments and voltage outputs logged online using Labview® data acquisition software (National Instruments, UK). All experiments were conducted at 30 ± 0.5°C using a water-bath (Fisher Scientific, UK).

2.5 Analytical methodology and data analysis

2.5.1 Phenanthrene analysis

Phenanthrene was analysed by HPLC (Dionex GS50, USA) using a Photo-diode Array (PDA) detector (DIONEX, PDA-100) at 254 nm. The injected volume was 20 μL. The analytical column was a reversed phase column, Supelcosil™ LC-PAH column (150 mm × 4.6 mm). The mobile phase (80% acetonitrile and 20% deionized water) flow rate was 0.5 mL/min. The column oven temperature was set at a constant temperature of 25°C. The minimum detectable concentration for phenanthrene was 5 μg/L. Phenanthrene extraction procedures employed were adopted from Kermanshahi pour et al. [19] but with slight modification: 1 mL of aliquots were withdrawn at intervals from the MFC and transferred to 2 mL eppendorf tubes. Subsequently, 1 mL of methanol was added to make up to 2 mL and the eppendorf tubes (which were placed in a 200 mL glass beaker) were incubated in an incubator shaker for 1 h at 25°C and 150 rpm. The tubes were then centrifuged at 10 000 g for 10 min and 500 μL of the supernatant was carefully transferred into 1.5 mL HPLC glass vials prior to analysis by HPLC. In order to quantify the total amount of phenanthrene degraded, the amount of phenanthrene adsorbed on the anode was determined by soaking the anode electrodes in 20 mL methanol at the end of each experiment for 1 h at 200 rpm. Aliquots were transferred into 2 mL eppendorf, immediately followed by centrifugation at 10 000 g for 10 min. All liquid samples were immediately analysed within few hours after sampling in order to minimize adsorption onto the wall of the sample vials. Degradation efficiencies and rates were determined based on the remaining phenanthrene in solution and that adsorbed on the anode at the end of MFC operation.

2.5.2 COD removal measurement

The COD of the samples was determined using the closed reflux titrimetric method as described in the Environment
Agency (UK) Standard method 5220D (APHA Standard Methods for the Examination of Water and Wastewater, 20th ed.). Appropriately diluted 1 mL samples were used for each determination.

The COD of samples was expressed as percentage COD removal and COD removal rate. The percentage COD removal was calculated as follows:

\[
\text{Percentage COD removal} = \left( \frac{\text{COD}_i - \text{COD}_f}{\text{COD}_i} \right) \times 100
\]

where \( \text{COD}_i \) and \( \text{COD}_f \) are initial COD and final COD values, respectively.

\[
\text{COD removal rate} = \left( \frac{\text{COD}_i - \text{COD}_f}{t} \right)
\]

where \( \text{COD}_i \) and \( \text{COD}_f \) are initial COD and final COD values respectively and \( t \) is time taken for each experiment when the voltage had dropped below 5 mV.

2.5.3 Electrochemical analysis

The performance of the MFCs for all inoculum types was assessed based on voltage and current outputs. Polarisation readings were taken by connecting various external resistances (ranging from 1 \( \Omega \) to 1 M\( \Omega \)) across the external circuit at average interval of about 5 min after the MFC reached a stable cell potential. The current flowing through each external load, internal resistance of the MFC and power produced were calculated as described by Logan [20]. Power density (Wm\(^{-2}\)) and current density (Am\(^{-2}\)) were normalized to the projected total surface area of the anode (40 cm\(^2\)). The coulombic efficiencies (CE) were calculated by integrating current over time and comparing to the total charge generated based on observed COD removal during the MFC operation [20].

2.5.4 Statistical analysis

Statistical analyses were performed using Prism Graph Pad 5.0 with \( \alpha = 0.05 \). All data are presented as means of duplicate experiments unless otherwise stated and the error bars represent the maximum error of the mean. One-way analysis of variance was used to compare MFC performances and degradation rates among all treatments. All one-way analysis of variances showing significant differences were followed by post-hoc Tukey’s comparison tests. Correlation analyses were also conducted (using Prism Graph Pad 5.0) to measure the degree of association between data collected. The modeling of adsorption data was done in Microsoft Excel using the solver add-in.

3 Results and discussion

3.1 Adsorption of phenanthrene onto the carbon anode

Adsorption studies revealed that the anode (carbon) electrode has a high affinity for phenanthrene with a maximum adsorption capacity of 0.45 mg/cm\(^2\) (within the range of concentrations tested). The adsorption isotherm (Fig. 1) was of Type II typical of non-porous solids or solids with large pores (macropores of size >50 nm). Adsorption of phenanthrene appears to proceed layer by layer with chemisorption (which follows the Langmuir isotherm) taking place in the first instance (up to the inflection point A) and physisorption (to form multi-layers) taking place later on (point B) [21]. The adsorption isotherm has been modelled onto the Brunauer, Emmett and Teller model of multilayer adsorption (Eq. (3), as modified for solutes in solutions [22]) and it was found that \( q_m = 0.088 \) mg/cm\(^2\), \( K_b = 1.38 \) L/mg, \( K_L = 0.018 \) L/mg for non-polarized electrode. For the polarized electrode, the corresponding values were \( q_m = 0.079 \) mg/cm\(^2\), \( K_b = 0.34 \) L/mg and \( K_L = 0.011 \) L/mg. Notably, the adsorption data for polarized electrode were lower than the non-polarised electrode as observed in this study. A possible explanation for this could be due to the negative anode potential leading to an increase in the repulsive forces between the electrode’s surface and the partially negatively charged phenanthrene molecules.
Figure 2. (A) Phenanthrene concentration in the bulk anode solution as a function of time and (B) phenanthrene (average) degradation rates by different inocula used in Experiment 1. (C) Comparison of power densities outputs and coulombic efficiencies for different inoculum types (External resistance, $R_{\text{ext}} = 1000 \ \Omega$). The error bars represent the maximum error of the mean.

thereby limiting affinity of the phenanthrene molecules for the electrode [23].

$$q_e = q_m \frac{K_s C_e}{(1 - K_L C_e) (1 + K_s C_e)} \quad (3)$$

($q_e$ = amount of adsorbate adsorbed on the solid surface, mg/cm$^2$; $q_m$ = amount of phenanthrene corresponding to complete monolayer adsorption, mg/cm$^2$; $K_s$ = equilibrium constant of adsorption for first layer, L/mg; $K_L$ = equilibrium constant of adsorption for upper layers in Brunauer, Emmett and Teller isotherm, L/mg and $C_e$ = equilibrium concentration of adsorbate in the liquid phase, mg/L).

The shape of the adsorption isotherm is related to the texture of the solid (pore shape and size, percent porosity, specific surface area etc.). The isotherm observed for phenanthrene on carbon felt shows that the electrode is porous with adsorption taking place in two stages: a fast stage followed by a slow one [15]. Various mechanisms may contribute to adsorption of phenanthrene onto the carbon electrode, e.g., weak intermolecular forces such as $\pi-\pi$ interactions, H-bonding and electron donor–acceptor interactions [15].

Walters and Luthy [24] demonstrated that PAHs adsorbed strongly onto porous carbon materials over soils, sediments and other suspended organic matters, suggesting the suitability of carbon materials for co-localisation or temporary removal of PAHs (and benzene, toluene, ethylbenzene and xylene) compounds from aquatic environments. Adsorption of petroleum hydrocarbons by the anode in MFCs was not considered in the work of Morris et al. [10] and Wang et al. [9].

The fact that the carbon-felt electrode is a good adsorbent for phenanthrene may suggest its use in treatment of petroleum hydrocarbons in both liquid and particulate systems. It is also suggested that kinetic studies of hydrocarbon degradation in MFC studies ought to include amounts adsorbed on the electrode instead of relying only on what is in solution. The question of whether adsorption of hydrocarbons on anodes interferes with electron transfer is discussed in Section 3.3.

### 3.2 Phenanthrene biodegradation in MFCs

#### 3.2.1 Role of adsorption in biodegradation of phenanthrene during MFC operation

Phenanthrene concentrations in the bulk solution for all seven inocula decreased rapidly by about 90% within 24 h followed by a gradual decrease afterwards (Fig. 2A). The sharp decrease in all cases observed was attributed to phenanthrene
adsorption by the carbon-felt electrode. The disappearance of phenanthrene from solution appears to occur via two stages: the first stage (fast) is controlled by adsorption while the second stage (slow) depends mainly on microbial action. Zhang et al. [3] and Xia et al. [25] reported that microorganisms can degrade aromatic hydrocarbons on an electrode and in solution but initially the hydrocarbons are adsorbed. Adsorption of hydrocarbons on carbon electrodes appears to be a good thing in this case as it negates mass transfer resistances of substrate/redox mediator from the bulk solution to the microorganisms existing as a biofilm on the electrode. The mechanism of microbial degradation of adsorbed phenanthrene on the electrode is not fully understood. Phenanthrene may desorb from the electrode followed by biodegradation or the biodegradation process may occur directly on the electrode [26]. Considering the fact that the microbes used are electrode respiring bacteria, electrons produced as a result of substrate utilisation could be transferred either directly, or through any mediated process, that facilitates electron transfer [12]. Figure 3A indicates that phenanthrene adsorbed on the electrode was probably utilised by anode-respiring bacteria as the amount adsorbed in connected MFCs was significantly different ($p < 0.05$) from that adsorbed on electrodes in disconnected MFCs. This suggests that MFC technology could enhance phenanthrene degradation in contaminated anaerobic environments.

### 3.2.2 Influence of inoculum type on degradation of phenanthrene

The degradation of phenanthrene for different inocula is shown in Fig. 2A and B. The reported degradation rates for phenanthrene using different inocula were determined based on their total concentrations (phenanthrene remaining in the aqueous solution and that adsorbed onto carbon electrode after the experiment) in comparison to the starting concentration. All the seven inocula (in connected MFCs) showed very high potential for phenanthrene degradation with minimum degradation efficiency of 97% compared to the controls (Fig. 3B). Abiotic loss in abiotic control might result from adsorption of phenanthrene on glassware used for the experiments [27]. The marked differences in degradation efficiency between the connected MFCs and the disconnected MFCs or non-MFCs (in all inoculum types) indicates an enhancement of phenanthrene degradation via electron transfer through a temporary electron acceptor (i.e., the anode) to the cathode where oxygen is used as terminal electron acceptor. Notably, phenanthrene removal in disconnected MFCs was much higher than anaerobic controls (non-MFCs); this could be attributed to oxygen intrusion from the anode (leading to aerobic degradation) and/or sorption of phenanthrene on the electrode possibly improving microbe-substrate interaction. Similar observations were made by Huang et al. [27] in a soil MFC. They suggested that the "electrons produced by the oxidation of..."
organic carbon in the open-circuit MFC might be transferred from the anode to the cathode via an internal soil matrix, such as bacterial nanowires and conductive minerals, resulting in higher COD and contaminant (phenol) removal in the open-circuit MFC compared with those in non-MFC [27].

A marked variation in the phenanthrene biodegradation rate was observed as a function of inoculum type during the MFC operation; P. aeruginosa gave the highest degradation rate of 54.70 μM/d followed by MCO; the anaerobic sludge with S. oneidensis MR1 gave the lowest degradation rate of 25.20 μM/d. The two strains, P. aeruginosa and S. oneidensis MR1 may possess PAH degrading enzymes that enabled them to co-metabolically biodegrade phenanthrene.

Pseudomonas species have been reported by several authors to have a potential to degrade PAHs compounds [28, 29]. Ma et al. [28] successively isolated P. aeruginosa strain PAH-1 that had the ability to anaerobically degrade phenanthrene with anthraquinone−2, 6−disulphonate as the sole electron acceptor. The authors reported 56.7% phenanthrene removal in the presence of a co-substrate, fructose. Pseudomonas species have also been found in MFC anodes and can be classified as electrochemically active bacteria [20]. They respire anaerobically via the production of phenazines and pyocyanin, electron shuttling compounds, which aid transfer of electrons to the anode [20]. These redox shuttling compounds aid in facilitating enhanced microbial oxidation of organic compounds like phenanthrene via electron transfer to the anode. Since these redox electrons shuttling compounds enhance electron transfer, high degradation rates and power densities would be expected. High degradation rates were observed as presented in this study. However, due to large internal resistances, the power density appears to be very small (Fig. 4). P. aeruginosa also secretes biosurfactants that may be expected to solubilize both suspended phenanthrene in anolyte and the phenanthrene adsorbed on the anode. Higher degradation rates observed with P. aeruginosa (relative to other inocula tested) might be due to the synergistic effect of its PAH degrading enzymes, biosurfactant self-production and the involvement of soluble shuttlers for the redox powers.

Anaerobic biodegradation of phenanthrene has previously been reported to occur via carboxylation followed by cleavage of the aromatic ring putatively at the K region of the phenanthrene ring [30]. In this study, a decrease in pH (from 7 to 5.8) was observed at the end of MFC operation, indicating that some acidic intermediates were probably produced during the phenanthrene degradation for all inocula tested. However, due to the range of inocula tested and the presence of co-substrate, identification of
these acidic metabolites becomes more complex and time consuming. Nevertheless, further work is underway to identify these metabolites with the aim of mapping out the metabolic pathways for phenanthrene degradation.

Degradation efficiencies recorded in this study are relatively higher than those reported in the literature (as shown in Table 2) where other alternative electron acceptors (e.g., nitrate and sulphate) were used to enhance anaerobic degradation of certain PAH compounds. This is the very first report that demonstrated that pure strains such as S. oneidensis and P. aeruginosa can oxidise phenanthrene in a microbial fuel cell. Morris et al. [10] investigated enhanced anaerobic biodegradation of diesel using MFCs and subsequently conducted microbial community analysis in order to identify the microorganisms that catalysed the anodic reactions. S. oneidensis was found among electrochemically active bacteria found in the anode of the MFCs.

The degradation rate obtained for CO (Table 2) was significantly lower than that for P. aeruginosa (p < 0.05). This implies that the degradation performance of P. aeruginosa in the co-culture (CO) was inhibited, suggesting possible negative microbial interactions between the two microorganisms under tested conditions. The reason for this is unknown and requires further investigation. One possible explanation for this could be due to differences in the redox conditions at which both strains thrive. S. oneidensis have been reported to thrive under anaerobic conditions more favourably than P. aeruginosa; S. oneidensis, a dissimilatory metal-reducing bacteria, can couple metal reduction with their metabolism whereas P. aeruginosa cannot [20,34].

As a result, there is a possible population shift occurring within the anode chamber, especially on the anode biofilm when both strains are used as co-culture (as in this study).

In this study, we have demonstrated that P. aeruginosa gave a high degradation rate and biodegradation efficiency of 0.055 mM/d and 98% respectively in a MFC reactor. It could potentially be used for bioaugmentation purposes. Bioaugmentation involves the introduction of foreign strains with the metabolic capability to degrade the hydrocarbon of interest in an environment where the indigenous microbes are incapable and is one of the bioremediation techniques that have been used in field applications [35]. Nasseri et al. [29] reported the influence of bioaugmentation on biodegradation of phenanthrene-contaminated soil in a bio-slurry reactor. They demonstrated that the bioaugmented bioreactor (using P. aeruginosa) showed higher degradation efficiency of over 85% compared to 17% observed in a non-augmented reactor (indigenous microorganisms). Others authors such as Hamdi et al. [36] and Gao et al. [37], all reported enhanced PAHs biodegradation with the aid of bioaugmentation. Our results suggest P. aeruginosa as a possible choice for enhanced bioremediation of PAHs in contaminated environments.

### 3.3 MFC performance during phenanthrene degradation

A marked variation in electrochemical performances of different inocula (Fig. 4) is an implication of differences in the electrochemical behaviour of each inoculum type. The bacteria consortia MCP gave the highest power density of 1.25 mW/m² while similar power densities of 1.1 and 1.05 mW/m² were recorded for CO and MCS respectively (cf. 0.37 mW/m² for MC). Power densities from MFCs with cosubstrate only (i.e., glucose or pyruvate) was higher than treatments (data not shown). This is likely expected as high power densities could be as a result of the presence of readily oxidisable compounds (like glucose or pyruvate) and their non-toxic effect compared to MFCs with phenanthrene (i.e., treatment). As previously discussed, P. aeruginosa could be forced to produce more redox electron shuttling molecules (i.e., pyocyanin or phenazines) from its cells under anaerobic conditions; its positive interaction with electrochemically active microbes present in the anaerobic sludge could have likely contributed to high power density observed for bacteria consortia MCP. Figure 4B and C indicates that from a power density perspective, synergistic microbial interactions exist in the CO, MCS and MCP, which perhaps, may have contributed to differences in electrochemical performances of different strains investigated. One possible explanation for this could be the possible transfer (e.g., via horizontal gene transfer) of “electrogenic activity” to non-electrochemically active or redundant strains. In light of this, findings from this study suggest bioaugmentation could play an important role in enhancing electrochemical performances of the system in which it is being implemented.

Notably, there was a no correlation between power density and degradation rates (r = 0.07, p < 0.05); a similar observation was reported by Hu et al. [38]. A number of factors, e.g., oxygen intrusion into the anode, substrate conversion to biomass etc. may confound the correlation between degradation rate and power density. The cell voltages and power densities for all inocula were inversely correlated with internal resistances of the cells (Fig. 4) in accordance with Ohm’s law.

Figure 2C shows that there is no correlation between power density and coulombic efficiency (r = 0.50, p < 0.05). Coulombic efficiency recorded for all inocula tested were generally lower (<1.4%), indicating a substantial loss of electrons within the system. The observed low coulombic efficiency may be due to oxygen diffusion into the anode chamber or a result of presence of other alternative electron acceptors such as sulfates (0.25 g L⁻¹ MgSO₄ was used in this study) that make up the anolyte medium. Adsorption of phenanthrene may not be expected to interfere with electron transfer from bacterial cells to the electrode.

<table>
<thead>
<tr>
<th>Electron acceptor</th>
<th>Inoculum</th>
<th>Degradation rate (μM/d)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>Marine sediment</td>
<td>3.3</td>
<td>[31]</td>
</tr>
<tr>
<td>Sulfate</td>
<td>Marine sediment</td>
<td>0.1</td>
<td>[32]</td>
</tr>
<tr>
<td>Sulfate</td>
<td>Marine sediment</td>
<td>0.1</td>
<td>[33]</td>
</tr>
<tr>
<td>Carbon-felt electrode&lt;sup&gt;41&lt;/sup&gt;</td>
<td>MC</td>
<td>35.7</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>PA</td>
<td>54.7</td>
<td></td>
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<tr>
<td></td>
<td>S.O</td>
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<tr>
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<td>CO</td>
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</tr>
<tr>
<td></td>
<td>MCP</td>
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<td></td>
</tr>
<tr>
<td></td>
<td>MCS</td>
<td>27.7</td>
<td></td>
</tr>
</tbody>
</table>

<sup>41</sup>Carbon-felt electrode used in an MFC as anode.
Table 3. Evaluation of system performances by different inocula based on three main parameters

<table>
<thead>
<tr>
<th>Inoculum type</th>
<th>Degradation rate, x (μM/d)</th>
<th>Max. power density, $P_{max}$, y (mW/m²)</th>
<th>COD removal, z (%)</th>
<th>System performance index, $A = xyz/1000$</th>
</tr>
</thead>
<tbody>
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<td>S.O</td>
<td>25.20 ± 5.15</td>
<td>0.51 ± 0.03</td>
<td>70.43 ± 0.42</td>
<td>0.97</td>
</tr>
<tr>
<td>P.A</td>
<td>54.70 ± 0.60</td>
<td>0.19 ± 0.05</td>
<td>60.61 ± 1.69</td>
<td>0.63</td>
</tr>
<tr>
<td>CO (P.A and S.O)</td>
<td>26.20 ± 2.81</td>
<td>1.11 ± 0.07</td>
<td>28.98 ± 0.99</td>
<td>0.87</td>
</tr>
<tr>
<td>MC</td>
<td>35.70 ± 2.73</td>
<td>0.37 ± 0.05</td>
<td>17.47 ± 0.14</td>
<td>0.24</td>
</tr>
<tr>
<td>MCO</td>
<td>36.50 ± 0.60</td>
<td>0.44 ± 0.02</td>
<td>46.63 ± 0.71</td>
<td>0.74</td>
</tr>
<tr>
<td>MCS</td>
<td>27.70 ± 0.20</td>
<td>1.05 ± 0.14</td>
<td>61.02 ± 2.55</td>
<td>1.67</td>
</tr>
<tr>
<td>MCP</td>
<td>27.30 ± 1.10</td>
<td>1.25 ± 0.11</td>
<td>65.60 ± 5.52</td>
<td>2.21</td>
</tr>
</tbody>
</table>

Values are means of duplicate experiments.

Substrate oxidation by anodophiles with release of electrons occurs both in solution and on the anode. Electrons released in the solution could be shuttled to the electrode by redox mediators secreted by the microorganism while electrons released on the anode can be transferred directly with the aid of c-type cytochromes [20, 39]. In this study, we have demonstrated that closed circuit MFC systems were better than open circuit and anaerobic systems in terms of higher degradation rates and efficiency (Figs. 2 and 3). This suggests that adsorption may not be detrimental to electron transfer. However, the power densities and coulombic efficiencies, as previously discussed, do appear to indicate otherwise; but this can be explained by the large internal resistances measured in the MFC systems (Fig. 4D).

COD removal for different inocula during the MFC operation is shown in Fig. 3C and 3D. Cultures P.A, S.O, MCP and MCS gave COD removal percentages in excess of 50% with COD removal rates above 8 mg/L/h. This points to the utility of *Pseudomonas aeruginosa* and *Shewanella oneidensis* as good candidates for MFC-based bioremediation as pure cultures or as supplements to mixed cultures.

### 3.4 Selection of the best performing strain(s)

The criterion for the assessment of MFC performance (using different inocula) is based on the degradation performance and electrochemical performance. Table 3 shows a summary of performances of different strains measured by a system performance index ($A$) that consists of three parameters lumped together namely: degradation rate, % COD reduction and maximum power density. A high $A$ index indicates good system performance. Data from Table 3 identifies MCP to be the best inocula while MC is least in order of overall system performance. This suggests the use of MCP for bioaugmentation purposes in treatment of phenanthrene-contaminated sites based on MFC technology.

### 4 Concluding remarks

This study demonstrated the possibility of using MFCs, utilising a range of inocula, to enhance the biodegradation of phenanthrene through co-metabolism with concomitant, albeit meagre electricity production. The best overall performing inoculum was MCP, a mixed culture supplemented with *P. aeruginosa*. The culture gave a phenanthrene degradation rate of 27.30 μM/d, a maximum power density of 1.25 mW/m² and a COD removal of 65.6%. Adsorption studies showed that phenanthrene exhibits a strong affinity for the carbon felt anode electrode conforming to a Type II isotherm and having a monolayer capacity of 0.088 mg/cm². It is suggested that *Pseudomonas aeruginosa* NCTC 10663 may offer good prospects for bioaugmentation of mixed cultures in MFCs for bioremediation of hydrocarbons.

**Practical application**

This article has demonstrated the feasibility of using an innovative technology, MFC, in the treatment of a model PAH compound, phenanthrene. This MFC technology can potentially be used as an independent system instead of other bioremediation technologies (e.g., pump and treat or permeable reactive barriers) for the treatment of petroleum hydrocarbons in contaminated subsurface environments or industrial effluents.

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### 5 References


[36] Hamdi, H., Renzerti, S., Manusadzianas, L., Aoyama, I. et al., Bioaugmentation and biostimulation effects on PAH.

