Earthline Journal of Chemical Sciences
ISSN (Online): 2581-9003
Volume 6, Number 1, 2021, Pages 37-63
https://doi.org/10.34198/ejcs.6121.3763

Enhanced Electrochemical Treatment of Phenanthrene-polluted Soil using Microbial Fuel Cells

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Abstract

In this study, tubular microbial fuel cells (MFCs) were inserted into phenanthrene-contaminated water-logged soil in order to evaluate their treatment efficiency and overall system performance within 60 days' incubation period. At day 10, phenanthrene degradation rates were found to decrease with increasing distance from the anodes from 50-55 % at 2 cm to 38-40 % at 8 cm. Bromate (used as a catholyte) removal in both MFCs was about 80-95 % on average which is significantly higher than the open circuit controls (15-40 %) over the 60day period. Total chemical oxygen demand removal (72.8 %) in MFCs amended with surfactants was significantly higher than MFCs without surfactant (20 %). This suggests that surfactant addition may have enhanced bioavailability of not only phenanthrene, but other organic matter present in the soil. The outcomes of this work has demonstrated the simultaneous removal of phenanthrene (86%) and bromate (95%) coupled with concomitant bioelectricity generation (about 4.69 mWm^{-2}) using MFC systems within a radius of influence (ROI) up to 8 cm. MFC technology may be used for *in situ* decontamination of soils due to its potential detoxification capacity and could be deployed directly as a prototype-MFC design in field applications.

1.0. Introduction

Increasing global demand for petroleum hydrocarbons and its products is often

Received: March 24, 2021; Accepted: April 22, 2021

Keywords and phrases: bromate, degradation efficiency, microbial fuel cells, phenanthrene, radius of influence.

associated with soil contamination resulting from waste disposal and leakage from storage tanks or during its transportation from one point to another. Petroleum hydrocarbons are known to adversely affect human health and render soils hazardous through contamination with BTEX and PAH compounds (Wang *et al.* [1], Guo and Zhou [2], Sarkar *et al.* [3], Zhou *et al.* [4]). A recent report on oil contamination in Ogoniland in Nigeria revealed the benzene and PAHs levels was 1800 and 500 times higher than WHO standards respectively. Two spills in Ogoniland required about $30 billion for clean-up operations over 30 years (UNEP [5]).The cleanup of these contaminants is expensive, especially using physical and chemical methods. The use of biological methods such as *in situ* bioremediation, is a relatively inexpensive, non-intrusive and eco-friendly methods of treating such contaminants in sediments and soil environments.

Soil microbial fuel cells (sMFC) are a new technology for remediation of soils contaminated with organic compounds without need for any introduction of donor or acceptor into the soil or subsurface environment (Morris and Jin [6], Zhang *et al.* [7]). Electrodes in sMFCs can provide a less-expensive and easy passage of electrons from the anode to the cathode (which does not corrode over long-term deployment) thus stimulating the anaerobic oxidation pollutants (Morris *et al.* [8], Zhang *et al.* [7],Wang *et al.* [1]). Moreover, electricity production during MFC operation is an indication of substrate biodegradation which can be used to provide energy needed for operating online monitoring wireless sensors (Donovan *et al.* [9]).

A few studies on bioelectrochemically-assisted soil/sediment bioremediation have been reported for phenol (Huang *et al.* [24]), BTEX compounds (Zhang *et al.* [7]), petroleum hydrocarbons (Morris and Jin [6]) and PAHs (Wang *et al.* [1]). Morris and Jin [6], reported TPH degradation rates in the sediment was about 12 times higher than the baseline control. Wang *et al.* [1] also observed a significant increase in TPH degradation (i.e. from 6.9% to 15.2 %) in U-tube like soil MFC, especially water-logged close to the anode but a decrease in system performance was observed with increasing distances from the anode and decreasing moisture content. Despite the previous studies on petroleum hydrocarbon removal using MFC of various configurations, to the best of our knowledge, there has been no report that explored the possibility of coupling the use of cathodic electron acceptors other than oxygen such as hydrogen peroxide, potassium persulfate among others, with petroleum hydrocarbon degradation using a tubular soil MFC bioreactor design. Bromate has previously been demonstrated in previous studies (Adelaja [10], Adelaja *et al.* [11]) to be a potential electron acceptor at the cathode in lieu of platinum due to the high cost of platinum catalyst and its limited application in subsurface anoxic environments. Bromate, a toxic pollutant, has been reportedly found in wastewater treatment effluents, groundwater, stagnant ponds/lakes and the marine environment with high chloride ions concentrations (Zhao *et al.* [12], Bao *et al.* [13]).

The radius of influence (ROI), which is a distance at which enhancement of biodegradation takes place from the anode, could to a large extent determine the practical deployment of MFCs for in situ bio-treatment of oil-contaminated soil. ROI could largely depend on MFC architecture, physical and bio-chemical characteristics of both pollutants and soil.

In this study, newly modified column-type MFCs were developed for to facilitate phenanthrene degradation (a model PAH compound) in soil by varying phenanthrene concentrations relative to the distance from the MFC anodes with concomitant bioelectricity production. The effect of surfactant addition on MFC performance was also investigated. Influence of changes of soil ionic strength and pH were also monitored in order to check their effect on the degradation of phenanthrene.

2.0. Materials and Methods

2.1. Chemicals and reagents

All Chemicals were purchased from Sigma Aldrich (Dorset, UK), Acros (UK) and QiaGen Ltd (Crawley, UK). HPLC Grade solvent reagents including Methanol, Acetonitrile (ACN), already prepared COD reagent (Ficodox Plus™) used was gotten from Fisher Scientific (Loughborough, UK). No further purification was done on chemicals prior to use and reagents employed for this work were of analytical grade (≥ 99.98 % purity).

2.2. Soil sample collection and characterization

Contaminated soil samples used for soil MFC studies were obtained from Barking, London, UK with a known history of petroleum hydrocarbon contamination. Soil auger was used to collect soil samples at the above location from 5 to 10 cm beneath the soil top layer surface. The soil was collected in airtight plastic bags, transported directly to the lab, spread to dry at ambient temperature for 72hrs (25 ± 3°C) and the debris of plant/animal origin and clay-like materials were removed by sieving with 2mm sieve and clayey material. The samples were thereafter stored at 4°C prior use. A complete physicochemical analysis of the aliquots sample of the soil was carried out by Forest Research, Surrey, UK. The original soil used is a sandy loam soil with a background phenanthrene levels of 1.950 mgkg^{-1} DS (dry soil). The main physiochemical and mineral analysis of the soil sample is described in the supplementary information.

2.3. Reactor design and set-up for tubular soil MFCs

The soil MFCs were tubular-like MFC reactors which were constructed using PVC tubes with one sealed end as shown in Figure 1.

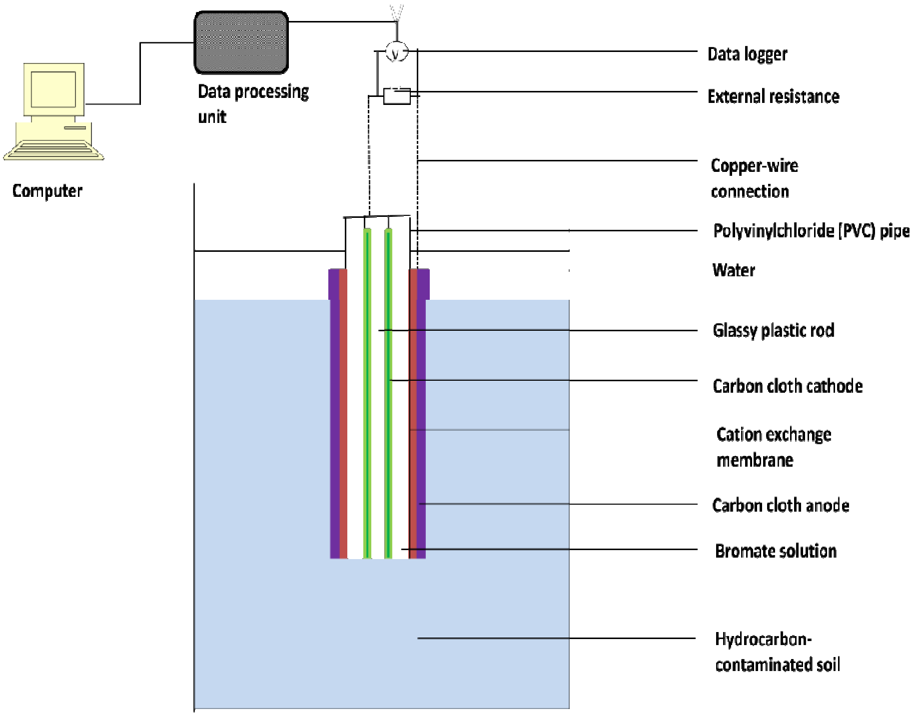

Figure 1. The schematic diagram of the tubular soil MFC reactor experimental set up employed for treating a model PAH-contaminated soil.

The inner chamber of the 0.5 cm thick PVC tube (4.5 cm diameter x 40 cm length) made up the cathode chamber (with operating volume of 200 mL) while the anode was fastened firmly onto the outer section of the PVC tube (which had evenly distributed holes of 1 cm diameter) using plastic cable ties, thus leaving the anode side exposed to the hydrocarbon-contaminated waterlogged soil. The evenly distributed holes on the PVC tube allowed cation exchange between the anode and the cathode with a cation exchange membrane, CMI-7000 (Membranes International, USA). The cathode chamber was filled with the catholyte potassium bromate solution (1000 mg L^{-1} at pH 5), and sterile deionized water was added to the cathode camber at intervals to make up for water loss due to evaporation. The soil MFC was embedded MFCs in a rectangular PVC storage container (18 cm x 7.6 cm x 20 cm) containing approximately 15 kg of waterlogged soil immersed in 2 cm of deionized water above the soil.

Carbon felt was used as both anode and cathode in the experiment (C-TEX 27; Mast Carbon Inc, Basingstoke, UK) with estimated surface area of 156 cm^2 and 96 cm^2 (measured) respectively. All electrical connections were insulated and subsequently coated will a silicone rubber in order to prevent system short circuit and corrosion due to immersion in aqueous medium. External load of 1000 Ω was connected to the MFC and the MFC was incubated at ambient temperature (25 ± 5°C) for 60 days and protected from sunlight.

Voltage outputs from the MFCs were monitored in real-time using a data acquisition system (Picolog ADC-24, Pico Technology, UK) which captured voltage outputs every 10 mins throughout the experiments. Water lost via evaporation and during samples collections was duly made up by sterile deionized water at intervals (2-3 days) to maintain the saturated condition.

2.4. Experimental design

In this study, four tubular MFC units (of same design) with two units each installed on two similar rectangular PVC storage containers was used in the experiment setup. In one of the storage containers, the two units installed were different tests (one with surfactant, MFC+S and the other without surfactant, MFC-S) while the other two in the second storage container, were two sets of open-circuit MFC controls each for MFC+S and MFC-S respectively.

Two non-MFC (or anaerobic) reactors were used as baseline controls; one with surfactant, E+S and the other without surfactant, F-S. These reactors were operated as previously described in Section 2.2. The original soil (previously described in Section 2.1) was spiked with phenanthrene and manually homogenised using an iron paddle to a final phenanthrene concentration of 1000 mg kg^{-1} dry soil. Prior to MFC operations, the spiked soils in each storage container were incubated for 14 days for partial aging and enrichment of the indigenous microbial population. Phenanthrene release or bioavailability and migration to the anode's ROI from the soil was tested by adding a non-ionic surfactant Tween 80 (500 mg L^{-1}) to the soil. The catholyte (1000 ppm bromate solution) was replaced thrice a month during the operational period. Biological and chemical evaluations were conducted on soil samples by periodically sampling at 2cm, 4cm and 8cm from the anode of the top, middle and bottom layer of soil. The distance from the anode's outer surface to where phenanthrene concentrations are less to that of control is known as radius of influence, ROI. No external inoculum was introduced to the primary microbes in the soil.

2.5. Analytical methods

2.5.1. Chemical analysis

2.5.1.1. Petroleum hydrocarbon determination

For the analysis of anolytes samples for the determination of phenanthrene concentrations present in the samples, High-performance liquid chromatography (HPLC, Dionex GS50, USA) fitted with a Photo-diode Array (PDA) detector (DIONEX, PDA-100) at 254nm was employed. HPLC conditions employed for analysis of the samples taken from the anode chamber were similar as earlier described in previous studies (Adelaja *et al.* [11]). The analytical column was a reverse phase column, Supelcosil^{TM} LC-PAH column (150 mm × 4.6 mm). A method as described by Kermanshahi pour *et al.* [14] was employed in the extraction of phenanthrene present in the soil MFC samples.

Degradation efficiencies were evaluated based on the residual phenanthrene (PHE) concentration at the end of MFC operation.

In order to determine the amount of phenanthrene present in the solid phase of the soil MFCs phenanthrene extraction was conducted by adding 5 mL of acetonitrile (ACN) to 2 g of collected soil samples in a centrifuge tube as previously described by Coates *et al.* [15] and vortexed for 5 mins. The soil-solvent mixture was sonicated for 1 h and subsequently centrifuged at 12000 g for 15 mins. The supernatant liquid was filtered through 0.22 μm filter units into 2 mL glass vial before HPLC analyses as described above.

2.5.1.2. Determination of bromate and COD removal in MFCs

Spectrophotometric method was employed in the quantitative determination of the removal of bromate at the end of MFC operation (Emeje *et al.* [16]). Procedure for sample preparations prior analysis at 620nm using a UV-Vis spectrophotometer M 6300 model (Jenway Staffordshire, UK) as followed as described by Adelaja *et al.* [17]. The percentage bromate removal was calculated based on residual bromated when experiment ended. COD titrimetric procedure was used for determination of the chemical oxygen demand (COD) of the samples in line with Environment Agency (UK) Standard method 5220 D (APHA, [18]).

2.5.1.3. pH, conductivity and total dissolved solid (TDS) measurements

The pH of the anodic medium during MFC operations and after each cycle was determined with a Mettler Toledo MP220 pH meter (UK). pH changes in the cathode chamber of MFCs containing bromate as catholyte were also monitored. Oakton PC-700 (Oakton Instruments, UK) conductivity meter was used to measure conductivity and TDS. 1:5 (w/v) soil-deionized mixture of water was used to detect soil conductivity and TDS.

2.5.1.4. Electrochemical characterisation

The soil MFCs performance was assessed by measuring the cell voltage and electric current across an external resistance of 1000 Ω with a multimeter connected to a personal computer by a data acquisition system on an hourly basis under normal operating conditions as described by Logan [19]. Polarisation curves were determined by gradually increasing the external resistances from 1 Ω to 1 MΩ with the pseudo steady-state voltage recorded at about 5 minutes. The total internal resistance (Rint) of the soil MFC were determined using the polarisation slope method while current and power densities were executed using standard methods (Logan *et al.* [19], Fan *et al.* [20], Sleutels *et al.* [21]).

2.5.1.5. Cyclic voltammetry analysis

The bioelectrochemical behaviour of soil MFCs was examined using cyclic voltammetry with the aid of a Potentiostat-Galvanostat (PG 581, Uniscan Instruments, Buxton UK). The scanned potential was between -600 and +200 mV (Vs Ag/AgCl reference electrode), at a scan rate of 10 mV/s. The working electrode was anode, cathode served as a counter electrode while Ag/AgCl was used as reference electrode (BASi, Germany, 4M KCI, +196 mV versus standard hydrogen electrode (SHE) at 25°C) in a sealed chamber was used as a reference electrode.

The bioelectrochemical cell was kept at 30°C unless otherwise stated. The device was operated remotely through a personal computer (PC) using UIE Chem v3.54 software.

2.5.1.6. Bioluminescence toxicity assays

The Microtox standard acute toxicity method was employed in conducting toxicity assays on samples drawn from the soil MFCs before and after MFC operation (Gaudet [22]). Soil samples were centrifuged at 13.2 x g and subsequently a Whatmanfilter (0.22 µm) was used to remove suspended biomass and soil particles. The bioluminescent marine bacteria used for this assay was *Vibrofischeri* (13938) which was grown, harvested and re-suspended in a sterile 2% w/v NaCl solution before use as described by Adelaja *et al.* [17] for the assay using a Fluostar Optima luminometer.

2.6. Data analysis

Statistical analyses were carried out using MATLAB software at significant level, P = 0.05. All experiments were done in duplicates and error bars is a function of the standard deviation of the mean. Data were treated through using correlation analysis to determine the degree of data association.

3.0. Results and Discussion

3.1. Pollutants removal and ROI determination during MF Cooperation

Phenanthrene degradation at different distances (2, 4 and 8 cm) from each MFC anode was monitored on day 10, 20, 30, 40, 50 and day 60 during the 60 days MFC operation as shown in Figures 2 and 3.

At day 10, phenanthrene removal from soil at 2 cm from the anode’s outer surface were 55 % and 50 % for MFC+S and MFC-S reactors respectively, which was 120-293 % higher than the non-MFC reactors (E-S and F+S) respectively (Figure 2). The observed rapid decrease in phenanthrene after MFC start up may be attributed to the adsorption of phenanthrene near the MFC anodes. Adsorption of phenanthrene near MFC anodes after MFC start up may be attributed to observed the rapid decrease in phenanthrene concentration in the soil. This observation corroborates previous studies conducted by Zhang *et al.* [7] and Lu *et al.* [23] where similar observations reported were linked to hydrocarbon adsorption on the electrode’s surface.

Phenanthrene degradation rates decreased with increasing distance from the anodes from 50-55 % at 2 cm to 38-40 % at 8 cm among MFC reactors. The negative steep slope between the ROI and phenanthrene removal at day 10, as shown in Figure 3, indicates a smaller ROI by MFC reactors. The decrease in the degradation rates may possibly be due to mass transfer limitations and lower activity of electrochemically active microorganisms. However, this limitation was gradually overcome with time from 10 to 60 days of operation as phenanthrene removal increased, especially at locations further away from MFC anodes. The creation of concentration gradient, as indicated by the removal of phenanthrene closer to the anode increased with time, and could have driven mass movement in the bulk electrolyte towards electrode by increasing ROI.

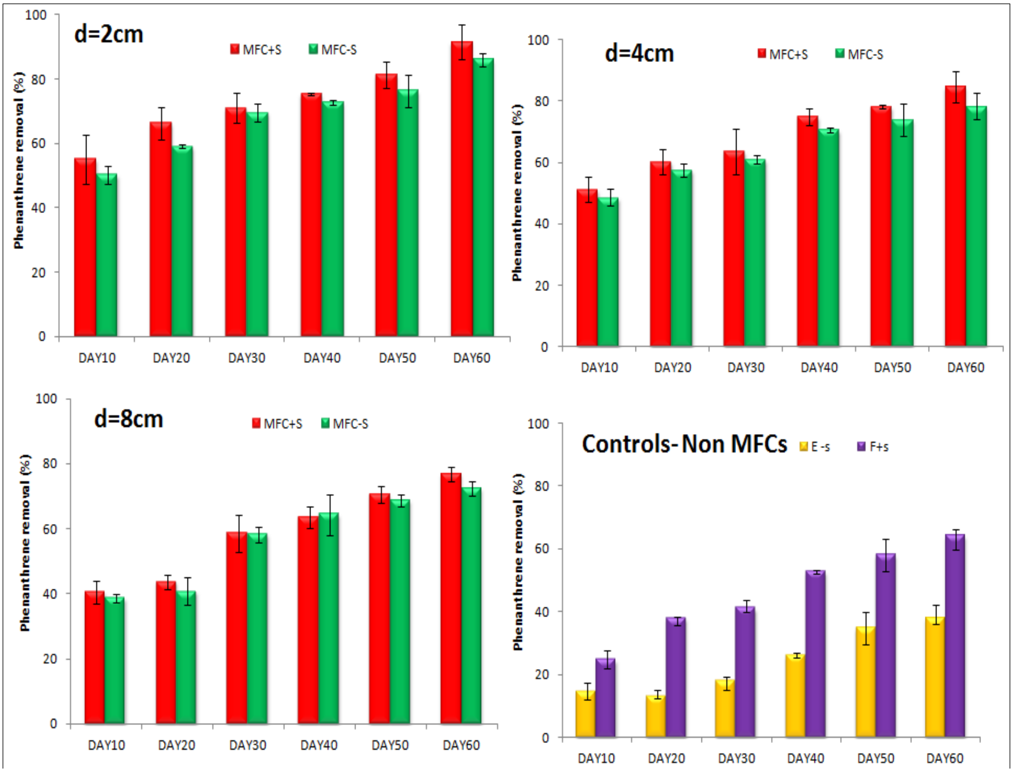

Figure 2. Phenanthrene removal in soil at distance of 2 - 8 cm from the anode during the operating period in MFC with no surfactant (MFC-S) and MFC with surfactant (Tween 80, 250 mg L^{-1}) amendment (MFC+S) using indigenous soil microorganism as inoculum source. Controls were prepared in the same reactors but with no electrodes. Error bars ± SD are based on triplicate measurements. E-S and F+S means controls without and with surfactant addition respectively.

There was continuous current production in MFCs over the experimental period which perhaps was sustained through steady mass transfer of the substrate towards the electrode. However, the phenanthrene degradation speeds in test MFCs is more than that of the control systems with better impact of MFCs on degradation regardless of their distances from the anode’s outer surface.

1. Consequentially, this may have led to improved mass transfer to the electrode and supported faster degradation rates.

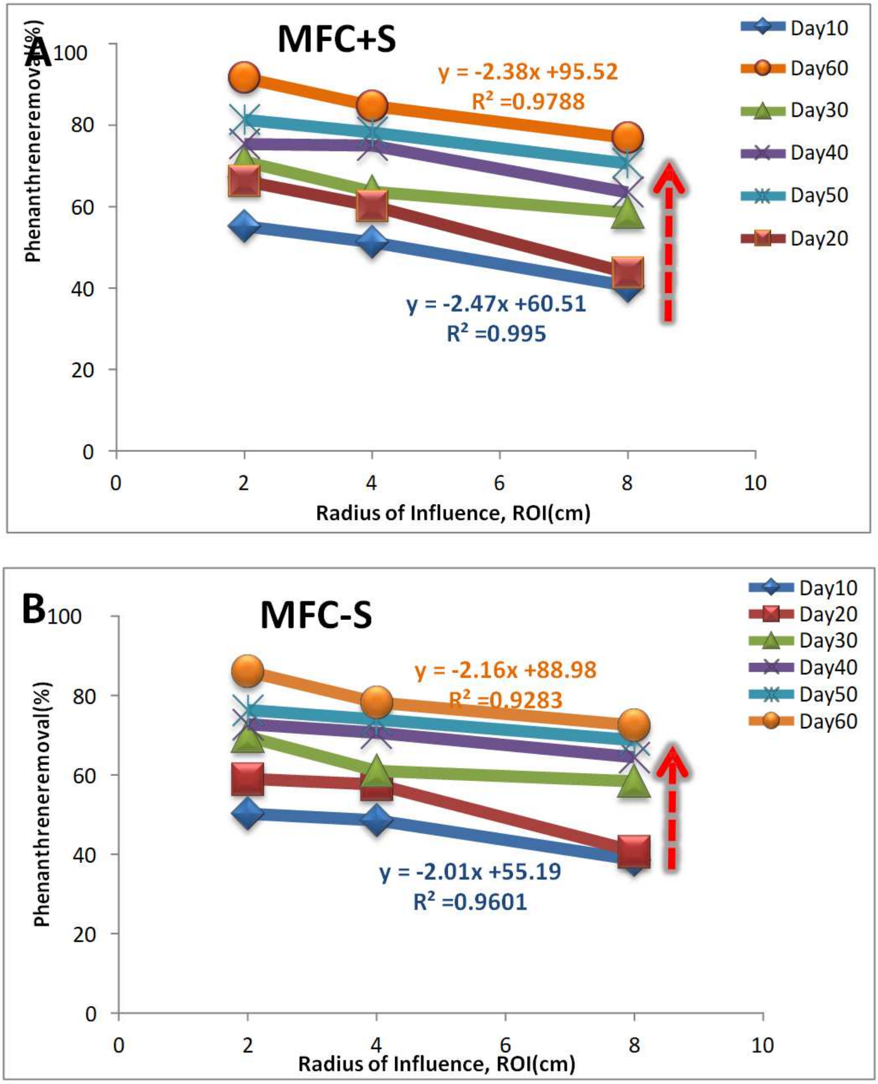

Figure 3. Relationship between phenanthrene removal and the radial distance from the MFC anodes at different sampling times. Dashed arrows indicate that maximum ROIs were expanding as reflected by the flattening of the slopes.

Phenanthrene depletion rates increased with period and attained 84.5-91.6 % in MFC+S and 78.3-86.1 % in MFC-S respectively (compared to 37.9-64.1 % in controls) with the phenanthrene fraction remaining in the soil being about the same for entire radial distances from anodes (Figure 2). There was a statistically substantial difference (at p=0.001) among the MFC+S and MFC-S reactors at all distances from the anode over the incubation period. The enhanced degradation performance observed in MFC+S reactor compared to MFC-S reactor may be attributed to the contribution from the surfactant added in enhancing phenanthrene availability in the soil.

The amount of surfactant used up during the study (250 mg L^{-1}) seems to enhance the surfactant sorption on soil, which may have caused increased in phenanthrene separating on soil particles. The result of this study conforms with former findings conducted by Lu *et al.* [23] on the influence of ROI on improved petroleum-hydrocarbon bioremediation in polluted soil in MFCs by means of two different cheap electrodes-biochar and graphite electrodes.

In this study, the configuration of the lab-based MFC bioreactor dictates the determination of the ROI for the soil MFCs. However, since MFC performance is a function of measured ROI, therefore calculated ROIs can be extrapolated based on such experimentally-obtained data. The linear regression equations derived for active MFCs at day 10 and day 60 can be used to descriptively explain and predict the extension of ROIs with respect to time in this saturated soil environment (Figure 3A and 3B). The maximum ROI is the maximum distance from the MFC anode at which phenanthrene removal efficiency is zero percent in reference to the baseline (anaerobic) control. Since day 10 up to 60th day, the estimated maximum ROI augmented from 24-27 cm to 39-41 cm with further extension in maximum ROI predicted with increase in time of soil MFC operation.

Lu *et al.* [23] demonstrated a further increase in maximum ROI at longer periods (about 120 days) of MFC operation under similar operating conditions corroborating the findings of this study. The radius of influence of a particular remediation technology significantly determines its remediation efficiency and cost effectiveness over other technologies in line with environmental considerations and therefore it is pivotal for its selection as a preferred remediation strategy. The knowledge of the ROI could also be very useful in the determining adequate anode electrode size and MFC reactor spacing in large scale field applications.

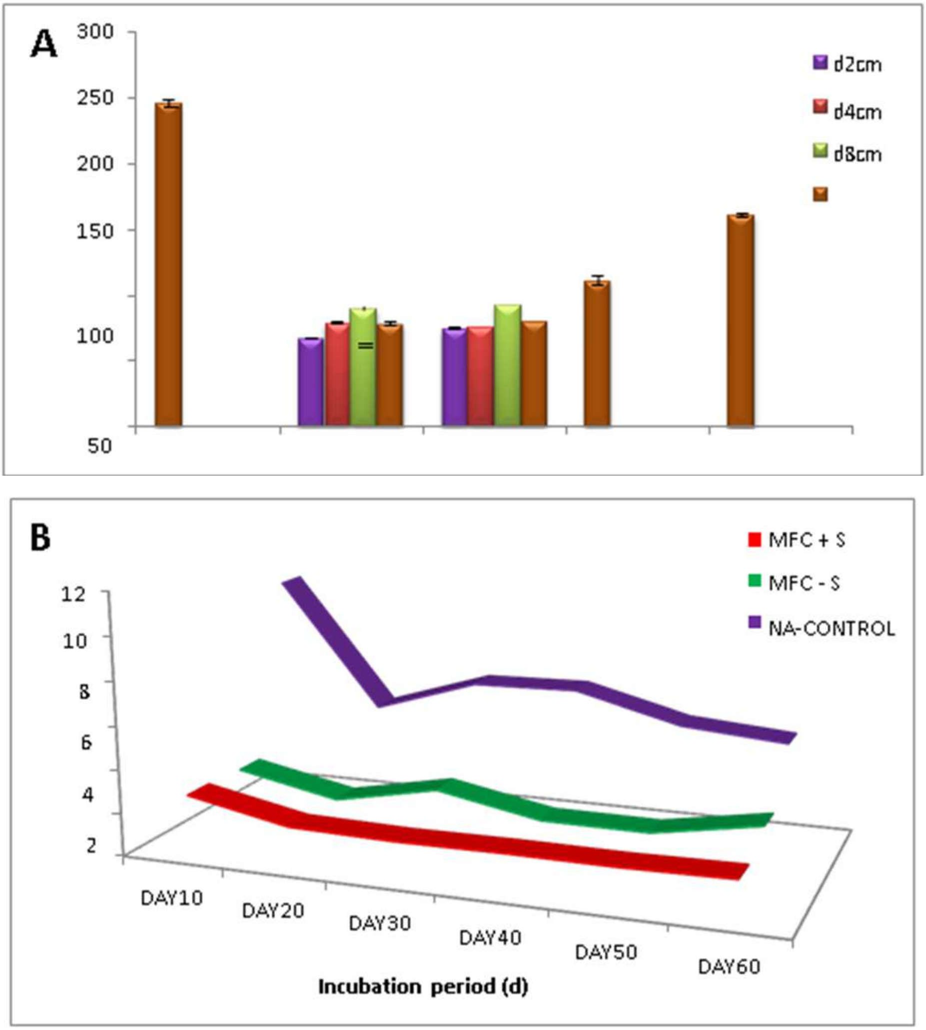

Figure 4. (A) Total phenanthrene removal at different distances from the anode at the end of testing period in MFC with no surfactant (MFC-S) and MFC with surfactant (Tween 80, 250 mg L^{-1}) amendment (MFC+S). (B) Phenanthrene pore water concentration in the overlying water in the saturated soil MFC. Controls were prepared in the same reactors but with no electrodes. Ravg is the average of removal efficiencies at different radial distance from the anode. The results are the mean of triplicate samples and error bars represent standard deviation of the mean. E-S and F+S means controls without and with surfactant addition respectively. NA-CONTROL is the anaerobic (non-MFC) control.

The phenanthrene concentration in the aqueous phase of the soil MFCs was relatively constant across both test MFCs (i.e. MFC+S and MFC-S reactors) over the period of MFC operation but was significantly lower than the control reactors indicating better degradation efficiency (Figure 4B). The insignificant change in pore water phenanthrene concentration (especially in MFC+S and MFC-S reactors) might be due to the dynamic balance in the phenanthrene partitioning between the soil-phase and the aqueous phase. The dynamic balance in phenanthrene partitioning between the aqueous-soil interface suggests a possible balance between the phenanthrene degradation and desorption rates in the MFC.

Figures 2 and 4A clearly demonstrate that phenanthrene degradation near the electrode was significantly enhanced relative to the control reactors. Phenanthrene fractions remaining on all MFC anodes at the end of the test period were less than 10 %, indicating that the majority of the PHE adsorbed by the electrodes were biodegraded by anodic microbial respiration rather than chemical/physical adsorption and that the adsorption process merely enhanced faster biodegradation rates.

The removal of phenanthrene was similar to that of TCOD, as shown in Figure 5.

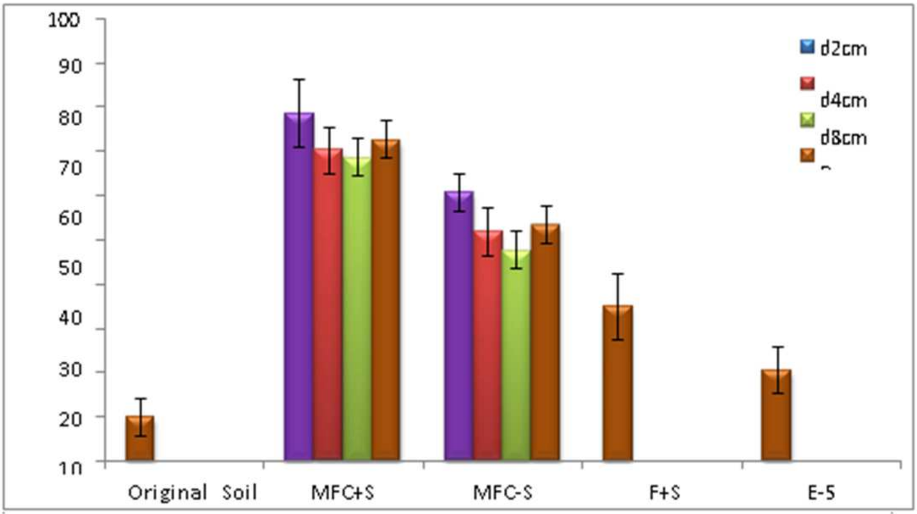

Figure 5. Percentage total COD (TCOD) removal at different distances from the anode for the active MFCs and control reactors at the end of 60 days of MFC operation at ambient temperature. Ravg is the average of removal efficiencies at different radial distance from the anode. Values are means of triplicate measurements ± SD. E-S and F+S mean controls without and with surfactant addition respectively.

Figure 5 shows the TCOD removal under different MFC conditions at varying radial distance from the anode. TCOD removal (which is 72.8 % on the average across all radial distances from the anode) in MFC with surfactant amendment, MFC+S, was significantly higher (20 %) than in MFC with no surfactant (MFC-S). This suggests that surfactant addition may have enhanced bioavailability of not only phenanthrene, but other organic matter present in the soil.

There was a negative linear relationship between the phenanthrene removal efficiency and the radial distance from the MFC anodes (Figure 3). The slope of the ROI gradually becomes more positive relative to time, indicating a steady ROI’s expansion with respect to time of reactor operation.

In this study, bromate removal in the cathode chamber coupled with phenanthrene degradation was monitored over the test period. Bromate removal in both MFCs was about 80-95 % on average which is significantly higher than the open circuit controls (15-40 %) over the 60day period of MFC operation (Figure 6). In the open circuit MFCs, the cathode and the anode terminals are physically separated and thus there is no transfer of electrons to the cathode which are needed for the electrochemical reduction of bromate to bromide ions (that are non-toxic). However, the small bromate reduction (15-40 %) observed in the open circuit MFC, as in this study, could be due to possible electron transfer across the permeable membrane from the anode to the cathode. This interstitial electron transfers especially in soil systems accounted for possible reduction or oxidation of pollutants in open circuit MFCs reported previously by Huang *et al.* [24] and Nielsen *et al.* [25].

Findings of this study have for the very first time demonstrated the simultaneous removal of two pollutants at both chambers and notably, using bromate as terminal electron acceptor in the cathode in lieu of Pt-catalysed oxygen reduction. Previous studies on soil MFCs had used Pt-coated cathodes which are expensive and cannot be deployed in deep subsurface environments where air/oxygen is largely limited.

Moreover, this study demonstrated that a tubular MFC configuration can significantly enhance phenanthrene biodegradation up to 293 % of that from the baseline reactor with the enhanced biodegradation from the MFC anodes extending even to an ROI of 8 cm (Figure 2). This results underpins the deplorability of this MFC design in real field practices to boost significantly, the biodegradation of petroleum-polluted soils coupled with bromate removal. This passive remedial technology is environmentally friendly and can significantly reduce clean-up time in a cost effective manner.

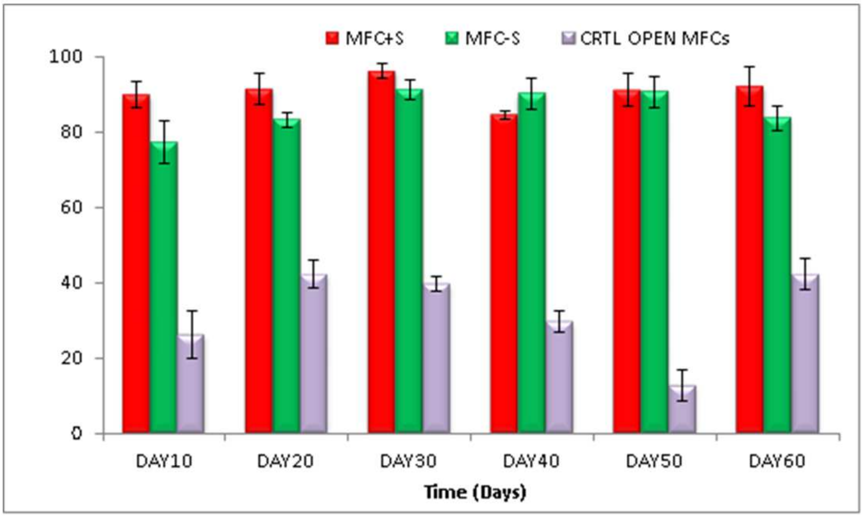

Figure 6. Bromate removal efficiency in the tubular MFC reactor during 60 days operation at incubation temperature between 25 and 30 °C. The error bars ±SD were based on averages measured in triplicate.

3.2. Voltage generation and electrochemical characterisation of performance of the soil MFC

Phenanthrene and bromate removal during tubular MFC operation over the test period was accompanied with concomitant biogenic electricity generation as observed in Figure 7. Current density reached approximately 60 mAm^{-2} and 53 mAm^{-2} for MFC+S and MFC-S respectively during MFC operation (across a 1000 Ω resistor). There was a good relationship between electricity generation and phenanthrene microbial degradation during the MFC operation, after the completion of the lag phase. The maximum power density obtained for MFC+S and MFC-S were 4.69 mWm^{-2} and 4.06 mWm^{-2} respectively, during the experimental period. These voltage generation outcomes were like the former reports on the retorts of waterlogged-contaminated soils in electricity generation by MFCs (Huang *et al.* [24], Wang *et al.* [1], Lu *et al.* [23]). The gradual increase in current generation may be due to bacterial acclimation and marked rise in the activity of the electrochemically-active microbial population in the soil.

Current output during reactor operation was erratic which could be probably due to the production of biotransformed intermediate products resulting from phenanthrene degradation and mass transfer limitations during the long operational periods (Lu *et al.* [23]; Huang *et al.* [24]).

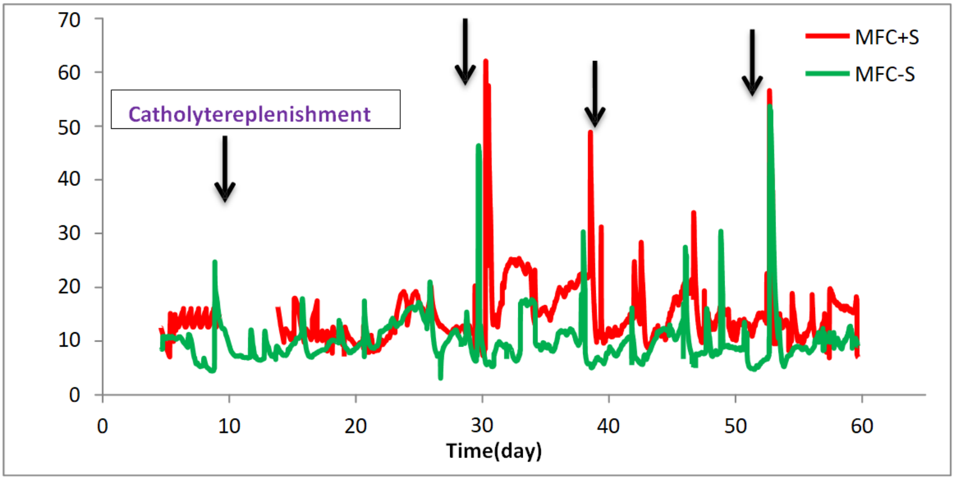

Figure 7. Current densities of MFC+S and MFC-S during the tubular MFC operations over 60 days incubation period (Rext = 1000 Ω). Black arrows indicate the points for catholyte replenishment.

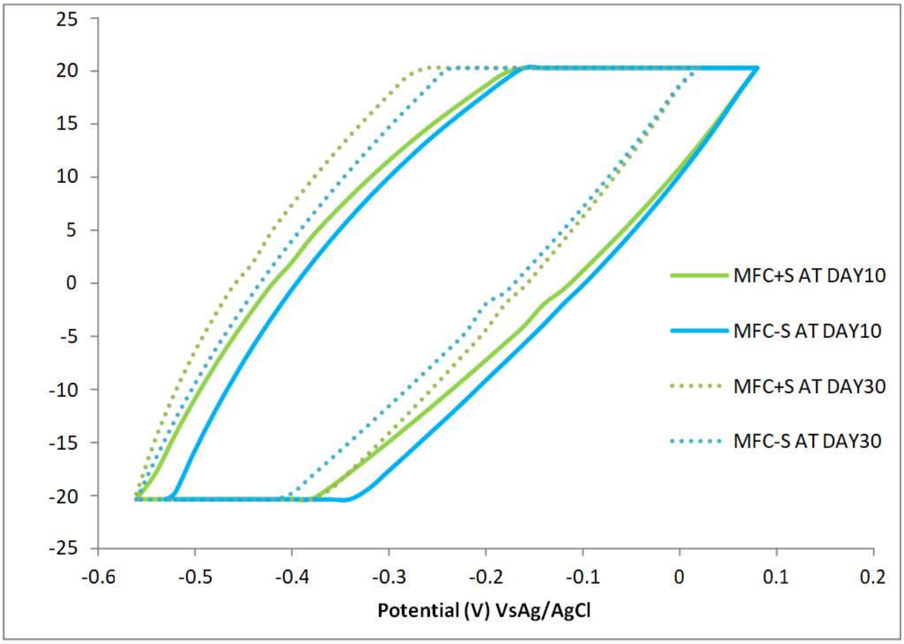

Figure 8. The CV curve for phenanthrene in MFC with no surfactant (MFC-S) and MFC with surfactant (Tween 80, 250 mg L^{-1}) amendment (MFC+S) at day 10 (solid line) and day 30 (dashed line) at sweep rate of 10 mVs^{-1}.

The CV (cyclic voltammograms) of the anode chamber of the MFC was analyzed during incubation at day 10 and 30 (Figure 8). The cyclic voltammograms for both MFC+S and MFC-S showed a substantial oxidation/reduction peak potential shift as phenanthrene microbial degradation proceeded in the MFCs from day 10 to 30, indicating a detectable drop in anode/oxidation potential resulting from the increasing microbial electrochemical oxidation processes occurring at the anode. However, there was more slight shift in redox potential in MFC with surfactant amendment (MFC+S) than in MFC with no surfactant as shown in Figure 8. This perhaps, might indicate positive impact of surfactant addition by possibly increasing phenanthrene bioavailability and mobility within the soil matrices or could act as redox electron shuttle for ferrying electrons to the anode.

The addition of surfactant to MFCs, as shown in this study, could enhance phenanthrene removal and improve electrochemical performance of MFC. Similarly, Wu *et al.* [26] reported observed enhanced toluene degradation and power generation in MFCs amended with a surfactant, pycocyanin which also corroborates findings from this study.

Findings of this study have demonstrated the potential practical application of this tubular-type soil MFC system for degradation of hydrocarbon contaminated subsurface soil environments coupled with concomitant bioelectricity production. Electrical outputs generated in soil MFCs could be employed in the monitoring contaminant's degradation profile, reduce the frequency of soil samples (in field applications) and electricity generated during the biodegradation can be used as power for remote sensors.

3.3. Changes in physicochemical characteristics of soil

Changes in physicochemical properties of the soils such as pH, electrical conductivity and total dissolved solid (TDS) are one of the key parameters for quantification and validation of hydrocarbon removal driven by microbial action (Figure 9). The soil pH values for all the MFCs except the controls, decreased up to 0.23 pH units in the first 10 d at a radial distance of 2 cm from the BES's anodes each (relatively lower than those obtained at radial distances 4 and 8 cm, demonstrating a minor proton build-up was noticed near the anode). A rise in electrical conductivity and TDS of about 25-54 % and 17-37 % was closely associated with decrease in pH. A possible explanation for the observed trend might be the adsorption of ions present in the soil matrix and accumulated hydrogen ions very close to the anode.

From day 10 to 60, fluctuations in pH, EC and TDS were observed at all radial distances from the anode for active MFCs at each sampling point. The observed fluctuation in physicochemical properties of the soil may possibly be due to the dynamic formation of intermediate readily oxidisable organic acids from phenanthrene metabolism and its subsequent consumption which in turn, resulted into dynamic changes in microbial population distribution and redox potentials at the anode during the test period (Du *et al.* [27], Allen *et al.* [28]). Notably, development of ionic species such as intermediate compounds during the biodegradation pathways and dissolution of minerals may lead to an increase of conductivity and TDS in soil, especially near the anodes (Allen *et al.* [28], Wang *et al.* [1]). Soil microbial activity declined as EC increased and this might greatly influence other soil chemical activities such as respiration, nitrification advection/adsorption, denitrification, and residue decomposition (Allen *et al.* [28], Johnsen *et al.* [29]).

Findings from the data analysed above vividly indicated that radial distance from the anode of active MFCs was directly related to increase in phenanthrene removal catalysed by high microbial activity at distances close to the anode. As presented in Table 1, phenanthrene removal is correlated negatively with TDS in the soil ($p < 0.05$) an electrical conductivity ($p < 0.05$) but positively correlated with total COD (TCOD). However, from statistical analysis based on the findings in the study, there is no statistically significant correlation between phenanthrene removal, bromate removal and pH, indicating that phenanthrene removal does not necessarily depend on the pH or bromate removal rates. Notably, bromate removal on the other hand is negatively correlated with pH ($p < 0.05$). Such correlations, associated with phenanthrene degradation and bromate removal, gives a holistic view on potential of MFC systems in improving phenanthrene removal coupled with bioelectricity generation and supports the restoration of the contaminated soil to its natural ecological status.

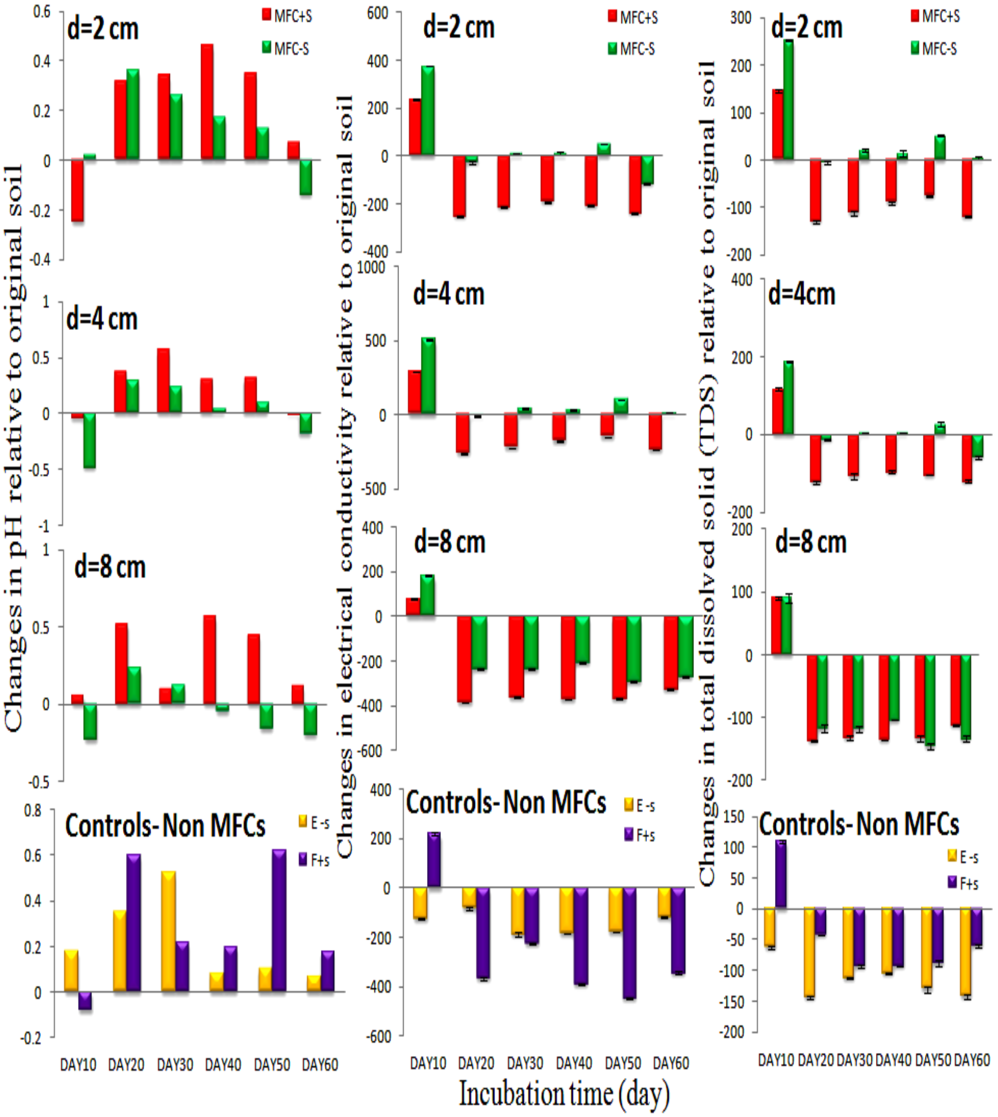

Figure 9. Changes in soil pH, TDS and electrical conductivity in MFC at different distance from anodes and control reactors over the tested period. The error bars ±SD were based on averages measured in triplicates. E-S and F+S mean controls without and with surfactant addition respectively.

Table 1. Correlation matrix between selected physicochemical parameters, phenanthrene and bromate removal over 60 days experimental period in MFC-S and MFC+S reactors. Data analysed at a level of significance, $p = 0.01$.

	Phenanthrene	**Bromate**	**TDS**	**EC**	**TCOD**
Bromate	0.004	-			
TDS	-0.365	-0.008	-		
EC	-0.39	-0.011	0.998	-	
TCOD	0.804	0.001	-0.099	-0.117	-
pH	0.033	-0.298	-0.445	-0.431	0.007

Correlation: Strong (positive, 0.5 to 1.0 or negative, −1.0 to −0.5). Medium (positive, 0.3 to 0.5 or negative, −0.5 to −0.3). Small (positive, 0.1 to 0.3 or negative, −0.3 to −0.1). None (positive, 0.0 to 0.09 or negative, −0.09 to−0.0).

3.4. Toxicity determinations in contaminated soil after MFC treatment

The disposal of phenanthrene-contaminated soil can be performed only when the pollutant levels and other toxic organic intermediate products are within permissible concentration levels set by relevant regulatory agencies. This ensures it is environmentally safe and pose no immediate danger to human health and ecosystems; the ultimate goal of any successful remediation process (Liu *et al.* [30]; Melo *et al.* [31], Ayed *et al.* [32]). Microbial pollutants degradation usually leads to partial mineralization, thus the formation of degradation products with unknown chemical and toxicological characteristics which sometimes may even be more toxic than the parent pollutant. The percentage relative inhibition of the growth of bioluminescent marine bacteria, *V. fischeri*, in soil extract taken at the start and end of MFC operational period is shown in Figure 10.

Bioluminescence based acute toxicity assays conducted using *V. fischeri* indicated a significant ($p < 0.01$, t-test) decrease in toxicological level by 65 % and 35 % in MFC amended with surfactant (MFC+S) and MFC with no surfactant (MFC-S) respectively compared to baseline controls. From Figure 10, the MFC reactors and baseline controls after 60 days of incubation were generally less toxic than at the start of treatment. A possible explanation the observed decrease in toxicity level may be attributed to the absence or very small amount of phenanthrene and the formation of lower molecular weight intermediate products of no or less toxic effect. The findings of the current study are in agreement with previous studies under similar soil conditions (Rodrigo *et al.* [33], Hamdi *et al.* [34]).

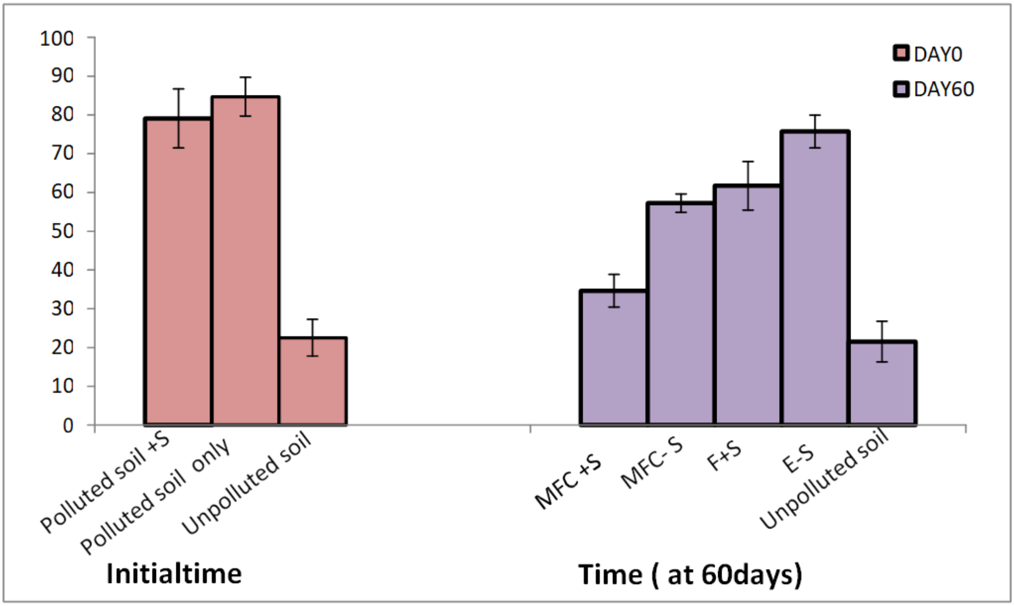

Figure 10. Toxicity levels in soil extractions of polluted soil with and without MFC treatment operated at 25-30°C over 60 days incubation period. The error bars represent 5% of deviation of the mean value for triplicate measurements. MFC-S and MFC+S are MFC with no surfactant and MFC with surfactant (Tween 80, 250 mg L^{-1}) amendment respectively. E-S and F+S mean controls without and with surfactant addition respectively.

Eco-toxicity testing is one of the remediation techniques employed in the assessment of the ecological profile of treated sites and may inform decisions for on-site treatments towards a successful reclamation of the contaminated site (Hankard *et al.* [35], Vogt *et al.* [36], Sarkar *et al.* [3]).

Therefore, this study has demonstrated the detoxification capability of MFC system over natural attenuation (i.e. a do-nothing scenario) in the treatment of phenanthrene-contaminated soil in a timely and effective manner under the same environmental condition.

4.0. Conclusion

In this study, a tubular MFC system performance in phenanthrene-contaminated soil was investigated. This MFC system significantly enhanced the biodegradation efficiency of phenanthrene (86 %) in the soil within a ROI up to 8 cm compared to non-MFCs control with a projected maximum ROI up to 40 cm. The findings of this study established for the first time, the simultaneous removal of phenanthrene and bromate (95%) coupled with concomitant bioelectricity generation using MFC systems.

MFC technology may be used for in situ decontamination of soils due to its potential detoxification capacity and could be deployed directly as a prototype-MFC design in field applications or integrated with existing infrastructure. Electricity generated could be used to power wireless sensors for remote site monitoring and as an indicator for real-time contaminant degradation profiling thus greatly reducing the cost of frequent soil samples analysis for pollutant degradation monitoring as usually demanded while using other non-bioelectrochemical, conventional remediation technologies.

Acknowledgements

Financial support for this research was provided by the Petroleum Development Technology Fund (PTDF), Nigeria. Their financial support is duly acknowledged.

Conflict of Interest

The authors have declared no conflict of interest.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

Table 2. Baseline characterization of the original soil sample.

Parameter	Value
pH	7.6
Soil conductivity (µs/cm)	664
Soil bulk density (g/cm^3)	1.1
Particle size distribution:	
% Clay (0-2µm)	9
% Silt (2-63µm)	28
% Sand (63 µm - 2mm)	63
Textural class of soil:	Sandy Loam
Total Nitrogen(%)	0.297
Total carbon(%)	4.497
Total organic carbon (%)	3.947
Total inorganic carbon (%)	0.549
Organic matter(%)	6.8
Carbon: Nitrogen (C:N) ratio Background	15
phenanthrene (mg/kgDS)	1.950
Water soluble anions (mg/kgDS):	
Cl	27.67
N(NO$_3$)	7.85
S(SO$_4$)	7.89
P(PO$_4$)	15.90
N(NO$_2$)	2.38
Water extractable metallic ions (mg/kgDS):	
K	440
Ca	3124
Mg	92
Na	9.7
Al	0.61
Fe	0.93
Exchangeable cations cmol(+)/kg DS):	
K	1.124
Ca	15.590
Mg	0.754
Na	0.042
Al	0.007
Fe	0.005

DS: Dry soil

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